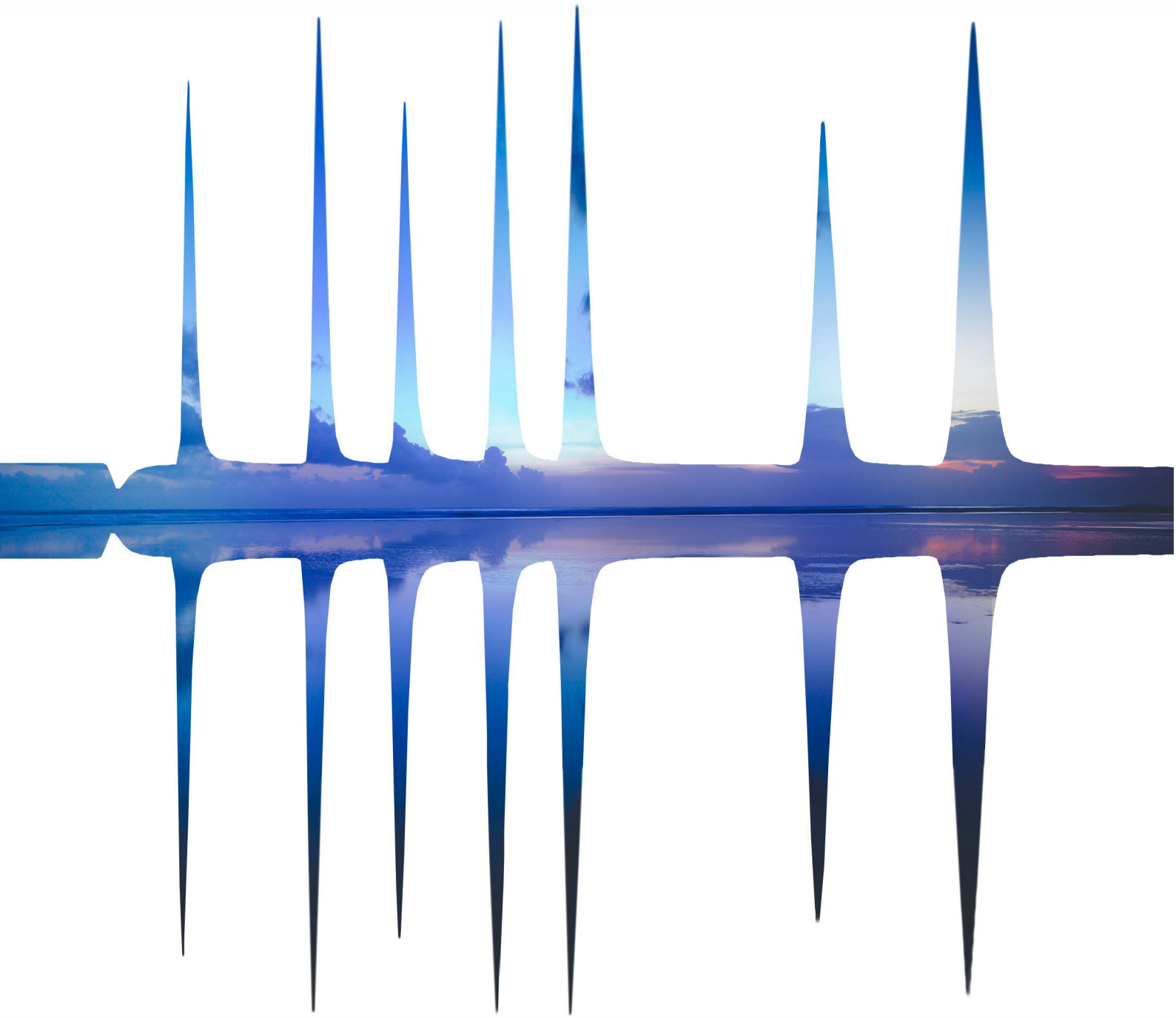
Dr. Joachim Weiss

BASICS OF ION CHROMATOGRAPHY



Separation Mechanisms, Stationary Phases,
Detection Methods, and Applications

ThermoFisher
SCIENTIFIC

Basics of ion chromatography

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Preface

With the publication of Hamish Small's legendary paper in Analytical Chemistry in 1975, the next year will mark the 45th anniversary of the introduction of *Ion Chromatography*. Over these four decades, ion chromatography not only became the most dominant method in ion analysis but also developed into a significant chromatographic technique within the field of separation science. While in its earliest embodiments, IC was focused primarily on the analysis of inorganic anions; today IC has an important role in the analysis of organic and inorganic anions and cations. Although separations of ions by ion-exchange chromatography prevail, other liquid chromatographic techniques, such as ion-exclusion chromatography, reversed-phase liquid chromatography in the ion-suppression mode, and even hydrophilic interaction and mixed-mode liquid chromatography, are also used today. Thus, the definition of the term ion chromatography became much broader over the years to be an umbrella term today for all liquid chromatographic techniques that are suitable for separating and detecting ionic and ionizable species.

The objective for this eBook is to address analytical chemists who are looking for an introduction to ion chromatography that can help provide an overview on the existing methods, stationary phases, detection principles, and applications offered by Thermo Fisher Scientific.

At this point, I would like to express my sincere gratitude to my many colleagues in all parts of the world who contributed their experience and knowledge to the preparation of this eBook and to my family.

Niedernhausen, September 2019 Joachim Weiss

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1
Introduction

Ion chromatography (IC) was introduced in 1975 by Small, Stevens, and Bauman [1] as a new analytical method. Within a short period of time, ion chromatography evolved from a new detection scheme for a few selected inorganic anions and cations to a versatile analytical technique for ionic species in general. Separation is usually carried out via ion-exchange chromatography, although other separation modes, such as ion-exclusion chromatography, ion-pair-chromatography, reversed-phase liquid chromatography in the ion-suppression mode, and mixed-mode liquid chromatography, are also used. Electrolyte solutions must be used to elute ions from an ion exchanger; the respective retention times of the ions are determined by their different affinities to the stationary phase. For the sensitive detection of ions via their electrical conductance, the separator column effluent is passed through a suppressor device, which chemically reduces the eluent background conductance while at the same time enhancing the analyte conductance. The applicability of ion chromatography was expanded by combining it with other detection methods, such as UV/Vis, amperometry, fluorescence, ICP, and mass spectrometry (MS).

In the following, the basics of the various separation and detection modes are discussed. In addition to the theoretical introduction of the various ion chromatography techniques, real-world application examples are the main focus of this eBook. The applications being chosen represent a cross section of the various application areas. A summary of common sample preparation techniques is summarized in Section 8.9.

2
Basics of ion chromatography

Ion chromatography (IC) is a physical-chemical separation method, which is based on the partition of an analyte between a liquid mobile phase and a solid stationary phase. The enormous improvement in the performance of modern ion chromatography is attributed to the increasingly better understanding of the stationary phases being used and the type of interactions being observed at the stationary phase surface.

The basic components of an ion chromatograph are shown schematically in Figure 2.1. It resembles the setup of conventional HPLC systems. The most important part of the chromatographic system is the separator column. The pioneering work of Small et al. was the development of low-capacity ion-exchange resins of high chromatographic efficiencies, which could be prepared reproducibly. The most commonly employed detector in ion chromatography is the conductivity detector, which is typically used in combination with a suppressor device ("suppressed conductivity detection"). The main function of the suppressor system is to chemically reduce the high background conductivity of the electrolytes in the eluent, and to convert the sample ions into a more conductive form. In this way, sensitivity and selectivity of the method are significantly enhanced.

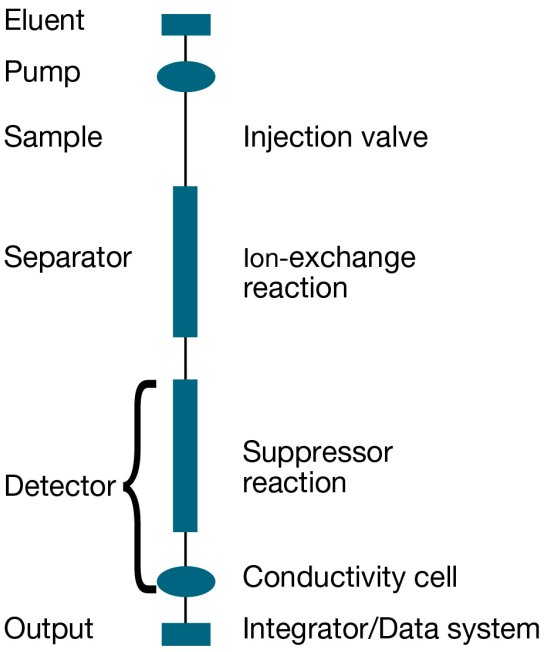


Figure 2.1 Basic components of an ion chromatograph.

2.1 Types of ion chromatography

Modern ion chromatography as an element of liquid chromatography is based on three different separation mechanisms, which also provide the basis for the nomenclature in use.

Ion-exchange chromatography (HPIC)
This separation method is based on ion-exchange processes occurring between the mobile phase and the ion-exchange groups bonded to the support material. Ion-exchange chromatography is used for the separation of both inorganic and organic anions and cations. Separation of anions is accomplished with quaternary ammonium groups attached to the polymer, whereas sulfonate-, carboxyl-, phosphonate, or mixtures of these groups are used as ion-exchange sites for the separation of cations.

Ion-exclusion chromatography (HPICE)
The separation mechanism in ion-exclusion chromatography is governed by Donnan exclusion, steric exclusion, sorption processes, and, depending on the type of separator column, by hydrogen bonding. Ion-exclusion chromatography is particularly useful for the separation of weak inorganic and organic acids from completely dissociated acids, which elute as one peak within the void volume of the column.

Ion-pair chromatography (MPIC)
The dominating mechanism in ion-pair chromatography is adsorption. Ion-pair chromatography is particularly suited for the separation of surface-active anions and cations, sulfur compounds, amines, and transition metal complexes.

Alternative methods

In addition to the three classical separation methods mentioned above, reversed-phase liquid chromatography (RPLC) in the ion-suppression mode [2] can also be used for the separation of polar and ionic species including phenols and long-chain fatty acids.

Moreover, applications of multidimensional ion chromatography utilizing mixed-mode phases are very interesting. In those separations, ion-exchange and reversed-phase interactions equally contribute to the retention mechanism of ionic and ionizable species [3].

3
Anion-exchange chromatography (HPIC)

In anion-exchange chromatography, quaternary ammonium bases are generally used as ion-exchange groups. When the counter ion of the ion-exchange site is replaced by a solute ion, the latter is temporarily retained by the fixed charge. The various sample ions remain for different periods of time within the column due their different affinities toward the stationary phase, and thus separation is brought about.

For example, if a solution containing bicarbonate anions is passed through an anion-exchange column, the quaternary ammonium groups attached to the resin are exclusively in their bicarbonate form. If a sample with the anions A^- und B^- is injected onto the column, these anions are exchanged for bicarbonate ions according to the reversible equilibrium processes given by Eqs. (3.1) and (3.2):

$$\text{Resin-NR}_3^+\ \text{HCO}_3^- + \text{A}^- \ \leftrightarrows\ \text{Resin-NR}_3^+\ \text{A}^- + \text{HCO}_3^- \qquad (3.1)$$

$$\text{Resin-NR}_3^+\ \text{HCO}_3^- + \text{B}^- \ \leftrightarrows\ \text{Resin-NR}_3^+\ \text{B}^- + \text{HCO}_3^- \qquad (3.2)$$

The separation of the anions is determined by their different affinities toward the stationary phase. The constant determining the equilibration process is the selectivity coefficient, K, which is defined as follows:

$$K = \frac{[\text{X}^-]_\text{s}\ \ [\text{HCO}_3^-]_\text{m}}{[\text{HCO}_3^-]_\text{s}\ \ [\text{X}^-]_\text{m}} \qquad (3.3)$$

$[\text{X}^-]_{\text{m,s}}$	Concentration of the sample ion in the mobile (m) or stationary (s) phase
$[\text{HCO}_3^-]_{\text{m,s}}$	Bicarbonate concentration in the mobile (m) or stationary (s) phase

3.1
Stationary phases

3.1.1
Nanobead-agglomerated anion exchangers

A special type of pellicular anion exchangers was first introduced in 1975 by Small et al. [1] in their introductory paper on ion chromatography. These stationary phases, which were originally called latex-based anion exchangers, have been further developed by Dionex Corporation (now part of Thermo Fisher Scientific). The structure of nanobead-agglomerated anion exchangers is depicted schematically in Figure 3.1.

Nanobead-agglomerated anion exchangers are composed of a surface-sulfonated poly(styrene-*co*-divinylbenzene) substrate with particle diameters between 5 and 10 µm and fully aminated, high-capacity porous polymer beads made of polyvinylbenzyl chloride or polymethacrylate, which are called nanobeads. The latter have a much smaller diameter (about 0.1 µm) and are agglomerated to the surface by electrostatic and van der Waals interactions. The pellicular structure of these anion exchangers is responsible for their relatively high chromatographic efficiencies. This complex system offers several advantages compared to other column packings, such as silica-based anion exchangers. The advantages are as follows:

- The inner substrate provides mechanical stability and a moderate back pressure.
- The small size of the nanobeads ensures fast ion-exchange processes and thus a high chromatographic efficiency of the separator column.
- Swelling and shrinkage are considerably reduced due to the surface-functionalization.

Nanobead-agglomerated anion exchangers are chemically very stable. With the exception of methacrylate-based resins, they are stable between pH 0 and 14. Even 4 mol/L sodium hydroxide is unable to cleave the ionic bond between the substrate particle and the nanobeads.

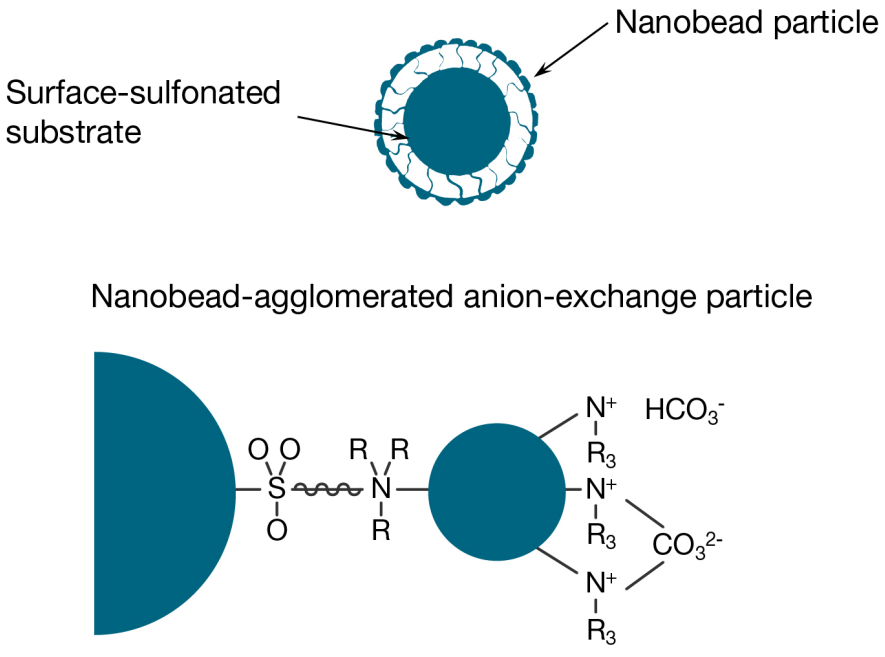


Figure 3.1 Structure of a nanobead-agglomerated anion exchanger.

The selectivity is altered by changing the chemical nature of the quaternary ammonium base. Since the nanobeads are synthesized in a separate step, it is possible to optimize the selectivity of a separator column for a specific analytical problem, either by varying the functional groups bonded to the nanobeads or by changing the degree of cross-linking.

Carbonate-selective nanobead-agglomerated anion exchangers At present, Thermo Fisher Scientific offers four different carbonate-selective, nanobead-agglomerated anion exchangers with different selectivities. The structural and technical characteristics of these separator columns are summarized in Table 3.1. Special columns for the separation of poly-valent anions, carbohydrates, amino acids, and oligonucleotides are also available.

Introduced in 1986, the Thermo Scientific™ Dionex™ IonPac™ AS4A-SC column is a universally applicable anion exchanger. For a long period of time it represented the state-of-the-art in ion chromatographic anion analysis. The elution profile in Figure 3.2 shows that a baseline-resolved separation of the seven most important inorganic anions can be obtained in less than 10 min. The Dionex IonPac AS4A-SC column is characterized by a high chromatographic efficiency and sample loadability. Due to the high degree of cross-linking of the nanobeads of 55%, the Dionex IonPac AS4A-SC resin is 100% compatible with typical HPLC solvents such as methanol and acetonitrile. Although the Dionex IonPac AS4A-SC column was recommended in the original US Environmental Protection Agency (EPA) Method 300.0 [4], fluoride is not separated from the system void and thus cannot be analyzed under standard chromatographic conditions. In addition, co-elution problems of disinfection by-product anions, such as bromate and chlorate with inorganic anions such as chloride and nitrate, are observed. Therefore, the resin is somewhat outdated and should only be used today for analyzing standard anions (except fluoride) in less contaminated samples.

Table 3.1 Structural and technical properties of carbonate-selective, nanobead-agglomerated anion exchangers.

Separator	Particle diameter (µm)	Degree of cross-linking (%)	Size of nanobead (nm)	Capacity (µequiv/col.)	Dimensions (length × i.d.) (mm)	Applicability
Dionex IonPac AS4A-SC	13	0.5	160	20 5	250 × 4 250 × 2	Universal high-performance separator
Dionex IonPac AS9-SC	13	20	110	30	250 × 4	Universal high-performance separator, especially suited for oxyhalides
Dionex IonPac AS9-HC	9	15	90	190 47.5	250 × 4 250 × 2	Universal high-capacity, high-performance separator, especially suited for oxyhalides
Dionex IonPac AS12A	9	0.2	140	52 13	200 × 4 200 × 2	High-performance separator, especially suited for fluoride and oxyhalides

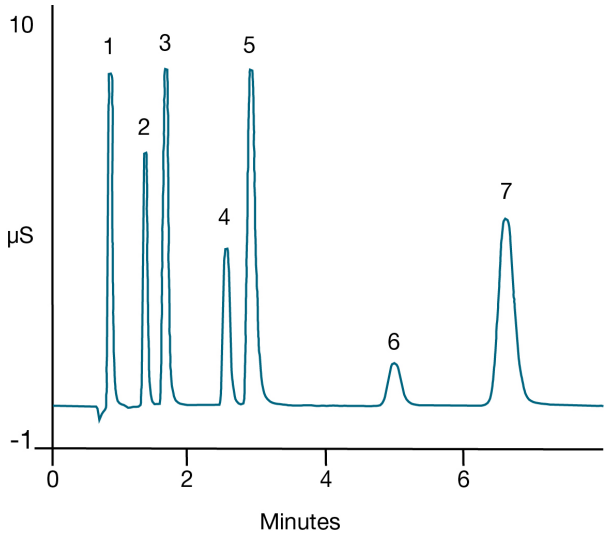


Figure 3.2 Elution profile of the Dionex IonPac AS4A-SC separator column for standard inorganic anions. Eluent: 1.7 mmol/L $NaHCO_3$ + 1.8 mmol/L Na_2CO_3; flow rate: 2 mL/min; detection: suppressed conductivity; injection volume: 50 µL; peaks: 3 mg/L fluoride (1), 4 mg/L chloride (2), 10 mg/L each of nitrite (3) and bromide (4), 20 mg/L nitrate (5), 10 mg/L ortho-phosphate (6), and 25 mg/L sulfate (7).

By agglomerating acrylate-based nanobeads on a 55% cross-linked ethylvinylbenzene/divinylbenzene substrate, a separation of chlorate and nitrate can be achieved; this was previously not possible using conventional anion exchangers. These two anions exhibit the same interactions with both polyvinylbenzene-based nanobead-agglomerated anion exchangers and directly aminated substrates, and thus co-elute at these stationary phases. This selectivity problem was solved with the Dionex IonPac AS9-SC anion exchanger, which was developed specifically for the separation of chlorate and nitrate. With 35 µequiv/g, the ion-exchange capacity of the Dionex IonPac AS9-SC column is slightly higher than that of the Dionex IonPac AS4A-SC column. The nanobeads are functionalized with a medium hydrophobic tertiary amine. Figure 3.3 shows a standard chromatogram obtained using a carbonate/ bicarbonate eluent.

As can also be seen in Figure 3.3, sulfite and sulfate are very well separated on the Dionex IonPac AS9-SC column. Even large concentration differences between these two anions do not prohibit their determination. Therefore, this stationary phase is especially suited for the analysis of flue gas scrubber solutions in desulfurization plants, where similar analytical problems occur. Compared to conventional anion exchangers, such as the Dionex IonPac AS4A-SC column, acrylate-based nanobeads are less pH stable.

A remarkable property of the Dionex IonPac AS9-SC column is the relatively short retention time of polarizable anions, such as iodide, thiocyanate, and thiosulfate. On the Dionex IonPac AS9-SC column, these anions, together with mineral acids, can be separated within 20 min using a sodium carbonate eluent (Figure 3.4). Although retention times are very different for nonpolarizable and polarizable anions, the latter ones do not exhibit tailing.

Using a macroporous support with a pore size of 200 nm instead of a microporous one, the acrylate-based nanobeads can also be agglomerated electrostatically inside the pores after sulfonation. Based on this technology, a solvent-compatible, pellicular packing material with a relatively high ion-exchange capacity of about 190 µequiv/column (250 mm × 4 mm i.d.) is obtained. This development was commercialized under the trade name Dionex IonPac AS9-HC. Figure 3.5 schematically shows the structure of such a macroporous support material. Both the Dionex IonPac AS9-SC column and the Dionex IonPac AS9-HC column are suitable for

the analysis of standard anions and oxyhalides in potable water and groundwater. Due to its higher capacity, the Dionex IonPac AS9-HC column is operated with a 9 mmol/L carbonate eluent. A representative standard chromatogram is shown in Figure 3.6.

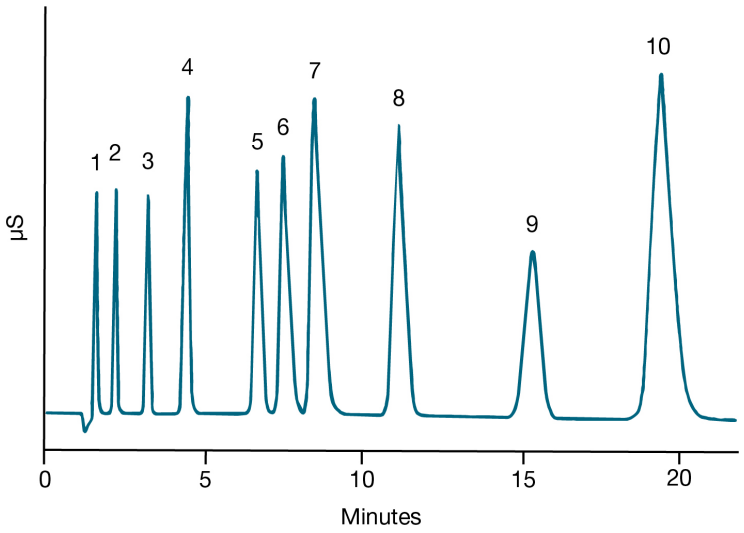


Figure 3.3 Separation of standard inorganic anions on the Dionex IonPac AS9-SC column. Eluent: 1.7 mmol/L $NaHCO_3$ + 1.8 mmol/L Na_2CO_3; flow rate: 1 mL/min; detection: suppressed conductivity; injection volume: 50 µL; peaks: 1 mg/L fluoride (1), 5 mg/L chlorite (2), 1.5 mg/L chloride (3), 6 mg/L nitrite (4), 10 mg/L bromide (5), 15 mg/L each of chlorate (6) and nitrate (7), 20 mg/L each of orthophosphate (8) and sulfite (9), and 25 mg/L sulfate (10).

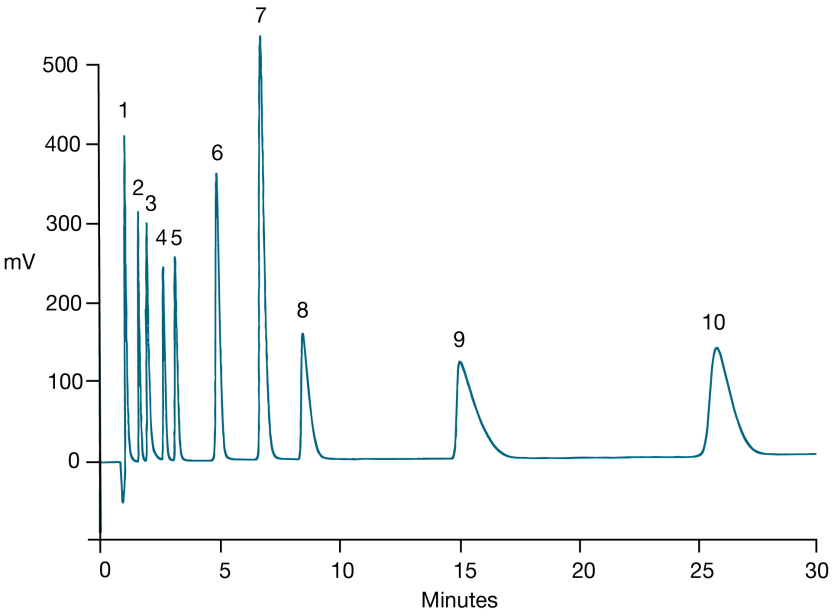


Figure 3.4 Separation of polarizable and nonpolarizable anions on the Dionex IonPac AS9-SC column. Eluent: 3 mmol/L Na_2CO_3; flow rate: 2 mL/min; detection: suppressed conductivity; injection volume: 25 µL; peaks: 2 mg/L fluoride (1), 2 mg/L chloride (2), 5 mg/L each of nitrite (3), bromide (4), and nitrate (5), 20 mg/L orthophosphate (6), 10 mg/L sulfate (7), 20 mg/L iodide (8), 20 mg/L thiocyanate (9), and 30 mg/L thiosulfate (10).

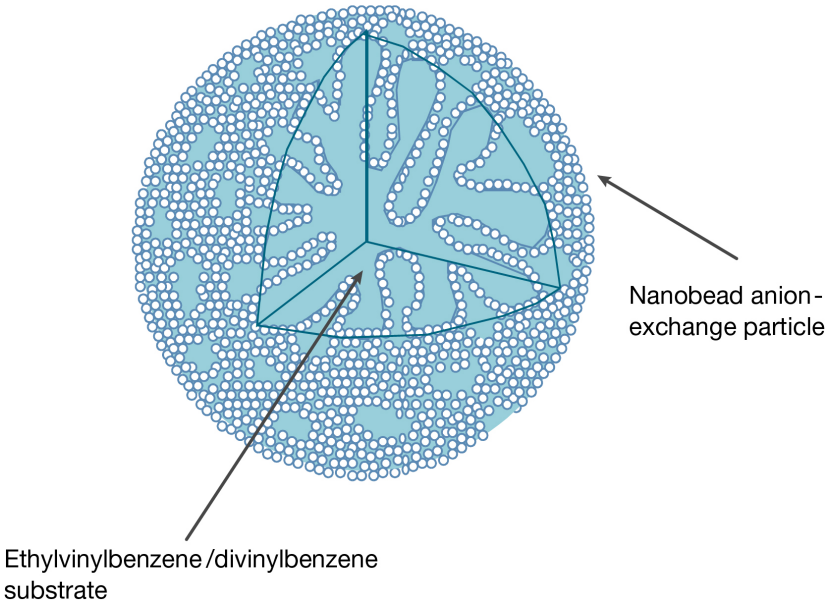


Figure 3.5 Schematic representation of the structure of a pellicular, macroporous support material.

The Dionex IonPac AS12A column was developed for the chromatographic analysis of fluoride and other mineral acids in water samples, allowing the separation of fluoride under isocratic conditions using a carbonate/bicarbonate eluent. For separating fluoride from the system void, nanobeads based on vinylbenzyl chloride with a degree of cross-linking of 0.15% have been synthesized. For solvent compatibility, the nanobeads were agglomerated on a highly cross-linked (55%) 9 µm macroporous EVB/DVB polymer. The ion-exchange capacity of this column (52 µequiv/column) is twice as high as that of the Dionex IonPac AS4A-SC column, which is somewhat remarkable considering the high water content of the nanobeads. As can be seen from the standard chromatogram in Figure 3.7, fluoride is well separated from the system void and can be analyzed together with other mineral acids and oxyhalides in less than 15 min.

Hydroxide-selective nanobead-agglomerated anion exchangers The structural and technical characteristics of the eleven different hydroxide-selective, nanobead-agglomerated columns with diverse selectivities, currently being offered by Thermo Fisher Scientific, are summarized in Table 3.2.

The development of hydroxide-selective anion exchangers was the prerequisite for the 1987 introduction of the gradient elution of anions in combination with suppressed conductivity detection. The objective of a gradient elution technique is to analyze anions having a wide range of retention characteristics, e.g., monovalent, divalent, and trivalent anions, within the same chromatographic run via a gradual increase of the ionic strength of the mobile phase.

Considering that a suppressor system is essential for the subsequent conductivity detection, a successful gradient elution is only feasible using a hydroxide eluent. Carbonate/bicarbonate mixtures are impractical for gradient elution. While a carbonate-based concentration gradient would lead to an outgassing of carbon dioxide in the suppressor, hydroxide eluents are converted to water that does not contribute to background conductivity. On the other hand, hydroxide as a monovalent eluent ion exhibits a much lower elution power. Therefore, the functionality and dimension of the nanobeads are different in comparison with carbonate-selective ones. The enormous power of anion-exchange chromatography with nanobead-agglomerated anion exchangers is illustrated in Figure 3.8, showing the gradient elution of a large variety of inorganic and organic anions on a Dionex IonPac AS11 column.

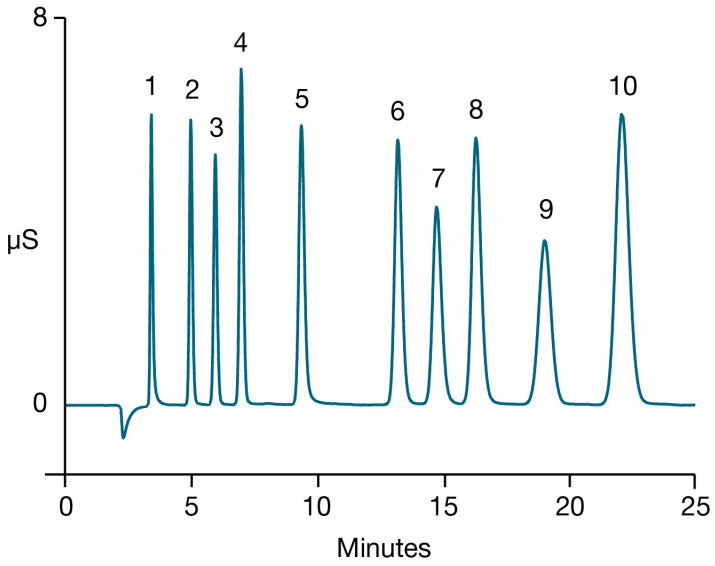


Figure 3.6 Separation of standard anions and oxyhalides on the Dionex IonPac AS9-HC column. Eluent: 9 mmol/L Na_2CO_3; flow rate: 1 mL/min; detection: suppressed conductivity; injection volume: 25 µL; peaks: 3 mg/L fluoride (1), 10 mg/L chlorite (2), 20 mg/L bromate (3), 6 mg/L chloride (4), 15 mg/L nitrite (5), 25 mg/L each of bromide (6), chlorate (7), and nitrate (8), 40 mg/L orthophosphate (9), and 30 mg/L sulfate (10).

Like the Dionex IonPac AS9-HC column, the Dionex IonPac AS11 column is also available in a high-capacity version, the Dionex IonPac AS11-HC column. The relatively high capacity of the Dionex IonPac AS11-HC column allows the injection of more concentrated samples that otherwise would lead to column overloading and peak broadening.

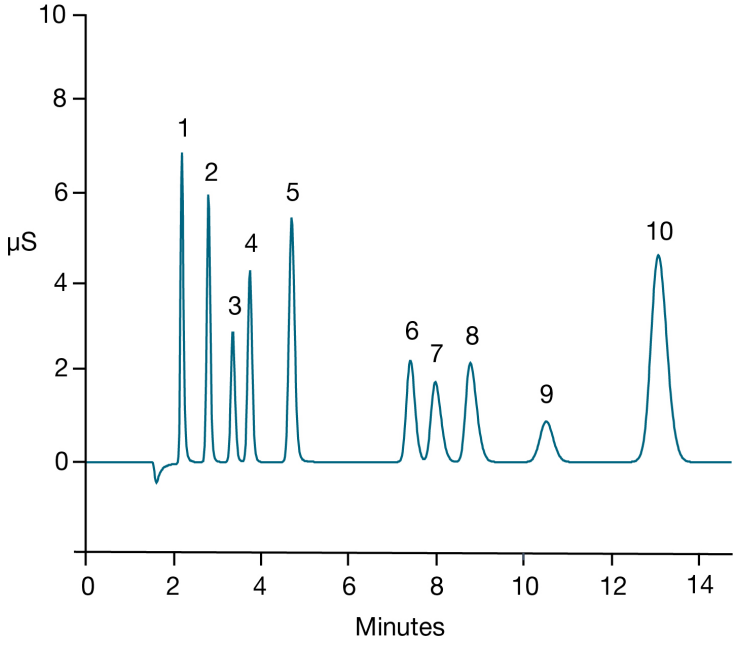


Figure 3.7 Separation of various inorganic anions on the Dionex IonPac AS12A column. Eluent: 0.3 mmol/L $NaHCO_3$ + 2.7 mmol/L Na_2CO_3; flow rate: 1.5 mL/min; detection: suppressed conductivity; injection volume: 25 µL; peaks: 3 mg/L fluoride (1), 10 mg/L chlorite (2), 10 mg/L bromate (3), 4 mg/L chloride (4), 10 mg/L each of nitrite (5), bromide (6), chlorate (7), nitrate (8), and orthophosphate (9), and 20 mg/L sulfate (10).

Table 3.2 Structural and technical properties of hydroxide-selective, nanobead-agglomerated anion exchangers.

Separator	Particle diameter (µm)	Degree of cross-linking (%)	Size of nanobead (nm)	Capacity (µequiv/col.)	Dimensions (length × i.d.) (mm)	Applicability
Dionex IonPac AS10	8.5	5	65	170 42.5	250 × 4 250 × 2	High-capacity separator with high affinity to bromide and nitrate
Dionex IonPac AS11	13	6	85	45 11	250 × 4 250 × 2	High-performance separator for gradient elution
Dionex IonPac AS11-HC	9	6	70	290 72.5 2.9	250 × 4 250 × 2 250 × 0.4	High-capacity, high-performance separator for gradient elution
Dionex IonPac AS11-HC-4µm	4	6	70	290 72.5 2.9	250 × 4 250 × 2 250 × 0.4	High-capacity, high-performance separator for gradient elution
Dionex IonPac AS16	9	1	200	170 42.5 1.7	250 × 4 250 × 2 250 × 0.4	High-performance separator for polarizable anions
Dionex IonPac AS16-4µm	4	1	200	170 42.5 1.71	250 × 4 250 × 2 250 × 0.4	High-performance separator for polarizable anions
Dionex IonPac AS17-C	10.5	6	75	30 7.5	250 × 4 250 × 2	High-performance separator for gradient elution of standard anions
Dionex IonPac AS18	7.5	8	65	285 75 2.85	250 × 4 250 × 2 250 × 0.4	High-capacity, high-performance separator for gradient elution of standard anions
Dionex IonPac AS18-4µm	4	8	65	171 45 1.71	150 × 4 150 × 2 150 × 0.4	High-capacity, high-performance separator for gradient elution of standard anions
Dionex IonPac AS18-Fast	7.5	8	65	171 45 1.71	150 × 4 150 × 2 150 × 0.4	High-performance column for fast analysis of common anions
Dionex IonPac AS18-Fast-4µm	4	8	65	171 45 1.71	150 × 4 150 × 2 150 × 0.4	High-performance column for fast analysis of common anions

The Dionex IonPac AS11-HC column is also available in the 0.4 mm format for capillary operation to offer the advantage of reduced operating costs due to the 100-fold reduction in eluent consumption and waste disposal. Capillary operation is ideal for limited sample volumes because of the higher mass sensitivity. Applications based on 4 mm columns can be directly transferred to the 0.4 mm format by reducing flow rate and injection volume, but the use of capillary ion chromatography hardware with a specifically designed fluidic system is mandatory to avoid extensive band broadening. Because the packing material of a capillary column is identical to that of an analytical column, the resulting chromatograms look essentially the same if operational parameters are scaled down correctly.

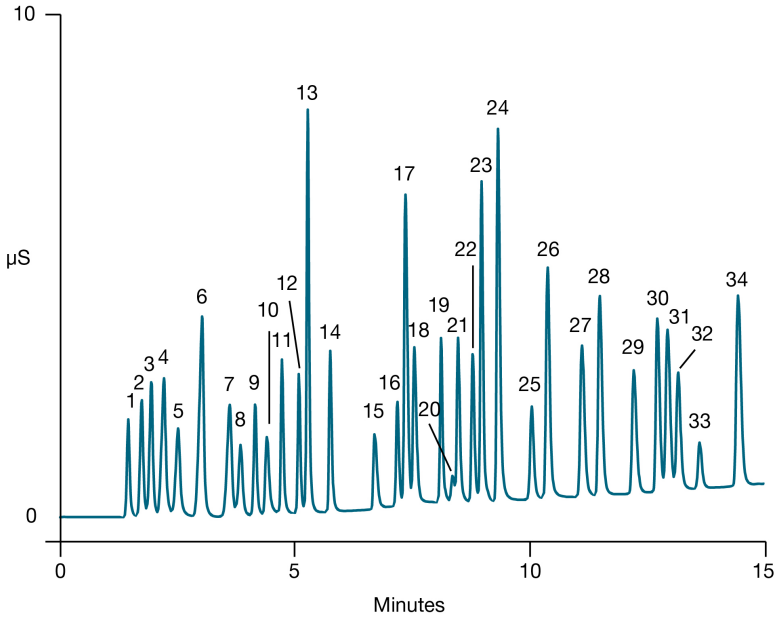


Figure 3.8 Gradient elution of inorganic and organic anions on the Dionex IonPac AS11 column. Eluent: (A) water, (B) 5 mmol/L NaOH, (C) 0.1 mol/L NaOH; gradient: 2 min 90% A + 10% B isocratically, then linearly to 100% B in 3 min, then linearly to 65% B + 35% C in 10 min; flow rate: 2 mL/min; detection: suppressed conductivity; injection volume: 25 µL; peaks: 5 mg/L each of isopropylethyl-phosphonate (1) and quinate (2), 1 mg/L fluoride (3), 5 mg/L each of acetate (4), propionate (5), formate (6), methylsulfonate (7), pyruvate (8), chlorite (9), valerate (10), mono-chloroacetate (11), and bromate (12), 2 mg/L chloride (13), 5 mg/L each of nitrite (14) and trifluoroacetate (15), 3 mg/L each of bromide (16), nitrate (17), chlorate (18), and selenite (19), carbonate (20), 5 mg/L each of malonate (21), maleate (22), sulfate (23), and oxalate (24), 10 mg/L each of ketomalonate (25), tungstate (26), phthalate (27), orthophosphate (28), chromate (29), citrate (30), tricarballylate (31), isocitrate (32), *cis*-aconitate (33), and *trans*-aconitate (34).

The selectivity and capacity of the recently introduced Dionex IonPac AS11-HC-4µm column are similar to the Dionex IonPac AS11-HC column but provide higher resolution due to the smaller particle size of the substrate. Available in 4 mm, 2 mm, and 0.4 mm formats, this column supports flow rates between 10 µL/min and 1.5 mL/min. Figure 3.9 illustrates the comparison of the Dionex IonPac AS11-HC and IonPac AS11-HC-4µm capillary columns using the separation of a large number of inorganic and organic anions with a potassium hydroxide gradient as an example. In general, all the peaks in the upper chromatogram are more efficient, providing better resolution and sensitivity when using the Dionex IonPac AS11-HC-4µm column.

The Dionex IonPac AS16 column was developed for the analysis of polarizable anions such as iodide, thiocyanate, thiosulfate, and perchlorate. The substrate material is also based on a 55% cross-linked macroporous EVB/DVB polymer with a particle diameter of 9 µm. To minimize sorption interactions between the polarizable anions and the stationary phase, the nanobeads carry extremely hydrophilic ion-exchange groups, so that even perchlorate anions, which exhibit an enormous affinity toward conventional anion exchangers, elute as completely symmetrical peaks. Its high capacity allows large-volume injections up to 2 mL for low microgram/liter level analysis of polarizable anions without using preconcentration techniques. When employing a gradient elution technique, a variety of other inorganic and organic anions can be separated together with polarizable anions. The only exceptions are bromide and nitrate, which cannot be separated because of the marked hydrophilicity of the resin. An important field of application for such a stationary phase is the analysis of polyphosphates. With a pure aqueous hydroxide eluent, polyphosphates up to P_{20} can be eluted from this column.

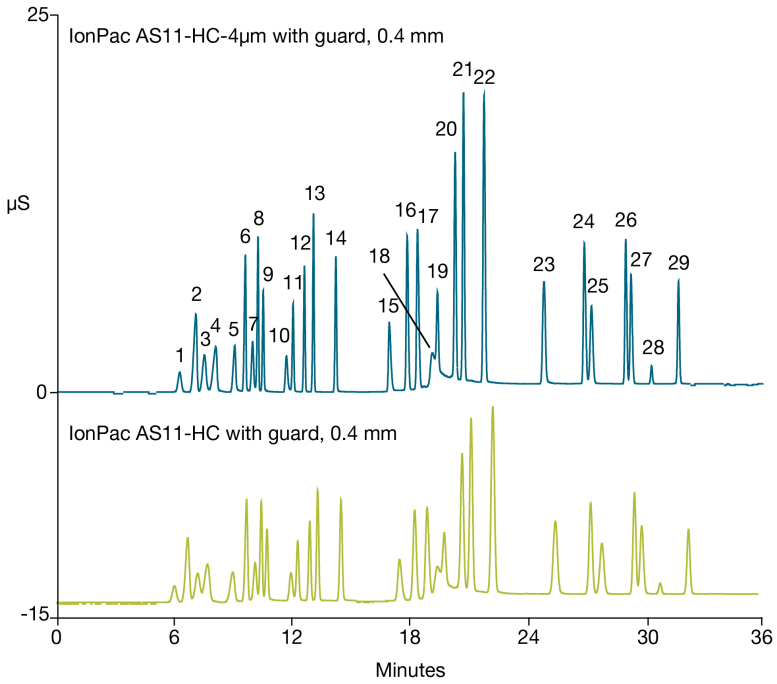


Figure 3.9 Comparison of Dionex IonPac AS11-HC and IonPac AS11-HC-4µm capillary columns exemplified by the separation of various inorganic and organic anions. Column temperature: 30 °C; eluent: KOH (EG); gradient: 1 mmol/L isocratically for 5 min, then linearly to 15 mmol/L from 5 to 14 min, then linearly to 30 mmol/L from 14 to 23 min, then linearly to 60 mmol/L from 23 to 31 min; flow rate: 15 µL/min; detection: suppressed conductivity; injection volume: 0.4 µL; peaks: 5 mg/L quinate (1), 1.5 mg/L fluoride (2), 5 mg/L each of lactate (3), acetate (4), propionate (5), formate (6), butyrate (7), methanesulfonate (8), pyruvate (9), valerate (10), monochloroacetate (11), and bromate (12), 2.5 mg/L chloride (13), 5 mg/L each of nitrite (14), trifluoro-acetate (15), bromide (16), and nitrate (17), carbonate (18), 7.5 mg/L each of malonate (19), maleate (20), sulfate (21), and oxalate (22), 10 mg/L each of tungstate (23), ortho-phosphate (24), phthalate (25), citrate (26), chromate (27), *cis*-aconitate (28), and *trans*-aconitate (29).

The Dionex IonPac AS17-C column has been designed for fast gradient elution of common inorganic anions in potable water, wastewater, and soil extracts. Thus, it is an analogue to the Dionex IonPac AS22 column (see Figure 3.16), which uses mostly carbonate/bicarbonate eluents under isocratic conditions for the same analyses. The nanobeads of the Dionex IonPac AS17-C column carry strongly hydrophilic ion-exchange groups. A special characteristic of the Dionex IonPac AS17-C column is the high resolution between fluoride and the system void, allowing fluoride quantitation at very low concentrations. Short-chain fatty acids such as formic, acetic, and propionic acid are also separated to baseline and elute behind fluoride. Standard anions are separated on this column in about 8 min with an electrolytically generated hydroxide eluent under gradient conditions. Including the time for re-equilibration, the total analysis time from injection to injection (cycle time) is approximately 10 min.

The Dionex IonPac AS18 column represents the latest development in the field of hydroxide-selective, nanobead-agglomerated stationary phases. It is ideal for use with isocratic hydroxide eluents for the fast separation of common inorganic anions in simple sample matrices. The high capacity of the Dionex IonPac AS18 column also allows the use of hydroxide gradients and large-loop injections to determine low levels of inorganic anions in complex matrices, including drinking water and wastewater samples. The Dionex IonPac AS18 column selectivity provides excellent separation between fluoride and the water dip as well as baseline resolution of fluoride, acetate, and formate. Solvent compatibility permits easy column cleanup after the analysis of samples with a high concentration of molecular organics. In a way, the Dionex IonPac AS18 column can be regarded as the high-capacity version of the Dionex IonPac AS17-C column, although the selectivities of both columns are not identical. The Dionex

IonPac AS18 column provides excellent separation of a variety of environmental anions using a hydroxide gradient. With a fast, dual hydroxide gradient, these analytes are easily separated in less than 15 min (Figure 3.10).

A new addition to the Dionex IonPacAS18 column line, the Dionex IonPac AS18-4µm column, is a high-capacity, high-efficiency column that provides excellent resolution of inorganic anions in diverse sample matrices. The new Dionex IonPac AS18-4µm column uses smaller resin particles for more efficient separations resulting in faster run times. Its selectivity is no different from the Dionex IonPac AS18 column, but with the reduced particle size, this column can separate common inorganic anions in less time without a significant loss of resolution. The Dionex IonPac AS18-4µm column is the choice for compliance monitoring of inorganic anions in drinking water and wastewater in accordance with US EPA Methods 300.0 (A) and 300.1.

Another member of the Dionex IonPac AS18 column family, the Dionex IonPac AS18-Fast column, is suitable for separations requiring higher flow rates for the fast analysis of inorganic anions in simple matrices. It has the same selectivity as the Dionex IonPac AS18 column, but the shorter column length of 150 mm allows higher flow rates to provide higher sample throughput. Using a higher flow rate in a shorter column format, the overall run time can be reduced to approximately 5 min maintaining optimal resolution of the common inorganic anions. Using a Dionex IonPac AS18-Fast column in the 2 mm format allows greater linear velocities of the mobile phase and reduced eluent consumption compared to a 4 mm format. Figure 3.11 compares the fast separation of common inorganic anions on a Dionex IonPac AS18-Fast column with the more efficient Dionex IonPac AS18-Fast-4µm column in the microbore formats.

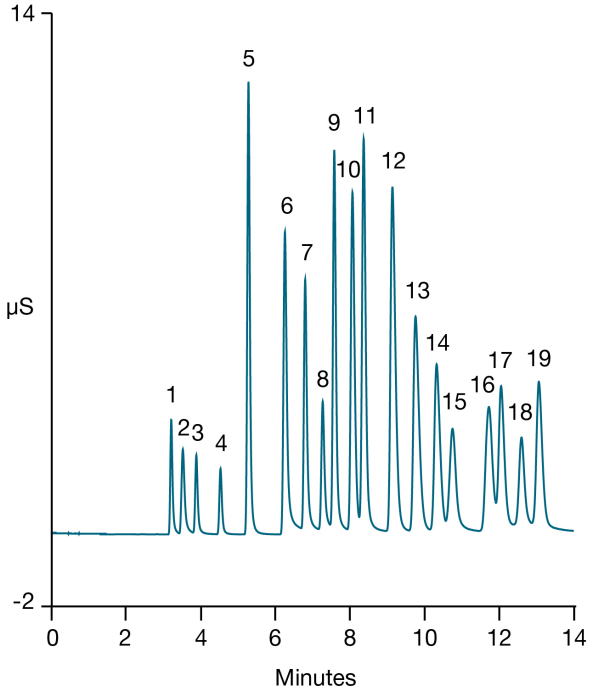


Figure 3.10 Gradient separation of environmental anions on a Dionex IonPac AS18 column. Column temperature: 30 °C; eluent: KOH (EG); gradient: 12–44 mmol/L from 0 to 5 min, then 44–52 mmol/L from 8 to 10 min; flow rate: 1 mL/min; detection: suppressed conductivity; injection volume: 25 µL; peaks: 0.5 mg/L fluoride (1), 2.5 mg/L acetate (2), 1 mg/L formate (3),5 mg/L chlorite (4), 3 mg/L chloride (5), 6 mg/L nitrite (6), 10 mg/L each of selenite (7), sulfite (8), sulfate (9), bromide (10), selenate (11), nitrate (12), chlorate (13), ortho-phosphate (14), molybdate (15), tungstate (16), arsenate (17), thiosulfate (18), and chromate (19).

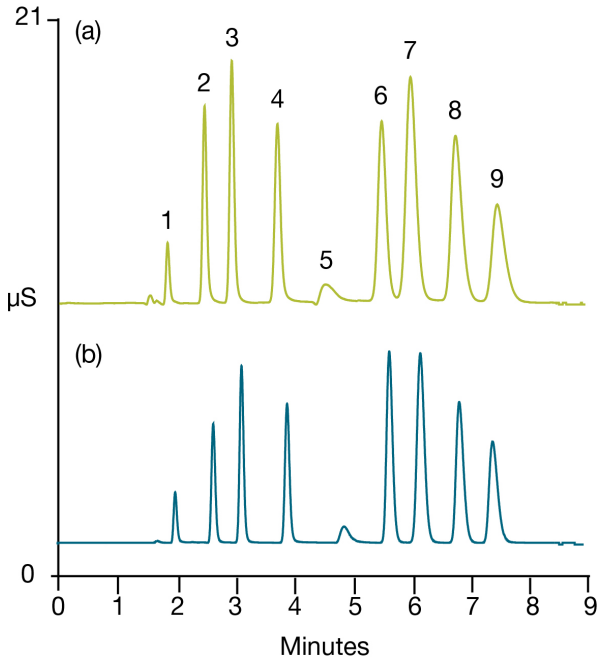


Figure 3.11 Comparison of (a) Dionex IonPac AS18-Fast and (b) Dionex IonPac AS18-Fast-4µm columns. Column dimensions: 250 mm × 2 mm i.d.; column temperature: 30 °C; eluent: 23 mmol/L KOH (EG); flow rates: 0.25 mL/min; detection: suppressed conductivity; injection volumes: (a) 5 µL, (b) 2.5 µL; peaks: 0.5 mg/L fluoride (1), 5 mg/L chlorite (2), 3 mg/L chloride (3), 5 mg/L nitrite (4), 20 mg/L carbonate (5), 10 mg/L each of bromide (6), sulfate (7), nitrate (8), and chlorate (9).

Specialty columns With the introduction of a special anion exchanger, the Dionex IonPac AS7 column, it became possible to separate and determine a variety of polyvalent anions, such as amino-polycarboxylic and aminopolyphosphonic acids. As an example, Figure 3.12 shows the separation of common aminopolyphosphonic acids. When 0.03 mol/L nitric acid is chosen as an eluent, the degree of analyte dissociation is pushed back via partial protonation, which is caused by the high acid strength of the eluent. Thus, the effective charge of the analytes is reduced, and their elution is possible. In addition, the choice of nitric acid is based on the known high elution strength of the nitrate ion. Conductivity detection of the analytes is not possible owing to the high acid concentration in the mobile phase. Thus, complexing agents are detected via post-column derivatization with ferric nitrate in acidic solution and subsequent measurement of the UV absorption in the wavelength region between 310 and 330 nm [5].

3.1.2 Surface-aminated ethylvinylbenzene/divinylbenzene polymers

All currently available surface-functionalized polymers based on ethylvinylbenzene/divinylbenzene (EVB/DVB) are grafted materials. In polymer chemistry, *grafting* describes a process in which two or more separately synthesized polymers are attached covalently. The goal is to produce a material with properties that cannot be obtained with the individual components or with mixtures of them.

The use of grafting technologies for the preparation of stationary phases in chromatography is not a new application; grafting was first used in the preparation of stationary phases more than 20 years ago when Schomburg et al. successfully used this technology to introduce weak acid cation exchangers based on polymer-coated silica. This process enabled the simultaneous

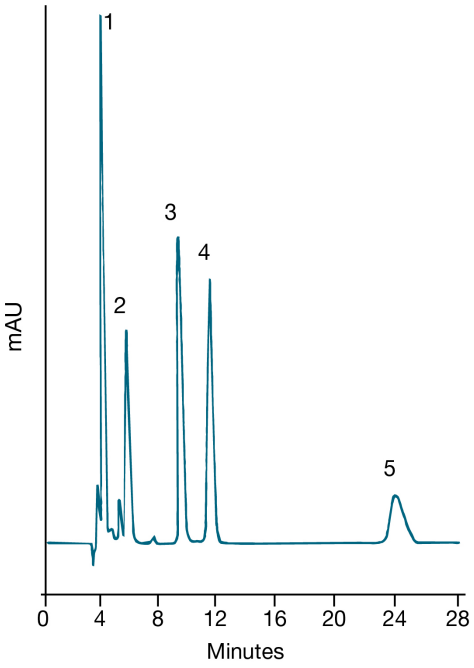


Figure 3.12 Separation of aminopolyphosphonic acids of the DEQUEST™ type. Separator column: Dionex IonPac AS7; eluent: 30 mmol/L HNO_3; flow rate: 1 mL/min; detection: UV (330 nm) after reaction with iron(III) nitrate in acidic solution; injection volume: 50 µL; peaks: 50 mg/L each of 1-hydroxy-ethylidene-1,1-diphosphonic acid (1), hexa-methylenediamine-tetra(methylenephosphonic acid) (2), amino-tri(methylenephosphonic acid) (3), ethylenediamine-tetra(methylenephosphonic acid) (4), and diethylenetriamine-penta(methylene-phosphonic acid) (5).

separation of alkali- and alkaline-earth metals (see Section 4.1.2), a feature that revolutionized cation-exchange chromatography. A schematic representation of such a grafted anion-exchange material is shown in Figure 3.13.

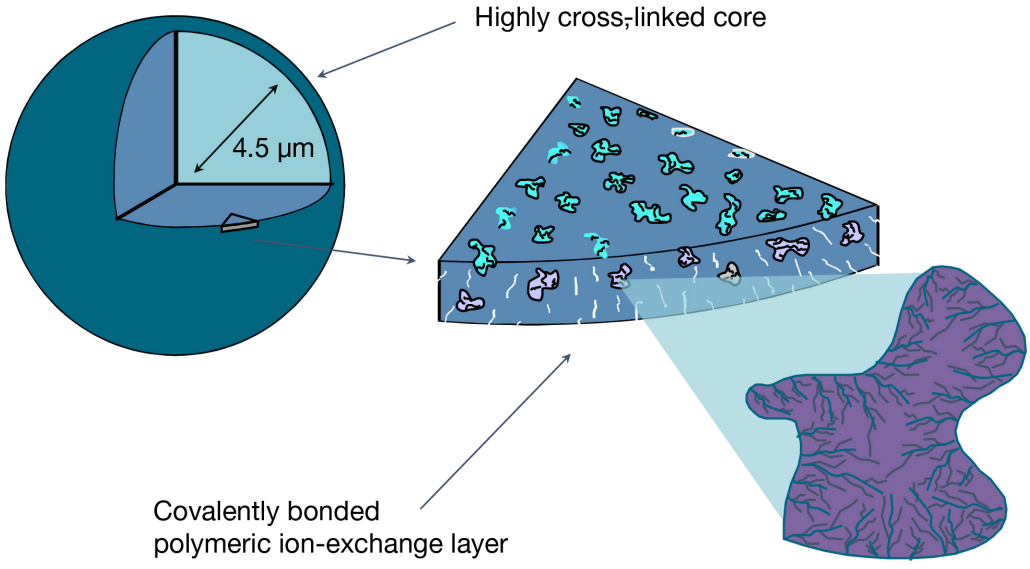


Figure 3.13 Schematic representation of a grafted anion-exchange material.

Carbonate/bicarbonate-selective grafted polymers The most common surface-functionalized, carbonate/bicarbonate-selective grafted polymer from Thermo Fisher Scientific is available under the trade name Dionex IonPac AS14, a universal anion exchanger for the analysis of inorganic anions. The Dionex IonPac AS14 polymer resolves fluoride from the negative water dip and provides good resolution among fluoride, acetate, and formate without increasing the total analysis time for the seven standard anions. This was achieved by grafting an anion-exchange polymer to the surface of a highly cross-linked support material that has a particle diameter of 9 µm [6]. The support material used for the Dionex IonPac AS14 column consists of ethylvinylbenzene cross-linked with 55% divinylbenzene, so it is completely solvent compatible. Three different monomers are attached to this support material via *cografting*. Each of these monomers contributes its specific properties to the selectivity of the stationary phase. The extremely small and controlled thickness of the anion-exchange polymer layer results in rapid mass-transfer characteristics and, consequently, in a high chromatographic efficiency. A respective chromatogram with the separation of standard inorganic anions is shown in Figure 3.14. No chromatographic interference of fluoride with short-chain fatty acids such as acetate and formate is observed, so that this column is suitable for the isocratic analysis of fluoride even in complex matrices. Samples with large concentration differences between the individual analytes can easily be analyzed with the Dionex IonPac AS14 column because of its sufficient ion-exchange capacity of 65 µequiv/column. The resulting analysis time for sulfate can be reduced to about 8 min by increasing the ionic strength or the flow rate of the eluent [7].

A further improvement was obtained when using a support material with a smaller particle diameter, as in the 5 µm Dionex IonPac AS14A column. The 150 mm × 3 mm i.d. column dimensions reduce the pressure drop along this separator column. Operating the column with an eluent consisting of 8 mmol/L sodium carbonate and 1 mmol/L sodium bicarbonate not only reduces the run time, but also improves sensitivity for the standard inorganic anions.

Hydroxide-selective grafted polymers A hydroxide-selective, surface-functionalized EVB/DVB resin featuring a covalently attached anion-exchange polymer has also been introduced under the trade name Dionex IonPac AS15. This material is suitable for gradient elution of inorganic and organic anions. As an improvement of the Dionex IonPac AS11-HC column (see Section 3.1.1), the Dionex IonPac AS15 column is specifically designed for the separation of fluoride, glycolate, acetate, and formate using a hydroxide concentration gradient. The support material of the Dionex IonPac AS15 column is identical to that of the Dionex IonPac AS14 column. Meanwhile, the Dionex IonPac AS15 column has been replaced by the more modern Dionex IonPac AS28-Fast-4µm column (see Section 3.1.3). Table 3.3 summarizes the characteristic structural and technical properties of surface-aminated EVB/DVB polymers.

3.1.3 Hyperbranched condensation polymers

Colloidal anion-exchange particles (so-called nanobeads or latex particles) that are electrostatically bound to the surface of nonporous surface-sulfonated or surface-carboxylated PS/DVB and EVB/DVB copolymer beads as well as grafted materials provide a number of advantages but also disadvantages**.** Thus, Pohl and Saini [8] were searching for a methodology to synthesize an anion-exchange material free of any π electron character in the polymer backbone. A particularly interesting system for synthesizing a cationic condensation polymer involves the reaction of diepoxides with primary amines. When diepoxides and primary amines are allowed to react in a 1:1 molar ratio, the product is a water-soluble polymer with a predominantly linear architecture.

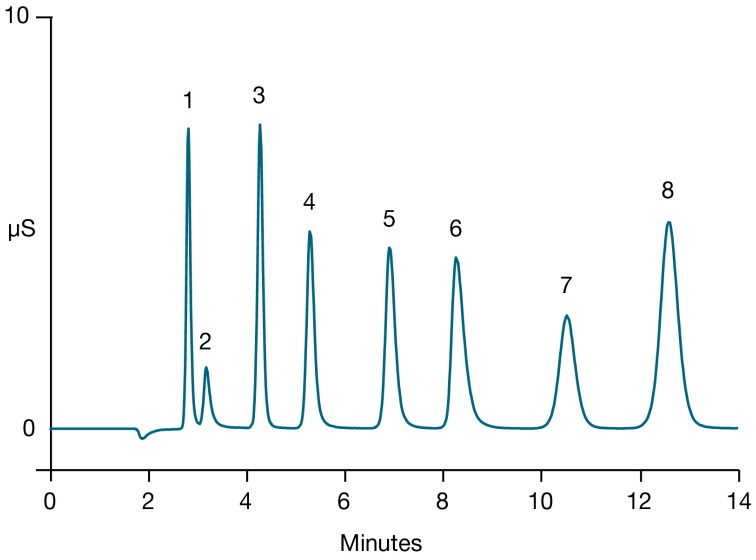
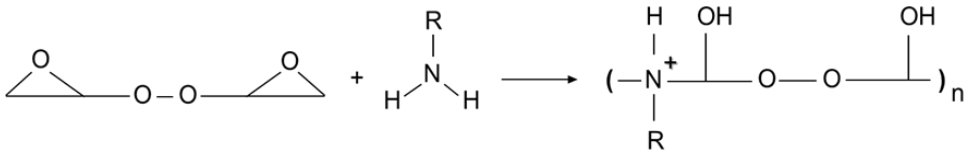


Figure 3.14 Separation of common inorganic anions on a Dionex IonPac AS14 column. Eluent: 3.5 mmol/L Na_2CO_3 + 1 mmol/L $NaHCO_3$; flow rate: 1.2 mL/min; detection: suppressed conductivity; injection volume: 10 µL; peaks: 5 mg/L fluoride (1), 20 mg/L acetate (2), 10 mg/L chloride (3), 15 mg/L nitrite (4), 25 mg/L each of bromide (5) and nitrate (6), 40 mg/L orthophosphate (7), and 30 mg/L sulfate (8).

Table 3.3 Structural and technical properties of ethylvinylbenzene/divinylbenzene copolymers.

Column	**Dionex IonPac AS14**	**Dionex IonPac AS14A**	**Dionex IonPac AS15**
Dimension (length × i.d.) (mm)	250 × 2 250 × 4	150 × 3 250 × 4	250 × 2 250 × 4 150 × 3
pH range	2–11	0–14	0–14
Maximum pressure (MPa)	27	27	27
Maximum flow rate (mL/min)	2	2	2
Solvent compatibility (%)	100	100	100
Capacity (µequiv/column)	16 65	40 120	56 225 97
Particle diameter (µm)	9	5 7	9 5
Type of column packing	EVB/DVB with quaternary ammonium functions	EVB/DVB with quaternary ammonium functions	EVB/DVB with quaternary ammonium functions



(3.4)

The polymer thus formed is polycationic. If this polymer is produced in the presence of substrate particles with a negatively charged surface, it will form a molecular film on that surface (see Figure 3.15a). Since most of the cationic sites will be tertiary amines, it also provides the opportunity to further react these remaining tertiary amines with the diepoxide monomer in the absence of the primary amine to produce pendant epoxide moieties forming a linear polymer chain (see Figure 3.15b). This pendant epoxide functional group is available for a subsequent reaction step with a primary amine to convert the epoxide groups to secondary amines (see Figure 3.15c). Repeating this process results in doubling the number of pendant chains for each cycle of treatment with diepoxide followed by treatment with a primary amine (see Figures 3.15d and 3.15e). The ability to double the capacity of the growing film with each reaction cycle allows the production of ion-exchange stationary phases with significantly higher capacities than those produced using other synthetic pathways. In addition, with each reaction cycle the density of polymer strands growing off the substrate surface increases progressively. This, in turn, increases the probability of cross-links between adjacent strands as illustrated in Figure 3.15e. Such phenomenon results in a progressively higher cross-link density with each reaction cycle, altering the selectivity of the stationary phase as well as increasing its capacity.

Carbonate-selective hyperbranched condensation polymers The synthesis method described above was used for the development of four carbonate-selective hyperbranched condensation polymers, the Dionex IonPac AS22, the Dionex IonPac AS22-Fast, the Dionex IonPac AS22-Fast-4µm, and the Dionex IonPac AS23. The technical characteristics of these columns are summarized in Table 3.4.

The Dionex IonPac AS22 column is a universal purpose anion-exchange column for the determination of inorganic anions and low-molecular weight organic acids, such as formate and acetate in diverse sample matrices, including drinking water, wastewater, and process streams. The Dionex IonPac AS22 column is typically used with a carbonate/bicarbonate eluent under isocratic conditions separating common inorganic anions and acetate in approximately 12 min as shown in Figure 3.16. It meets the performance requirements specified in US EPA Method 300.0 (A). In comparison to the carbonate-selective, grafted surface-functionalized Dionex IonPac AS14 column (see Figure 3.14), the Dionex IonPac AS22 column is characterized by higher peak efficiencies and less rounding at the peak bases, indicating the absence of interpenetrating networks below the stationary phase surface. Thus, the Dionex IonPac AS22 column is currently the most modern carbonate-selective, universal purpose anion exchanger. The Dionex IonPac AS22 column can also be used in combination with an eluent generator and an electrolytic pH modifier (EPM), which automatically produce potassium carbonate/ bicarbonate eluents from deionized water. Apart from standard anions, the Dionex IonPac AS22 column can be used to separate a wide variety of environmentally relevant anions using a carbonate/bicarbonate eluent under isocratic conditions.

The Dionex IonPac AS22-Fast column was designed to have sufficient resolution of common inorganic anions in simple sample matrices. The shorter column length of 150 mm allows higher linear velocities of the mobile phase without exceeding the maximum column back pressure of 3000 psi (27 MPa), so that the overall run time can be reduced to less than 8 min.

The latest addition to this family of columns is the Dionex IonPac AS22-Fast-4µm column, which was designed for compliance monitoring with US EPA Methods 300.0 (A) and 300.1. Exhibiting higher peak efficiencies, the selectivity of the Dionex IonPac AS22-Fast-4µm column does not differ from the Dionex IonPac AS22-Fast column because the same functionality is attached to the smaller resin particles used in the Dionex IonPac AS22-Fast-4µm column. Fluoride is well separated from the water dip and common inorganic anions are separated under isocratic conditions. The Dionex IonPac AS22-Fast-4µm column is available

in standard bore, microbore, and capillary formats; its length of 150 mm allows fast analysis with moderate ionic strength.

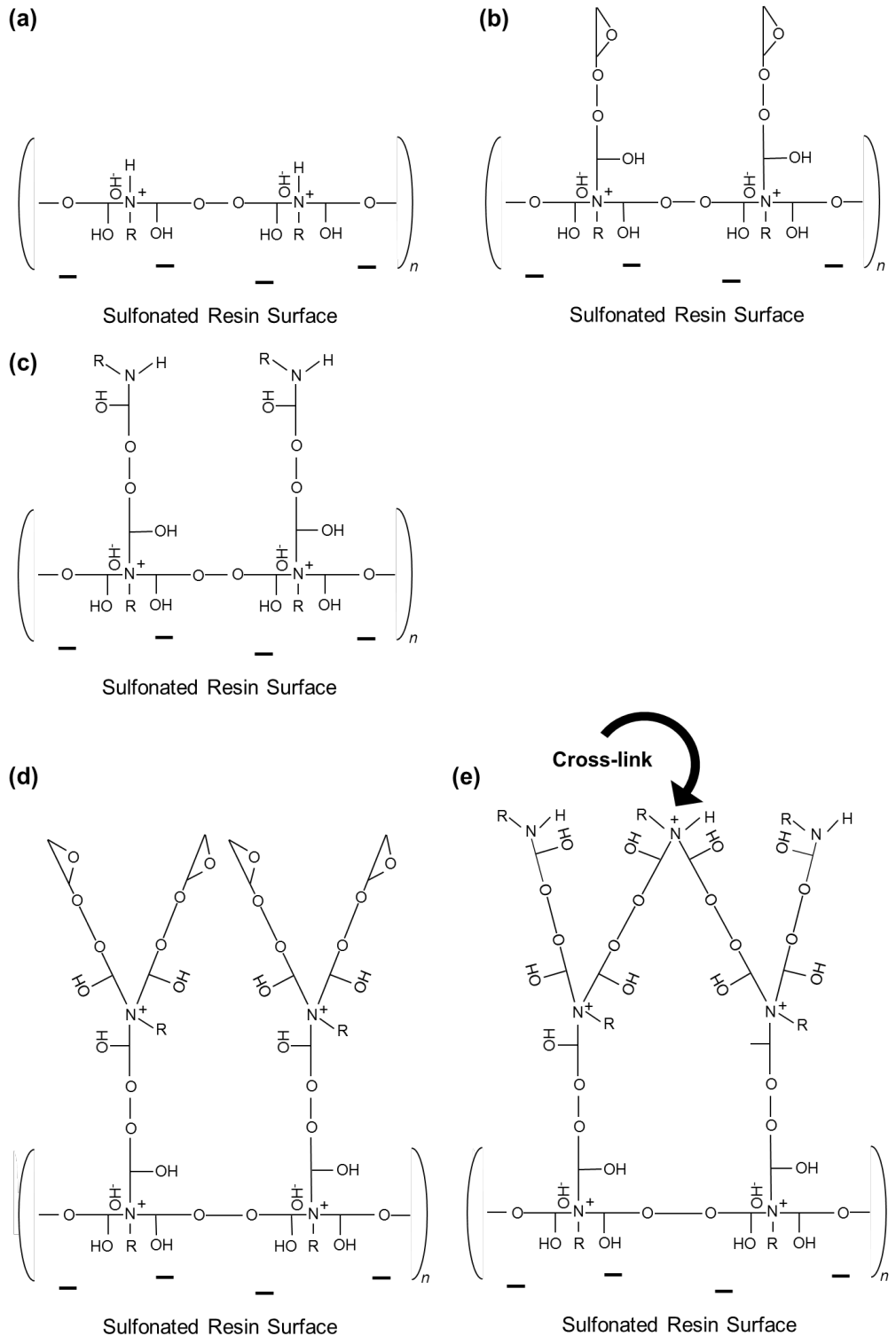


Figure 3.15 (a) Condensation polymer after deposition of a basement condensation polymer, (b) condensation polymer after deposition of basement condensation polymer and first reaction with a diepoxide monomer, (c) condensation polymer after deposition of basement condensation polymer, first reaction with a diepoxide monomer, and first reaction with a primary amine, (d) condensation polymer after deposition of basement condensation polymer, first reaction with a diepoxide monomer followed by a primary amine, and second reaction cycle with a diepoxide monomer, (e) condensation polymer after deposition of basement condensation polymer, first reaction with a diepoxide monomer followed by a primary amine, and second reaction cycle with a diepoxide monomer followed by a primary amine.

Table 3.4. Structural and technical properties of carbonate/bicarbonate-selective, hyperbranched condensation polymers.

Separator	Particle diameter (µm)	Max. pressure (MPa)	Solvent compatibility (%)	Capacity (µequiv/col.)	Dimensions (length × i.d.) (mm)	Applicability
Dionex IonPac AS22	6.5	27	100	210 52.5	250 × 4 250 × 2 250 × 0.4	Universal high-performance separator for standard inorganic anion analysis
Dionex IonPac AS22-Fast	6.5	27	100	126 31.5	150 × 4 150 ×2	Universal high-performance separator for fast analysis of common anions
Dionex IonPac AS22-Fast-4µm	4.0	34	100	126 31.5 1.26	150 × 4 150 × 2 150 × 0.4	Universal high-performance separator for fast analysis of common anions
Dionex IonPac AS23	6.0	27	100	320 80	250 × 4 250 × 2	High-capacity separator for standard inorganic anion and oxyhalide analysis

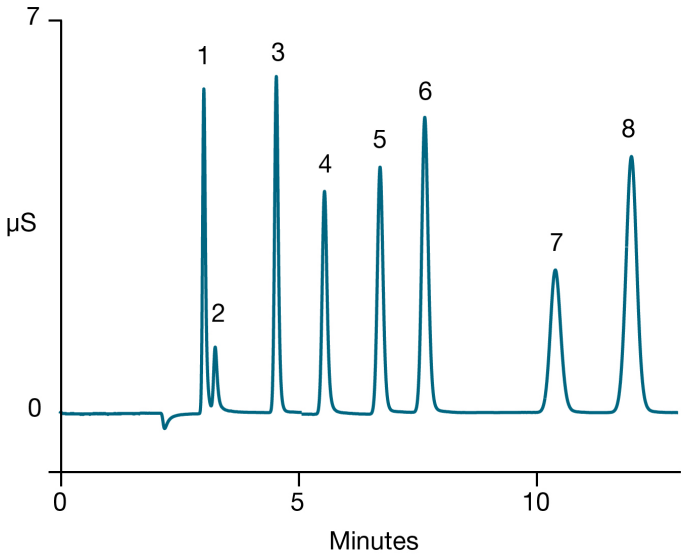


Figure 3.16 Separation of common inorganic anions and acetate on the Dionex IonPac AS22 column. Column: Dionex IonPac AS22 plus guard column; column dimensions: 250 mm × 4 mm i.d.; column temperature: 30 °C; eluent: 4.5 mmol/L Na_2CO_3 + 1.4 mmol/L $NaHCO_3$; flow rate: 1.2 mL/min; detection: suppressed conductivity; injection volume: 10 µL; peaks: 5 mg/L fluoride (1), 20 mg/L acetate (2), 10 mg/L chloride (3), 15 mg/L nitrite (4), 25 mg/L bromide (5), 25 mg/L nitrate (6), 40 mg/L orthophosphate (7), and 30 mg/L sulfate (8).

The high-capacity Dionex IonPac AS23 column is a carbonate-selective anion exchanger designed for the analysis of common inorganic anions and oxyhalides in diverse sample matrices including drinking water, groundwater, and wastewater in less than 25 min as shown in Figure 3.17. Thus, it practically replaces the Dionex IonPac AS9-HC column (see Figure 3.6), which was a very popular column for this type of application in the past. However, the Dionex IonPac AS9-HC column exhibited two major deficiencies: limited pH stability due to the methacrylate substrate and lack of resolution between fluoride and the water dip being a characteristic property of all nanobead-agglomerated anion exchangers. With the development of the Dionex IonPac AS23 column, these deficiencies were overcome.

Hydroxide-selective hyperbranched condensation polymers As an alternative to carbonate-selective hyperbranched condensation polymers described above, which are typically used for conventional anion-exchange chromatography of common inorganic anions, a larger number of hydroxide-selective stationary phases have been developed following the same synthetic method. The technical characteristics of these columns are summarized in Table 3.5.
The high-capacity Dionex IonPac AS19 column is the hydroxide-selective analog of the carbonate-selective Dionex IonPac AS23 column and designed for the analysis of common inorganic anions and oxyhalides in diverse sample matrices, including drinking water, groundwater, and wastewater as shown in Figure 3.18. It is the column of choice for trace bromate analysis in drinking water matrices when using a potassium hydroxide eluent (isocratic or gradient) in combination with suppressed conductivity detection. Like its carbonate-selective counterpart, it meets the performance requirements specified by the US EPA Methods 300.0 and 300.1 for the determination of oxy-halide by-products from drinking water disinfection with ozone at single-digit microgram/liter concentrations in the presence of much larger concentrations of chloride, sulfate, and carbonate. The Dionex IonPac AS19 column is also available in a capillary format containing the same packing material as the equivalent standard bore and microbore versions.

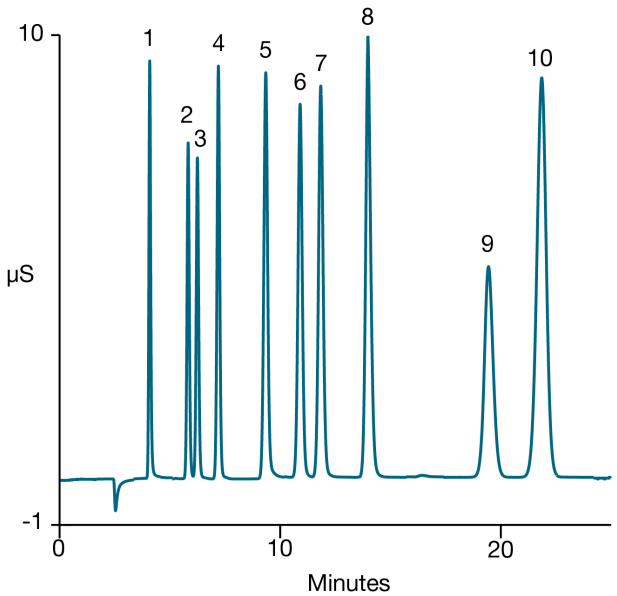


Figure 3.17 Separation of common inorganic anions and oxyhalides on the Dionex IonPac AS23 column. Column: Dionex IonPac AS23 plus guard column; column dimensions: 250 mm × 4 mm i.d.; column temperature: 30 °C; eluent: 4.5 mmol/L Na_2CO_3 + 0.8 mmol/L $NaHCO_3$; flow rate: 1.0 mL/min; detection: suppressed conductivity; injection volume: 25 µL; peaks: 3 mg/L fluoride (1), 10 mg/L chlorite (2), 20 mg/L bromate (3), 6 mg/L chloride (4), 10 mg/L nitrite (5), 25 mg/L chlorate (6), 25 mg/L bromide (7), 25 mg/L nitrate (8), 40 mg/L ortho-phosphate (9), and 30 mg/L sulfate (10).

Table 3.5 Structural and technical properties of hydroxide-selective hyperbranched condensation polymers.

Separator	Particle diameter (µm)	Max. pressure (MPa)	Solvent compatibility (%)	Capacity (µequiv/col.)	Dimensions (length × i.d.) (mm)	Applicability
Dionex IonPac AS19	7.5	35 45	100	240 60 2.4	250 × 4 250 × 2 250 × 0.4	High-capacity separator for standard inorganic anion and oxyhalide analysis
Dionex IonPac AS19-4µm	4	35	100	240 60 2.4	250 × 4 250 × 2 250 × 0.4	High-capacity separator for standard inorganic anion and oxyhalide analysis
Dionex IonPac AS20	7.5	27 45	100	310 77.5 3.1	250 × 4 250 × 2 250 × 0.4	High-capacity separator for trace analysis of perchlorate in environmental samples
Dionex IonPac AS21	7.0	27	100	45	250 × 2	Separator for fast analysis of perchlorate with MS or MS/MS detection
Dionex IonPac AS24	7.0	27	100	140	250 × 2	High-capacity separator for haloacetic acid analysis with MS/MS
Dionex IonPac AS24A	7.0		100	520 5.2	250 × 4 250 × 0.4	High-capacity separator for haloacetic acids by IC × IC
Dionex IonPac AS25	7.5	27 45	100	350 87.5 3.5	250 × 4 250 × 2 250 × 0.4	High-capacity separator for the analysis of multi-valent and polarizable anions including sulfur speciation
Dionex IonPac AS26	7.5	27	100	250 62.5 2.5	250 × 4 250 × 2 250 × 0.4	High-capacity separator for haloacetic acid analysis with IC × IC
Dionex IonPac AS27	6.5	21	100	220 55 2.2	250 × 4 250 × 2 250 × 0.4	Trace bromate analysis in drinking water preserved with ethylenediamine
Dionex IonPac AS28-Fast-4µm	4	35	100	230 57 2.4	150 × 4 150 × 2 150 × 0.4	Separator for fast, high-resolution analysis of common inorganic anions and low-molecular weight organic acids
Dionex IonPac AS31	6	35	100	116	250 × 2	Fast separation of haloacetic acids, bromate, and dalapon in drinking water prior to MS or MS/MS detection
Dionex IonPac AS32-Fast-4µm	4	35	100	126 32	250 × 4 250 × 2	Separator for fast analysis of low-polarity anions, aromatic dyes, poly-thionates, persulfate, and perchlorate

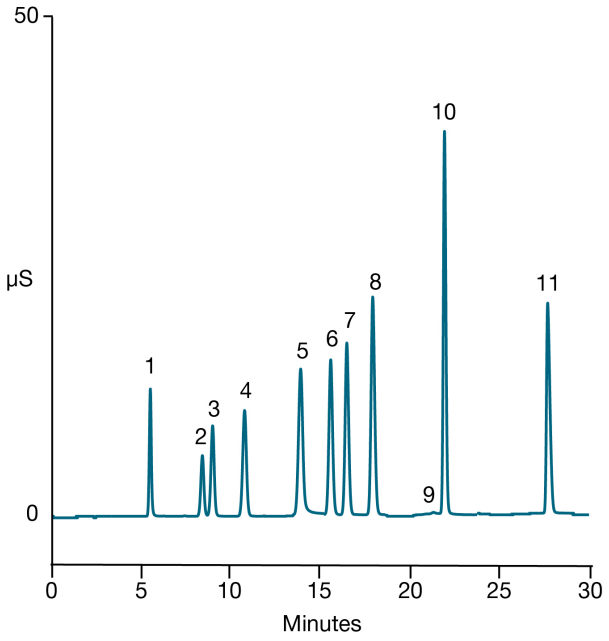


Figure 3.18 Separation of common inorganic anions and oxyhalides on the Dionex IonPac AS19 column. Column: Dionex IonPac AS19 plus guard column; column dimensions: 250 mm × 4 mm i.d.; column temperature: 30 °C; eluent: KOH (EG); gradient: 10 mmol/L from 0 to 10 min, 10–45 mmol/L from 10 to 25 min; flow rate: 1.0 mL/min; detection: suppressed conductivity; injection volume: 25 µL; peaks: 2 mg/L fluoride (1), 10 mg/L chlorite (2), 20 mg/L bromate (3), 6 mg/L chloride (4), 15 mg/L nitrite (5), 25 mg/L chlorate (6), 25 mg/L bromide (7), 25 mg/L nitrate (8), carbonate (9), 25 mg/L sulfate (10), and 40 mg/L orthophosphate (11).

The Dionex IonPac AS19 column is recommended for use with electrolytic eluent generation (RFIC-EG) producing high-purity potassium hydroxide from deionized water. Because the KOH eluent is free of any carbonate contamination, baseline shifts during hydroxide concentration gradients are minimized. In addition, hydroxide eluents provide lower background conductance and, in turn, lower detection limits for target analytes in comparison to carbonate-based eluents, even if they are combined with a carbonate removal device (Thermo Scientific™ Dionex™ Carbonate Removal Device CRD 300).

The Dionex IonPac AS19-4µm column is the latest addition to the Dionex AS19 column family, exhibiting higher peak efficiency while maintaining the same selectivity. The same functionality is attached to the smaller resin particles used in this column.

The Dionex IonPac AS20 column is a high-capacity anion exchanger designed for trace analysis of perchlorate in drinking water, groundwater, and surface water samples. The capacity and selectivity of the Dionex IonPac AS20 column ensure the separation of perchlorate at sub-microgram/liter levels using suppressed conductivity detection, even in the presence of much larger concentrations of chloride, sulfate, and carbonate. It meets the performance requirements of the US EPA Method 314.1 for the determination of perchlorate in drinking water.

Prior to the introduction of the Dionex IonPac AS20 column, perchlorate analysis was carried out on the Dionex IonPac AS16 column (see Section 3.1.1). However, aromatic sulfonates such as 4-chlorobenzene sulfonate, which can be found in leachates from some hazardous waste sites, can potentially interfere with perchlorate determination on this column. The presence of 4-chlorobenzene sulfonate in an environmental sample may result in false positives for perchlorate. The Dionex IonPac AS20 column solves this problem with an aliphatic backbone of the substrate coating in contrast to the aromatic backbone of the Dionex IonPac AS16 substrate. Figure 3.19 illustrates this selectivity difference between the Dionex IonPac AS16 and AS20 columns, exemplified by the separation of common inorganic anions, polarizable anions, and 4-chlorobenzene sulfonate. Figure 3.19a shows that 4-chloro-benzene

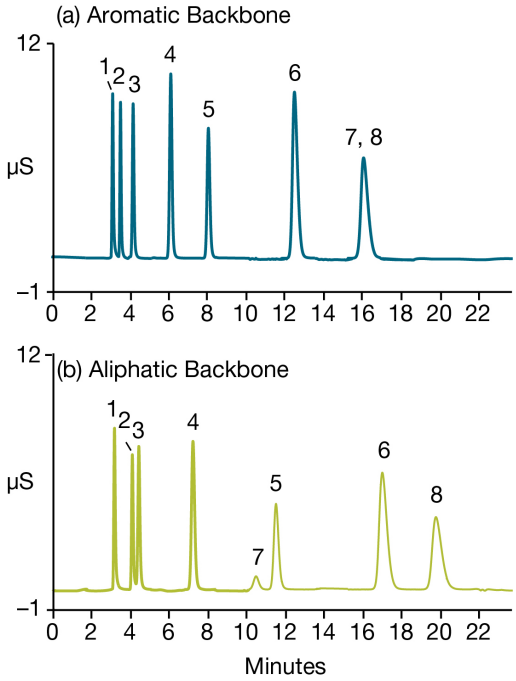


Figure 3.19 Selectivity comparison between Dionex IonPac AS16 and AS20 columns for the separation of polarizable anions. Separator columns: (a) Dionex IonPac AS16, (b) Dionex IonPac AS20; column dimensions: 250 mm × 2 mm i.d.; column temperature: 30 °C; eluent: 35 mmol/L KOH (EG); flow rate: 250 µL/min; detection: suppressed conductivity; injection volume: 2.5 µL; peaks: 2 mg/L fluoride (1), 3 mg/L chloride (2), 5 mg/L sulfate (3), 10 mg/L thiosulfate (4), 20 mg/L iodide (5) and thiocyanate (6), 5 mg/L 4-chloro-benzene sulfonate (7), and 30 mg/L perchlorate (8).

sulfonate coelutes with perchlorate on the Dionex IonPac AS16 column, while both components are widely separated on the Dionex IonPac AS20 column (Figure 3.19b).

The Dionex IonPac AS21 column is a moderate-capacity anion exchanger designed for the fast analysis of perchlorate in drinking water matrices prior to detection with MS or MS/MS, specified in the US EPA Method 331.0 for the determination of trace perchlorate using LC–MS or LC–MS/MS. The hyperbranched condensation polymer of the Dionex IonPac AS21 column is extremely hydrophilic, allowing the use of both hydroxide and volatile eluents such as methyl amine. The moderate capacity of 45 µequiv/column in the microbore format was chosen to elute perchlorate with a relatively low eluent concentration in about 10 min. The Dionex IonPac AS21 column is also compatible with electrolytic hydroxide eluent generation (RFIC-EG) in combination with suppressed conductivity detection prior to MS detection. This method combines matrix diversion of common salts prior to MS detection. With both IC-MS/MS based on hydroxide eluents and LC–MS/MS based on methyl amine as the mobile phase, minimum reliable quantification levels of perchlorate using a Dionex IonPac AS21 column are typically less than 50 ng/L.

The Dionex IonPac AS24 column is a high-capacity anion exchanger designed for the separation of haloacetic acids in drinking water matrices prior to MS or MS/MS detection. The microbore format of this column was specifically developed for MS detection and is specified in the US EPA Method 557. The unique selectivity and high capacity of the Dionex IonPac AS24 column makes it an ideal column for specialized applications, providing excellent separation of environmentally relevant anions including common inorganic anions, oxyhalides, oxyanions, and organic acids. The most important application for this column, however, is the separation of haloacetic acids that are formed during the disinfection of drinking water via chlorination. The Dionex IonPac AS24 column can separate all nine compounds including MCAA, DCAA, TCAA, MBAA, DBAA, TBAA, BCAA, DBCAA, and DCBAA. Column selectivity is optimized for an operational temperature of 15 °C to ensure reproducible

recoveries for all brominated acetic acids, especially for mono- and tribromoacetic acid, which exhibit significant on-column degradation (>80%) at a standard column temperature of 30 °C. The high ion-exchange capacity of the Dionex IonPac AS24 column allows haloacetic acid analysis in high-ionic strength matrices utilizing MS/MS detection in combination with matrix diversion. Figure 3.20 shows the separation of haloacetic acids in drinking water using a potassium hydroxide gradient delivered by an eluent generator. Low microgram/liter concentrations of haloacetic acids can easily be determined by IC–MS/MS.

The Dionex IonPac AS24A column is the standard bore and capillary format of the Dionex IonPac AS24 column. During the development of these column formats it was determined that an extra step in the procedure of the resin production was required to insure the recovery of MBCC. This extra step means that the Dionex IonPac AS24 and AS24A columns are slightly different and cannot be called the same name. The Dionex IonPac AS24A (4 mm) column can be used for trace analysis of bromate in drinking water matrices by IC × IC as the high-capacity column in the first channel or as a high-capacity capillary column for environmentally relevant anions.

The Dionex IonPac AS25 column is a high-capacity anion exchanger designed for the analysis of sulfur components, such as sulfite, sulfate, thiocyanate, and thiosulfate as well as other polarizable and multivalent anions in complex sample matrices such as process effluents, wastewater, and scrubber solutions. Due to the extremely hydrophilic character of the hyperbranched condensation polymer, the analytes mentioned above can be separated under isocratic conditions in less than 30 min without the use of solvents. The selectivity of the Dionex IonPac AS25 column has been optimized for sulfur speciation in complex samples. Sulfur-containing inorganic anions are commonly present in soil sediments, hot springs, and lake water. They are also frequently encountered in the monitoring of process liquors and wastewaters from paper mills, mining sites, offshore oil drilling operations, alkaline scrubbers, and in the analysis of food and beverages. Many sulfur-containing anions can readily react with each other, decompose over time, or be oxidized in the presence of oxygen. They are also very sensitive to eluent pH. All these make sulfur speciation a very challenging task. While the most difficult separation of sulfite and sulfate is best performed with nanobead-agglomerated methacrylate resins such as Dionex IonPac AS9-SC (see Figure 3.3), using carbonate/ bicarbonate eluents, hydroxide-selective stationary phases traditionally exhibited problems

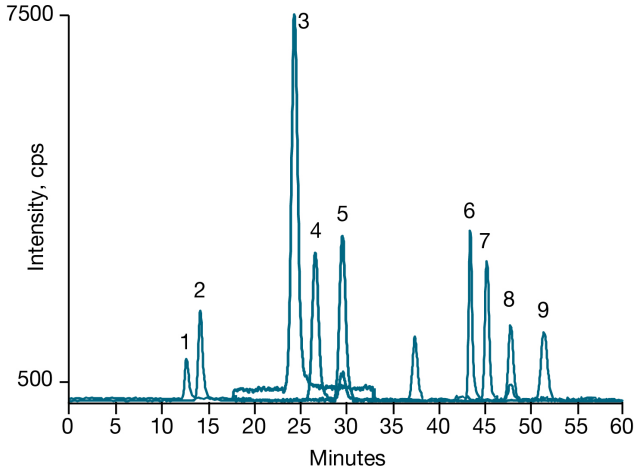


Figure 3.20 Analysis of haloacetic acids on a Dionex IonPac AS24 column using MS/MS detection. Separator column: Dionex IonPac AS24; column dimensions: 250 mm × 2 mm i.d.; column temperature: 15 °C; eluent: KOH (EG); gradient: 7 mmol/L in 0 to18 min, 7–18 mmol/L in 18 to 36.5 min, 18–60 mmol/L in 36.5 to 36.8 min; flow rate: 0.3 mL/min; detection: –ESI, MRM mode; postcolumn solvent: 0.2 mL/min MeCN; injection volume: 100 µL; peaks: 1 µg/L each of monochloroacetic acid (1), monobromoacetic acid (2), dichloroacetic acid (3), monobromomonochloroacetic acid (4), dibromoacetic acid (5), trichloroacetic acid (6), monobromodichloroacetic acid (7), monochlorodibromoacetic acid (8), and tribromoacetic acid (9).

with baseline-resolved separations of these two anions. As can be seen from Figure 3.21, baseline separation between sulfite and sulfate can be achieved with a Dionex IonPac AS25 column, separating polarizable sulfur components such as thiocyanate and thiosulfate in the same run. However, it should be noted that other anion exchangers designed for polarizable anions such as the Dionex IonPac AS16 or Dionex IonPac AS20 columns are recommended if higher peak efficiencies for these anions are required.

A common property of all stationary phases suitable for the analysis of polarizable anions including the Dionex IonPac AS25 column is the limited resolution between bromide and nitrate. This is not surprising as all these resins are extremely hydrophilic. The separation of nitrate from bromide, however, is merely based on adsorption, which requires a more hydrophobic substrate. Bromide/nitrate separation can be improved by applying a gradient elution technique. From all hydroxide-selective anion exchangers, the Dionex IonPac AS25 column is by far the best column to separate carbonate and sulfate. This is important for environmental water samples containing a high degree of dissolved bicarbonate as well as for caustic scrubber solutions to remove acid gases such as hydrochloric acid, phosgene, chlorine, and hydrogen sulfide in industrial processes. The Dionex IonPac AS25 column is also available in a capillary format, packed with the same material as the equivalent analytical scale column, providing reduced eluent costs and the ability to continuously operate the ion chromatography system.

The Dionex IonPac AS26 column is a high-capacity anion exchanger designed for the separation of haloacetic acids in high-ionic strength matrices using IC × IC. In this approach, a standard bore Dionex IonPac AS24 column is typically used as the separator in the first channel to provide the initial separation of the haloacetic acids from the major matrix ions, such as chloride, sulfate, and carbonate. For the second channel separation, aliquots of the suppressed eluent that contains haloacetic acids are concentrated on a Thermo Scientific™ Dionex™ IonSwift™ MAC-200 concentrator column and then separated on a capillary Dionex IonPac AS26 column to benefit from the concentration effect. In this way, low microgram/liter concentrations of haloacetic acids can easily be determined. Like the Dionex IonPac AS24 column, column selectivity has been optimized for an operational temperature of 15 °C to ensure reproducible recoveries for the brominated acetic acids. Temperature has a very strong effect on the selectivity of the Dionex IonPac AS26 column. At a temperature of 25 °C, for

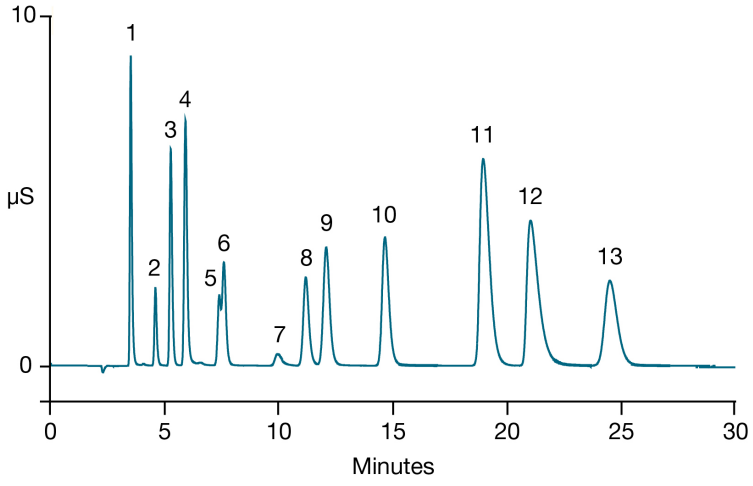


Figure 3.21 Isocratic analysis of nonpolarizable and polarizable inorganic anions on a Dionex IonPac AS25 column. Separator column: Dionex IonPac AS25; column dimensions: 250 mm × 4 mm i.d.; column temperature: 30 °C; eluent: 37 mmol/L KOH (EG); flow rate: 1 mL/min; detection: suppressed conductivity; injection volume: 25 µL; peaks: 2 mg/L fluoride (1), 5 mg/L bromate (2), 3 mg/L chloride (3), 3 mg/L nitrite (4), 3 mg/L bromide (5), 5 mg/L nitrate (6), 5 mg/L carbonate (7), 1 mg/L sulfite (8), 5 mg/L sulfate (9), 20 mg/L iodide (10), 20 mg/L thiocyanate (11), 30 mg/L perchlorate (12), and 10 mg/L thiosulfate (13).

instance, there is almost no resolution between sulfate and chloride, while orthophosphate and nitrite reverse their elution order. Because of this sensitivity to temperature changes, it is important to keep the column temperature constant and, if necessary, to adjust it to obtain optimal resolution. The Dionex IonPac AS26 column can also be used to analyze haloacetic acids at higher concentrations using direct injection and suppressed conductivity detection. The ultrahigh capacity of this column is responsible for the long overall analysis time.

The column is also suitable for the determination of oxyhalides such as chlorite, bromate, and chlorate in drinking water without any sample pretreatment other than filtration and without preconcentration. Figure 3.22 shows the respective separation using a potassium hydroxide gradient and suppressed conductivity detection. The ultrahigh resolution between chlorite, bromate, and chloride under these conditions is remarkable.

The Dionex IonPac AS27 column is designed for the analysis of inorganic anions and oxyhalides in the presence of 50 mg/L ethylenediamine (EDA) using an isocratic or gradient hydroxide eluent. Ethylene-diamine is sometimes added to drinking water samples as a preservative; however, it can react with carbonate and produce artifacts that interfere with early eluting analytes. The Dionex IonPac AS27 column minimizes this interference by increasing the separation between artifacts and bromate and allowing better integration of the bromate peak. In comparison with the Dionex IonPac AS19 column, the Dionex IonPac AS27 column has similar selectivity and capacity and therefore most applications can be performed on either column. However, the Dionex IonPac AS27 column is recommended for samples preserved with EDA. Using an isocratic or gradient hydroxide eluent, inorganic anions can be easily separated in a variety of sample matrices, including drinking water, groundwater, wastewater, process streams, and scrubber solutions.

The Dionex IonPac AS28-Fast-4µm column is a high-resolution, high-capacity anion-exchange column that has been designed to retain fluoride from the water dip and to separate common anions and low-molecular weight organic acids encountered in high-purity water matrices. Thus, the selectivity of this column is very similar to that of the Dionex IonPac AS15 column (see Section 3.1.2). The high resolution provides better peak identification and high

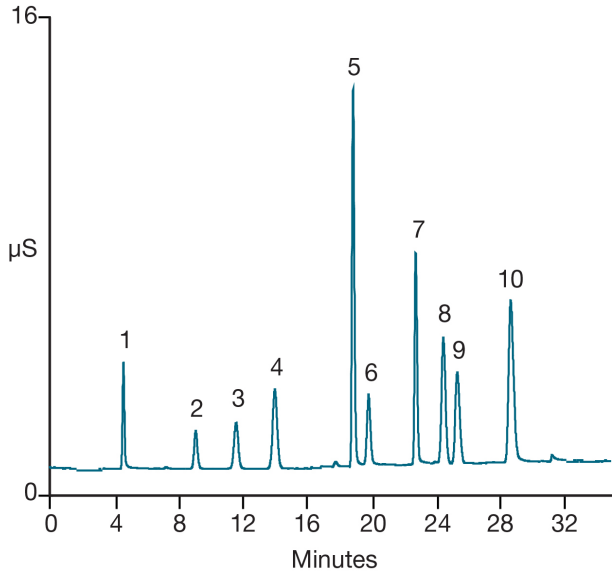


Figure 3.22 Gradient separation of oxyhalides and common inorganic anions on a Dionex IonPac AS26 column. Separator column: Dionex IonPac AS26; column dimensions: 250 mm × 2 mm i.d.; column temperature: 15 °C; eluent: KOH (EG); gradient: 12 mmol/L from 0 to 10 min, 12–65 mmol/L from 10 to 25 min; flow rate: 0.25 mL/min; detection: suppressed conductivity; injection volume: 2.5 µL; peaks: 3 mg/L fluoride (1), 10 mg/L chlorite (2), 20 mg/L bromate (3), 6 mg/L chloride (4), 30 mg/L sulfate (5), 15 mg/L nitrite (6), 40 mg/L ortho-phosphate (7), 25 mg/L each of bromide (8), chlorate (9), and nitrate (10).

capacity allows injection of more concentrated samples without overloading the column. Electrolytically generated hydroxide is normally used for gradient elution to minimize background shift. The chromatograms in Figure 3.23 illustrate the separation of weakly retained anions such as fluoride, acetate, glycolate, and formate on the Dione IonPac AS28-Fast-4µm column using a hydroxide gradient at a controlled temperature of 30 °C. Analysis time can be reduced by using a slightly higher flow rate, but in this case, gradient steps must be reduced by approximately the same factor as the flow rate increase.

The Dionex IonPac AS31 column has been designed for faster separation of haloacetic acids (HAAs), dalapon, and bromate in drinking water. As a low-bleed column, it can be used for ion chromatography separations coupled with mass spectrometry (IC–MS or IC–MS/MS). Its high capacity allows the injection of relatively large sample volumes. Water samples can be directly injected into an ion chromatography system coupled to a mass spectrometer for sensitive detection. The use of the Dionex IonPac AS31 column for IC–MS and IC–MS/MS eliminates the need for sample pretreatment and preconcentration that is typically required to eliminate sample matrix effects on analyte peak efficiency and resolution, such as in HAA determination or other environmental ions in relatively high-ionic strength samples. The Dionex IonPac AS31 column provides good separation of all nine haloacetic acids, bromate, and dalapon using a potassium hydroxide gradient with approximately 40% shorter run times as compared to the Dionex IonPac AS24 column.

The Dionex IonPac AS32-Fast-4µm column has been designed for the analysis of low-polarity anions, polythionates, polysulfonated aromatics, aromatic dyes, and pigments. Its selectivity allows for sub-microgram/liter quantification of perchlorate in matrices with high chloride and sulfate concentrations using either suppressed conductivity or mass spectrometry detection. This column is available in standard bore and microbore formats, each 150 mm long.

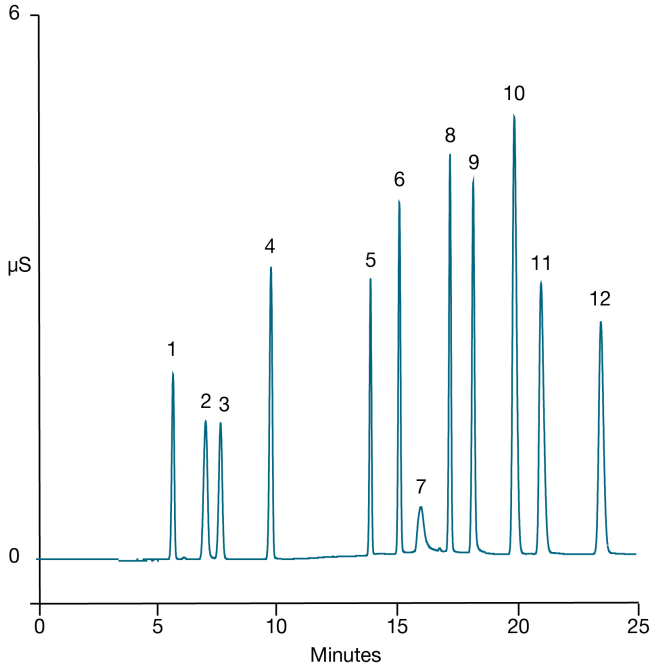


Figure 3.23 Gradient elution of common inorganic anions and low-molecular weight organic acids on a Dionex IonPac AS28-Fast-4µm column. Column dimensions: 150 mm × 2 mm i.d.; column temperature: 30 °C; eluent: KOH (EG); gradient: 5 mmol/L from 0 to 6 min, 5–20 mmol/L from 6 to 8 min, 20–72 mmol/L from 8 to 13 min, 72 mmol/L from 13 to 25 min; flow rate: 0.3 mL/min; detection: suppressed conductivity; injection volume: 2.5 µL; peaks: 1 mg/L fluoride (1), 5 mg/L each of acetate (2), glycolate (3), and formate (4), 2.5 mg/L chloride (5), 5 mg/L nitrite (6), 25 mg/L carbonate (7), 5 mg/L sulfate (8), 5 mg/L oxalate (9), 10 mg/L bromide (10), 10 mg/L nitrate (11), and 15 mg/L ortho-phosphate (12).

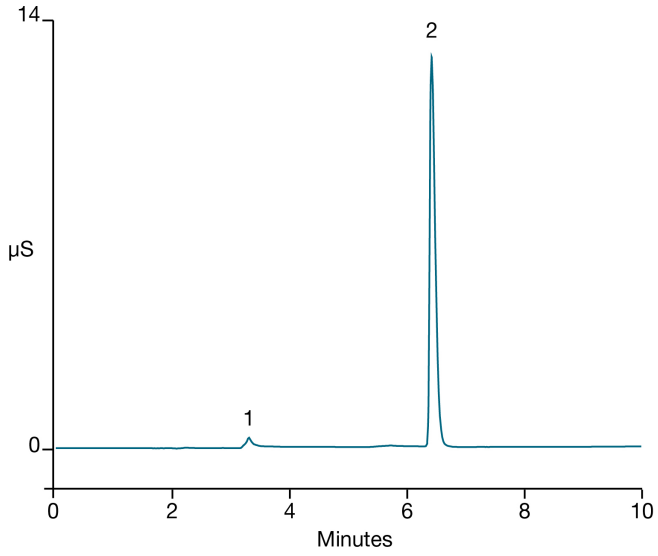


Figure 3.24 Analysis of peroxodisulfate on a Dionex IonPac AS32-Fast-4µm column. Column dimensions: 150 mm × 2 mm i.d.; column temperature: 30 °C; eluent: KOH (EG); gradient: 10 mmol/L from 0 to 3 min, 10–30 mmol/L from 3 to 5 min, 30 mmol/L from 5 to 10 min; flow rate: 0.3 mL/min; detection: suppressed conductivity; injection volume: 2.5 µL; peaks: sulfate (1) and 25 mg/L peroxodisulfate (2).

As an example of the fast elution of strongly polarizable anions, Figure 3.24 shows the separation of peroxodisulfate, which is a strong oxidizing agent used in various applications such as surface preparation for plating and coating processes and measurement of total organic carbon (TOC). Chromatographic analysis of peroxodisulfate used to be carried out by ion-pair chromatography using either silica-based [9] or polymer-based [10] reversed-phase columns with tetrabutylammonium hydroxide as the ion-pair reagent. In 2011, Khan and Adewuyi [11] were the first to report the elution of peroxodisulfate from a Dionex IonPac AS11-HC column, but a very high hydroxide concentration close to c = 200 mmol/L was required to elute peroxodisulfate within 50 min. As can be seen from Figure 3.24, the Dionex IonPac AS32-Fast-4µm column elutes peroxodisulfate extremely fast using a moderate hydroxide eluent concentration. Under similar chromatographic conditions, polythionates such as di-, tri- and tetrathionate can also be eluted in less than 15 min.

3.2 Anion exchangers for the analysis of carbohydrates, amino acids, proteins, and oligonucleotides

Carbohydrates Carbohydrates are weak acids with pK values between 12 and 14 [12]; they can be converted into their anionic form in a superalkaline environment and thus be separated by anion-exchange chromatography [13]. Sodium hydroxide proved to be a suitable eluent in the concentration range between 0.001 mol/L and 0.75 mol/L. The hydroxide ions have two functions: (1) they act as eluent ions and (2) they determine the pH value of the mobile phase. Thus, a change in the hydroxide ion concentration in the mobile phase has two different effects on the retention behavior of carbohydrates. While carbohydrate dissociation and, consequently, retention increases with increasing pH value, the associated higher eluent ion concentration results in a retention decrease. As long as the carbohydrates are not fully dissociated, the two effects compensate each other. At complete dissociation of carbohydrates, a further increase in the hydroxide ion concentration merely results in decreased retention. Carbohydrates with a high affinity toward the stationary phase can be analyzed by adding sodium acetate to the eluent, which is not detected by the pulsed amperometry detection system [14].

In preparing sodium hydroxide-based eluents, extreme care must be taken that they are carbonate-free. Since carbonate ions exhibit a much higher elution strength than hydroxide ions, the presence of even small amounts of carbonate reduces resolution. Therefore, eluents should be prepared from a 50% NaOH concentrate that contains only small amounts of carbonate. The deionized water being used to make up the mobile phase must be degassed thoroughly with helium prior to adding the concentrate. The eluent itself must be stored under helium. For the separation of monosaccharides and amino sugars, which is usually carried out with hydroxide concentrations $c << 0.1$ mol/L, electrolytic hydroxide generation with an eluent generator is mandatory to obtain reproducible retention times (see Section 3.3). The structural and technical properties of all the anion exchangers for carbohydrate analysis currently offered by Thermo Fisher Scientific are summarized in Table 3.6.

Introduced in 1983, the Thermo Scientific™ Dionex™ CarboPac™ PA1 column is the oldest separator column, designed as a universal-purpose column for various sugar alcohols and saccharides. It is used to the present day for samples with complex matrices such as food because of its ruggedness. Baseline-resolved separations of various sugar alcohols are obtained on a Dionex CarboPac MA1 column, which consists of a fully aminated, macroporous substrate functionalized with a quaternary alkyl amine. Since this material is not very stable mechanically, the separator column must be operated at the relatively low flow rate of 0.4 mL/min. At this stationary phase, the most important sugar alcohols present in foodstuffs and physiological samples can be separated under isocratic conditions (Figure 3.25). Due to the extremely high ion-exchange capacity of the Dionex CarboPac MA1 column, a highly concentrated NaOH eluent is required. The column can also be utilized under gradient conditions. As an example, Figure 3.26 shows the gradient separation of weakly retained anhydrosugars [15], such as levoglucosan, galactosan, and mannosan, which originate from the combustion of cellulose and hemicelluloses. Optimum resolution between levoglucosan and arabitol is achieved with an initial NaOH concentration of $c = 480$ mmol/L, increasing it later to 650 mmol/L to elute disaccharides in the same chromatographic run.

Dionex CarboPac PA10 and PA20 columns are typically employed for the separation of various neutral and acidic monosaccharides as well as disaccharides. The Dionex CarboPac PA20 column is the successor to the Dionex CarboPac PA10 column, following the general trend in ion-exchange chromatography toward higher sample throughput without compromising resolution. Based on 6.5 µm resin technology, the Dionex CarboPac PA20 column provides improved resolution, peak shapes, and efficiencies over the Dionex CarboPac PA10 column for the separation of the six monosaccharides found in glycoprotein hydrolysates. The respective anion-exchange material is packed in a 150 mm × 3 mm i.d. column for fast separations. As illustrated in Figure 3.27, optimal resolution between the six analytes is achieved with a relatively low hydroxide eluent concentration of $c = 10$ mmol/L that requires the use of electrolytic eluent generation (see Section 3.3) for reproducible retention times. The Dionex CarboPac PA20 column is also available in a capillary format that offers additional advantages of higher mass sensitivity and significantly lower eluent consumption.

The latest addition to the Dionex CarboPac column family for fast mono- and disaccharide analysis in biofuels and foods is the Dionex CarboPac SA10 column. It is composed of a unique 6 µm macroporous substrate that is coated with a layer of nanobeads functionalized with alkanol quaternary ammonium groups. The wide-pore resin structure permits fast mass transfer, resulting in high-resolution separations. The highly porous nanobeads have a large surface area, providing higher loading capacity. The combination of the high capacity provided by the morphology of the porous substrate and the new chemistry of the nanobead functionality delivers high resolution and short analysis times for common sugars in biofuel and food applications.

Table 3.6 Structural and technical properties of anion exchangers suitable for carbohydrate analysis.

Separator	Dionex CarboPac PA1	Dionex CarboPac SA10	Dionex CarboPac PA10	Dionex CarboPac PA20	Dionex CarboPac PA100	Dionex CarboPac PA200	Dionex CarboPac MA1	Dionex CarboPac PA210-Fast-4µm
Dimensions (length × i.d.) (mm)	250 × 2 250 × 4	250 × 4	250 × 2 250 × 4	150 × 3 150 × 0.4	250 × 2 250 × 4 50 × 9 250 × 22	250 × 3	250 × 4	150 × 2 150 × 4
Support material	PS/DVB	EVB/DVB	EVB/DVB	EVB/DVB	EVB/DVB	EVB/DVB	VBC/DVB	EVB/DVB
Particle diameter (µm)	10	6	10	6.5	8.5	5.5	7.5	4
Degree of nanobead crosslinking (%)	5	4.5	5	5	6	6	15	6
Nanobead diameter (nm)	500	55	460	130	275	43	--	43
Ion-exchange capacity (µequiv/col.)	100	290	100	65 1.16	90	35	1450	17 66
Recommended flow rate (mL/min)	0.25 1	1.5	0.25 1	0.5 0.01	0.25 1	0.5	0.4	0.2 0.8
Max. pressure (MPa)	27.5	24.1	27.5	24.1	27.5	18.6	9.6	27.5
pH stability	0–14	0–14	0–14	0–14	0–14	0–14	0–14	1–14
Solvent compatibility (%)	2	100	90	100	100	100	0	100
Application	Separation of acidic, basic, and neutral mono-saccharides	Fast separation of mono- and di-saccharides in biofuels, food and beverages	Separation of mono- and disaccharides derived from glycoproteins	Rapid separation of mono- and disaccharides derived from glycoproteins	Separation of oligo-saccharides	High-resolution separation of oligo-saccharides	Special column for sugar alcohols and alditols	High-resolution separation of mono- through tetra-saccharides in food

Both the biofuel and the food and beverage industries are processing a large number of samples and thus require high-throughput capabilities for mono- and disaccharide analysis. Rapid separation of the eight most common biofuel mono- and disaccharides can be achieved on a Dionex CarboPac SA10 column in less than 10 min, as shown in Figure 3.28. The same type of separation on a Dionex CarboPac PA20 would require almost 30 min. The column is also capable of separating the six common food sugars, sucrose, glucose, fructose, lactose, cellobiose, and maltose, in the exceptionally fast time of only 10 min under isocratic conditions.

Dionex CarboPac PA100 and PA200 columns are nonporous, high efficiency, nanobead-agglomerated anion exchangers that have been designed for high-resolution oligosaccharide mapping and analysis. With these two columns, the resolution between the various oligo-saccharides can be improved significantly over the Dionex CarboPac PA1 column. Using high-performance anion-exchange chromatography, all classes of oligosaccharides according to structural features, such as size, charge, composition, anomericity, and linkage isomerism, can be separated. Like with the Dionex CarboPac PA10 and PA20 columns, the Dionex CarboPac PA100 and PA200 columns differ in substrate particle diameter and column dimensions. The smaller particle diameter (5.5 µm) and the smaller column format (250 mm × 3 mm i.d.) of the Dionex CarboPac PA200 column, the successor of the Dionex CarboPac PA100 column, leads

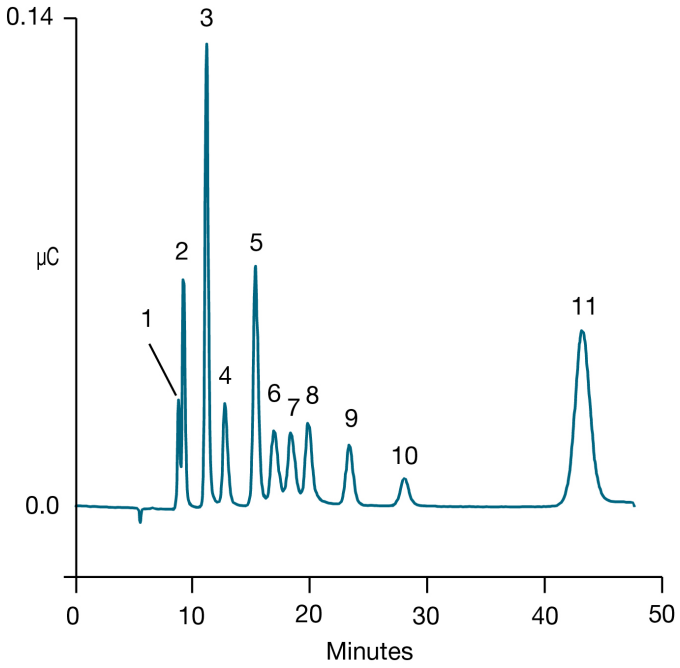


Figure 3.25 Separation of various sugar alcohols and saccharides on a Dionex CarboPac MA1 column. Eluent: 0.5 mol/L NaOH; flow rate: 0.4 mL/min; detection: pulsed amperometry on a gold working electrode; injection volume: 10 µL; peaks: 2 nmol each of *myo*-inositol (1), glycerol (2), *i*-erythritol (3), xylitol (4), arabitol (5), sorbitol (6), dulcitol (7), mannitol (8), glucose (9), fructose (10), and sucrose (11).

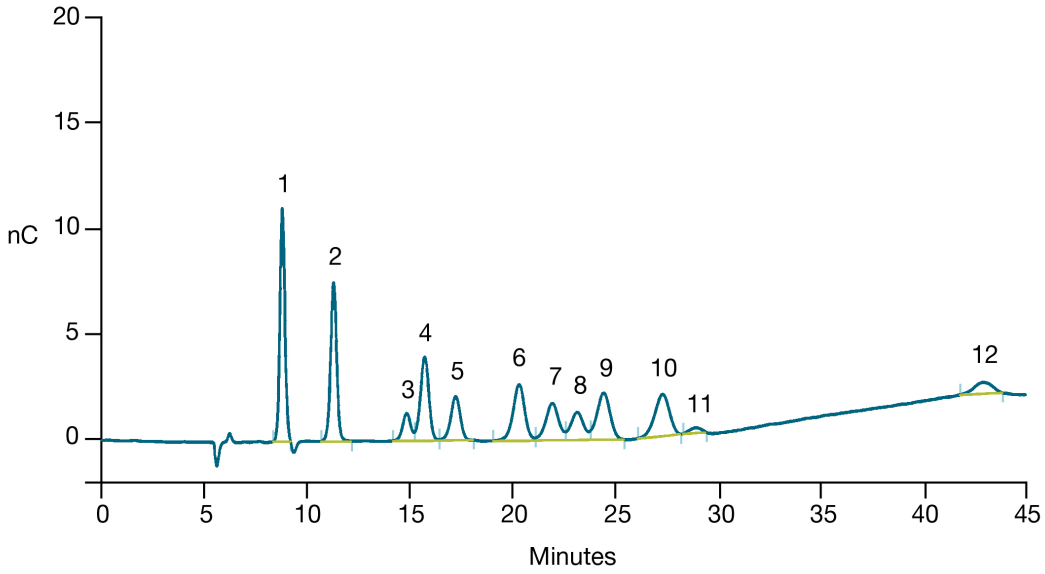


Figure 3.26. Gradient separation of sugar alcohols, anhydrosugars, and mono- and disaccharides. Separator column: Dionex CarboPac MA1; column dimensions: 250 mm × 4 mm i.d.; column temperature: ambient; eluent: NaOH; gradient: 480 mmol/L from 0–20 min, then linearly to 650 mmol/L in 15 min; flow rate: 0.4 mL/min; detection: pulsed amperometry on a gold working electrode; injection volume: 25 µL; peaks: 5 mg/L each of inositol (1), erythritol (2), levoglucosan (3), arabitol (4), mannosan (5), mannitol (6), mannose (7), galactosan (8), glucose (9), galactose (10), fructose (11), and sucrose (12).

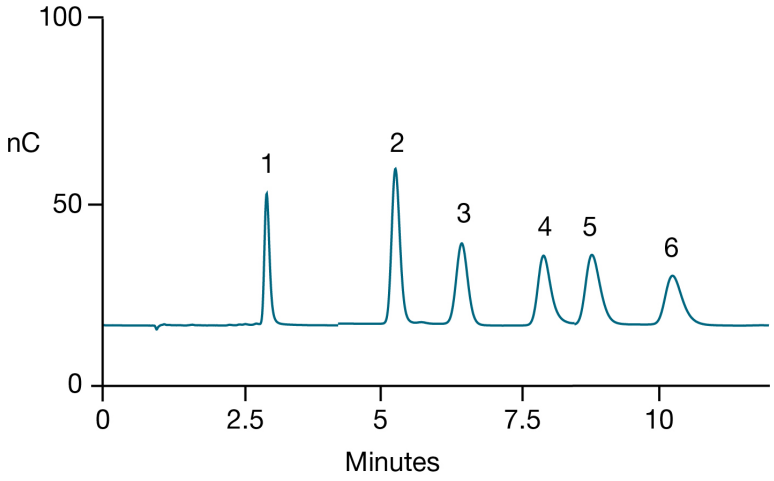


Figure 3.27 Separation of the six monosaccharides found in glycoprotein hydrolysates on a Dionex CarboPac PA20 column. Eluent: 10 mmol/L KOH (EG); flow rate: 0.5 mL/min; detection: pulsed amperometry on a gold working electrode; peaks: 100 pmol each of fucose (1), galactosamine (2), glucosamine (3), galactose (4), glucose (5), and mannose (6).

to higher resolution separations of oligosaccharides. Unlike sugar alcohols, mono- and disaccharides, the elution of the more strongly retained oligosaccharides cannot be accomplished with pure NaOH eluents. If pulsed amperometry is used for detection, linear or curved composition gradients consisting of sodium hydroxide and sodium acetate are typically employed. Sodium acetate accelerates the elution of strongly bound species and offers further selectivity control, without interfering with pulsed amperometric detection. Since the optimum pH value for pulsed amperometry of carbohydrates is pH 13, the NaOH concentration of $c = 0.1$ mol/L is held constant, to which increasing amounts of sodium acetate are added during the chromatographic run. If convex gradient profiles are applied, an improved resolution in the higher molecular weight range is obtained, especially when analyzing polysaccharides [16].

Until recently, NaOH/NaOAc eluents had to be prepared manually. facing enormous problems inherent to the required purity of the chemicals and the related carbon dioxide

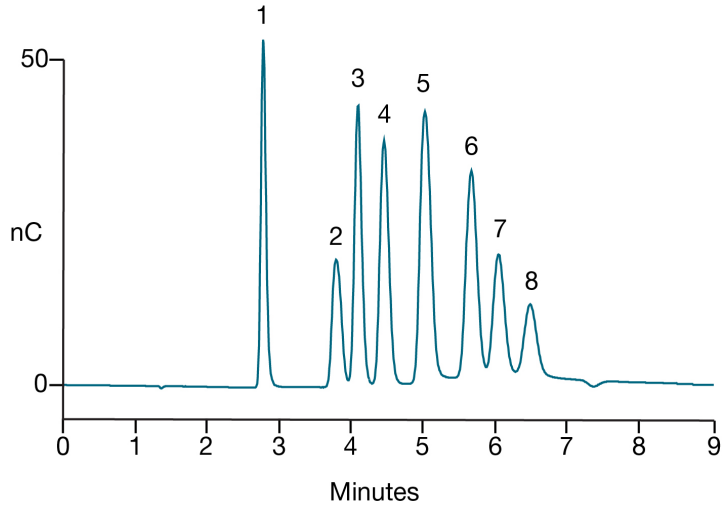


Figure 3.28 Fast separation of biofuel sugars on a Dionex CarboPac SA10 column. Column temperature: 45 °C; eluent: 1 mmol/L KOH (EG); flow rate: 1.5 mL/min; detection: pulsed amperometry on a gold working electrode; peaks: 20 mg/L each of fucose (1), sucrose (2), arabinose (3), galactose (4), glucose (5), xylose (6), mannose (7), and fructose (8).

intrusion. With the novel operating mode for Reagent-Free™ IC systems (RFIC™), called Dual Eluent Generation Cartridge (Dual EGC) mode, manual preparation of the NaOH/NaOAc eluents required for analyzing oligosaccharides can be replaced. In Dual EGC mode, methanesulfonic acid (MSA) and potassium hydroxide (KOH) EGC Eluent Generator Cartridges are employed in series to electrolytically generate KOH/KMSA eluents. As shown in Figure 3.29, deionized water is pumped into the MSA cartridge to generate MSA. The MSA solution is then passed into the KOH cartridge to titrate the potassium hydroxide to potassium methanesulfonate. By balancing the concentration of the two cartridges, pure KMSA can be generated. By generating an excess of KOH compared to MSA, a basic solution of KMSA plus KOH can be generated (Basic Eluent Mode). By generating an excess of MSA compared to KOH, an acidic solution of KMSA plus MSA can be generated (Acidic Eluent Mode). The system can be switched between the two modes as needed. For applications of analyzing oligosaccharides, the system is operated under Basic Eluent Mode. This extension of RFIC technology enables the analyst to run gradient methods using an isocratic pump, while offering the analyst the flexibility to modify or change the eluent concentrations any time through the Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS).

The demand for oligosaccharide analysis is apparent, in the food industry where the need for fast, high-resolution profiling of homologous sugar series, such as inulins, amylopectins, and malto-oligosaccharides, is evident. Using anion-exchange chromatography with pulsed amperometric detection (HPAE-PAD), polymers up to DP70 may be analyzed. As an example, Figure 3.30 shows the separation of inulin, a polyfructan that is used as a component of functional foods on Dionex CarboPac PA100 and PA200 columns. The structure of inulin corresponds to a poly-β-(2–1)-fructofuranosan. It contains about 30 D-fructose residues and a terminal sucrose molecule. As can be seen, the chain length distribution of inulin is captured with much higher resolution on a Dionex CarboPac PA200 column as compared to a Dionex CarboPac PA100 column.

Another important application area is the analysis of oligosaccharides released from glycoprotein therapeutics. Until the 1960s, protein chemists did not give much credit to carbohydrates. Today, we know that most of the eukaryotic proteins exist in a glycosylated form. The carbohydrate moieties, enzymatically coupled to the protein during or after the translation (co- or post-translational), are responsible for a certain biological or therapeutic activity. For these reasons, there has been great interest in investigating the composition and

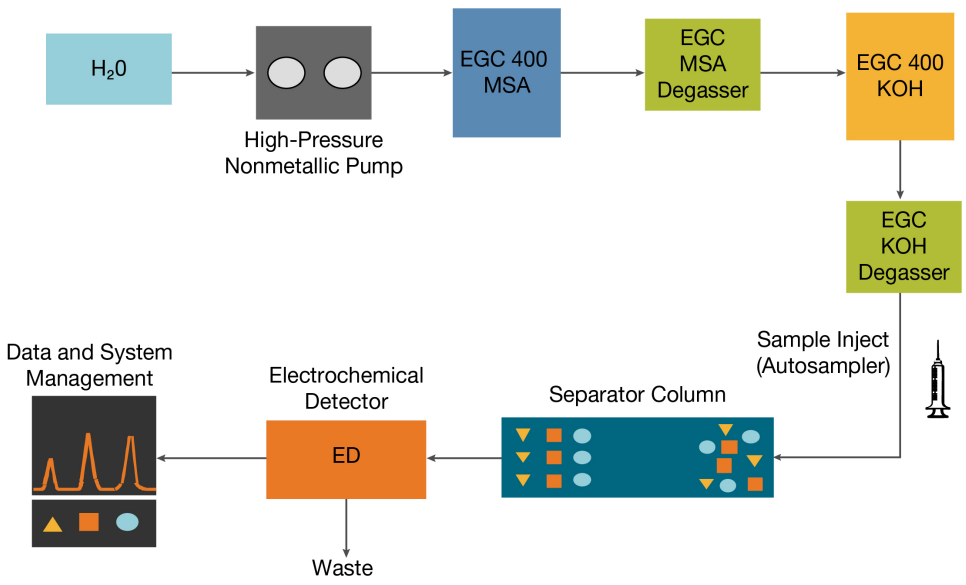


Figure 3.29 Ion chromatography system configuration under Dual EGC mode.

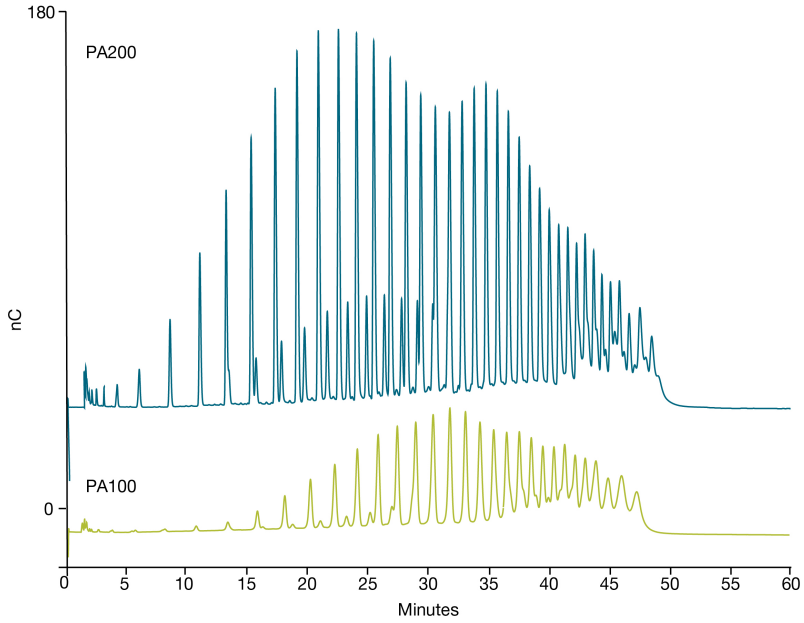


Figure 3.30 HPAE-PAD analysis of inulin. Separator columns: Dionex CarboPac PA100 and PA200. Column formats: 250 mm × 4 mm i.d. (PA100) and 250 mm × 3 mm i.d. (PA200); eluent: NaOH/ NaOAc; gradient: 120–320 mmol/L NaOAc in 100 mmol/L NaOH in 40 min; flow rates: 1 mL/min (PA100) and 0.5 mL/min (PA200); detection: pulsed amperometry on a gold working electrode; sample: inulin from chicory (Sigma-Aldrich).

structure of carbohydrates. The empirical correlation between oligosaccharide structures and their retention behavior has been documented in great detail by Rohrer [17]. As an example, Figure 3.31 shows a high-resolution separation of two mannose 7-linkage isomers on a Dionex CarboPac PA200 column.

The Dionex CarboPac PA210-Fast-4µm column is the latest addition to the Dionex CarboPac family of columns for carbohydrate separations. The new column was developed to provide fast, high-resolution separations for most mono- through tetrasaccharides in a variety of applications including food and beverage analyses. Numerous oligosaccharides, consisting of 3–5 monosaccharide units, occur in foods. These oligosaccharides are generally considered nondigestible. The raffinose family oligosaccharides (RFOs) include raffinose (a trisaccharide), stachyose (a tetra-saccharide), and verbascose (a pentasaccharide). They are found in vegetables such as peas, beans, and lentils. Unlike other sugars, raffinose, stachyose, and verbascose are not digested by human gastrointestinal enzymes in the small intestine and are poorly absorbed by most people. As such, they are referred to as prebiotic carbohydrates. Interest in fructans and RFOs has increased during the last decade due to their health-promoting abilities to selectively stimulate beneficial bacteria. Aside from their prebiotic characteristics, fructans and RFOs are also emerging as important immune stimulators in animals and humans. The unique pellicular resin of the Dionex CarboPac PA210-Fast-4µm column offers exceptional selectivity, separating 20 mono- through tetrasaccharides in approximately 20 min with a pure hydroxide eluent.

Amino acids Although amino acids are traditionally separated on totally sulfonated cation exchangers, pellicular anion exchangers are also used today, especially in combination with integrated pulsed amperometric detection [18]. The Thermo Scientific™ Dionex™ AminoPac™ PA10 column was developed for anion-exchange chromatography of amino acids.

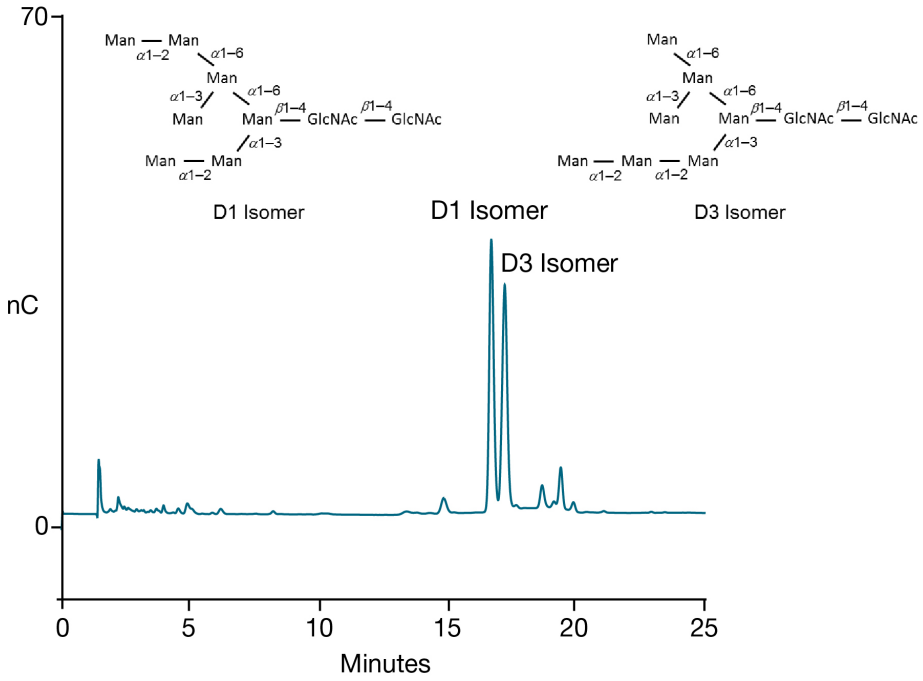


Figure 3.31 Gradient elution of mannose-7-isomers on a pellicular anion exchanger. Separator column: Dionex CarboPac PA200; column format: 250 mm × 3 mm i.d.; eluent: NaOH/NaOAc; gradient: 0–200 mmol/L NaOAc in 100 mmol/L NaOH in 110 min; flow rate: 0.5 mL/min; detection: pulsed amperometry on a gold working electrode; sample: mannose-7-isomers (Dextra Labs, Reading, UK).

The resin consists of a poly(styrene-*co*-divinylbenzene) substrate with a particle diameter of 8.5 µm and a degree of cross-linking of 55%. This stationary phase is compatible with typical HPLC solvents, although solvents are not necessary for separating amino acids by anion-exchange chromatography. The nanobeads, which are functionalized with an alkylamine, have a diameter of 80 nm and a relatively low degree of cross-linking of 1%. To achieve high mass sensitivity, the Dionex AminoPac PA10 column is only available in the microbore format (250 mm × 2 mm i.d.).

Nanobead-agglomerated anion exchangers are an alternative to cation exchangers. Figure 3.32 illustrates a typical elution profile of hydrolysate amino acids on a Dionex AminoPac PA10 column; the gradient program being used is graphically highlighted. It was obtained in less than 30 min with a NaOH/NaOAc gradient utilizing pulsed amperometric detection. As seen from the chromatogram in Figure 3.32, anion-exchange chromatography allows the complete separation of phenylalanine and tyrosine, which is not possible on conventional cation-exchange resins. The elution order is reversed in comparison with cation exchange: arginine and lysine elute first, and threonine and serine are separated much better. *O*-phosphorylated amino acids such as P-Arg, P-Ser, P-Thr, and P-Tyr, which are only weakly retained on a cation exchanger, elute toward the end of the chromatographic run in the retention range of tyrosine when using an anion exchanger. Nevertheless, they are separated very well from hydrolysate amino acids. The separation of amino acids on anion exchangers with integrated pulsed amperometric detection is compatible with all common hydrolysis protocols based on hydrochloric acid, performic acid, methanesulfonic acid, and sodium hydroxide.

Proteins Proteins are isolated and characterized with different chromatographic techniques. Depending on the protein, ion-exchange (IEX), size-exclusion (SEC), affinity (IMAC), hydrophobic interaction (HIC), and reversed-phase chromatography (RPLC) may be applied.

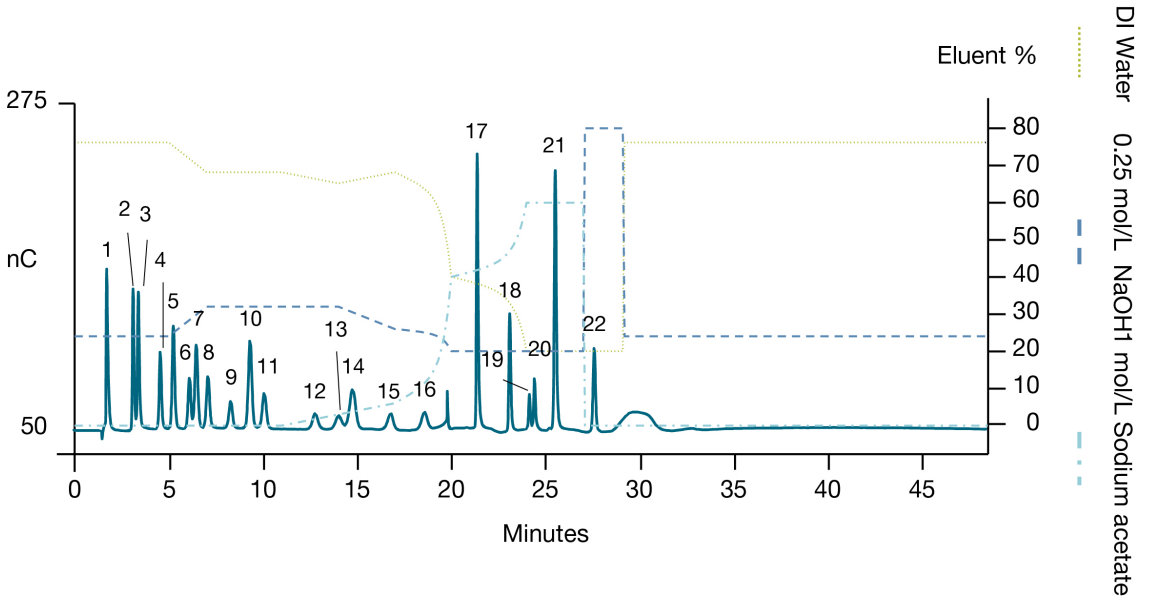


Figure 3.32 Separation of amino acids on a Dionex AminoPac PA10 column. Eluent: NaOH/NaOAc; gradient: graphically highlighted; flow rate: 0.25 mL/min; detection: integrated pulsed amperometry on a gold working electrode; analytes: 100 pmol each of arginine (1), ornithine (2), lysine (3), glutamine (4), asparagine (5), alanine (6), threonine (7), glycine (8), valine (9), serine (10), proline (11), isoleucine (12), leucine (13), methionine (14), norleucine (15), taurine (16), histidine (17), phenylalanine (18), glutamate (19), aspartate (20), cystine (21), and tyrosine (22).

Traditionally, separation materials with low mechanical stability and limited resolution were used. In recent years, HPLC materials were developed that offered the separation power required by the protein chemist. Now, pellicular ion-exchange resins as well as organic polymer monoliths exhibit high resolution at relatively short retention times, while maintaining the biological activity of the molecule.

Ion-exchange chromatography is widely used in the analysis of protein variants that may include glycosylated [19], phosphorylated [20], deamidated [21], truncated [22]. and oxidized [23] forms. Chromatographic separations of closely related variants are challenging because of the physicochemical properties associated with proteins. The high molecular masses of proteins and their large Stokes radii are responsible for hydrophobic (nonspecific) interactions with the stationary phase and poor mass-transfer properties, even in free solution. In conjunction with the anfractuous nature of the pore structure found in conventional porous particles, these low diffusivities can result in excessive band broadening under chromatographic conditions that would be suitable for small analytes. A simple solution to this problem is to utilize tentacle-type support materials [24]. With this type of stationary phase, the contact between the protein and the substrate material is markedly reduced, which minimizes hydrophobic interaction between the protein and the stationary phase.

Tentacular ion exchangers exhibit minimal band broadening in conjunction with extremely high selectivity. The high selectivity is due to the flexibility of the charge arrangement between charged sites on the protein surface and the functional groups located on the grafted tentacle strands. The general structure of a tentacular ion exchanger shown in Figure 3.33 consists of a rigid and impervious 10 µm styrenic substrate particle, which is highly cross-linked (55%) and completely coated with a base stable hydrophilic neutral polymer. Tentacles bearing the ion-exchange functional groups are then grafted to the hydrophilic surface. The length and surface distribution of these covalently bonded polymer chains are well-defined. The selectivity of this column packing differs significantly from tentacles attached to silica particles where free silanol groups can contribute to undesirable interactions with the protein analyte.

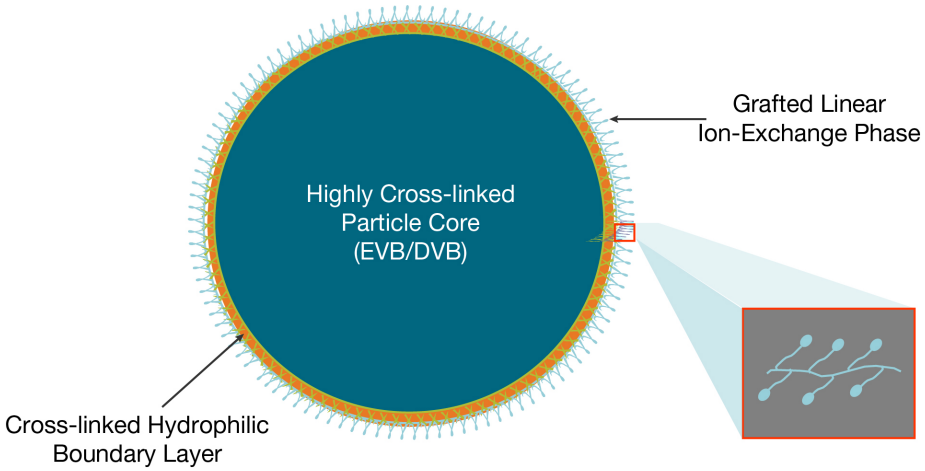


Figure 3.33 Schematic structure of a tentacular material for protein separation.

Thermo Scientific™ ProPac™ columns rely on polymer-based tentacular materials for anion-exchange chromatography of proteins. The specifications of the two different ProPac anion exchangers currently available are summarized in Table 3.7. Tentacular anion exchangers are predominantly used for the following separation problems:

- Acidic proteins
- Glycosylation variants
- Phosphorylation variants
- Protein isoforms

Protein phosphorylation is one of the most studied post-translational modifications because it provides a rapid, reversible means of regulating protein activity and numerous other cellular processes including cell differentiation, proliferation, and migration. Changes in levels of phosphorylated isoforms often signal developmental or pathological disorders. To better understand the role of phosphorylation for clinical diagnostics, it is important to differentiate phosphorylated forms of protein from their dephosphorylated forms. Ovalbumine is a good example of this. As a 43 kDa phosphoglycoprotein, it is an essential constituent of avian egg white. It carries *N*-linked oligosaccharides of the hybrid- and mannose type, and phosphate residues on two serine amino acids at positions 68 and 344. Hence, the degree of phosphorylation can vary quite a bit. The ProPac SAX-10 strong basic, tentacular anion exchanger separates ovalbumine in nine prominent peaks (Figure 3.34, lower trace), two of which could be identified as the mono- and diphosphorylated forms. The other signals probably represent further microheterogeneic forms or ovalbumine variants that differ in amino acid composition. The upper trace in Figure 3.34 shows the dephosphorylated form of ovalbumin after treatment with alkaline phosphatase. As a result, an earlier elution of peaks is observed, which is consistent with a loss of negative charge. Ovalbumine has been reported to have four isoforms with varying phosphate content, one with zero, two with one, and one with two phosphates [25]. Comparing the two traces in Figure 3.34, the treatment with alkaline phosphatase reduces the number of peaks from nine in the native sample to one large and three smaller peaks in the dephosphorylated sample.

Pellicular anion exchangers can also be used for profiling dairy milk caseins. Caseins are a group of phosphoproteins that represent about 80% of the total protein in bovine milk and between 20 and 45% of the proteins in human milk. Caseins are insoluble at their isoelectric point, pH 4.6, and exist in solution as micelles. However, in sharp contrast to surfactant micelles, the interior of a casein micelle is highly hydrated. The caseins in the micelles are held

Table 3.7 Specifications of Thermo Scientific ProPac anion exchangers.

Parameter	ProPac WAX-10	ProPac SAX-10
Base matrix material	Ethylvinylbenzene 55% cross-linked with divinylbenzene	Ethylvinylbenzene 55% cross-linked with divinylbenzene
Substrate bead diameter (µm)	10	10
Functional group	Tertiary amine	Quaternary amine
Temperature range	Ambient to 60 °C	Ambient to 60 °C
Flow rate range (mL/min)	0.2–2	0.2–2
Max. operating pressure	21 MPa (3000 psi)	21 MPa (3000 psi)
pH range	2–12	2–12
Protein binding capacity per mL volume	15 mg BSA	15 mg BSA
Typical buffers	Sodium and potassium salts of phosphate, MES, Tris	Sodium and potassium salts of phosphate, MES, Tris
Solvent compatibility	80% MeCN, acetone 80% MeOH	80% MeCN, acetone 80% MeOH
Detergent compatibility	Nonionic, cationic, zwitterionic	Nonionic, cationic, zwitterionic

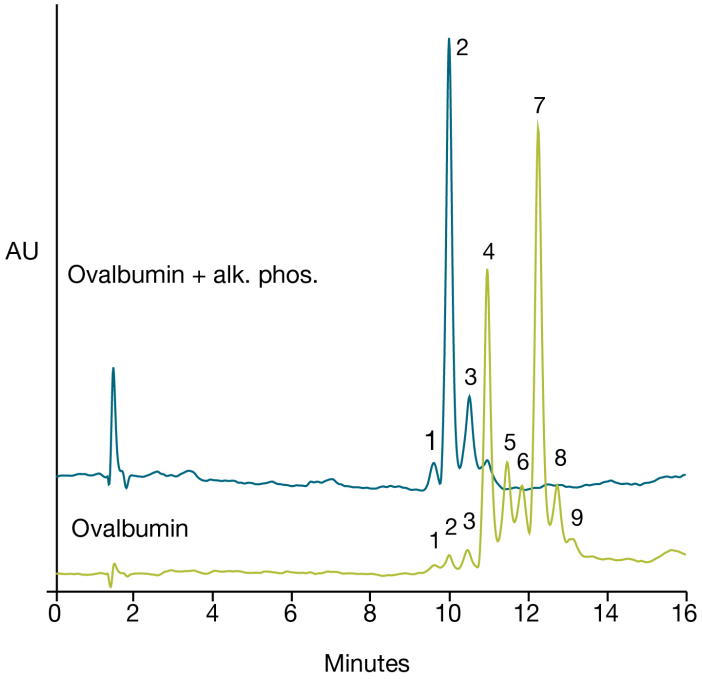


Figure 3.34 Separation of ovalbumine before and after treatment with alkaline phosphatase. Separator column: ProPac SAX-10; column dimensions: 250 mm × 4 mm i.d.; column temperature: 25 °C; eluent: (A) 0.02 mol/L Tris/HCl, pH 8.5, (B) 20 mmol/L Tris/HCl + 0.5 mol/L NaCl, pH 8.5; gradient: linear, 0% B to 50% B in 15 min, 50% B to 96% B in 2 min; flow rate: 1 mL/min; detection: UV (280 nm); injection volume: 10 µL; sample: 2 mg/mL ovalbumine and dephosphorylated ovalbumine.

together by calcium ions and hydrophobic interactions. In the dairy industry, milk protein profiling is used to assess adulteration and the effects of processing. It is known that milk protein profiling is depending on the type of animal as well as on the state of lactation and the nutritional status of the animal. Figure 3.35 shows a high-resolution separation of caseins from bovine and goat milk. The disruption of the micelles was achieved by dissolving the milk proteins and chromatographing them with a mobile phase containing urea and 2-mercapto-ethanol. This figure reveals that caseins are very complex samples with considerable heterogeneity and that some of their retention is due to phosphorylation.

Introduced by Svec and Fréchet in the early 1990s [26], porous polymer monoliths are successfully used for the separation of biomolecules, such as peptides and proteins. The relatively low surface area as a result of the high porosity of the material is one of the reasons for its extensive use with larger analytes. Svec et al. focused their research on monolithic capillary columns for nano-HPLC with well-defined flow-through properties and surface chemistries [27]. They are prepared in tubes by *in situ* polymerization and can be used directly as separator columns. Thermo Scientific™ ProSwift™ columns of this type are commercially available from Thermo Fisher Scientific (Waltham, MA, USA). The most widely used polymer monoliths are poly(styrene-*co*-divinylbenzene)-based monoliths and moderately polar monoliths based on methacrylic acid esters.

The ProSwift SAX-1S and ProSwift WAX-1S anion-exchange monoliths are specifically designed to provide high-resolution, high-efficiency separations of proteins at elevated flow rates with a loadability exceeding that of nonporous beads. ProSwift media are methacrylate-based monoliths with a unique morphology, pore structure, and pore size distribution to offer the optimum performance for the separation of proteins and other biomolecules. The monoliths consist of flow-through pores and cauliflower-shaped globules with meso- and micropores.

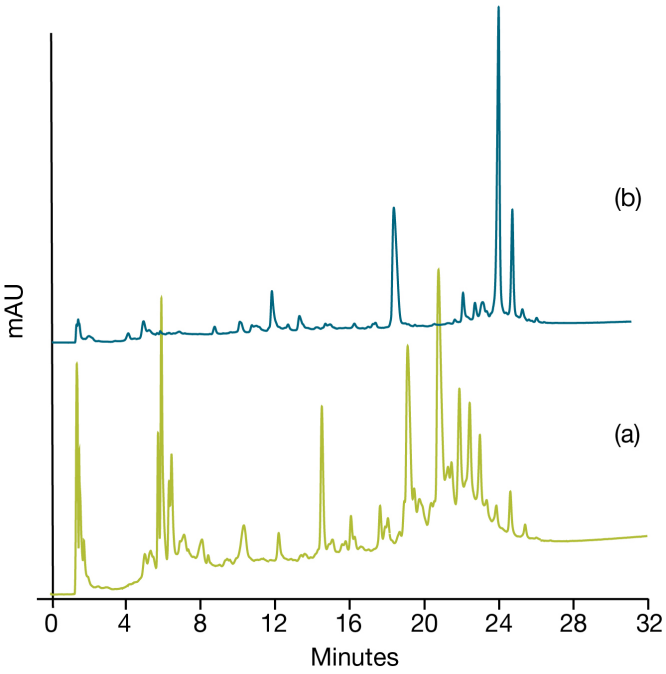


Figure 3.35 Separation of caseins from bovine and goat milk. Separator column: ProPac SAX-10; column dimensions: 250 mm × 4 mm i.d.; eluent: (A) 4 mol/L urea + 0.1 mol/L 2-mercaptoethanol + 0.01 mol/L imidazole, pH 7, (B) 4 mol/L urea + 0.1 mol/L 2-mercaptoethanol + 0.01 mol/L imidazole + 1 mol/L NaCl, pH 7; gradient: linear, 0% B for 3 min, 5% B to 30% B in 27 min; flow rate: 1 mL/min; detection: UV (280 nm); injection volume: 50 µL; sample: 1 mg/mL caseins from (a) goat milk and (b) bovine milk obtained by precipitation at pH 4.5 of whole milk.

Micropores, with sizes smaller than 2 nm, contribute most significantly to the overall surface area, followed by the mesopores ranging from 2 to 50 nm. The flow-through pores are essential to allow liquid to flow through the monolith at reasonably low back pressure, but do not contribute much to the surface area. Mass transfer is primarily driven by convective flow through these large pores instead of much slower molecular diffusion. This allows higher flow rates to be used with little effect on resolution. Diffusion-controlled mass transfer is minimized because the globules are essentially nonporous. Table 3.8 summarizes the specifications of ProSwift columns. The high loading capacities of the 4.6 mm columns allow research-scale semipreparative analyses to be carried out. Large amounts of protein can be loaded while maintaining resolution to allow the detection and/or collection of low-abundant proteins. In case sample is limited, the higher mass sensitivity of the 1 mm column is ideal because it allows better detection with less sample loading.

ProSwift WAX-1S columns can be used to separate complex protein mixtures obtained, for instance, from the lysate of *Escherichia coli* cells, which is typically carried out by multidimensional chromatography. In top-down proteomics, the use of a high-capacity ion exchanger in the first dimension is a very common first step toward the identification of individual proteins. Figure 3.36 shows such separation on a 50 mm × 1 mm i.d. ProSwift WAX-1S column. Its small column volume in combination with its relatively high capacity favors this column for use as a first dimension in multidimensional chromatography.

Table 3.8 Specifications of ProSwift anion-exchange columns.

Parameter	**ProSwift WAX-1S**	**ProSwift WAX-1S**	**ProSwift SAX-1S**	**ProSwift SAX-1S**
Column dimensions (length × i.d.) (mm)	50 × 4.6	50 × 1	50 × 4.6	50 × 1
Base matrix material	Polymethacrylate	Polymethacrylate	Polymethacrylate	Polymethacrylate
Functional group	Tertiary amine	Tertiary amine	Quaternary amine	Quaternary amine
Protein binding capacity per mL of polymer	18 mg BSA	18 mg BSA	18 mg BSA	18 mg BSA
Protein binding capacity per mL of polymer	13 mg BSA	0.7 mg BSA	13 mg BSA	0.7 mg BSA
Bed height (mm)	44	50	45	50
Bed volume (mL)	0.73	0.039	0.75	0.039
Recommended flow rate (mL/min)	0.5–1.5	0.05–0.25	0.5–1.5	0.05–0.25
Max. flow rate (mL/min)	2	0.3	2	0.3
pH range	2–12	2–12	2–12	2–12
Operating pressure	<500 psi 3.4 MPa	<1500 psi 10.4 MPa	<500 psi 3.4 MPa	<1500 psi 10.4 MPa
Max. pressure	1000 psi 6.8 MPa	2000 psi 13.8 MPa	1000 psi 6.8 MPa	2000 psi 13.8 MPa
Temperature	60 °C	60 °C	70 °C	70 °C
Solvent compatibility	Most common organic solvents			

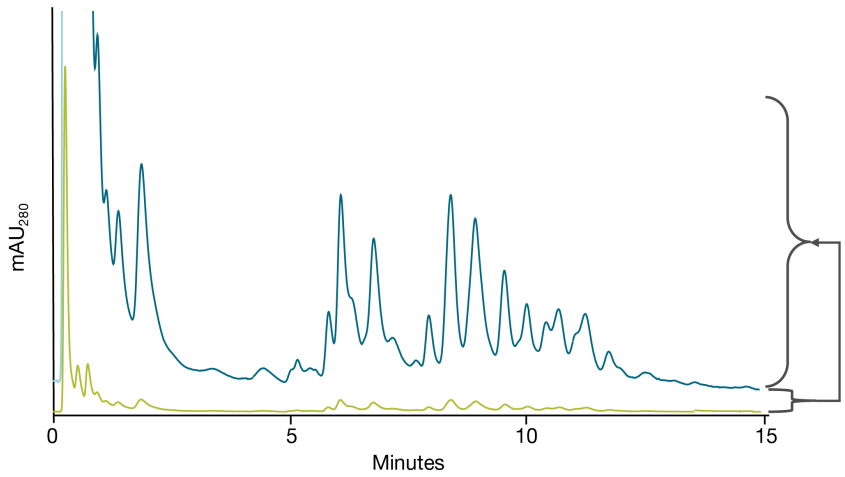


Figure 3.36 Separation of *E. Coli* proteins on ProSwift WAX-1S column. Column dimensions: 50 mm × 1 mm i.d.; column temperature: 30 °C; eluent: (A) 10 mmol/L Tris, pH 7.6, (B) 10 mmol/L Tris + 1 mol/L NaCl, pH 7.6: gradient: linear, 5% B to 55% B in 13 min; flow rate: 0.2 mL/min; detection: UV (280 nm); injection volumes: 1.3 µL; sample: 1.25 mg/mL *E. Coli* proteins.

Nucleic acids In recent years, the demand for synthetic oligonucleotides has drastically increased because their medical–therapeutic and molecular–biological use, especially in genetic engineering, has gained more and more importance. Oligonucleotides are used for gene sequencing, as linkers for recombinant DNA technology, as primers for sequencing, as substrates for enzymatic and structural analysis, or as sensors for detecting DNA and RNA sequences in the diagnosis of genetic defects. Another increasingly important function of oligonucleotides is their use as *antisense* therapeutics for suppressing the expression of cancer and other genes. *Antisense* oligonucleotides exist in a modified form as phosphorothioates.

Oligonucleotides have a number of properties that can be utilized in their chromatographic separation. As polyvalent anions, they differ in net charge depending on their chain length, so that they can be separated in a simple way on anion exchangers. Modern applications of modified oligonucleotides as diagnostic sensors or therapeutic agents require the highest possible resolution (*N*,*N*-1 with oligonucleotides >DP30) and short retention times (<30 min). Only pellicular ion exchangers can meet these demands because their ion-exchange groups are directly accessible to the analytes. In this way, their small diffusion rate is compensated; normally the small diffusion rate has a negative influence on the efficiency of the separation when using macroporous resins.

The Thermo Scientific™ DNAPac™ PA100 and Thermo Scientific™ DNAPac™ PA200 columns are the two pellicular anion exchangers for nucleic acid analysis currently available from Thermo Fisher Scientific. The latter is the successor of the former [28]. Both columns have been developed for a wide range of DNA samples, including ssDNA and RNA. The structural and technical properties of these two columns are summarized in Table 3.9.

A very common application is the analysis of synthetic oligonucleotides, which are usually 10 to 30 bases long and constructed using automated DNA synthesizers. The delivered product typically includes truncated sequences, some partially depurinated oligomers, deprotected oligomers, and the target, full-length, fully dimethoxytrityl (DMT)-protected oligonucleotides [29]. Since the nonporous, nanobead-agglomerated resins used in anion-exchange chromatography exhibit very few hydrophobic properties, elution with NaCl or $NaClO_4$ at pH 8 results in the target oligonucleotide being separated in a single peak that elutes after truncated or other

Table 3.9 Structural and technical properties of nanobead-agglomerated anion exchangers for oligonucleotide separations.

Parameter	DNAPac PA100	DNAPac PA200
Particle diameter (µm)	13	8
Degree of crosslinking (%)	5	6
Size of nanobead (nm)	100	130
Capacity (µequiv/col.)	40	40
Dimensions (length × i.d.) (mm)	250 × 2 250 × 4 250 × 9 250 × 22	250 × 4
Recommended flow rate (mL/min)	1.2	1.2
Max. operating pressure (psi/MPa)	4000/27.6	4000/27.6
Max. operating temperature (°C)	90	85
pH range	2–10 (unrestricted) 10–12.5 (with equimolar ratio of hydroxide to Cl^- or ClO_4^-)	3–11 (unrestricted) 2.5–12.5 (with equimolar ratio of hydroxide to Cl^- or ClO_4^-)
Chaotropic agent limit	40% formamide or 6 mol/L urea	40% formamide or 6 mol/L urea
Solvent compatibility (%)	100	100

undesirable components. A corresponding chromatogram of a –20 sequencing primer (17-mer) on a DNAPac PA100 column is shown in Figure 3.37. Failure sequences are separated from the main product in less than 20 min.

The latest addition to the family of oligonucleotide separators is the Thermo Scientific™ DNASwift™ SAX-1S column, which combines a pressure and chemically stable monolithic support coated with functionalized nanobeads. These nanobeads with quaternary ammonium functional groups, optimized for oligonucleotide separations, are similar to those of the DNAPac columns and contribute to the column's high resolution and selectivity control. In analogy to the ProSwift columns for protein separations, the monolithic support of the DNASwift column contributes to its high capacity, maintaining high resolution even at elevated flow rates. This makes the DNASwift column ideal for DNA and RNA oligonucleotide therapeutic and diagnostic research. The structural and technical properties of the DNASwift SAX-1S column are summarized in Table 3.10.

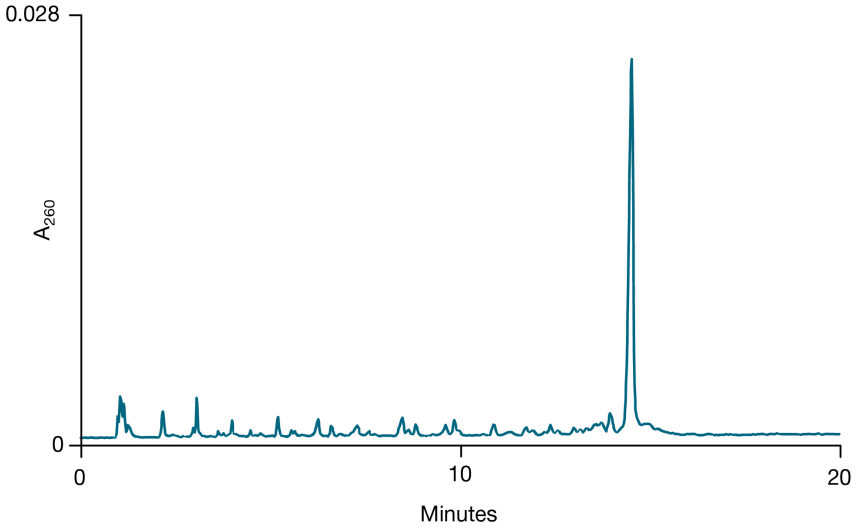


Figure 3.37 Separation of a crude synthetic -20 sequencing primer (17-mer) from its failure sequences. Separator column: DNAPac PA100; column dimensions: 250 mm × 4 mm i.d.; eluent: nonlinear gradient of 7.5 to 124 mmol/L $NaClO_4$ in 25 mmol/L Tris-Cl, pH 8; flow rate: 1.5 mL/min; detection: UV (260 nm).

Table 3.10 Structural and technical properties of DNASwift SAX-1S column for oligonucleotide separations.

Parameter	**DNASwift SAX-1S**
Column dimension (length × i.d.) (mm)	150 × 5
Base matrix material	Poly(meth)acrylate
Surface modification	Nanobeads with quaternary ammonium functional groups
Oligonucleotide binding capacity per mL of polymer	20 mg, 20-mer
Total binding capacity per column	50 mg, 20-mer
Bed height (mm)	130
Bed volume (mL)	2.5
pH range	6–12.4 (for operation)
	2–14 (for cleaning) (equimolar ratio of hydroxide to Cl^- or ClO_4^- required)
Recommended flow rate (mL/min)	0.5–2.5
Max. flow rate (mL/min)	3
Operating pressure (psi/MPa)	<1500/10.3
Solvent compatibility (%)	100

3.3
Eluents for conventional anion-exchange chromatography

The kind of eluent that is used for anion-exchange chromatography depends mainly on the detection system being employed. In many cases, detection of inorganic and organic anions is performed with suppressed conductivity detection. Eluents for suppressed conductivity detection include the salts of weak inorganic acids, which exhibit a low background conductivity after chemical modification within a suppressor system (see Section 7.1.1).

For many years, the versatile mixture of sodium carbonate and sodium bicarbonate has found widespread application because the elution power and the selectivity resulting therefrom are determined solely by the concentration ratio of these two compounds. A great variety of inorganic and organic anions can be separated with this eluent combination under isocratic conditions. As the product of the suppressor reaction, carbonic acid (carbon dioxide dissolved in water) is formed and is only weakly dissociated, so that the background conductivity is much lower than that of the carbonate salts used as the eluent. However, carbonate-based concentration gradients are impractical due to the limited solubility of carbon dioxide in water, which will result in outgassing effects. Even in combination with a carbonate removal device (Dionex CRD 300), significant baseline shifts would be observed.

In the past, carbonate/bicarbonate eluents were usually prepared manually, for instance, by diluting the respective eluent concentrate with deionized water. Today, an eluent generator can also be used to prepare a high-purity carbonate-based eluent online at the point of use, utilizing deionized water as the carrier. The use of electrolytic eluent generation (RFIC-EG) offers the significant advantage of eliminating manual eluent preparation and all the uncertainties that potentially may result from it. Moreover, the use of high-purity eluents can significantly improve the performance of the IC methods. The heart of the electrolytic carbonate eluent generation process is the potassium carbonate eluent generator cartridge in combination with an electrolytic pH modifier (EPM) [30].

In the past, the application of pure hydroxide eluents was regarded to be disadvantageous. Although water as the suppressor product produces virtually no background conductance, carbonate impurities in any manually prepared hydroxide solutions were the reason for nonreproducible retention times. Because hydroxide ions exhibit only a small affinity toward the stationary phase, it is necessary to work with relatively high concentrations to elute anions with more than one negative charge. This has an adverse effect on the background conductance because early suppressor generations did not provide sufficient suppression capacity. Only after the introduction of high-capacity micromembrane and self-regenerating suppressors did it become possible to use hydroxide eluents with concentrations up to 0.1 mol/L at analytical flow rates up to 1 mL/min. With modern capillary IC, the maximum hydroxide concentration that can be suppressed could even be increased to 0.2 mol/L due to the small eluent flow rates of only 10 µL/min. At these high concentrations, it is possible to elute even polyvalent analyte ions. Therefore, hydroxide is perfectly suited for gradient elution of anions in combination with suppressed conductivity detection and predominantly used for this purpose today.

When preparing hydroxide eluents manually, care must be taken that they are, to a high degree, carbonate-free! Because carbonate ions exhibit a much higher elution strength than hydroxide ions, even traces of carbonate in the eluent results in a lower resolution, especially at the beginning of a gradient run. In addition, the dissociation of the carbonic acid formed in the suppressor leads to a strong baseline drift during the gradient run. Therefore, NaOH-based eluents should be prepared in a conventional way from a 50% concentrate in which the carbonate, originally adsorbed at the NaOH pellets, precipitates as a fine slurry. The deionized water used for eluent preparation should have an electrical conductance of 0.05 µS/cm and should be degassed with helium prior to adding the NaOH concentrate. After preparation, eluents are to be kept under inert gas. As an additional precaution, a short anion trap column

(ATC) is placed between the analytical pump and the injection valve, which retains anionic impurities from the eluent being used at the beginning of an analysis. As the eluent strength increases during the gradient run, the anionic impurities retained on the ATC are released again but do not appear as regular peaks in the chromatogram due to the low efficiency of the material.

A much more elegant way to prepare hydroxide eluents has been introduced by Liu et al. [31]. The respective device was commercialized under the trade name Eluent Generator™. The most important part of this module is a cartridge, in which potassium hydroxide is generated by means of electrolysis. As schematically depicted in Figure 3.38, such a cartridge consists of an electrolyte reservoir, which is filled with a concentrated KOH solution and connected to an electrolysis chamber via a stack of cation-exchange membranes. While the electrolyte reservoir contains a perforated anode, the respective cathode is placed inside the electrolysis chamber. For generating high-purity KOH, deionized water is pumped through the electrolysis chamber and an electric field is applied between the two electrodes. Under the influence of the electric field, hydroxide ions are generated at the cathode and combine in the electrolysis chamber with the potassium ions migrating through the membranes to yield KOH. The concentration of KOH is directly proportional to the applied current and inversely proportional to the flow rate. On its way to the injection valve, the KOH must pass a high-pressure degas unit for removing the hydrogen gas formed at the cathode.

The greatest advantages of electrolytic KOH generation are that only deionized water is used as a carrier for the ion chromatograph and that KOH is generated free of any carbonate contaminants because it is generated in a closed system. This is especially important for very low KOH concentrations ($c << 0.1$ mol/L) because time-consuming postanalytical rinsing steps to remove accumulated carbonate from the stationary phase are no longer necessary. Moreover, being able to adjust the KOH concentration via the applied current and the flow rate is also a real advantage. While the eluent flow rate is usually kept constant by programming the analytical pump, the applied current can be programmed electrically with minimal delay. Thus, gradient elution techniques are realized by electrical current gradients. Because the Eluent Generator is software-controlled via the Chromeleon CDS, gradient separations, programmed per mouse click, are as easy to perform as isocratic ones.

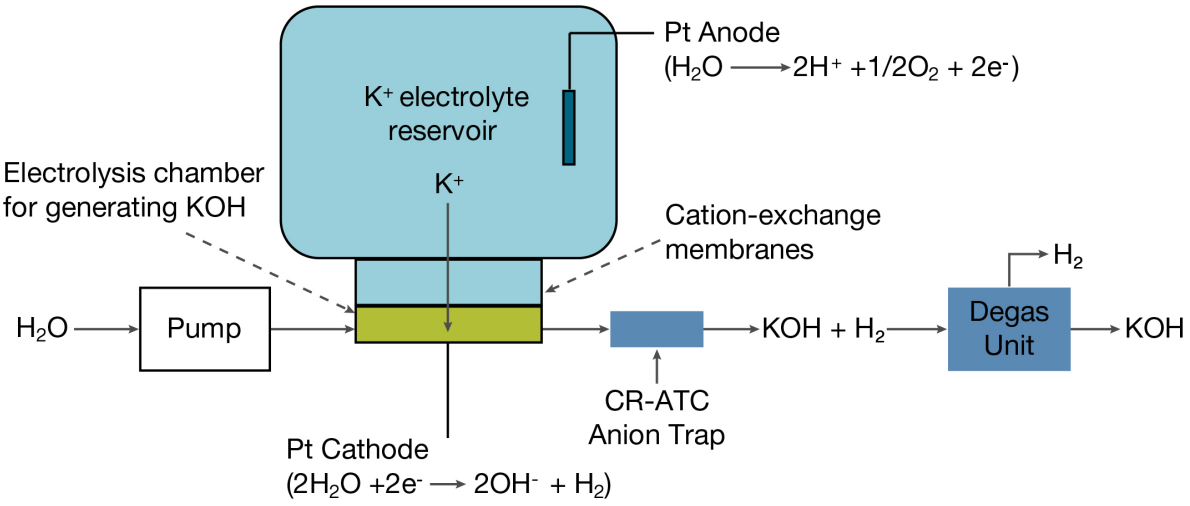


Figure 3.38 Schematic illustration of a cartridge for a contamination-free generation of KOH.

4
Cation-exchange chromatography

Small et al. [1] devoted a significant part of their pioneering work in ion chromatography to the separation and determination of cations. The required hardware was identical to that used for anion analysis. It comprised a low-capacity cation exchanger and a suppressor column containing a strong basic anion-exchange resin in the hydroxide form. With the exception of the suppressor column, which has since been replaced by modern membrane suppressors, the principle setup remains unchanged. As in anion analysis, the application of a suppressor system is not necessarily a prerequisite for the cation determination by means of conductivity detection but is highly recommended.

The exchange reaction with a cation M^+ that occurs at the stationary phase of a cation exchanger can be expressed as follows:

$$\text{Resin}\text{–}SO_3^- H^+ + M^+A^- \leftrightarrows \text{Resin}\text{–}SO_3^- M^+ + H^+A^- \quad (4.1)$$

The separation of cations is determined by their different affinities toward the stationary phase.

4.1
Stationary phases

As with anion exchangers, cation exchangers are classified according to the type of substrate material. Although cation exchangers are usually made using organic polymers, a variety of substrate materials are appropriate. Because dilute acids serve as the eluent in cation separations, the stability over the whole pH range (a condition provided by organic polymers) is not really required. Therefore, silica-based cation exchangers are also used.

4.1.1
Nanobead-agglomerated cation exchangers

While the concept of nanobead agglomeration for anion exchangers (see Section 3.1.1) was realized with the introduction of ion chromatography, it was not until 1986 that its usefulness for cation exchangers was recognized. The reason for the long development time was the lack of suitable surface-aminated substrates. Based on experiences gained in the manufacturing of nanobead-agglomerated anion exchangers, it eventually became possible to cover the anion-exchange beads with a second layer of totally sulfonated nanobeads. These materials are called nanobead-agglomerated cation exchangers. Their structure is schematically depicted in Figure 4.1.

The only nanobead-agglomerated cation exchangers that are currently available and still in use for special applications consist of weakly sulfonated ethylvinylbenzene/divinylbenzene substrates with particle sizes between 8 and 8.5 µm. Totally aminated nanobeads with a much smaller diameter (about 50 nm) are agglomerated on their surface by both electrostatic and van der Waals interactions. The anion-exchange substrate that results is covered by a second layer of nanobeads that carry the actual ion-exchange functional groups, namely, sulfonate groups. In contemporary practice, such separator columns are only used for the sequential analysis of alkali or alkaline-earth metals because the simultaneous analysis of common inorganic cations is almost exclusively carried out on weak acid cation exchangers today. The structural and

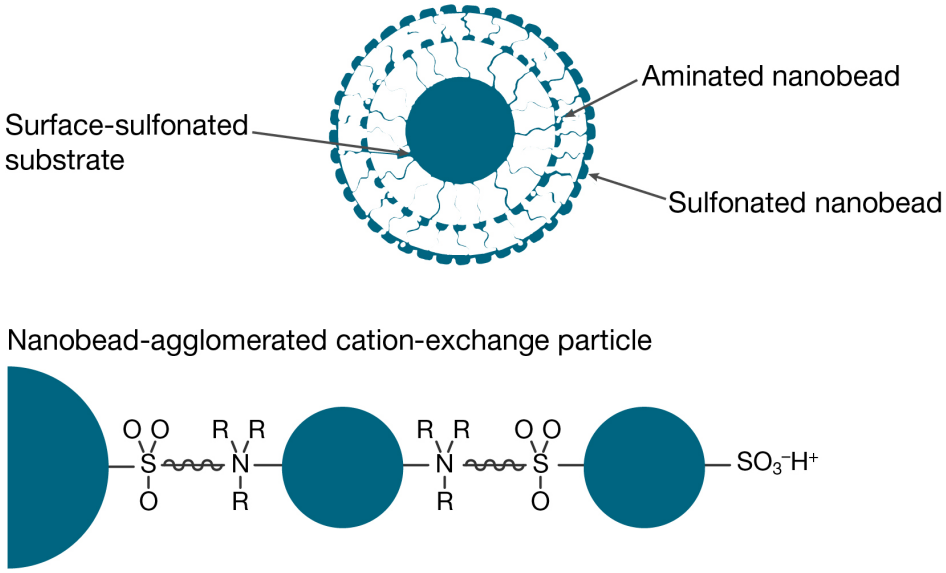


Figure 4.1 Schematic representation of a nanobead-agglomerated cation-exchange particle.

technical properties of the two nanobead-agglomerated cation exchangers available today are summarized in Table 4.1.

The Dionex IonPac CS10 nanobead-agglomerated cation exchanger, originally designed for the simultaneous analysis of alkali and alkaline-earth metals under isocratic conditions, was introduced in the early 1990s. It is offered in the standard dimensions of 250 mm × 4 mm i.d.; the sulfonated nanobeads have a diameter of 200 nm and a degree of cross-linking of 5%. Its support material is composed of a highly cross-linked ethylvinylbenzene/divinylbenzene copolymer with a particle size of 8.5 µm. Nanobead-agglomerated cation exchangers are 100% solvent compatible, which results from the high degree of cross-linking of the microporous support.

Cation separations on the Dionex IonPac CS10 column are characterized by a very limited resolution between monovalent cations, which elute within a short time close to the void volume due to the low ion-exchange capacity of the column. Nevertheless, concentration ratios of sodium to ammonium as high as 100:1 can be quantified. The extreme resolution between divalent cations is another characteristic of this column; the resolution is much higher than it is with weak acid cation exchangers (see Section 4.1.2), which is advantageous for the analysis of manganese, eluting just between potassium and magnesium under standard chromatographic conditions. Because of the introduction of weak acid cation exchangers, the Dionex IonPac CS10 column is no longer used for the simultaneous analysis of mono- and divalent cations.

Both Dionex IonPac CS10 and CS11 columns are still being used for petrochemical applications when high resolution between alkanolamines and alkali metal ions is required. Figure 4.2 shows the isocratic elution of monovalent cations and ethanolamines on a Dionex IonPac CS11 column, the microbore version of the Dionex IonPac CS10 column with a slightly higher cation-exchange capacity. Under the chromatographic conditions used, a baseline-resolved separation of mono-, di-, and triethanolamine as well as *N*-methyldiethanolamine is achieved within 30 min. Wastewaters from oil refineries, for example, contain these amines, which can be present in widely different concentration levels.

Table 4.1 Structural and technical properties of nanobead-agglomerated cation exchangers.

Column	Dionex IonPac CS10	Dionex IonPac CS11
Dimensions (length × i.d.) (mm)	250 × 4	250 × 2
Substrate material	Ethylvinylbenzene/ Divinylbenzene	Ethylvinylbenzene// Divinylbenzene
Nanobead diameter (nm)	200	200
Nanobead crosslinking degree (%)	5	5
pH range	0–14	0–14
Maximum pressure	27.6 MPa 4000 psi	27.6 MPa 4000 psi
Maximum flow rate (mL/min)	1.5	2.0
Solvent compatibility	100	100
Capacity (µequiv/column)	80	35
Particle diameter (µm)	8.5	8
Functionality	Sulfonate groups	Sulfonate groups

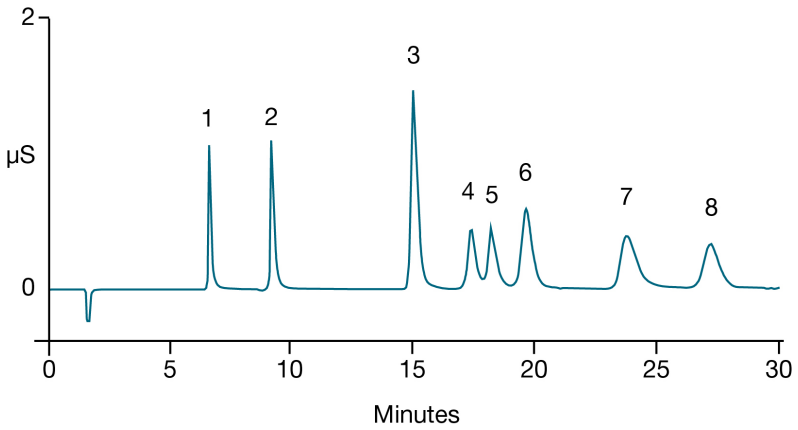


Figure 4.2 Isocratic separation of ethanolamines and alkali metals on a Dionex IonPac CS11 column. Eluent: 35 mmol/L methanesulfonic acid; flow rate: 0.25 mL/min; detection: suppressed conductivity; peaks: 0.25 mg/L lithium (1), 1 mg/L sodium (2), 2 mg/L ammonium (3), 2 mg/L monoethanolamine (4), 1 mg/L potassium (5), 10 mg/L diethanolamine (6), 100 mg/L triethanolamine (7), and 10 mg/L *N*-methyl-diethanolamine (8).

4.1.2

Grafted ethylvinylbenzene/divinylbenzene copolymers

The simultaneous analysis of alkali and alkaline-earth metals can be realized only with weak acid cation exchangers using carboxyl groups as ion-exchange functional groups. Schomburg et al. [32] were first to report an isocratic separation of mono- and divalent cations within an acceptable time frame of 20 min using a silica phase coated with poly(butadiene-maleic acid) (PBDMA). Because a weak acid complexing agent is used as an eluent, cation-exchange interactions as well as complexation processes contribute to the retention mechanism. As with many polymer-coated silica columns, reproducible manufacturing is extremely difficult. This stability problem was solved with the development of a cation exchanger based on a polymeric substrate material. This Dionex IonPac CS12 [33] column was introduced in 1993 and replaced later by the Dionex IonPac CS12A column. As schematically depicted in Figure 4.3, it consisted of a highly cross-linked mesoporous ethylvinylbenzene/divinylbenzene copolymer with a pore size of 60 Å, a particle diameter of 8 µm, and a specific surface area of 300 m²/g, which was grafted at the surface with a thin film (5 nm) of an ion-exchange polymer. This ion-exchange polymer contained the ion-exchange functional groups, namely, carboxyl groups (pK < 3). The resulting high ion-exchange capacity of 2.8 mequiv/column (250 mm × 4 mm i.d.) is necessary because in carboxylate-based weak acid cation exchangers only a fraction of the ion-exchange groups are available for the cation-exchange process due to the low acid strength. Today, those mesoporous substrates with high specific surface areas are used for the simultaneous separation of alkali- and alkaline-earth metals, ammonium, and a wide variety of organic amines.

Based on this concept, a number of grafted weak acid cation exchangers have been developed. The structural-technical properties of these stationary phases are summarized in Table 4.2.

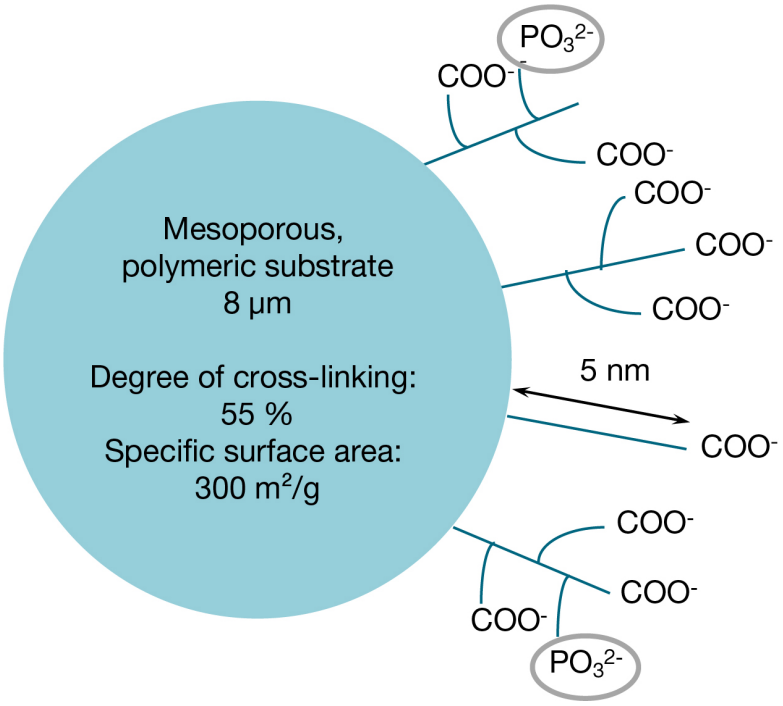


Figure 4.3 Schematic representation of a grafted, carboxylate-based weak acid cation-exchange particle.

Table 4.2 Structural and technical properties of grafted weak acid cation exchangers.

Column	Dionex IonPac CS12A	Dionex IonPac CS14	Dionex IonPac CS15	Dionex IonPac CS16	Dionex IonPac CS16-4µm	Dionex IonPac CS17	Dionex IonPac CS18	Dionex IonPac CS19	Dionex IonPac CS19-4µm
Dimensions (length × i.d.) (mm)	250 × 4 250 × 2 250 × 0.4 150 × 3 150 × 0.4 100 × 2	250 × 4 250 × 2	250 × 4 250 × 2	250 × 5 250 × 3 250 × 0.5	250 × 2 250 × 0.4	250 × 4 250 × 2 250 × 0.4	250 × 2	250 × 4 250 × 2 250 × 0.4	250 × 4 250 × 2 250 × 0.4
Degree of cross-linking	55	55	55	55	55	55	55	55	55
Maximum pressure (MPa)	27.6 27.6 34.5	27.6	27.6	27.6 27.6 34.5	34.5	27.6	27.6	20.7	34.5
Solvent compatibility	100*)	100*)	100*)	100*)	100*)	100*)	20*)	100*)	100*)
Capacity (mequiv/col.)	2.8 0.7 0.028 0.94 0.0094 0.28	1.3 0.325	2.8 0.7	8.4 3.0 0.084	1.34 0.05	1.45 0.363 0.0145	0.29	2.41 0.6 0.024	2.41 0.6 0.024
Particle diameter (µm)]	8 8 8 8 5 5	8	8.5	5	4	7	6	5.5	4
Functionality	Carboxyl and phosphonate groups	Carboxyl groups	Carboxyl, phospho-nate and crown ether groups	Carboxyl groups	Carboxyl groups	Carboxyl groups	Carboxyl groups	Carboxyl groups	Carboxyl groups

*) aprotic solvents such as acetonitrile

A limitation of all cation exchangers suitable for simultaneous analysis of mono- and divalent cations is the maximal concentration ratio between sodium and ammonium that allows a separation of both components. Under isocratic conditions, universal purpose cation exchangers separate ammonium in the presence of a 250–500-fold excess of sodium. Higher concentration differences are only applicable when applying high-capacity cation exchangers such as the Dionex IonPac CS16 column.

Standard separations of alkali metals, alkaline-earth metals, and ammonium are typically performed on a Dionex IonPac CS12A column [34], which contains phosphonate *and* carboxyl groups in the ion-exchange polymer. This modification allows the separation of manganese together with alkali and alkaline-earth metals. Manganese(II) that elutes between magnesium and calcium is regarded as a corrosion indicator in boiler waters and thus of great importance for the power generating industry. Figure 4.4 shows the respective separation of all alkali and alkaline-earth metals under isocratic conditions with a methanesulfonic acid eluent. The efficiencies of the divalent cations can even be improved by increasing column temperature. Thus, by optimizing eluent concentration and column temperature, the analysis time for a baseline-resolved separation of the six standard cations can be significantly reduced. Even faster separations are obtained with the Dionex IonPac CS12A-5µm column, which is

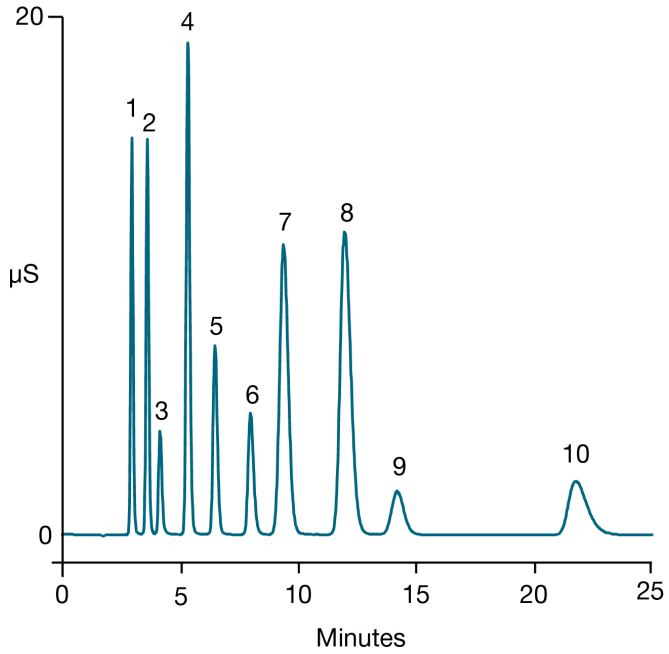


Figure 4.4 Separation of all alkali- and alkaline-earth metals and ammonium on a Dionex IonPac CS12A column. Eluent: 20 mmol/L methanesulfonic acid; flow rate: 1 mL/min; injection volume: 25 µL; detection: suppressed conductivity; peaks: 1 mg/L lithium (1), 4 mg/L sodium (2), 5 mg/L ammonium (3), 10 mg/L potassium (4), 10 mg/L rubidium (5), 10 mg/L cesium (6), 5 mg/L magnesium (7), 10 mg/L calcium (8), 10 mg/L strontium (9), and 10 mg/L barium (10).

commercialized in an analytical (150 mm × 3 mm i.d.) and a capillary format (150 mm × 0.4 mm i.d.) with an ion-exchange capacity of 0.94 and 0.0094 mequiv/column, respectively. The chromatographic efficiencies of these columns are so high that they can be operated with higher acid concentrations and/or higher flow rates, which reduces the total analysis time for the six standard cations to 6 min or less. The Dionex IonPac CS12A-5µm columns use the same carboxyl and phosphonate functional groups as the 8 µm counterparts. As a result, they provide the advantages of faster analysis times for inorganic cations, reduced eluent consumption, and increased sensitivity. The Dionex IonPac CS12A-MS column (100 mm × 2 mm i.d.) is ideally suited for fast elution and low flow rates required for interfacing with MS detection. With this column, inorganic cations, polar hydrophilic amines, and other small organic molecules, such as theophylline, caffeine, and uracil, can be separated prior to MS detection. Typically, the Dionex IonPac CS12A-MS column will be operated in an ion chromatography system containing a suppressor device for converting the acid eluent to water prior to entering the ESI interface.

The Dionex IonPac CS14 column is specifically designed for the analysis of aliphatic amines. The structure of this carboxylate-based weak acid cation exchanger is very similar to that of the Dionex IonPac CS12 column. A significant difference between the separators are the ion-exchange capacities. With 1.3 mequiv/column, the ion-exchange capacity of the Dionex IonPac CS14 column is only half of the capacity of the Dionex IonPac CS12 column. As expected, the required eluent concentration of 10 mmol/L methanesulfonic acid for a baseline-resolved separation of mono- and divalent cations is respectively lower, too. The separator columns also differ in the surface properties of their ion-exchange groups. Because even short-chain aliphatic amines exhibit a strong tailing when separated on the Dionex IonPac CS12A column, one can conclude that the ion-exchange functionality in the ion-exchange polymer of the Dionex IonPac CS14 column is much more hydrophilic. Therefore, aliphatic amines elute significantly faster and with better peak symmetries on the Dionex IonPac CS14 column;

however, the column technology is outdated as much better separation of amines are carried out on the more efficient Dionex IonPac CS17 through CS19 columns.

The isocratic separation between sodium, ammonium, monoethanolamine, and potassium is generally difficult. However, ammonium, monoethanolamine, and potassium are more strongly retained when a crown ether is added to the mobile phase; in this way, they can be separated from other mono- and divalent cations. 18-crown-6 is especially suitable for this purpose. A more elegant way to separate those cations is by functionalization of the ion-exchange polymer with crown ethers, implemented in the development of the Dionex IonPac CS15 column [35]. The primary structural and technical properties of this separator column are identical to those of the Dionex IonPac CS12A column. Only the ion-exchange polymer differs; the Dionex IonPac CS15 column contains additional crown ether groups besides the carboxyl- and phosphonate groups of the Dionex IonPac CS12A column. The resulting selectivity is a good fit for trace analysis of ammonium in environmental samples and sodium in amine-containing waters. Elevating the column temperature to 40 °C and adding small amounts of acetonitrile to the mobile phase are necessary to improve the peak efficiency of the late-eluting potassium. The Dionex IonPac CS15 column allows the separation of sodium and ammonium at very disparate concentration levels of up to 4000:1; larger concentration differences between these two cations can only be handled by a high-capacity cation exchanger such as the Dionex IonPac CS16 column.

The Dionex IonPac CS16 column is a carboxylate-based, weak acid high-capacity cation exchanger that provides excellent peak shape for alkali and alkaline-earth metals and amines. It is available in three column formats: 250 mm × 5 mm i.d., 250 mm × 3 mm i.d., and 250 mm × 0.5 mm i.d. The column packing consists of 5 µm diameter macroporous particles that are 100% solvent compatible due to the 55% degree of cross-linking. The substrate is functionalized with a hydrophilic carboxylic acid layer that permits the simultaneous elution of mono- and divalent cations using a dilute acid eluent such as methanesulfonic acid. The use of a smaller resin particle size improves peak efficiencies, while the grafting technology allows higher capacity (8.4 mequiv for the 250 mm × 5 mm i.d. column) via the incorporation of a much higher number of carboxylic acid cation-exchange sites. Common inorganic cations and ammonium can be resolved in about 20 min using a 30 mmol/L methanesulfonic acid eluent, as shown in Figure 4.5. The acid concentration can be optimized for the fast determination of mono- and divalent cations by using 48 mmol/L MSA; this is also illustrated in Figure 4.5.

The Dionex IonPac CS16 column is specifically designed for the determination of disparate concentration ratios of sodium and ammonium (up to 10,000:1) in diverse sample matrices utilizing isocratic elution at elevated temperature. Thus, it is commonly employed for the determination of low concentrations of ammonium in environmental water samples. The Dionex IonPac CS16 column is an alternative to the Dionex IonPac CS15 column when high-to-low-ratios of sodium to ammonium must be analyzed. It provides improved resolution of sodium from ammonium and alkanolamines, even for high-ionic strength samples. This is accomplished with an isocratic MSA eluent on the 5 mm Dionex IonPac CS16 column at an elevated temperature of 40 °C, utilizing suppressed conductivity detection. Ratios up to 20,000:1 can be resolved using an MSA gradient with the column in the 3 mm format. It can also be used to monitor the amine content in the quality control of chemical additives, process solutions, plating baths, and scrubber solutions, as well as for the determination of trace-level sodium in ammonium and amine-treated cooling waters.

The latest additions to the Dionex IonPac CS16 column family are the Dionex IonPac CS16-4µm and the Dionex IonPac CS16-Fast-4µm columns. Both have the same selectivity as the Dionex IonPac CS16 column but use smaller particles, producing higher peak efficiencies and better resolution. Due to the smaller column length of only 150 mm, the Dionex IonPac CS16-Fast-4µm column can be used for the fast determination of common inorganic cations and ammonium in about 12 min using 30 mmol/L MSA.

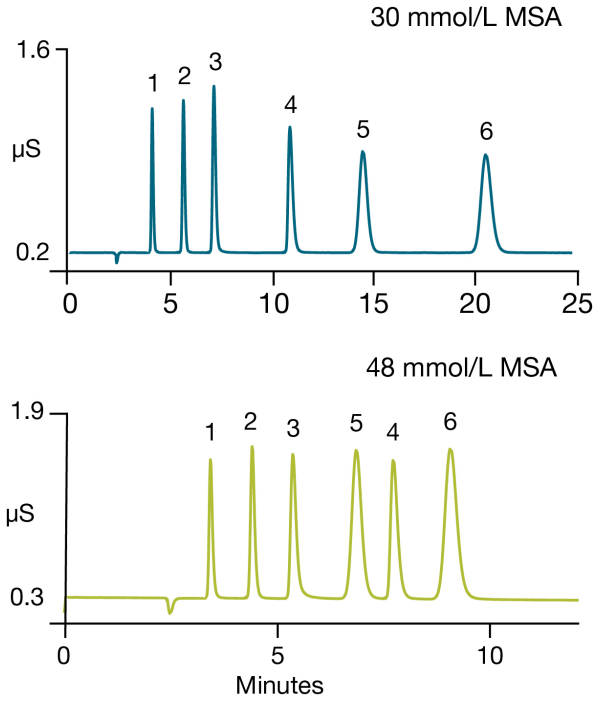


Figure 4.5 Isocratic separation of alkali- and alkaline-earth metals and ammonium on a Dionex IonPac CS16 column. Column dimensions: 250 mm × 5 mm i.d.; column temperature: 40 °C; eluent: see chromatograms; flow rate: 1 mL/min; detection: suppressed conductivity; injection volume: 25 µL; peaks: 0.1 mg/L lithium (1), 0.4 mg/L sodium (2), 0.5 mg/L ammonium (3), 1 mg/L potassium (4), 0.5 mg/L magnesium (5), and 1 mg/L calcium (6).

Dionex IonPac CS17, CS18, and CS19 columns belong to a group of columns that are designed for the determination of a wide variety of amines, including alkylamines, alkanolamines, hydrophobic amines, biogenic amines, diamines, and polyvalent amines in the presence of common inorganic cations, using simple aqueous eluents and elevated column temperature. All three columns are carboxylate-based weak acid cation exchangers. They offer improved performance for most Dionex IonPac CS14 and CS15 column amine applications. The resin beads of Dionex IonPac CS17 and CS18 columns are produced using a different grafting technique, which employs a grafted nonfunctional coating on the resin surface and pores with the cation-exchange polymer grafted onto this nonfunctional coating. Due to the hydrophilic character of the nonfunctional coating, hydrophobic interactions between the analytes and the resin surface are reduced, resulting in excellent mass-transfer characteristics and, consequently, high peak efficiencies (Figure 4.6). This novel grafting technique was originally used to produce the ProPac columns for high-resolution protein separations (see Sections 3.2 and 4.2) and has proved to be extremely successful for amine separations, too.

The Dionex IonPac CS17 column [36] is a hydrophilic cation exchanger with moderate capacity that provides excellent selectivity and peak shape for amines. The macroporous substrate of the resin with a particle diameter of 7 µm consists of ethylvinylbenzene cross-linked with 55% divinylbenzene to ensure solvent compatibility for column cleanup. The column is predominantly used for the separation of hydrophobic and polyvalent amines, including alkyl-amines, biogenic amines, and diamines.

A typical gradient application of the Dionex IonPac CS17 column is the analysis of alkanolamines. Mono-ethanolamine, for instance, serves as one of the most widely used additives to prevent corrosion in power plant waters. Hydrazine, on the other hand, is a powerful reducing agent also used in the power generating industry as an oxygen scavenger to reduce turbine corrosion.

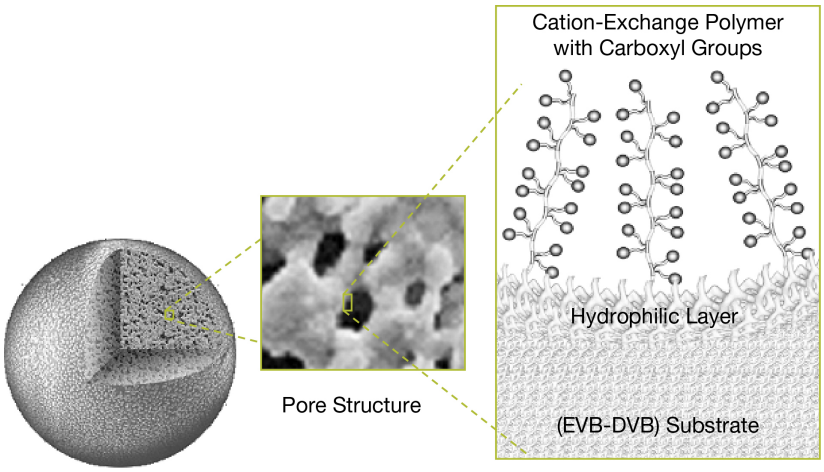


Figure 4.6 Schematic representation of a carboxylate-based grafted weak acid cation-exchange particle with a hydrophilic coating.

Traditionally, the separation of hydrazine from ammonium and monoethanolamine on conventional cation exchangers is a serious problem. However, the hydrophilic nature of the Dionex IonPac CS17 column in combination with its moderate ion-exchange capacity allows such separation, as illustrated in Figure 4.7. Cyclic amines such as morpholine and cyclohexylamine are also separated under these chromatographic conditions. Together with 2-diethylaminoethanol they are added to water as vapor-phase corrosion inhibitors in steam generator plants and for other boiler applications. The concentrations of these amines must be monitored to maintain optimum levels. Separation, detection, and quantitation can easily be achieved using the Dionex IonPac CS17 column, utilizing a methanesulfonic acid gradient and suppressed conductivity detection shown in Figure 4.7. The column also facilitates the analysis of biogenic amines, such as putrescine, cadaverine, spermine, spermidine, and histamine, because they can be separated together with common inorganic cations by gradient elution employing a purely aqueous acid eluent. Biogenic amines elute behind magnesium and calcium as very symmetric and efficient peaks. Like biogenic amines, diamines also elute behind the six common cations as symmetric and efficient peaks using a simple acid gradient at elevated temperature. However, alkyl diamines larger than 1,10-decanediamine are not soluble in water and, therefore, would require the addition of organic solvents to the mobile phase when they are present in samples.

The Dionex IonPac CS18 column is a weak acid cation exchanger of moderate hydrophobicity and low capacity, which is typically used for the isocratic and gradient elution of polar and moderately hydrophobic amines together with common inorganic cations. The macroporous CS18 substrate consists of ethylvinylbenzene cross-linked with 55% divinylbenzene and has a particle diameter of 6 µm. It is only offered in the microbore format (250 mm × 2 mm i.d.); the cation-exchange capacity is 290 µequiv/column. Figure 4.8 shows the separation of a variety of amines together with common inorganic cations that are monitored in the petrochemical industry. Using a simple MSA gradient at an elevated temperature of 50 °C, monoethanolamine (MEA), diethanolamine (DEA), and *N*-methyldiethanolamine (MDEA), which are used in recirculating systems for scrubbing acid gases like hydrogen sulfide, are very well separated from each other. Diethanolamine and potassium that coelute under these conditions can be resolved using a lower column temperature of 30 °C. Inorganic cations such as sodium and potassium have to be monitored as well because they can contaminate an amine scrubber solution and bind anions that are not stripped out. Also, at elevated temperature precipitation of solids can occur. Cyclic amines such as morpholine and *N*-methylmorpholine as well as aliphatic amines such as monomethylamine and monoethylamine are often used for

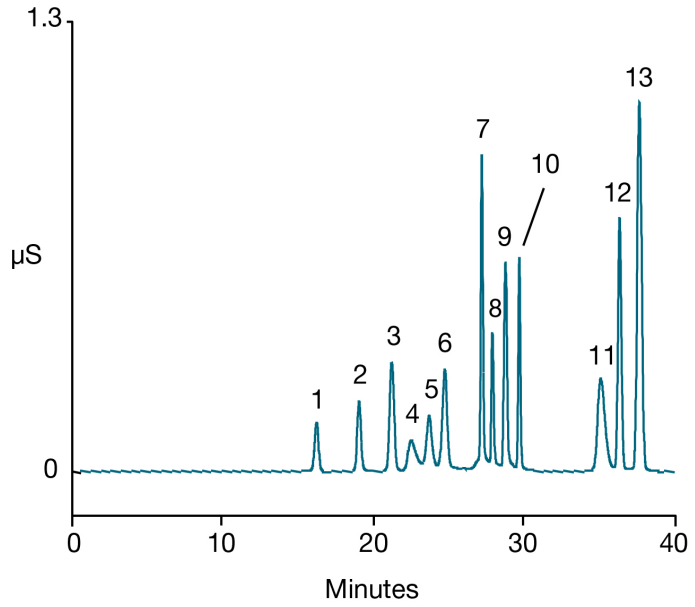


Figure 4.7 Separation of steam generator additives on a Dionex IonPac CS17 column. Column dimensions: 250 mm × 2 mm i.d.; column temperature: 30 °C; eluent: methanesulfonic acid (EG); gradient: 0.5 mmol/L linearly to 0.7 mmol/L in 25 min, then step change to 4 mmol/L and isocratic for 2 min, step change to 6.5 mmol/L and isocratic for 10 min; flow rate: 0.35 mL/min; detection: suppressed conductivity; injection volume: 25 µL; peaks: 25 µg/L lithium (1),100 µg/L sodium (2), 125 µg/L ammonium (3), 1000 µg/L hydrazine (4), 250 µg/L monoethanol-amine (5), 250 µg/L potassium (6), 1000 µg/L 2-(2-aminoethoxy)ethanol (7), 500 µg/L 5-amino-1-pentanol (8), 1000 µg/L each of morpholine (9), 2-diethylaminoethanol (10), and cyclohexylamine (11), 125 µg/L magnesium (12), and 250 µg/L calcium (13).

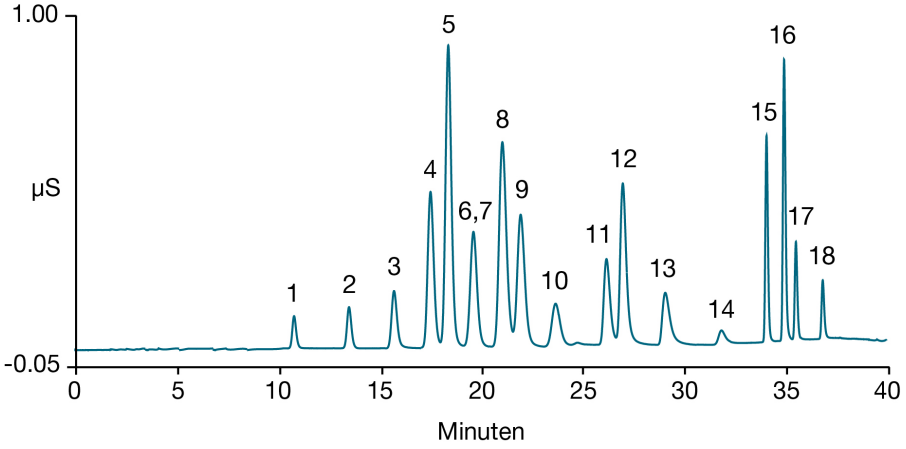


Figure 4.8 Separation of amines that are relevant for the petrochemical industry on a Dionex IonPac CS18 column. Column dimensions: 250 mm × 2 mm i.d.; column temperature: 50 °C; eluent: methanesulfonic acid (EG); gradient: 0.5–1 mmol/L in 20 min, then linear to 4 mmol/L in 8 min, then to 11 mmol/L in 6 min; flow rate: 0.3 mL/min; detection: suppressed conductivity; injection volume: 5 µL; peaks: 0.05 mg/L lithium (1), 0.2 mg/L sodium (2), 0.25 mg/L ammonium (3), 3 mg/L mono-ethanolamine (4), 3.6 mg/L monomethylamine (5), 3.6 mg/L diethanolamine (6), 0.5 mg/L potassium (7), 3 mg/L monoethylamine (8), 1.4 mg/L dimethylamine (9), 3 mg/L *N*-methyl-diethanolamine (10), 3.2 mg/L morpholine (11), 3.7 mg/L 1-dimethylamino-2-propanol (12), 7.5 mg/L *N*-methylmorpholine (13), 1.5 mg/L monobutylamine (14), 0.25 mg/L magnesium (15), and 0.5 mg/L each of calcium (16), strontium (17), and barium (18).

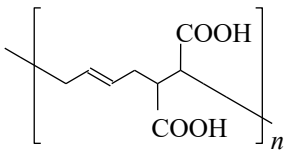
pH control; a variety of other amines are used for corrosion and foam control. All these amines of interest can be analyzed in one run using the Dionex IonPac CS18 column.

Like the Dionex IonPac CS18 column, the Dionex IonPac CS19 column is typically used for the isocratic and gradient elution of polar and moderately hydrophobic amines together with common inorganic cations. The supermacroporous Dionex IonPac CS19 substrate consists of ethylvinylbenzene cross-linked with 55% divinylbenzene and has a particle diameter of 5.5 µm. It is offered in analytical (standard bore and microbore) and capillary formats. In comparison to the other two columns with a hydrophilic layer on top of the substrate surface, the Dionex IonPac CS19 column offers the highest cation-exchange capacity of 2.4 mequiv for the 250 mm × 4 mm i.d. column, which facilitates the analysis of samples with complex matrices. It has a unique selectivity for ethanol-amines and, therefore, can resolve mixtures of all three compounds using a 5 mmol/L methanesulfonic acid eluent under isocratic conditions and suppressed conductivity detection in less than 30 min. Having the same selectivity as the Dionex IonPac CS19 column, the resin bead of the Dionex IonPac CS19-4µm column is produced using a novel low surface area substrate. All commercially available grafted cation-exchange materials make use of high-surface area substrates that provide good capacity but tend to preclude good peak shape, especially for polarizable cations that interact strongly with the substrate surface. The Dionex IonPac CS19-4µm resin bead architecture overcomes these issues by making use of a low-surface area substrate. The use of 4 µm particle size substrate allows for further improvements in the chromatographic performance compared to the original Dionex IonPac CS19 resin bead.

4.1.3 Silica-based cation exchangers

A great deal of interest has been shown in a polymer-coated, silica-based cation-exchange phase introduced by Schomburg et al. [32] in 1987. It belongs to the class of weak acid cation exchangers. The coating of the silica is performed with "prepolymers", which are synthesized in a separate step and then applied to the support material and immobilized. For a number of years, this method has been successfully employed in the synthesis of various stationary phases based on silica and alumina, creating resins with remarkable selectivities. Further information on this subject may be found in the review by Schomburg [37].

The prepolymer used for manufacturing this cation exchanger consists of a copolymer that is derived from a mixture of butadiene and maleic acid in equal parts:



Poly(butadiene-maleic acid), PBDMA

The structural formula reveals that this polymer contains two different types of carboxyl groups that have different dissociation constants. The first dissociation step is characterized by a p*K* value of 3.4; the p*K* value of the second step is about 7.4. The cation-exchange capacity of the finished stationary phase is directly proportional to its polymer content.

The PBDMA-coated silica cation exchanger from Thermo Fisher Scientific is offered under the trade name Dionex IonPac SCS 1 for nonsuppressed conductivity detection of cations only. It is available in a standard bore and a microbore format; the substrate particle diameter is 4.5 µm. Like all the other PBDMA-coated silica cation exchangers from different vendors, the

Dionex IonPac SCS 1 column can be used with eluents of pH 2–7 and is compatible with aprotic solvents such as acetonitrile and acetone up to a level of 100%. It is designed for the separation of common inorganic cations, selected alkanolamines, and transition metals such as zinc and copper using nonsuppressed conductivity detection. Thus, it is targeted for applications in the power generating and petrochemical industries. As an example, Figure 4.9 shows the separation of common inorganic cations and monoethanolamine on the Dionex IonPac SCS-1 column with c = 3 mmol/L methanesulfonic acid as the eluent.

4.1.4 Cation exchanger for transition metal analysis

The separation of transition metals with ion exchangers requires a complexation of the metal ions in the mobile phase to reduce their effective charge density. Monovalent cations such as H^+ are unsuitable as eluents. Because the selectivity coefficients for transition metals of the same valency are so similar, a selectivity change is obtained only by the introduction of a secondary equilibrium such as a complexation equilibrium, which is established by adding appropriate complexing agents to the mobile phase. Common complexing agents include weak organic acids that preferably form anionic or neutral complexes with metal ions such as citric acid, oxalic acid, tartaric acid, or pyridine-2,6-dicarboxylic acid (dipicolinic acid). Such complexes may be separated on anion or cation exchangers. Nowadays, stationary phases with a defined anion- *and* cation-exchange capacity are used. Often, two different organic acids are used as complexing agents to optimize a particular separation.

The separation of the various transition metals may be optimized by varying the pH value. If the above-mentioned weak organic acids are used as complexing agents, lowering pH leads to a decrease in the effective ligand concentration and, in turn, to an increase in the proton

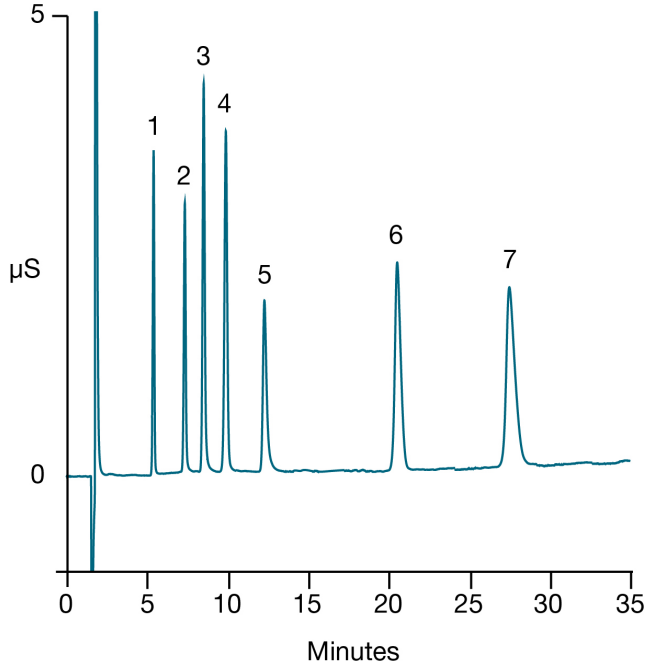


Figure 4.9 Separation of common inorganic cations and monoethanolamine on a Dionex IonPac SCS 1 column. Column dimensions: 250 mm × 4 mm i.d.; column temperature: 30 °C; eluent: 3 mmol/L methane-sulfonic acid; flow rate: 1 mL/min; detection: non-suppressed conductivity; injection volume: 25 µL; peaks: 0.4 mg/L lithium (1), 1.6 mg/L sodium (2), 2 mg/L ammonium (3) and monoethanolamine (4), 4 mg/L potassium (5), 2 mg/L magnesium (6), and 4 mg/L calcium (7).

concentration resulting in longer retention times. In contrast, a higher effective ligand concentration, raised by a higher concentration of the organic acid, leads to a higher concentration of the respective counter ion (H^+) as well. This, in turn, accelerates the displacement of metal ions from the cation-exchange functional groups and results in a severe loss of separation efficiency.

When low-capacity cation exchangers are used, the ion-exchange capacity depends on the kind of counter ion. Accordingly, lithium hydroxide is recommended for adjusting the pH value because lithium ions exhibit a low affinity toward the stationary phase.

When selecting a ligand for the separation and elution of transition metal ions from *cation exchangers* the following guidelines should be considered:

- Metal ions and ligands must form neutral or anionic complexes.
- Different complex formation constants for the various metals increase selectivity.
- The transition metal complexes being formed should be thermodynamically stable and kinetically labile; that is, the complex should have a high energetic and entropic formation tendency and the thermodynamic equilibrium should be established quickly and without restriction. This requirement is discussed in the following examples:

  a) If a transition metal complex has a high formation constant but is kinetically stable, then the cation-exchange process is impossible; that is, the anionic complex is not retained due to Donnan exclusion.
  b) If the formation constant is small but the complex is kinetically unstable, the retention mechanism is dominated by cation exchange. Correspondingly long retention times could result.
  c) If the formation constant is small and the complex is kinetically stable, the chromatographic signals are often tainted by a strong tailing.

- If the transition metal complexes are detected by postcolumn derivatization with a suitable metallochromic indicator, the constant of the formation reaction should be high. Also, the indicator complex, MeIn, should be kinetically stable.

$$MeL_x + In \leftrightarrows MeIn + x \cdot L \tag{4.16}$$

Me	Metal
L	Ligand
In	Indicator

This also applies for the separation of anionic transition metal complexes on *anion exchangers*. Here, the ligand molecules should be relatively small, so that the resulting complex is similar in size to the hydrated metal ion. In metal ions of the same valency that share the same coordination number and geometry with any given ligand, large ligands cause a loss in efficiency if the formation constants of these anionic complexes do not differ significantly.

Since both cation- and anion-exchange equilibria contribute to the separation of transition metals with complexing agents in the mobile phase, best separations are accomplished on bifunctional ion exchangers with defined anion- *and* cation-exchange capacities. The Dionex IonPac CS5 separator column [38], introduced in the mid-1980s, was the first bifunctional nanobead-agglomerated ion exchanger to be used for transition metal separations. It was based on a surface-sulfonated microporous poly(styrene-*co*-divinylbenzene) substrate with a particle size of 13 µm. Such stationary phase design opened up the possibility to use pyridine-2,6-dicarboxylic acid (PDCA) as a complexing agent in the mobile phase. With PDCA as the complexing agent iron(III) is more strongly retained than with an oxalic/citric acid eluent mixture used in the past. The retention behavior of the other metal ions is characterized by differences in the geometry, charge density, and hydrophobicity of the metal–PDCA complexes. However, bifunctional nanobead-agglomerated ion exchangers of the first generation

were characterized by a limited resolution between copper, nickel, cobalt, and zinc, which could not be improved much at that time, even under optimized chromatographic conditions. Further developments in the field of bifunctional ion exchangers to solve that problem resulted in the commercialization of the Dionex IonPac CS5A column in 1996. The separate steps in the manufacturing of such a stationary phase are illustrated in Figure 4.10. A 55% cross-linked microporous DVB substrate with a particle diameter of 9 µm serves as a starting material. As in carboxylate-based weak acid cation exchangers (see Section 4.1.2), the substrate surface is coated with an anion-exchange polymer. This positively charged surface is then electrostatically agglomerated with 10% cross-linked sulfonated nanobeads with a diameter of 140 nm. The third layer consists of 2% cross-linked aminated nanobeads with a diameter of 76 nm. The finished ion exchanger is available in a standard bore (250 mm × 4 mm i.d.) and a microbore (250 mm × 2 mm i.d.) format. In addition to optimizing the stationary phase, eluents have also been modified to improve selectivity, buffer capacity, and total analysis time.

Figure 4.11 illustrates the separation of eight different transition metals, including iron(II) and iron(III), with pyridine-2,6-dicarboxylic acid (PDCA) as a complexing agent in less than 15 min. Suppressed conductivity detection cannot be employed in transition metal analysis because transition metal hydroxides formed in the suppressor would not be dissociated. Moreover, the eluents used for transition metal separations are not compatible with suppressed conductivity detection. Thus, postcolumn derivatization with 4-(2-pyridylazo)-resorcinol (PAR) is typically used to detect transition metals. The metal ions eluting from the separator column are mixed with the PAR reagent to form purple colored chelate complexes that absorb in the wavelength range between 490 and 530 nm.

A common alternative to PDCA as the complexing agent is oxalic acid. When oxalic acid is used as the single complexing agent, a completely different elution order is observed. Iron cannot be analyzed under these conditions because it forms extremely stable complexes with oxalic acid that do not allow the formation of the iron–PAR complex for detection.

The PAR reagent is also suitable for the detection of lanthanides which can be separated by gradient ion-exchange chromatography on bifunctional ion exchangers [39].

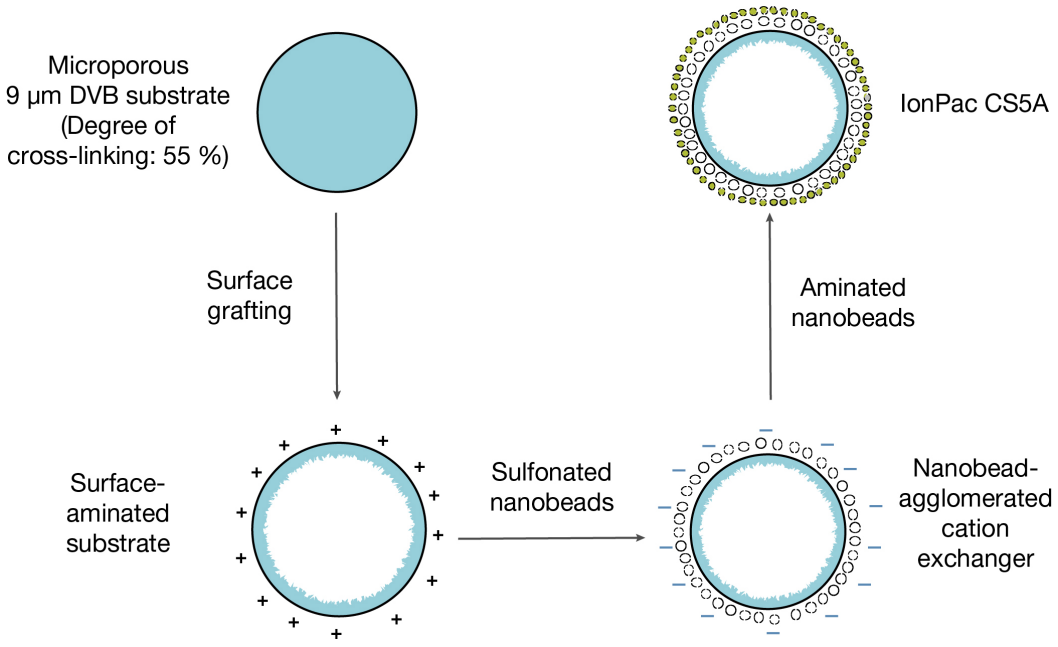


Figure 4.10 Schematic representation of the separate steps in the manufacturing of the Dionex IonPac CS5A bifunctional nanobead-agglomerated ion exchanger.

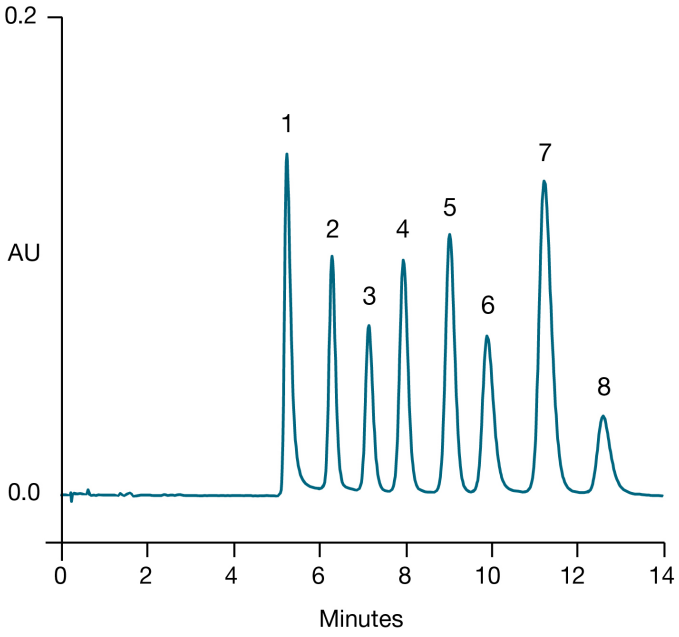


Figure 4.11 Separation of transition metals on the Dionex IonPac CS5A bifunctional nanobead-agglomerated ion exchanger. Column dimensions: 250 mm × 4 mm i.d.; eluent: 7 mmol/L pyridine-2,6-dicarboxylic acid + 66 mmol/L KOH + 74 mmol/L formic acid + 56 mmol/L K2SO4; flow rate: 1.2 mL/min; detection: photometry at 520 nm after reaction with PAR; injection volume: 50 µL; peaks: 1.3 mg/L iron(III) (1), 1.3 mg/L copper (2), 2.6 mg/L nickel (3), 1.3 mg/L zinc (4), 1.3 mg/L cobalt (5), 6 mg/L cadmium (6), 2.6 mg/L manganese (7), and 1.3 mg/L iron(II) (8).

The simultaneous analysis of chromium(III)/(VI) had been a difficult task for a long time. Numerous ion chromatographic methodologies for this analytical problem were described in the past, but all of them turned out to be rather impracticable. Although chromium(III) forms a chelate complex with PDCA, its ligand-exchange kinetics are too slow for postcolumn derivatization. Today, the most widely accepted ion chromatographic method for chromium speciation is based on anion-exchange chromatography. Using an anion exchanger and a potassium hydrogenphthalate eluent, for instance, chromium(VI) is retained while chromium(III) elutes in the column void. In this method, which was developed by Persits [40], a post-chromatographic oxidation of chromium(III) to chromium(VI) is employed, which is carried out with ammonium peroxodisulfate in acid solution, using silver nitrate as a catalyst. Complete conversion is achieved within a short time by raising the reaction temperature to 80 °C. Both chromium species can then be detected photometrically either directly at 520 nm or after derivatization with 1,5-diphenylcarbazide (1,5-DPC). In the most elegant method for chromium speciation, the effluent of a bifunctional ion exchanger such as the Dionex IonPac CS5A is hyphenated with ICP–MS. Measuring chromium via *m/z* 52, minimum detection limits for chromium(III) and chromium(VI) are about 80 ng/L and thus one order of magnitude lower than with photometry after derivatization with 1,5-DPC. Figure 4.12 shows a corresponding chromatogram with the separation of chromium(III) and chromium(VI) on a microbore Dionex IonPac CS5A column with a nitric acid gradient and detection via ICP–MS.

If only hexavalent chromium is to be analyzed, postcolumn derivatization with 1,5-DPC is unmatched for its sensitivity. Based on an injection volume of 1000 µL, it is possible to determine chromium(VI) at single-digit nanogram/liter levels without any problem [41]. Eluent pH should be kept neutral to ensure that all chromium(VI) exists in the chromate form.

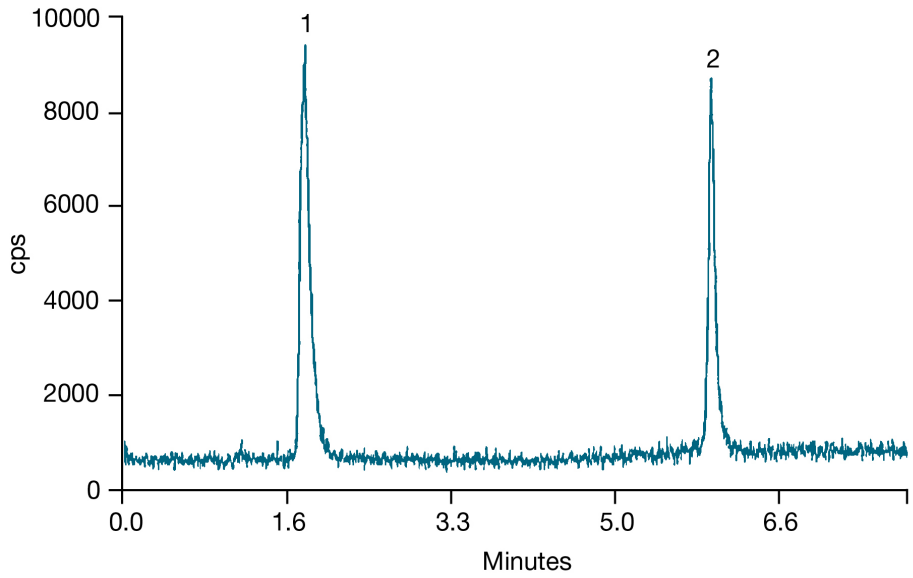


Figure 4.12 Chromium speciation on a Dionex IonPac CS5A column with ICP–MS detection. Column dimensions: 250 mm × 2 mm i.d.; eluent: 0.3–1 mol/L nitric acid; flow rate: 0.5 mL/min; detection: ICP–MS; injection volume: 100 µL; peaks: 2 µg/L each of chromium(VI) (1) and chromium(III) (2).

4.1.5 Cation exchangers for the analysis of proteins

The general structure of a tentacular ion exchanger has already been shown in Figure 3.33 (Section 3.2). It consists of a rigid and impervious 10 µm styrenic substrate particle, which is highly cross-linked (55%) and completely coated with a base stable hydrophilic neutral polymer. Tentacles bearing the ion-exchange functional groups are then grafted to the hydrophilic surface. The length and surface distribution of these covalently bonded polymer chains are well-defined. Polymer-based tentacular materials are offered under the trade name Thermo Scientific ProPac. The specifications of the two different ProPac cation-exchange columns currently available are summarized in Table 4.3.

Tentacular cation exchangers are predominantly used for the following separation problems:

- Basic proteins
- Monoclonal antibodies
- Deamidation products
- *N*-terminal pyroglutamate variants
- Protein isoforms

A special challenge in the development and production of therapeutic proteins is the characterization of the structural variants of monoclonal antibodies. Of the five classes of immunoglobulins in human blood plasma, the IgG globulins are the most common and the best understood. IgG antibodies are large molecules of about 150 kDa and consist of four polypeptide chains: two identical class γ heavy chains of about 50 kDa with 430 amino acid residues, and two identical light chains of about 25 kDa with 214 amino acid residues. The two heavy chains are characterized by a region with a constant amino acid sequence and a region with a variable sequence. They are linked to each other and to a light chain each by disulfide bonds. The resulting tetramer has two identical halves, which together form a Y-shaped, flexible structure.

Table 4.3 Specifications of ProPac cation-exchange columns.

Parameter	ProPac WCX-10	ProPac SCX-10
Base matrix material	Ethylvinylbenzene 55% cross-linked with divinylbenzene	Ethylvinylbenzene 55% cross-linked with divinylbenzene
Substrate bead diameter (μm)	10	10
Functional group	Carboxylate	Sulfonate
Temperature range	Ambient to 40 °C	Ambient to 40 °C
Flow rate range (mL/min)	0.2–2	0.2–2
Max. operating pressure	21 MPa (3000 psi)	21 MPa (3000 psi)
pH range	2–12	2–12
Protein binding capacity per mL volume	6 mg lysozyme	3 mg lysozyme
Typical buffers	Sodium and potassium salts of phosphate, MES, Tris	Sodium and potassium salts of phosphate, MES, Tris
Solvent compatibility	80% MeCN, acetone	80% MeCN, acetone 80% MeOH
Detergent compatibility	Nonionic, anionic, zwitterionic	Nonionic, anionic, zwitterionic

A frequent structural variation requiring thorough analysis [42] is the *C*-terminal processing of lysine residues at the heavy chain of monoclonal antibodies isolated from mammalian cell culture. Incomplete protein processing leads to charge heterogeneity due to the absence of *C*-terminal lysine residues, which can be identified by cation-exchange chromatography. As can be seen from the chromatogram in Figure 4.13, *C*-terminal lysine variants can be separated from the native humanized IgG antibody on a weak acid cation exchanger at neutral pH using a shallow NaCl gradient. To verify that the reason for the different retention times of the three peaks is the different content of heavy chain *C*-terminal lysine, the IgG sample is treated with carboxypeptidase B, an exopeptidase that specifically cleaves *C*-terminal lysine residues. Such treatment results in the quantitative disappearance of peaks **5** and **8** accompanied by a corresponding increase in the area of peak **3**. This can be taken as a proof that peaks **5** and **8** differ by one or two lysine residues at the *C*-terminal of the heavy chain. Humanized IgG not only produces the three major peaks shown in Figure 4.13 but also several other variants, both more acidic as well as more basic variants. 4-Morpholineethanesulfonic acid (MES)-based buffers resolve those additional details not seen when using traditional phosphate-based buffers [43]. Thus, cation-exchange chromatography can be applied to the quality control of therapeutic proteins. Even though the resolving power of cation-exchange separations of monoclonal antibodies is high, it takes a lot of method development time to fully optimize the separation. Parameters to be optimized include column selection, eluent pH, mobile-phase additives, and salt concentration gradient profiles. Once method development is completed, the method is usually product-specific and does not tolerate significant changes in chromatographic conditions. Nevertheless, cation-exchange chromatography utilizing salt gradients is currently one of the most popular methods for charge-sensitive antibody analysis as stated in a review article by Vlasek and Ionescu [44].

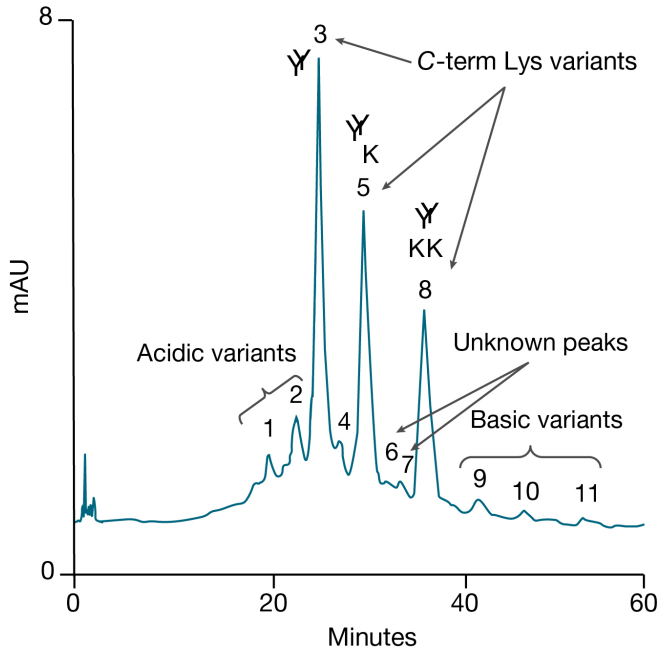
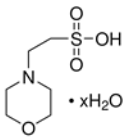


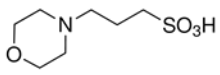
Figure 4.13 Separation of acidic and basic terminal lysine variants of an IgG monoclonal antibody. Separator column: ProPac WCX-10; eluent: (A) 0.02 mol/L MES + 0.06 mol/L NaCl, pH 5.5, (B) 0.02 mol/L MES + 0.18 mol/L NaCl, pH 5.5; gradient: linear; 55% B to 85% B in 50 min; flow rate: 1 mL/min; detection: UV (280 nm); sample: 10 µL of 0.5 mg of IgG per mL of eluent; peaks: (1) and (2) acidic variants, (3) mAb without heavy chain *C*-terminal lysine, (4) unknown, (5) mAb with a lysine residue on one *C*-terminal of the heavy chains, (6) and (7) unknown, (8) mAb with lysine residues on both *C*-terminals of the heavy chains, (9)–(11) basic variants.

The latest column development for separating closely related monoclonal antibody variants is the Thermo Scientific™ MAbPac™ SCX-10 column. In its 250 mm × 4 mm i.d. format, this column is complementary to the ProPac WCX-10 column, offering an alternative selectivity and providing higher resolution and efficiency for variant analysis of most monoclonal antibodies. The 55% cross-linked divinylbenzene-based MAbPac resin with nonporous 10 µm core particles is the basis for the superior performance of monoclonal antibody variant analysis. Like with the ProPac WCX-10 column, a hydrophilic layer surrounds the polymeric beads, preventing hydrophobic interactions between proteins and the stationary phase. A proprietary grafted cation-exchange surface with sulfonate groups provides pH selectivity control, resulting in high-resolution separations.

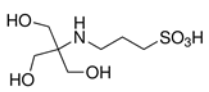
Farnan and Moreno [45] have shown that the use of a pH gradient to assess charge heterogeneity of monoclonal antibodies offers some key advantage over salt gradients as a single pH method can be applied to a series of mAbs having a very wide range of isoelectric points. However, it is extremely difficult to create a buffer system that provides a truly linear pH gradient. For this purpose, Thermo Fisher Scientific introduced a CX-1 buffer kit in 2013 [46] for pH gradients within a range from pH 5.6 to pH 10.2. Buffer A and B each contain a mixture of the following four Good buffers of zwitterionic nature:



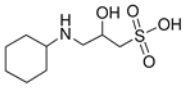
MES
p*K* 6.1



MOPS
p*K* 7.2



TAPS
p*K* 8.4



CAPSO
p*K* 9.6

Buffer A is titrated to pH 5.6, buffer B to pH 10.6. In this pH range, each buffer component is either neutral or negatively charged and thus not retained on the stationary phase of the cation exchanger. The application of linear regression results in a regression factor of $r^2 = 0.9996$. Thus, a truly linear gradient is performed with this buffer system.

Another advantage of pH gradients is the simple optimization of the separation of monoclonal antibodies whose p*I* values are typically between 6 and 10. The pH gradient method can serve as a platform for the separation of charge variants. Figure 4.14a shows the separation of a mAb sample when applying the total pH range from pH 5.6 to pH 10.2; in this case, the gradient slope is 0.153 pH unit per minute. Depending on the p*I* value of the antibody, further optimization can simply be achieved by running a shallower pH gradient over a narrower pH range. The respective separation profile from pH 5.6 to pH 7.9 with a pH gradient slope at 0.078 pH unit per minute is shown in Figure 4.14b. Optimal resolution is achieved in a pH range between pH 6.75 and pH 7.9 with a once again reduced gradient slope at 0.038 pH unit per minute (see Figure 4.14c). These three chromatograms impressively demonstrate that the pH gradient maintains linearity even though the gradient slope was reduced to one half or even one quarter of the initial run. Thus, the chromatographic behavior of the variants remains predictable when running a shallower gradient profile.

5
Ion-exclusion chromatography (HPICE)

The introduction of ion-exclusion chromatography is attributed to Wheaton and Bauman [47]. It is primarily employed for the separation of weak inorganic and organic acids. In addition, ion-exclusion chromatography can be used for the separation of alcohols, aldehydes, amino acids, and carbohydrates. Due to Donnan exclusion, fully dissociated acids are not retained at the stationary phase, eluting therefore within the void volume as a single peak. Undissociated compounds, however, can diffuse into the pores of the resin, since they are not subject to Donnan exclusion. In this case, separations are based on nonionic interactions between the solute and the stationary phase.

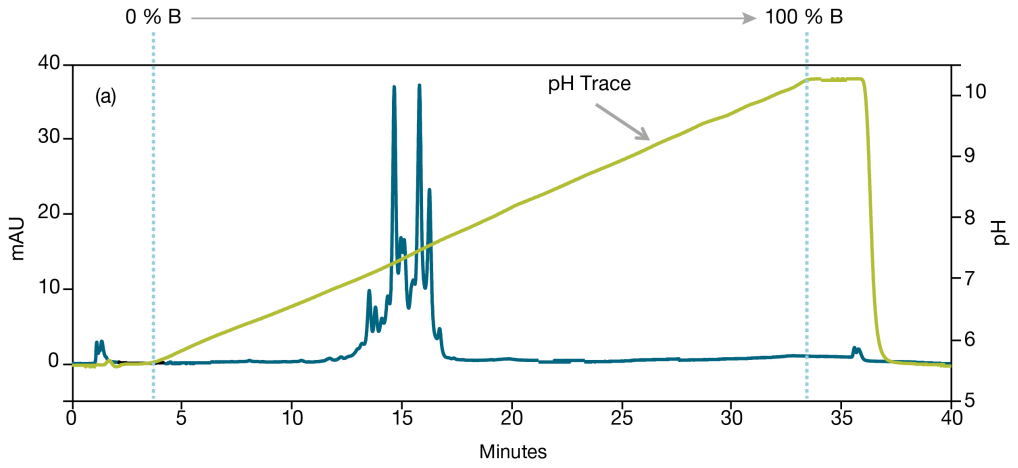
Detection is usually carried out by measuring the electrical conductivity. When combined with a suppressor system, this detection method is superior to all other detection methods (e.g., refractive index or UV detection at low wavelengths) with regard to selectivity and sensitivity.

5.1
The ion-exclusion process

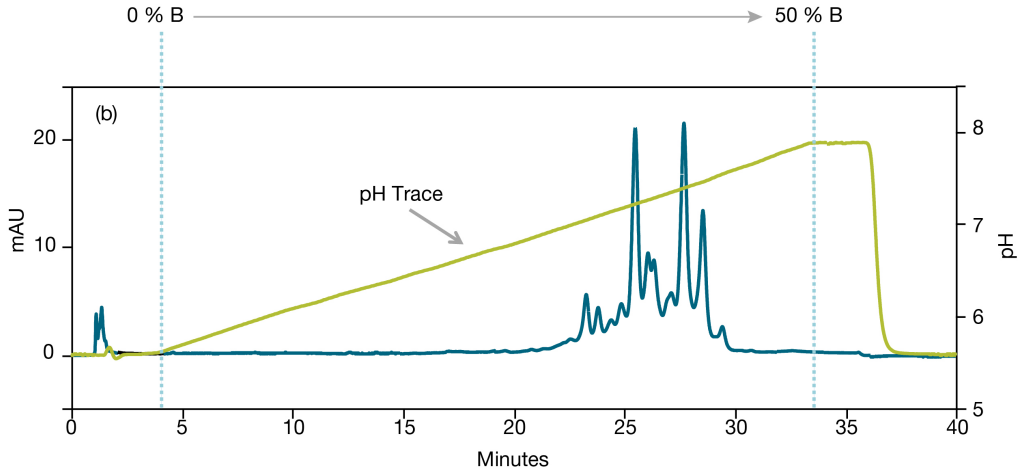
Typically, HPICE separator columns contain a totally sulfonated high-capacity cation-exchange resin. The separation mechanism occurring at this stationary phase is based on three phenomena:

- Donnan exclusion
- Steric exclusion
- Adsorption

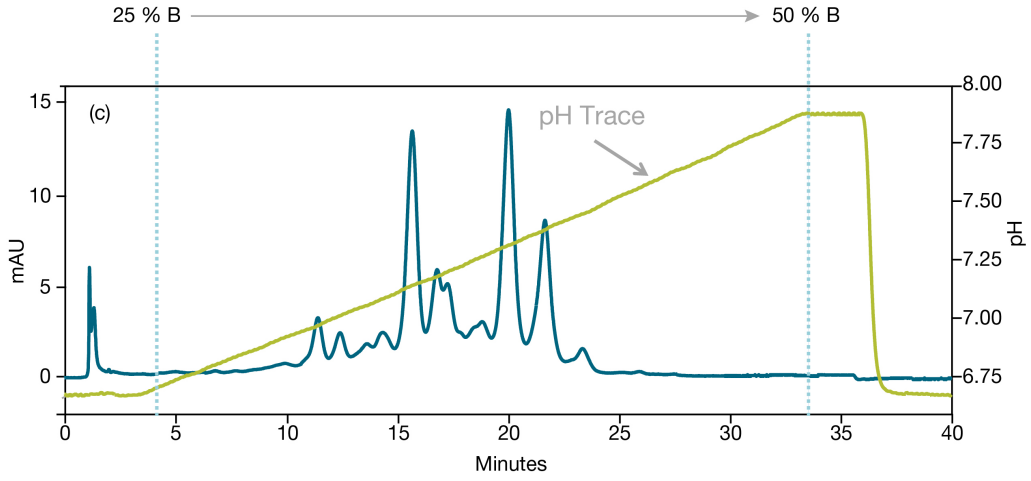
Figure 5.1 represents a schematic illustration of the separation process on an HPICE column. It shows the resin surface with its bonded sulfonic acid groups. If pure water is passed through the separator column, a hydration shell is formed around the sulfonic acid groups. Therefore, some of the water molecules are in a higher state of order compared to water molecules in the bulk mobile phase.



(a)



(b)



(c)

Figure 4.14 Optimization of the separation of mAb charge variants with a linear pH gradient. Separator column: MAbPac SCX-10, 10 µm; column format: 250 mm × 4 mm i.d.; eluent: pH gradient based on the CX-1 buffer system; gradient: (a) 0% B (pH 5.6) to 100% B (pH 10.2) in 30 min, (b) 0% B (pH 5.6) to 50% B (pH 7.9), (c) 25% B (pH 6.75) to 50% B (pH 7.9).

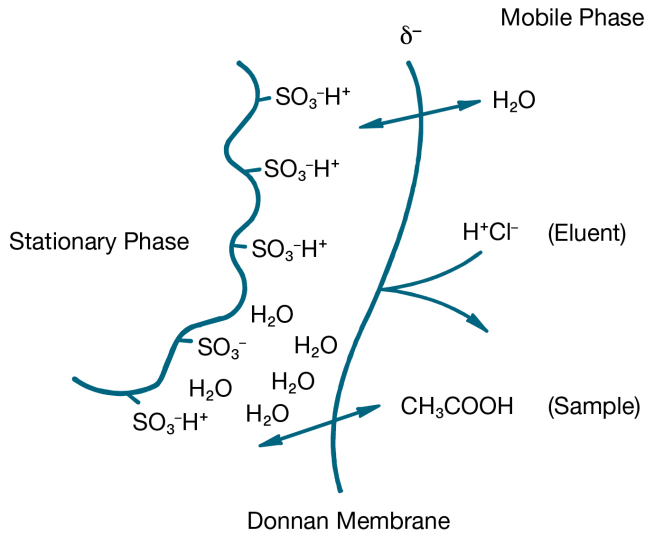


Figure 5.1 Schematic representation of the separation process on a HPICE column.

In this retention model, a negatively charged layer analogous to the Donnan membrane characterizes the interface between the hydration shell, which is only permeable by undissociated compounds, and the bulk mobile phase. Fully dissociated acids, such as hydrochloric acid acting as the eluent, cannot penetrate this layer because of the negative charge of the chloride ion. Thus, such ions are excluded from the stationary phase. Their retention volume is called the *exclusion volume* V_e. On the other hand, neutral water molecules may diffuse into the pores of the resin and back into the mobile phase. The volume corresponding to the "retention time" of water is called the *totally permeated volume* V_p.

Depending on the eluent pH, a weak organic acid (e.g., acetic acid) may be present after injection in partly undissociated form, which is not subject to Donnan exclusion. Although both acetic acid and water may interact with the stationary phase, a retention volume is observed for acetic acid that is higher than V_p. This phenomenon can only be explained by adsorption occurring on the surface of the stationary phase. The separation mechanism in the case of aliphatic monocarboxylic acids, therefore, is determined by Donnan exclusion and adsorption. Retention time increases with increasing length of the alkyl chain of the acid. By adding organic solvents such as acetonitrile or 2-propanol, the retention of aliphatic monocarboxylic acids may be reduced. This is due to: (1) adsorption sites being blocked by solvent molecules and (2) the solubility in the eluent being enhanced.

Di- and tricarboxylic acids such as oxalic acid, citric acid, and so on elute between the exclusion volume and the totally permeated volume. Apart from Donnan exclusion, the predominant separation mechanism is, in this case, steric exclusion. The retention is determined by the size of the sample molecule. Because the pore volume of the resin is determined by its degree of cross-linking, resolution can only be improved by utilizing another column or by coupling with another column.

In general, organic acid separations can be optimized by changing the pH because the eluent pH influences the degree of dissociation and, consequently, the solute retention.

5.2
Stationary phases

The selection of stationary phases for ion-exclusion chromatography of small-molecular weight anions is relatively limited. Typically, totally sulfonated poly(styrene-*co*-divinyl-benzene)-

based cation exchangers in the hydrogen form are used. The percentage of divinylbenzene – expressed as the degree of cross-linking – is of particular importance for the retention behavior of weak inorganic and organic acids. Depending on the degree of cross-linking, these acids may diffuse into the interior of the stationary phase to a greater or lesser degree, resulting in different retention times. Most of the materials offered today have a degree of cross-linking of 8%. This compromise takes into account the different retention behavior of weak and strong acids.

Particle diameters between 5 and 10 µm, which enable relatively fast diffusion processes, are characteristic of the stationary phases that are used today in ion-exclusion chromatography. The structural-technical properties of these phases are listed in Table 5.1.

Thermo Fisher Scientific currently offers two ion-exclusion columns: Dionex IonPac ICE-AS1 and AS6. The former is a moderately hydrophilic, microporous poly(styrene-*co*-divinylbenzene)-based cation exchanger with a particle diameter of 7.5 µm and an ion-exchange capacity of 27 mequiv/column, functionalized with sulfonate groups. The Dionex IonPac ICE-AS1 column is primarily used for the separation of weak inorganic acids, short-chain fatty acids, and alcohols. Difficulties are encountered, however, in the separation of aliphatic mono-, di-, and tricarboxylic acids. These acids elute from such stationary phases within the totally permeated volume. The selectivity of the separation in this retention range is usually very poor. Since the totally permeated volume is determined by the degree of cross-linking of the resin, selectivity can only be improved marginally by altering the chromatographic conditions. For example, Figure 5.2 shows a separation of short-chain fatty acids on a Dionex IonPac ICE-AS1 column using suppressed conductivity detection. The packing material of the column exhibits a 50% solvent compatibility toward common HPLC solvents such as methanol, 2-propanol, and acetonitrile, which can be used to drastically decrease retention of strongly retained compounds.

The Dionex IonPac ICE-AS6 column also contains a microporous resin with a particle diameter of 8 µm. In comparison to the Dionex ICE-AS1 column, it is more hydrophobic; this is due to the additional incorporation of polymethacrylates in the substrate, which is functionalized with sulfonate and carboxyl groups. The carboxyl groups are responsible for a higher selectivity that can be attributed to hydrogen bonding between the resin and the analyte acid. Thus, this stationary phase is very well suited for the analysis of aliphatic hydroxycarboxylic acids, which are not well separated on a conventional ion-exclusion column. Because nonionic adsorption interactions also contribute to retention, mono- and dicarboxylic acids with hydrophobic segments such as propionic acid, butyric acid, fumaric acid, and succinic acid are very strongly retained. The separation of aliphatic carboxylic acids and hydroxycarboxylic acids illustrated in Figure 5.3 impressively demonstrates the unique selectivity of this separator column.

Table 5.1 Structural and technical properties of poly(styrene-*co*-divinylbenzene)-based ion-exclusion columns offered by Thermo Fisher Scientific.

Column	Dimensions (length × i.d.) (mm)	Particle diameter (µm)	Max. pressure (MPa)	Ion-exchange functionality	Application
Dionex IonPac ICE-AS1	250 × 9 250 × 4	7.5	9.6	Sulfonate groups	High-performance separator for weak inorganic acids and short-chain fatty acids
Dionex IonPac ICE-AS6	250 × 9	8	5.6	Sulfonate and carboxyl groups	High-performance separator for aliphatic hydroxycarboxylic acids and alcohols

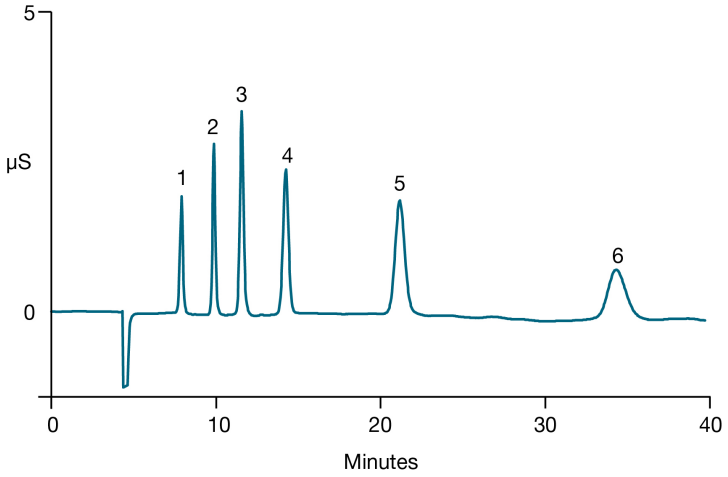


Figure 5.2 Separation of short-chain fatty acids on a Dionex IonPac ICE-AS1 column. Eluent: 0.4 mmol/L heptafluorobutyric acid; flow rate: 1 mL/min; detection: suppressed conductivity; injection volume: 50 µL; peaks: (1) formic acid, (2) acetic acid, (3) propionic acid, (4) butyric acid, (5) valeric acid, and (6) caproic acid.

Analyte pairs such as tartaric acid/citric acid, glycolic acid/lactic acid, lactic acid/formic acid, and succinic acid/formic acid, which are poorly separated or not at all separated on conventional ion-exclusion columns, are separated to baseline on a Dionex IonPac ICE-AS6 column under standard chromatographic conditions. It also has a limited solvent compatibility of 20% (*v/v*) toward methanol, 2-propanol, and acetonitrile.

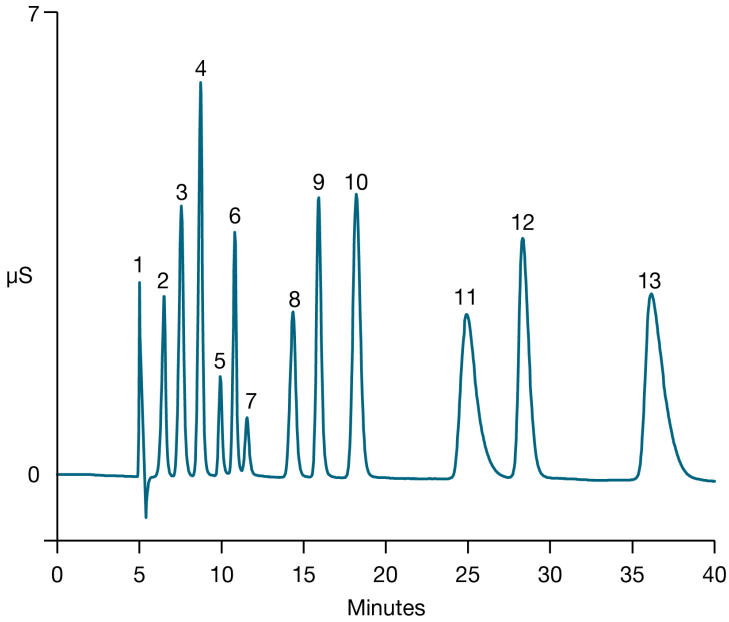


Figure 5.3 Separation of carboxylic acids and hydroxycarboxylic acids on a Dionex IonPac ICE-AS6 column. Eluent: 0.4 mmol/L heptafluorobutyric acid; flow rate: 0.4 mL/min; detection: suppressed conductivity; injection volume: 50 µL; peaks: 5 mg/L oxalic acid (1), 10 mg/L tartaric acid (2), 15 mg/L citric acid (3), 20 mg/L malic acid (4), 10 mg/L glycolic acid (5), 10 mg/L formic acid (6), 10 mg/L lactic acid (7), 30 mg/L hydroxyisobutyric acid (8), 25 mg/L acetic acid (9), 25 mg/L succinic acid (10), 35 mg/L fumaric acid (11), 50 mg/L propionic acid, (12), and 40 mg/L glutaric acid.

5.3
Eluents for ion-exclusion chromatography

In ion-exclusion chromatography, the selection of eluents is very limited. In the simplest case, pure deionized water can be utilized. However, acidifying the mobile phase suppresses dissociation, thus significantly improving peak shape. Nowadays, pure deionized water is recommended only for the analysis of carbonate, which can easily be determined in this way.

For the separation of organic acids, mineral acids and long-chain aliphatic carboxylic acids are usually employed as eluents. Among mineral acids, sulfuric acid is most commonly used, especially when applying direct UV detection [48]. Hydrochloric acid eluents are outdated, although they are compatible with modern membrane suppressors. However, the high equivalent conductance of the chloride ion results in an unnecessarily high background conductivity. Good separations are obtained with tridecafluoroheptanoic acid (perfluoroheptanoic acid), which is applied (like all other eluents) in a concentration range between c = 0.5 and 10 mmol/L. Octanesulfonic acid exhibits similar elution properties and is also suited for the analysis of borate and carbonate. For the sensitive detection of boric acid by electrical conductivity measurement, a mixture of octanesulfonic acid and mannitol should be used because boric acid is weakly dissociated. The sugar alcohol complexes boric acid, which leads to a significantly higher conductivity. On the other hand, for a Dionex IonPac ICE-AS6 separator column, short-chain perfluorinated fatty acids such as heptafluoropropanoic acid (perfluorobutyric acid) are recommended as the eluents. The high retention of aliphatic monocarboxylic acids (n_C > 4) and aromatic carboxylic acids can be lowered by adding small amounts of an organic solvent (10–30 mL/L) to the eluent. This blocks adsorption sites on the surface of the stationary phase [49]. Acetonitrile, 2-propanol, or ethanol are particularly suitable. If possible, methanol should not be used because the stationary phase is subject to strong volume changes when using this solvent.

5.4
Analysis of inorganic acids

The separator columns listed in Table 5.1 (see Section 5.1) allow the separation of a variety of weak inorganic acids. While fully dissociated mineral acids elute within the void volume because of Donnan exclusion, weak inorganic acids are more strongly retained. This includes the barely dissociated boric acid (pK_a = 9.23), which is detected by measuring the electrical conductivity. The analysis of boric acid in the milligram/liter range is carried out with a pure aqueous octanesulfonic acid eluent (c = 1 mmol/L) in combination with a membrane-based suppressor system. A chromatogram from such an analysis is shown in Figure 5.4. The chromatogram illustrates that the more strongly retained carbonate can be analyzed under the same chromatographic conditions. The minimum detection limits for both compounds are about 1 mg/L. For sensitive borate detection, a large excess of mannitol is added to a methanesulfonic acid eluent to increase the borate conductance via complexation. A special concentrator column (*t*race *b*orate *c*oncentrator, TBC-1) was developed for the ultratrace analysis of borate. The stationary phase of this concentrator is functionalized with a *cis*-diol, on which borate is selectively retained. Minimum detection limits in the lower nanogram/liter range are obtained when large sample volumes are preconcentrated, so that this method is suitable for the trace analysis of borate in deionized water. A Dionex IonPac ICE-Borate column was used as the separator, which is a Dionex IonPac ICE-AS1 column being especially conditioned for borate analysis.

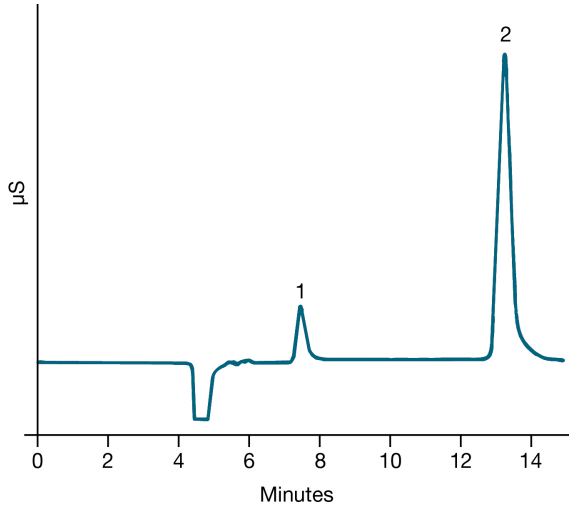


Figure 5.4 Separation of borate and carbonate by ion-exclusion chromatography. Separator column: Dionex IonPac ICE-AS1; eluent: 1 mmol/L octanesulfonic acid; flow rate: 1 mL/min; detection: suppressed conductivity; injection volume: 50 µL; peaks: 10 mg/L borate (1) and 50 mg/L carbonate (2).

In combination with an amperometric detection, ion-exclusion chromatography is suitable for the determination of sulfite and arsenite. Both ions can be oxidized at a platinum working electrode and thus be detected selectively and very sensitively. Introduced in 1986 by Kim and Kim [50], DC amperometry of sulfite is widely used in the food industry and accepted as a standard method by AOAC (*A*ssociation of *O*fficial *A*nalytical *C*hemists) [51]. The method is highly selective, so that a time-consuming sample preparation is not necessary. The only disadvantage is that the electrode surface changes its characteristics relatively quickly, resulting in a 40% decrease of the response factor within a time frame of about 8 h. When applying pulsed amperometry of sulfite [52] instead of DC amperometry, the surface characteristics and, consequently, the response factor remains constant due to the fast and repeating sequence of oxidizing and reducing potentials. The minimum detection limit for sulfite under these chromatographic conditions was calculated to be 40 µg/L. For the amperometric detection of arsenite on the platinum working electrode the applied oxidation potential of +0.95 V can be kept constant.

Another important application of ion-exclusion chromatography for weak inorganic acids is the determination of orthosilicate. Li and Chen [53] applied this method for the analysis of various types of water samples including mineral water, tap water, and sea water using an Aminex® HPX-87H separator column and nonsuppressed conductivity detection. A much higher selectivity is obtained by combining ion-exclusion chromatography with postcolumn derivatization with sodium molybdate in acidic solution and subsequent photometric detection of the yellow heteropoly acid $H_4[Si(Mo_3O_{10})_4 \cdot H_2O]$ at 410 nm. Orthophosphate may also be detected under these conditions. Because of its different acid strength, it elutes *prior* to orthosilicate, so this method is very selective for the determination of both anions.

5.5 Analysis of organic acids

In addition to a couple of inorganic acids, a wealth of organic acids may be separated by using the separator columns listed in Table 5.1.

As already mentioned, fully dissociated acids elute within the void volume due to Donnan exclusion. The retention behavior of weak organic acids, on the other hand, may be predicted based on the following criteria:

- Members of a homologous series elute in the order of decreasing acid strength and decreasing water solubility, that is, increasing solvophobicity. Taking aliphatic mono-carboxylic acids as an example, the elution order, according to Figure 5.2, is formic acid > acetic acid > propionic acid, and so on. The characteristics of such separations are similar to those of reversed-phase chromatography. Van der Waals forces between the solute and the polymeric resin material (mainly benzene rings) as well as the decrease in solubility of the solutes in the eluent influence the distribution of the solutes between the stationary and the mobile phases.
- Dibasic acids are eluted prior to the corresponding monobasic acids because of their higher solubility in polar eluents. Thus, it is observed that oxalic acid elutes prior to acetic acid, and malonic acid prior to propionic acid.
- Organic acids with a branched carbon skeleton such as *iso*-butyric acid are generally eluted prior to the nonbranched analogues such as *n*-butyric acid. Again, this corresponds to the retention behavior in RPLC.
- A double-bond in the carbon skeleton leads to a significantly higher retention because of π-π interactions with the aromatic rings of the polymer: acrylic acid is eluted *after* propionic acid.
- Aromatic acids are strongly retained because of the interactions described above. Thus, they should not be analyzed by ion-exclusion chromatography. The high retention of unsaturated and aromatic moieties on ICE stationary phases is unlike the behavior on ODS phases, where π-π interactions are not possible and where only the enhanced solubility in the eluent comes to fruition.

In the analysis of di- and polybasic acids (see Figure 5.3), the acid concentration in the mobile phase determines the retention. It affects the degree of dissociation and thus, the retention time of the carboxylic acid to be analyzed. In general, a higher resolution is observed with increasing acid concentration. Organic acids with low pK_a values such as glucuronic acid, tartaric acid, citric acid, and malonic acid are only separated to baseline at higher acid concentrations in the mobile phase. The retention of short-chain fatty acids, on the other hand, is not significantly affected by changes made to the mobile-phase pH. Because these acids are mainly retained by nonionic interactions resembling reversed-phase effects, retention times can only be reduced by adding small amounts of 2-propanol or acetonitrile (10 up to 30 mL/L). Later eluting acids such as shikimic acid, itaconic acid, and acrylic acid are most strongly affected by solvent addition: the addition of 10% (*v/v*) acetonitrile decreases their retention by 40%.

5.6
Size-exclusion chromatography of proteins

The biopharmaceutical industry has continued its focus on the development of biotherapeutic monoclonal antibody (mAb) drugs. mAbs produced from mammalian cell culture may contain significant amounts of dimers and higher-order aggregates that can compromise safety and efficacy. The formation of aggregates may originate from elevated temperatures, shear strain, surface adsorption, high protein concentrations, or other unknown reasons. Studies show that the aggregates present in drug products can cause severe immunogenic and anaphylactic reactions. Thus, protein aggregates are typically monitored throughout the production of a biotherapeutic drug as per the guidelines of the FDA and other regulatory agencies. While a

variety of analytical techniques have been used to analyze soluble aggregates, the dominant technique continues to be size-exclusion chromatography (SEC).

The MAbPac SEC-1 column has been especially designed for the analysis of mAbs and their aggregates. It is based on high-purity, spherical 5 µm silica particles with a pore size of 300 Å that are covalently modified with a hydrophilic diol layer. The column is available in three different formats (7.8, 4.0, and 2.1 mm i.d.) to accommodate different applications and sample loadings. The 7.8 mm i.d. column provides the highest resolution of mAb and aggregate separation for routine analysis, while the 4 mm i.d. column offers excellent resolution at greatly reduced mobile-phase consumption. The column with the smallest internal diameter is suitable for direct MS detection, providing the highest sensitivity for small sample loading. As an example, Figure 5.5 shows the separation of a monoclonal antibody and its aggregates on the 4 mm i.d. MAbPac SEC-1 column. The ionic strength of the mobile phase must be adjusted to minimize any secondary interaction between the column packing material and the protein. As commonly observed with size-exclusion packing materials, higher ionic strength mobile phases lead to decreased peak tailing and narrower peaks for the mAb monomer. The chromatogram in Figure 5.5 has been obtained with 300 mmol/L sodium chloride, a concentration that is high enough to minimize any secondary interactions. The ionic strength of the mobile phase also affects the recovery of the aggregates. However, at sodium chloride concentrations above 200 mmol/L, aggregate quantitation does not change significantly. Resolution in size-based separations are influenced by the linear velocity of the mobile phase. Although using lower flow rates results in longer analysis times, the increased resolution gives greater confidence in aggregate quantitation. If shorter analysis times are required, shorter columns with smaller particle sizes must be employed. Improvements in SEC resolution can only be gained by increasing column length. Because SEC separations are based on diffusion in and out of the pores of the column packing material, larger proteins cannot access the pores and elute early. The smaller the protein, the longer the residence time within the pores, which results in longer retention times. These principles allow for better resolution with longer columns, and are addressed with two different lengths for the MAbPac SEC-1 columns: 150 mm and 300 mm.

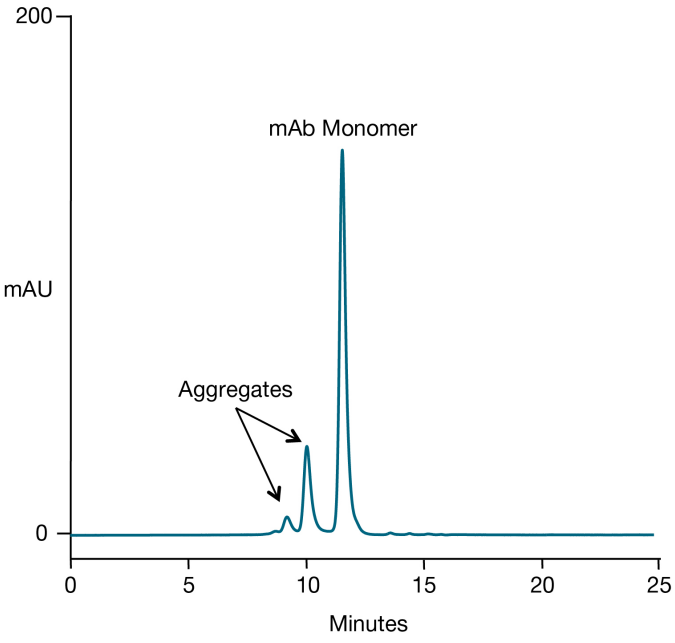


Figure 5.5 Size-exclsuion separation of a monoclonal antibody (mAb) and its aggregates. Separator column: MAbPac SEC-1, 5 µm; column dimensions: 300 mm × 4 mm i.d.; column temperature: 30 °C; Eluent: 50 mmol/L sodium phosphate, pH 6.8, in 300 mmol/L NaCL; flow rate: 200 µL/min; detection: UV (280 nm); injection volume: 5 µL; sample: 1 mg/mL mAb.

The commonly used buffer for separating mAbs by SEC is a phosphate buffer with 300 mmol/L NaCl. The nonvolatile nature of this buffer and the high concentration makes this buffer incompatible with online MS detection. Using a volatile buffer such as 20 mmol/L ammonium formate, a MAbPac SEC-1 column can be directly coupled to a mass spectrometer.

6
Ion-pair chromatography (MPIC)

In the early days of ion chromatography, ion-pair chromatography provided a useful alternative to ion-exchange chromatography for the analysis of polarizable and surface-active anions and cations, which were highly retained or not eluted at all on first generation pellicular ion exchangers. With the introduction of modern grafted or hyperbranched ion exchangers, which allow rapid elution of common polarizable ions (e.g., iodide, thiocyanate, thiosulfate, perchlorate, hydrophobic amines, and others), the practical use of ion-pair chromatography – with very few exceptions – has been reduced to the analysis of surface-active ions today. Nevertheless, ion-pair chromatography is universally applicable because the selectivity of the separation is mainly determined by the type of mobile phase. Thus, either anionic or cationic compounds can be separated using the same stationary phase.

The term "mobile-phase ion chromatography" (MPIC) describes a method that combines the major elements of reversed-phase ion-pair chromatography (RPIPC) with suppressed conductivity detection. Besides silica-based chemically bonded reversed phases, neutral divinylbenzene resins featuring a high surface area and a weakly polar character are also used as stationary phases.

The physicochemical phenomena of RPIPC, the basis for the retention mechanism, are still not fully understood. This mechanistic uncertainty is reflected by the many terms proposed for this kind of separation method in the past. Horvath et al. [54] took the view that solute ions form neutral ion pairs with the lipophilic ions in the aqueous mobile phase. These neutral ion pairs are retained at the nonpolar stationary phase. In contrast, Huber et al. [55] supported the ion-exchange model, where the lipophilic reagent first adsorbs at the surface of the stationary phase, giving it an ion-exchange character. Both hypotheses represent limiting cases. It is not to be expected that the retention process is fully described by just one of the two limiting cases.

In 1979, Bidlingmeyer et al. [56] introduced a third model they termed the *ion-interaction model*. It is based on conductivity measurements, the results of which rule out the formation of ion pairs in the mobile phase. This retention model, also used by Pohl [57] to interpret the retention mechanism on an MPIC phase, neither presupposes the formation of ion pairs nor is it based on classical ion-exchange chromatography.

According to the ion-interaction model, a high surface tension is generated between the nonpolar stationary phase and the polar mobile phase. From this, the stationary phase obtains a high affinity toward those components of the mobile phase that can reduce the high surface tension. Such components include, for example, polar organic solvents, surfactants with their respective counter ions, and quaternary ammonium bases. Moreover, the model concept of ion interaction provides for an electrically charged double layer at the surface of the stationary phase. This phenomenon is schematically represented in Figure 6.1, taking the analysis of surface-inactive anions as an example. As can be seen from Figure 6.1, the lipophilic ions (e.g., tetrabutylammonium cations) and acetonitrile (as the organic modifier) are adsorbed in the inner region at the surface of the nonpolar stationary phase. As all lipophilic cations are equally charged, the surface can be only partly covered with such ions because of the repulsive forces between these charges. The corresponding counter ions (typically OH^- ions when

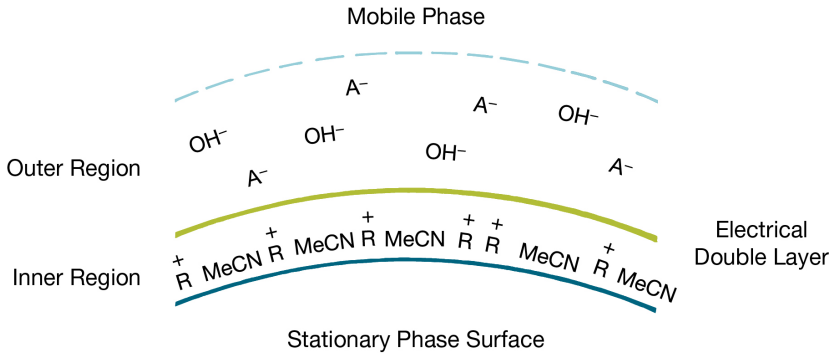


Figure 6.1 Schematic representation of the electrically charged double layer on the stationary-phase surface when separating surface-inactive anions.

applying conductivity detection), as well as the analyte ions A^-, are found in the diffuse outer region. When the lipophilic ion concentration in the mobile phase is increased, the concentration of ions adsorbed to the surface will also increase because of the dynamic equilibrium between the mobile and the stationary phases. The transfer of a solute ion through the electrical double layer is, therefore, a function of electrostatic and van der Waals forces. If a solute ion with an opposite charge is attracted by the charged surface of the stationary phase, retention is a result of Coulomb attractive forces and additional adsorptive interactions between the lipophilic part of the solute ion and the nonpolar surface of the stationary phase. Adding a negative charge to the positively charged inner region of the double layer is tantamount to removing one charge from this region. To reestablish electrostatic equilibrium, another lipophilic ion can be adsorbed at the surface. Finally, two oppositely charged ions (not necessarily an ion pair) are adsorbed at the stationary phase.

The separation of surface-inactive cations can be interpreted analogously. In this case, lipophilic anions are adsorbed at the resin surface, while the analyte cations are retained in the outer region of the double layer.

Unlike normal solute ions, surface-active ions may penetrate the inner region of the double layer, where they are adsorbed at the surface of the stationary phase. With this class of compounds, retention depends on the carbon chain length and thus on the degree of hydrophobicity. Retention increases with growing chain length. Acetonitrile as an organic modifier is also adsorbed at the resin surface and, therefore, is involved in a competing equilibrium with the lipophilic ions. When both surface-active and surface-inactive ions are being analyzed, the organic modifier serves to shorten retention by blocking adsorption sites on the resin surface. In the case of surface-active ions, this is achieved by direct competition; for surface-inactive ions it is achieved via competition with $R\text{-}SO_3^-$ and R_4N^+, the solvophobic counter ions.

The primary advantage of ion-pair chromatography over ion-exchange chromatography is its great flexibility, which allows the chromatographic conditions to be adjusted for a given separation problem. This flexibility results from the great variety of experimental parameters that affect retention. Thus, ion-pair chromatographic separations of ions on an MPIC phase are affected by the following parameters:

- Type of lipophilic counter ion in the mobile phase
- Concentration of lipophilic counter ion in the mobile phase
- Type of organic modifier
- Concentration of organic modifier in the mobile phase
- Type and concentration of inorganic additives
- Eluent pH
- Column temperature

For the ion-pair chromatographic separation of *anions*, quaternary ammonium bases are preferably added as lipophilic ions to the mobile phase; the kind of counter ion in the ion-pair reagent very much depends on the detection method being used. If suppressed conductivity detection is applied, the ion-pair reagent is used in its hydroxide form. The same applies to the ion-pair chromatographic separation of *cations*, which is performed either with long-chain alkanesulfonic acids or, in the simplest manner, with mineral acids. A survey of the most commonly used reagents is listed in Table 6.1 in the order of increasing hydrophobicity.

The choice of lipophilic ion depends solely on the degree of hydrophobicity of the analyte ion. For the separation of surface-inactive ions, a hydrophobic reagent is necessary; on the other hand, the separation of ions with long alkyl chains requires a strongly hydrophilic reagent. Another method for controlling retention is to vary the concentration of the ion-pair reagent. In the concentration range between 0 and 0.02 mol/L, analyte retention depends almost linearly on the concentration of the ion-pair reagent. Compounds listed in Table 6.1 are typically used in a concentration range between $5 \cdot 10^{-4}$ and 10^{-2} mol/L.

In analogy to reversed-phase chromatography, in ion-pair chromatography, organic solvents such as acetonitrile or methanol are added to the aqueous mobile phase as organic modifiers. Because the solvent molecules are adsorbed at the surface of the stationary phase, they are in a competing equilibrium with lipophilic ions, vying for active centers on the stationary phase available for adsorption. When replacing acetonitrile with methanol, the solvent content in the mobile phase must be enhanced to obtain comparable retention times. The selectivity difference obtained with methanol as the organic modifier is due to the capability of methanol to form hydrogen bonds. Compared with acetonitrile, the higher viscosity of methanol is a disadvantage because it leads to a larger pressure drop along the column.

Inorganic additives such as sodium carbonate are added to the mobile phase to control the retention of di- and multivalent anions. With the addition of sodium carbonate to the eluent, the retention decrease is much higher for divalent anions than for monovalent ones, which improves their peak shapes and avoids an unnecessarily high resolution between anions of different valency. The effect of sodium carbonate as an inorganic additive is mechanistically not completely clear. According to the dynamic ion-exchange model, it is to be assumed that carbonate ions are found in a competing equilibrium with solute ions for the ion-exchange groups that are adsorbed at the surface of the stationary phase. This is a plausible explanation for the strong effect of carbonate on the retention of di- and multivalent species.

When analyzing multivalent ions it is often necessary to change the pH value of the mobile phase by adding appropriate acids or bases. As the retention of multivalent ions increases with the degree of dissociation, the pH value affects retention by determining the degree of dissociation. In general, boric acid is especially suitable for lowering the pH value while an increase in the eluent pH is accomplished with sodium hydroxide. Both compounds only marginally contribute to the increase in background conductance because boric acid is only weakly dissociated and sodium hydroxide suppresses to water.

Table 6.1 Commonly used reagents for ion-pair chromatography on MPIC phases, in the order of increasing hydrophobicity.

Anion analysis		**Cation analysis**
Ammonium hydroxide	Hydrophobicity ↓	HCl, $HClO_4$
Tetramethylammonium hydroxide		Hexanesulfonic acid
Tetrapropylammonium hydroxide		Heptanesulfonic acid
Tetrabutylammonium hydroxide		Octanesulfonic acid

Unlike the eluent pH value, the column temperature is seldom relevant for optimizing the separation. Retention can be somewhat reduced by raising the column temperature. Generally speaking, the viscosity of the mobile phase will be reduced and the chromatographic efficiency will be increased when the column temperature is raised.

6.1 Examples of ion-pair chromatography separations

In the past, ion-pair chromatography has assumed a special position in the analysis of strongly polarizable anions, which today are separated on modern hyperbranched anion exchangers (see Section 3.1.3). Important polarizable anions are perchlorate and citrate, oxidic sulfur anions, and metal complexes. For the ion-pair chromatographic analysis of these compounds, TBAOH is typically used as the ion-pair reagent. In comparison to the separation of nonpolarizable anions, the acetonitrile content in the mobile phase must be increased. Remarkably, the trivalent citrate elutes prior to the monovalent perchlorate. The broader peak width of the citrate signal is caused by the higher valency of this compound and illustrates the limitations of ion-pair chromatography in the analysis of multivalent anions. In the field of inorganic sulfur compounds, ion-pair chromatography is applied to the analysis of dithionate [58], $S_2O_6^{2-}$, peroxodisulfate [10], $S_2O_8^{2-}$, and polythionates [59], $S_nO_6^{2-}$, as an alternative to anion-exchange chromatography. While dithionate and peroxodisulfate can be separated today on modern anion exchangers such as the Dionex IonPac AS32-Fast-4µm column using a hydroxide concentration gradient, the separation of the much more strongly retained polythionates still requires the application of ion-pair chromatography with TBAOH as the ion-pair reagent. Because these compounds are divalent anions, retention may be reduced with the addition of sodium carbonate. Figure 6.2 shows the separation of higher polythionates (n_S = 5–11), which was obtained under isocratic conditions by Steudel and Holdt [59]. Polythionates are sulfur chains bearing terminal sulfonate groups, so measuring the light absorption at 254 nm was used as the detection method.

Ion-pair chromatography is also suited for the analysis of metal complexes. To be separated chromatographically, the complexes must be thermodynamically *and* kinetically stable. This means that complex formation must be thermodynamically possible and irreversible. Metal-ETDA and metal-DTPA complexes exhibit similarly high stabilities. To separate the Gd-DTPA complex (Figure 6.3), which is of great relevance as a contrast agent in magnetic resonance imaging (MRI) [60], TBAOH was used as the ion-pair reagent [61]. Detection was carried out by measuring the electrical conductivity in combination with a suppressor system. More recent articles on the speciation of gadolinium complexes in a variety of biological and environmental samples describe the use of ICP–OES [62] and Orbitrap ESI–MS [63], as detection systems coupled to various modes of liquid chromatography. A comprehensive review about this subject has been published by Karst et al. [64].

In the field of polarizable cation analysis, ion-pair chromatography was an alternative method to cation-exchange chromatography for the separation of all types of amines. While short-chain aliphatic amines (C_1–C_3), alkanolamines, and some smaller aromatic amines are much more easily separated on modern cation exchangers, ion-pair chromatographic applications have been developed for the separation of structurally isomeric amines and quaternary ammonium compounds, as well as arylalkylamines, barbiturates, and alkaloids. With the introduction of mixed-mode liquid chromatography, however, ion-pair chromatography is barely used for these applications.

The same is true for the analysis of surface-active anions such as simple aromatic sulfonic acids, hydrotropes (toluene, cumene, and xylene sulfonates), alkane- and alkene sulfonates,

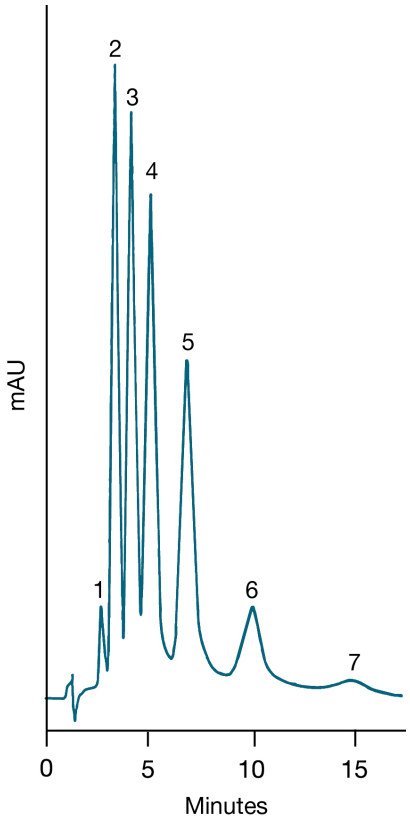


Figure 6.2 Ion-pair chromatographic separation of higher polythionates, $S_nO_6^{2-}$ (n_S = 5–11). Separator column: Dionex IonPac NS1, 10 µm; eluent: 2 mmol/L TBAOH + 1 mmol/L Na_2CO_3/MeCN (60:40 *v/v*); flow rate: 1 mL/min; detection: UV (254 nm); (reproduced from Ref. [35]. Copyright 1986, Elsevier).

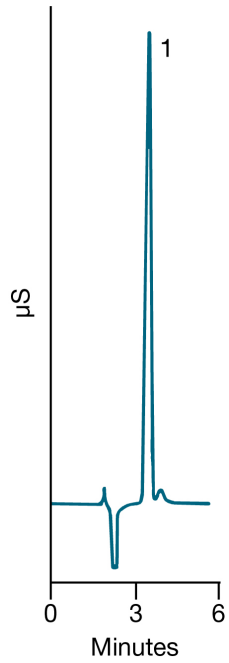


Figure 6.3 Separation of Gd-DTPA. Separator column: Dionex IonPac NS1, 10 µm; eluent: 2 mmol/L TBAOH + 1 mmol/L Na_2CO_3/MeCN (75:25 *v/v*); flow rate: 1 mL/min; detection: suppressed conductivity; injection volume: 50 µL; sample: 1:1000 diluted solution with Gd-DTPA (1).

fatty alcohol ether sulfates, alkylbenzene sulfonates, and α-sulfofatty acid methyl esters. Such compounds are relevant in the detergent and cleansing industries. Surface-active anions with aromatic backbones have long been separated by RPIPC and detected via their UV absorption. However, a chromatographic determination of the nonchromophoric compounds mentioned above was feasible only by conductivity detection. This situation has changed completely with the introduction of mixed-mode stationary phases and modern nonspecific detection methods such as charged aerosol detection (CAD) (see Section 6.2).

Today, surface-active anions such as anionic surfactants are separated on modern polar-embedded stationary phases such as the Thermo Scientific™ Acclaim™ Surfactant Plus column. This type of column belongs to the group of mixed-mode stationary phases, that is, the retention mechanism is based on both reversed-phase and anion-exchange interactions. The strongly hydrophilic aryl sulfonates can also be analyzed with this column. This is very problematic with conventional ODS (octadecyl silica) phases because such analyses require a highly aqueous mobile phase that often leads to the undesirable dewetting (desolvation) effect [65]. This phenomenon, which is commonly referred to as phase collapse, causes an unexpected loss of analyte retention, reduced efficiency, and changes in peak shape. Polar-embedded stationary phases incorporate a region of hydrophilic functional groups between the hydrophobic alkyl chain and the silica surface, which allows the bonded phase to remain wetted, even in purely aqueous mobile-phase condition. The novel surface chemistry of the Acclaim Surfactant Plus column provides adequate retention of xylene sulfonate and excellent resolution between its isomers.

Ethoxylation of fatty alcohols prior to their sulfonation leads to a class of compounds called fatty alcohol ether sulfates, which are contained in a variety of detergents and cleansing agents as active compounds.

$R–CH_2–O–(C_2H_4O)_n–SO_3Na$ $\quad R = C_{11}–C_{13}$, $n = 1–5$

Fatty alcohol ether sulfate

Depending on their degree of ethoxylation, fatty alcohol ether sulfates are extremely complex mixtures, for which the separation efficiency of a polymer phase is not sufficient. Good separations are obtained with ion-pair chromatography using silica-based, chemically bonded reversed phases. The chromatographic conditions must be adjusted accordingly. The free base ammonium hydroxide cannot be used as the ion-pair reagent because of the pH limitation of modified silica, so suppressor systems cannot be used for subsequent conductivity detection. Sodium acetate has proved to be a suitable ion-pair reagent for nonsuppressed conductivity detection. It exhibits a sufficiently low background conductance at the required concentration of $c = 1$ mmol/L. In combination with a solvent gradient, fatty alcohol ether sulfates can be separated according to their alkyl chain length and degree of ethoxylation. Figure 6.4 illustrates this with the analysis of Texapon® N25 (Henkel KGaA, Düsseldorf, Germany), a raw material that contains dodecyl ether sulfate and the corresponding tetradecyl homologues. Thus, the resulting peak pattern consists of two overlapping peak series of ethoxylated compounds whose concentration decreases as the EO content increases. However, even the high separation efficiency of the Thermo Scientific™ Hypersil™ 5 MOS stationary phase is insufficient to resolve all the compounds in this mixture. Coelution is observed for tetradecyl sulfate, indicated as C_{14}, and the higher ethoxylated dodecyl compound. Also, only a small retention difference exists between an alkyl ether sulfate with just one EO group and a pure alkyl sulfate with equal carbon chain length. This means that the retention increase, which usually goes along with chain elongation by two methylene groups, is compensated by solvation because another polar center is introduced around the oxygen atom in the EO group. The retention-increasing effect of an EO group is noticed only at higher degrees of ethoxylation. For comparison, Figure 6.5 shows

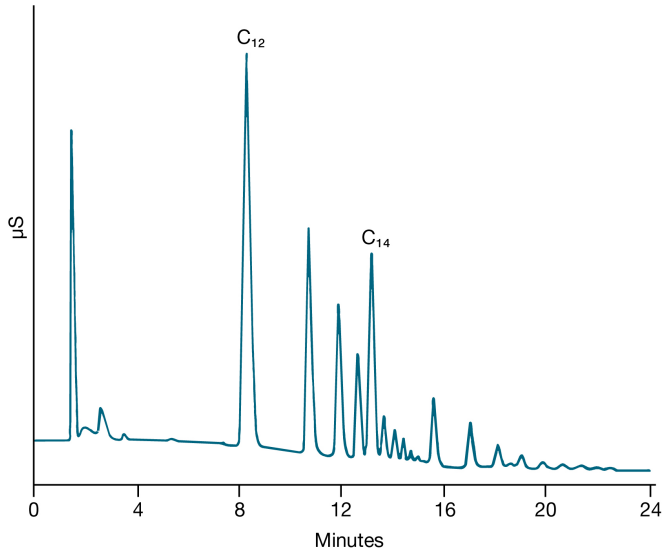


Figure 6.4 Separation of a lauryl ether sulfate (Texapon N 25). Separator column: Hypersil 5 MOS; eluent: (A) 1 mmol/L NaOAc/MeCN (70:30 *v/v*), (B) 1 mmol/L NaOAc/MeCN (60:40 *v/v*); gradient: linear, 100% A to 100% B in 10 min; flow rate: 1 mL/min; detection: nonsuppressed conductivity; injection volume: 50 µL; sample: 500 mg/L of the raw material.

the respective separation profiles of two lauryl ether sulfates that differ in the degree of ethoxylation ($n = 2$ and $n = 30$) on an Acclaim Surfactant Plus column using charged aerosol detection. In contrast to the Hypersil 5 MOS column, the elution order of the ethoxylated ether sulfates seems to be reversed, that is, retention increases with decreasing degree of ethoxylation.

In the field of surface-active cations, ion-pair chromatography is predominantly applied to the analysis of quaternary ammonium compounds, pyridine, pyrrolidine, and piperidine

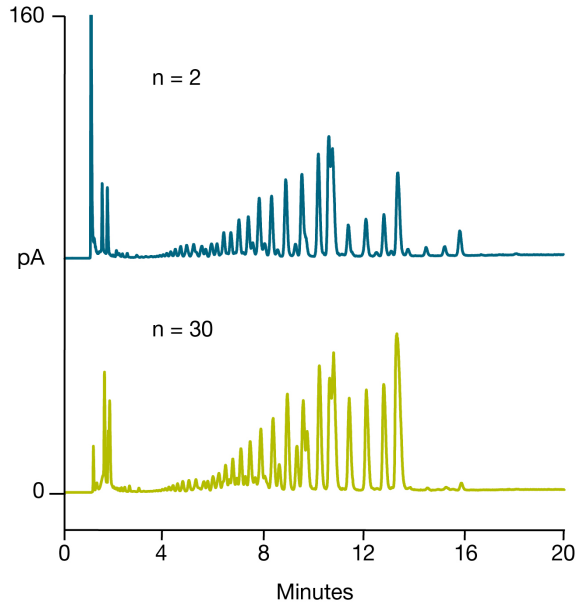


Figure 6.5 Separation of lauryl ether sulfates on a polar-embedded stationary phase. Separator column: Acclaim Surfactant Plus, 3 µm; column dimensions: 150 mm × 3 mm i.d.; column temperature: 30 °C; eluent: (A) 0.1 mol/L NaOAc, pH 5, (B) MeCN; gradient: linear, 45% B to 75% B in 15 min; flow rate: 0.6 mL/min; detection: Corona *ultra* (gain: 100 pA, filter: med, neb. temp.: 20 °C); injection volume: 2 µL; sample: 10 mg/mL each of the raw materials.

quaternisates, and for sulfonium, phosphonium, ammonium, and hydrazinium salts. In principle, polar-embedded silica columns may also be applied in all these separations. The high chromatographic efficiency of the Acclaim Surfactant Plus column in combination with an acetonitrile gradient allows the analysis of a variety of cationic surfactants in the same chromatographic run within a short time. Under the conditions used in Figure 6.6, even the very hydrophobic dehydrogenated tallow dimethyl ammonium chloride ($DHTDMA^+$) elutes in less than 15 min. This cationic surfactant contains a mixture of saturated C_{14}–C_{18} alkyl chains, with C_{16}–C_{18} being the most abundant.

6.2
Applications of the ion-suppression technique

Another application for neutral, nonpolar stationary phases based on silica or organic polymers is the separation of weak acids or bases in their molecular form. Their dissociation is suppressed at the appropriate pH value. The solute interactions with the stationary phase are determined solely by their adsorption and distribution behavior. The term *ion-suppression mode* has been coined for this technique. In some areas of analysis, it provides an alternative to ion-exchange chromatography.

One of the best known and most widely used applications of this technique is the separation of phenols, which are eluted on a nonpolar phase using acetonitrile/water or methanol/water mixtures. A phosphate buffer is added to the solution to suppress the small dissociation of

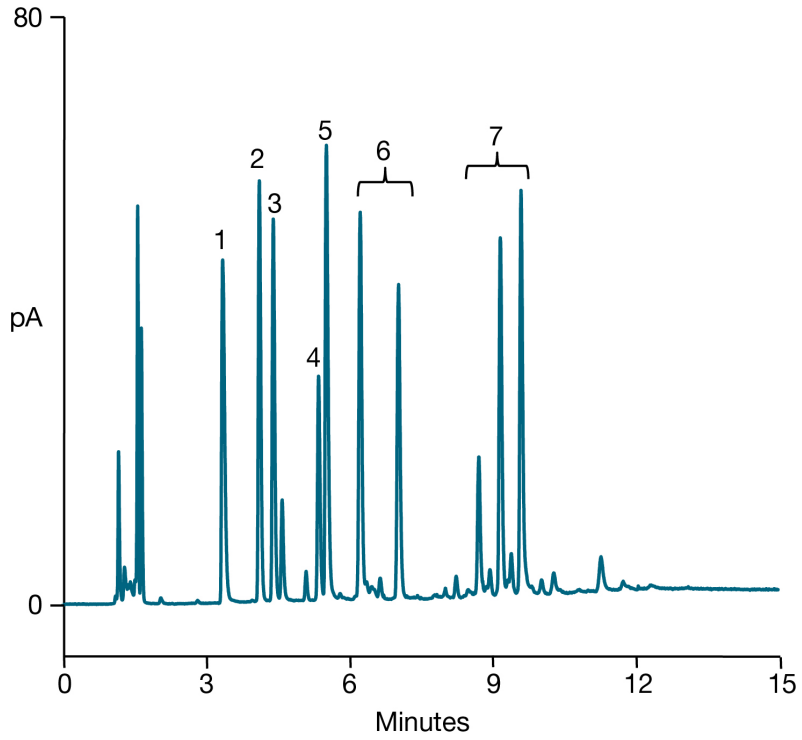


Figure 6.6 Separation of cationic surfactants on a polar-embedded stationary phase. Separator column: Acclaim Surfactant Plus, 3 µm; column dimensions: 150 mm × 3 mm i.d.; column temperature: 30 °C; eluent: (A) 0.1 mol/L NH_4OAc, pH 5, (B) MeCN; gradient: 35% B to 85% B in 8 min; flow rate: 0.6 mL/min; detection: Corona *ultra* (gain: 100 pA, filter: med, neb. temp.: 20 °C); injection volume: 5 µL; peaks: 200–400 mg/L each of laurylpyridinium chloride (1), lauryldimethylbenzylammonium chloride (2), octylphenoxyethoxyethyl-dimethyl-benzyl-ammonium chloride (3), cetyltrimethyl-ammonium chloride (4), cetylpyridinium chloride (5), diethyl-heptadecyl imizolinium chloride (6), and dihydrogenated tallow dimethylammonium chloride (7).

phenols into phenolate anions. Potassium dihydrogenphosphate at concentrations of about $c = 0.01$ mol/L are typically employed. Figure 6.7 shows a separation of various mono-, di-, and trivalent phenols on an organic polymer.

Another application of the ion-suppression technique is the separation of long-chain fatty acids described by Slingsby [66]. While short-chain monocarboxylic acids up to valeric acid may be separated via ion-exclusion chromatography (see Section 5.2), long-chain fatty acids exhibit unacceptably long retention times under these conditions. In a protonated state, they can easily be separated on a nonpolar stationary phase with a solvent/water-mixture. Figure 6.8 illustrates a respective separation of various fatty acids (butyric acid to palmitic acid); the separation was achieved on an organic polymer by applying a gradient technique. To ensure sufficient solubility of long-chain fatty acids in the mobile phase, a solvent mixture composed of acetonitrile *and* methanol was used. Small amounts of hydrochloric acid in the eluent served to suppress the dissociation of the compounds. If chemically modified silica (e.g., ODS) is used instead of an organic polymer, compensation for the enhanced solute interactions with such a material must be made. This is achieved both by raising the column temperature to 40 °C and by adding 2-propanol or THF to the mobile phase. The detection of long-chain fatty acids depends on the kind of carbon skeleton. If conjugated double bonds are present, as in the case of sorbic acid, a sensitive UV detection is feasible. On the other hand, if the carbon chain is purely paraffinic, conductometric detection must be utilized because aliphatic fatty acids do not exhibit any appreciable absorption even at low wavelengths around 200 nm. A prerequisite for conductivity detection is the transformation of fatty acids that are separated as molecular compounds into their dissociated form. For this, the separator column effluent is passed through a membrane-based suppressor before entering the conductivity cell. The fatty acid oxonium ions are exchanged for potassium ions. The solutes enter the detector cell as fully dissociated potassium salts and may be detected conductometrically. In addition, the background

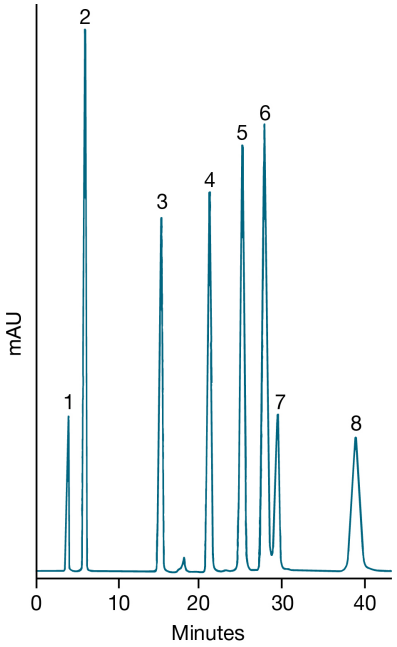


Figure 6.7 Separation of various mono- and polyvalent phenols. Separator column: Dionex IonPac NS1, 10 µm; eluent: (A) 10 mmol/L KH_2PO_4 (pH 4)/MeCN (90:10 *v/v*), (B) 10 mmol/L KH_2PO_4 (pH 4)/MeCN (20:80 *v/v*); gradient: linear, 15–55% B in 20 min; flow rate: 1 mL/min; detection: UV (280 nm); injection volume: 50 µL; peaks: 100 mg/L each of pyrogallic acid (1), resorcinol (2), phenol (3), *o*-cresol (4), 2,4-dimethylphenol (5), *β*-naphthol (6), 2,4-dichloro-3-nitrophenol (7), and thymol (8).

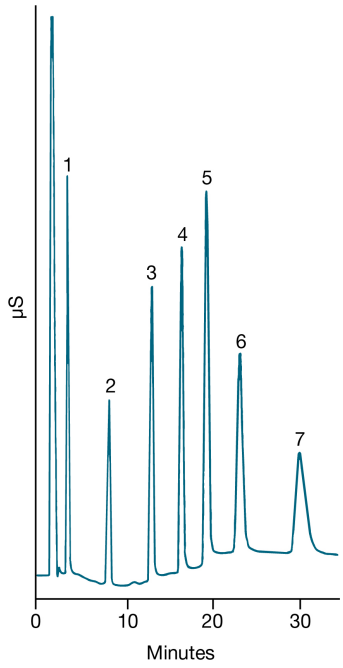


Figure 6.8 Separation of long-chain fatty acids utilizing an ion-suppression technique. Separator column: Dionex IonPac NS1, 10 µm; eluent: (A) $3 \cdot 10^{-5}$ mol/L HCl/MeCN/MeOH (70:24:6 *v/v/v*), (B) $3 \cdot 10^{-5}$ mol/L HCl/MeCN/MeOH (16:60:24 *v/v/v*); gradient: linear, 100% A to 100% B in 15 min; flow rate: 1 mL/min; detection: suppressed conductivity; injection volume: 50 µL; peaks: 100 mg/L butyric acid (1) and caproic acid (2), 200 mg/L caprylic acid (3) and capric acid (4), 300 mg/L lauric acid (5) and myristic acid (6), and 400 mg/L palmitic acid (7).

conductivity of the hydrochloric acid eluent is also reduced by converting it into the potassium salt. This is advantageous because it significantly improves the signal-to-noise ratio for the analyte compound. According to Slingsby [66], detection limits for this method are between 50 µg/L for butyric acid and 50 mg/L for stearic acid.

6.3 Applications of ion chromatography on mixed-mode stationary phases

The term *Mixed-Mode Chromatography, or multimodal chromatography,* summarizes the separation techniques that utilize more than one form of interaction between the stationary phase and analytes for their separation. Before mixed-mode chromatography was considered as a chromatographic approach, secondary interactions were generally believed to be the main cause for peak tailing. However, as early as in the mid-1980s, it was discovered that secondary interactions can be applied for improving separation power. In 1986, Regnier et al. were the first to synthesize a stationary phase that showed characteristics of anion-exchange and hydrophobic interactions for separating proteins [67]. Reversed-phase/ion-exchange mixed-mode liquid chromatography has been known for more than 20 years [68]. Columns supporting this kind of mixed-mode chromatography separate analytes by both hydrophobicity and charge differences, and provide a number of benefits, including adequate retention of ionic and ionizable analytes and adjustable selectivity [69]. This, in turn, results in greater flexibility for retention and selectivity tuning during method development and an expanded application range. Today, reversed-phase/ion-exchange mixed-mode columns are predominantly used in the pharmaceutical and food industries.

Depending on the combination of functional groups, mixed-mode columns can be classified into bimodal (RP/anion exchange and RP/cation exchange) and trimodal (RP/anion exchange/ cation exchange) materials. In contrast to blending two or more different types of stationary phases in a single column, chemical mixed-mode columns contain only one type of packing material with two or more functionalities. Mixed-mode chromatography should not be confused with multidimensional chromatography in which the sample is separated into its components on two or several stationary phases of different properties, using valve-switching or column-switching techniques, a change in the type of eluent, or even a change in eluent flow direction. In the following, the term *mixed-mode liquid chromatography* is used to describe separations in which ion-exchange and reversed-phase interactions occur at the same stationary phase and thus contribute to the separation of ionic, ionizable, and/or strongly polar species [70].

Polymer-based mixed-mode columns Thermo Scientific™ OmniPac™ polymer-based mixed-mode columns allow separations based on ion-exchange, ion-pair, reversed-phase, and ion-suppression interactions, depending on the type of eluent. Any combinations of these interactions, at the same time or in succession, can also be employed to separate analyte species.

The principle structure of these mixed-mode phases is very similar to that of a nanobead-agglomerated ion exchanger. In contrast to classical nanobead-agglomerated ion exchangers (see Sections 3.1.1 and 4.1.1), in which the nanobeads are electrostatically agglomerated on the surface-functionalized substrate, the highly cross-linked and therefore solvent-compatible ethylvinylbenzene/divinylbenzene substrate of mixed-mode phases has a particle diameter of 8.5 µm and is coated with a second polymer, containing carboxyl groups. The carboxyl groups, in turn, serve as anchor groups for the oppositely charged nanobeads. Even though electrostatic attractive forces initiate the agglomeration procedure, the true bonding of the nanobeads is based on pure adsorption. In contrast to traditional nanobead-agglomerated anion exchangers, OmniPac anion exchangers do not exhibit any cation-exchange capacity.

The various columns of the OmniPac series differ in the porosity of the substrate material. When nanobeads are bonded to the outer surface of a microporous, highly cross-linked substrate resin particle (specific surface area < 1 m^2/g), a pellicular packing material is produced that exclusively allows ion-exchange interactions in a solvent compatible matrix. Separators of that kind are available as the OmniPac PAX-100 anion exchanger and the OmniPac PCX-100 cation exchanger. When a macroporous substrate resin particle is used, the resulting packing material allows ion-exchange interactions at the substrate surface as well as adsorption processes in the interior of the core material due to the relatively high specific surface area of about 300 m^2/g (OmniPac PAX-500 column and OmniPac PCX-500 column). Because the nanobeads with a diameter of 60 nm are significantly larger than the pores of the substrate material (pore size: ~6 nm), they cannot penetrate the interior of the stationary phase. Therefore, only the substrate surface is coated with the nanobead colloid. The structural and technical properties of the OmniPac columns are summarized in Table 6.2.

Due to their ion-exchange and reversed-phase properties, polymer-based mixed-mode phases offer high flexibility for solving complex separation problems. In the past, differentiation between ion-exchange and reversed-phase chromatography was forcibly given because the different packing materials did not exhibit similar properties. However, when using mixed-mode phases, ion-exchange, ion-pair, and pure reversed-phase interactions can contribute to the separation process at the same time, complementing each other in an excellent way.

The solvent compatibility of OmniPac columns allows organic solvents to be added to the mobile phase in order to modify the selectivity. Organic solvents affect the degree of hydration of solute ions as well as ion-exchange groups. In general, retention times decrease with an

Table 6.2 Structural and technical properties of the OmniPac separator columns.

Separator	PAX-100	PAX-500	PCX-100	PCX-500
Substrate				
Porosity	Microporous	Macroporous	Microporous	Macroporous
Pore size (Å)	–	60	–	60
Particle diameter (µm)	8.5	8.5	8.5	8.5
Degree of crosslinking (% DVB)	55	55	55	55
Spec. surface area (m^2/g)	<1	300	<1	300
Nanobeads				
Particle diameter (nm)	60	60	200	200
Degree of crosslinking (% DVB)	4	4	5	5
Capacity (µequiv/column)	40	40	120	120
Functionality	Quaternary alkanolamine	Quaternary alkanolamine	Sulfonate groups	Sulfonate groups

increase in the amount of organic solvents in the mobile phase. Because a similar effect is achieved by decreasing the degree of cross-linking of the nanobeads, the addition of organic solvents to the mobile phase is synonymous with a change of the *effective* degree of cross-linking of the nanobeads. This effect can be utilized for separating aliphatic and aromatic carboxylic acids on an OmniPac PAX-100 column utilizing a sodium hydroxide gradient in combination with suppressed conductivity detection. If the analyte components carry chromophores that allow UV detection, a small amount of sodium hydroxide in the mobile phase is sufficient for complete dissociation of the solutes. Solute elution can then be carried out with a salt gradient.

The versatility of mixed-mode columns can be applied for the simultaneous separation of molecular and ionic organic compounds. An impressive example is shown in Figure 6.9. During the first 10 min, neutral species, free of any interferences by ionic components, are eluted with an acetonitrile/water mixture. After all neutral compounds are eluted, the organic solvent content in the mobile phase is dropped and ionic components are eluted with a salt gradient. This mode of operation can, of course, be reversed. Figuratively, one can think of two-dimensional chromatography at one and the same stationary phase. An interesting practical application of this technique is on-column sample preparation as reported by Slingsby and Rey [65] with the separation of acebutolol in rat urine on an OmniPac PAX-500 column. As a prerequisite, matrix components and analytes must differ in their hydrophobicity and their degree of dissociation. If this is the case, the gradient is programmed in such a way that the interfering matrix component elutes prior to the analyte. In the above-mentioned example, the matrix components of urine that are mostly of ionic nature are eluted with a high carbonate concentration after injection of the filtrated sample. Thereafter, the carbonate concentration drops drastically, followed by the elution of the drug component with an organic solvent gradient.

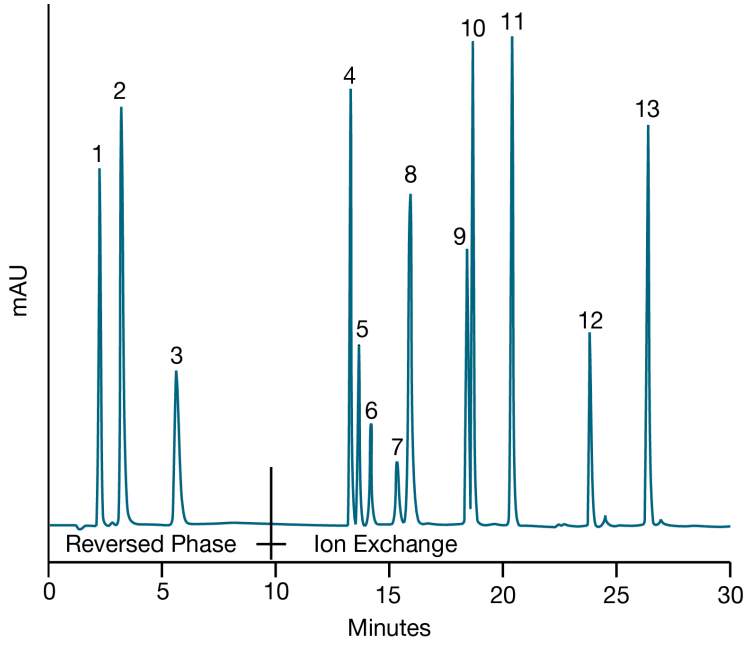
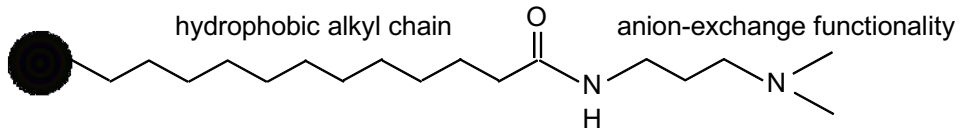


Figure 6.9 Simultaneous analysis of molecular and ionic organic compounds on an OmniPac PAX-500 column. Eluent: NaOH/NaCl/MeCN; gradient: MeCN/water (80:20 *v/v*) for 10 min isocratically, then linearly from 0.2 mmol/L NaOH + 50 mmol/L NaCl/MeCN (80:20 *v/v*) to 1.6 mmol/L NaOH + 400 mmol/L NaCl/MeCN (80:20 v/v) in 10 min; flow rate: 1 mL/min; detection: UV (254 nm); peaks: (1) benzyl alcohol, (2) diethyltoluolamide, (3) benzene, (4) benzoate, (5) benzenesulfonate, (6) toluene-sulfonate, (7) *p*-chlorobenzenesulfonate, (8) *p*-bromo-benzenesulfonate, (9) phthalate, (10) terephthalate, (11) *p*-hydroxybenzenesulfonate, (12) 1,3,5-benzene-tricsulfonatearboxylate, and (13) 1,2,4,5-benzene-tetracarboxylate (pyromellitate).

Polymer-based mixed-mode phases with anion-exchange properties are suitable for the analysis of a number of ionic and ionizable organic species, including the compound classes of barbiturates, anti-inflammatory drugs, acidic azo dyes, and others. Among organic cations, polymer-based mixed-mode phases with cation-exchange properties are used for the analysis of ephedrins, alkaloids, water-soluble vitamins, sulfonamides, cephalosporins, catecholamines, diuretics, xanthines, and a number of basic dyes.

Silica-based mixed-mode columns Depending on the combination of functional groups, silica-based mixed-mode columns are grouped into bimodal and trimodal materials. More than ten years ago, Thermo Fisher Scientific commercialized two mixed-mode columns that are based on an alkyl chain with terminal ion-exchange functional groups: the Acclaim Mixed-Mode WAX-1 column [71] and the Acclaim Mixed-Mode WCX-1 column [72].

The Acclaim Mixed-Mode WAX-1 column combines both hydrophobic and anion-exchange characteristics, which facilitates selectivity adjustment by changing mobile-phase ionic strength, pH, or organic modifier, either independently or concurrently.

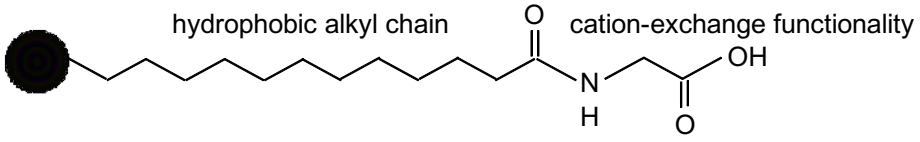


Acclaim Mixed-Mode WAX-1

The Acclaim Mixed-Mode WAX-1 column provides ideal selectivity for weakly dissociated anions such as hydrophilic monocarboxylic acids. Although these compounds can sometimes be separated on a reversed-phase column in ion-suppression mode at low pH, dewetting often causes a sudden irreversible loss of retention in highly aqueous conditions. Even aqueous-compatible, polar-embedded stationary phases fail to separate hydrophilic organic acids, primarily because hydrophobic interactions alone are inadequate to differentiate molecules with similar hydrophobicities. Likewise, it is very difficult if not impossible to separate these organic acids by anion-exchange chromatography, especially in samples with complex matrices. Combining RP and anion-exchange characteristics, the Acclaim Mixed-Mode WAX-1 column provides adequate retention for hydrophilic organic acids. This is especially true for the first five monocarboxylic acids shown in Figure 6.10. The baseline-resolved separation of ascorbic acid and isoascorbic acid under isocratic conditions is also remarkable.

Reversed-phase columns are commonly used to analyze active pharmaceutical ingredients (APIs). However, they perform very poorly in determining counter ions of basic drugs. The anionic counter ions are highly hydrophilic and elute close to or in the void. In addition, hydrophilic basic drugs might interfere with the counter ions, while hydrophobic basic drugs result in long analysis times. The adjustable selectivity of the Acclaim Mixed-Mode WAX-1 column overcomes these issues. As illustrated with the separation of trimipramine maleate in Figure 6.11, the basic drug elutes close to the void when using 40% acetonitrile and 24 mmol/L phosphate buffer as the eluent, while the acid counter ion exhibits a reasonable retention time that facilitates accurate and easy quantification (Figure 6.11a). By decreasing the organic solvent content in the mobile phase, both the API and counter ion can be determined (Figure 6.11b). It is also possible to elute the acid before the basic drug by increasing the ionic strength in the mobile phase (Figure 6.11c).

Analogously, the Acclaim Mixed-Mode WCX-1 column combines both hydrophobic and cation-exchange characteristics. The packing material consists of an alkyl chain with a terminal carboxyl group, so that its selectivity can be adjusted though changes in mobile-phase ionic strength, pH, or organic modifier, either independently or concurrently.



Acclaim Mixed - Mode WCX -1

The Acclaim Mixed-Mode WCX-1 column is ideally suited for the analysis of basic compounds, which are important in a variety of industrial applications, including pharmaceutical, chemical, food, beverages, and consumer products. The analysis of these compounds is often challenging when using silica-based reversed-phase columns. At neutral pH, basic compounds exhibit peak tailing because of the secondary interactions between the analytes and unreacted silanol groups on the stationary phase surface. Although this difficulty is minimized with polar-embedded stationary phases that separate a wide variety of basic compounds with optimal peak shape, strongly hydrophilic basic compounds are not adequately retained without the addition of ion-pair reagents to the mobile phase. The Acclaim Mixed-Mode WCX-1 column not only retains those compounds, ranging from very hydrophilic ones such as catecholamines to strongly hydrophobic ones such as antidepressant drugs, but also elutes them with symmetrical peak shapes and high chromatographic efficiencies. Quaternary ammonium compounds, used as bactericides and corrosion inhibitors, are also best analyzed with the Acclaim Mixed-Mode WCX-1 column as shown in Figure 6.12. In this case, the phosphate buffer in the mobile phase is changed for ammonium acetate, which is volatile

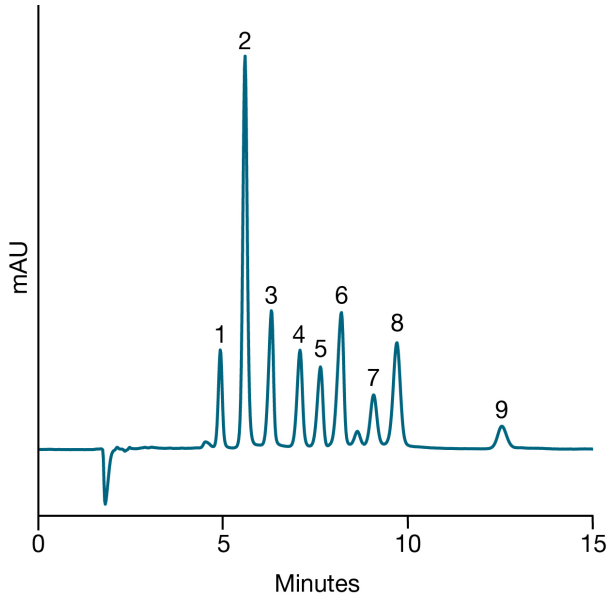


Figure 6.10 Separation of various monocarboxylic acids of similar hydrophobicities on an Acclaim Mixed-Mode WAX-1 column, 5 µm. Column dimensions: 150 mm × 4.6 mm i.d.; column temperature: 30 °C; eluent: 25 mmol/L phosphate buffer, pH 6; flow rate: 0.8 mL/min; detection: UV (210 nm); injection volume: 10 µL; peaks: (1) quinic acid, (2) shikimic acid, (3) glycolic acid, (4) lactic acid, (5), acetic acid, (6) formic acid, (7) ascorbic acid (Vitamin C), (8) isoascorbic acid, and (9) propionic acid.

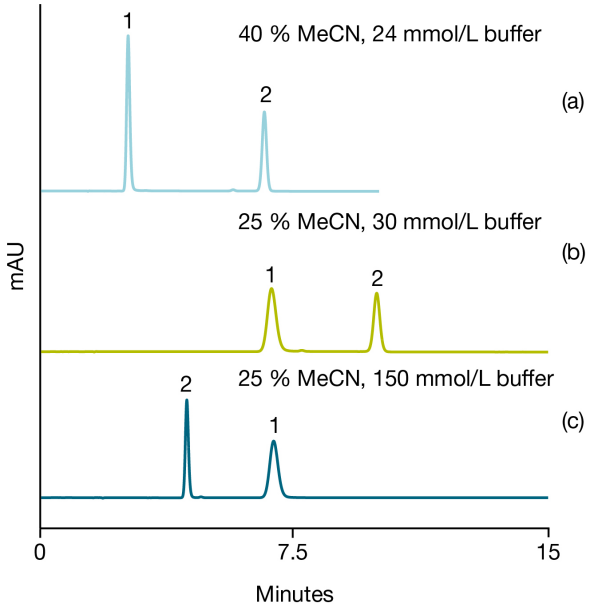


Figure 6.11 Selectivity adjustment for the separation of a basic drug and its counter ion on an Acclaim Mixed-Mode WAX-1 column, 5 µm. Column dimensions: 150 mm × 4.6 mm i.d.; column temperature: 30 °C; eluent: MeCN/phosphate buffer, pH 6; flow rate: 1 mL/min; detection: UV (220 nm); injection volume: 2.5 µL; sample: 0.5 mg/mL of trimipramine maleate; peaks: (1) trimipramine and (2) maleate.

enough to allow the use of nebulization detection techniques, such as evaporative light scattering (ELS) or charged aerosol detection (CAD) because both quaternary ammonium and phosphonium compounds are nonchromophoric.

Compared with RP/IEX bimodal columns, trimodal columns featuring reversed-phase, anion-exchange, and cation-exchange interactions, and have potential for an even broader application range as anionic, cationic, and neutral analytes could be retained and separated within a single analysis. This is especially important for the pharmaceutical industry. Pharmaceutical drugs are often developed in salt forms because of desirable physicochemical properties, such as solubility, dissolution rate, stability, and impurity profiles. A variety of inorganic and organic acids and bases have been used to form counter ions. Identification and quantification of counter ions and residual salts are critical to establish stoichiometry and to determine drug purity and mass balance. Therefore, ion analysis methods play a critical role in drug discovery, development, and manufacturing. Traditionally, APIs and counter ions are analyzed in two separate assays using different analytical techniques. For the separation and detection of counter ions a variety of chromatographic approaches are used. Ion chromatography with suppressed conductivity detection, using ion-exchange columns, is by far the most commonly used technique. On the other hand, APIs are usually analyzed by reversed-phase chromatography. Mixed-mode columns have become increasingly popular in the simultaneous separation of APIs and their counter ions. The introduction of the Thermo Scientific™ Acclaim™ Trinity P1 column has revolutionized this approach [73]. It is based on nanopolymer silica hybrid technology (NSH). As schematically depicted in Figure 6.13, the inner pore area of the 3 µm silica particles is covalently modified with an organic layer that provides both reversed-phase and anion-exchange properties. The silica particles are then coated with strong acid polymeric nanobeads (d_p ~100 nm). This arrangement ensures distinctive spatial separation of the anion and cation-exchange sites, which allows both retention mechanisms to function simultaneously and to be controlled independently. Like with all bimodal Acclaim columns

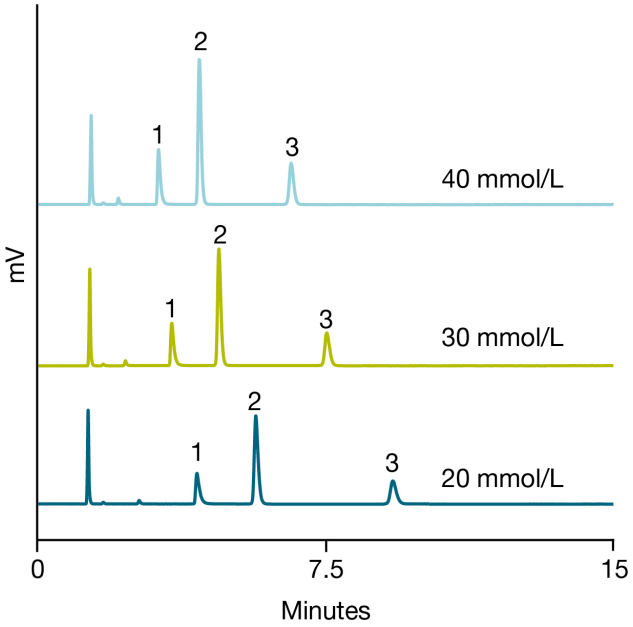


Figure 6.12 Separation of quaternary ammonium compounds on an Acclaim Mixed-Mode WCX-1 column, 5 µm. Column dimensions: 150 mm × 4.6 mm i.d.; column temperature: 30 °C; eluent: MeCN/ammonium acetate, pH 5.2 (50:50 *v/v*); flow rate: 1 mL/min; detection: ELS; injection volume: 5 µL; peaks: 300 mg/L each of tetrapropylammonium chloride (1), tetrabutylammonium chloride (2), and tetrapentylammonium chloride (3).

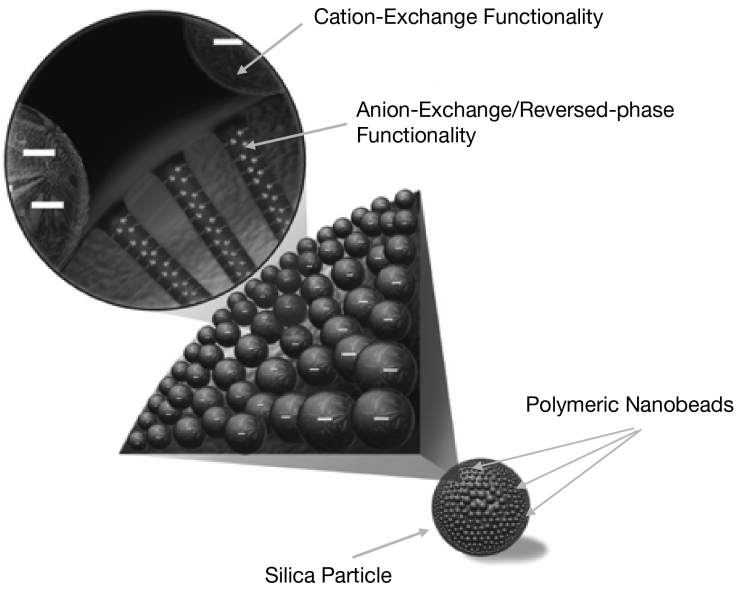


Figure 6.13 Schematic illustration of the Acclaim Trinity P1 packing material based on NSH technology.

mentioned above, selectivity can be optimized by adjusting the mobile-phase ionic strength, pH, and organic solvent content, independently or concurrently. The Acclaim Trinity P1 column provides optimum selectivity for various pharmaceutical counter ions [74] and drug substances using volatile buffers such as ammonium acetate and organic solvents as eluents. This type of mobile phase is compatible with a number of detection methods, including Corona™ charged aerosol detection (CAD), evaporative light scattering detection (ELSD), UV, and mass spectrometry (MS). Figure 6.14 illustrates the selectivity of the Acclaim Trinity P1 column for common anionic and cationic counter ions in an acetonitrile/ammonium acetate mobile phase system. Baseline separation of five anions and five cations is achieved within a single run on a 50 mm long column in less than 15 min. The column selectivity is designed such that cations elute before anions or elute in separate time frames.

In pharmaceutical development, the determination of APIs and counter ions are two important assays. Due to the charge and/or hydrophobicity differences, APIs and counter ions are usually analyzed by different chromatographic techniques that require different separator columns and/or detection methods. For example, reversed-phase liquid chromatography is most commonly used for analyzing APIs with intermediate to higher hydrophobicity, but it fails to provide adequate retention for hydrophilic counter ions. In contrast, ion chromatography provides a selective and highly sensitive solution for the analysis of counter ions, but lipophilic APIs cannot be eluted from the ion exchanger with aqueous mobile phases. Therefore, they must be removed from the sample by off-line or in-line SPE prior to the ion-exchange separation of counter ions. Alternatively, significant amounts of organic solvents must be added to the aqueous mobile phase to solubilize and finally elute the lipophilic APIs from the ion exchanger to avoid the loss of ion-exchange capacity over time.

The Acclaim Trinity P1 column along with a Thermo Scientific™ Dionex™ Corona™ *ultra* charged aerosol detection enables simultaneous analysis of hydrophilic and hydrophobic APIs and counter ions over four orders of magnitude. As a typical example of a hydrophilic acidic drug and its counter ion, Figure 6.15 shows the separation of the potassium salt of penicillin G, which can be achieved with baseline resolution and excellent peak shapes on a 50 mm Acclaim Trinity P1 column under isocratic conditions in less than 3 min.

The Acclaim Trinity Q1 column, a variation of the Acclaim Trinity P1 column, is also based on nanopolymer silica hybrid technology (NSH) as described above, but the agglomerated

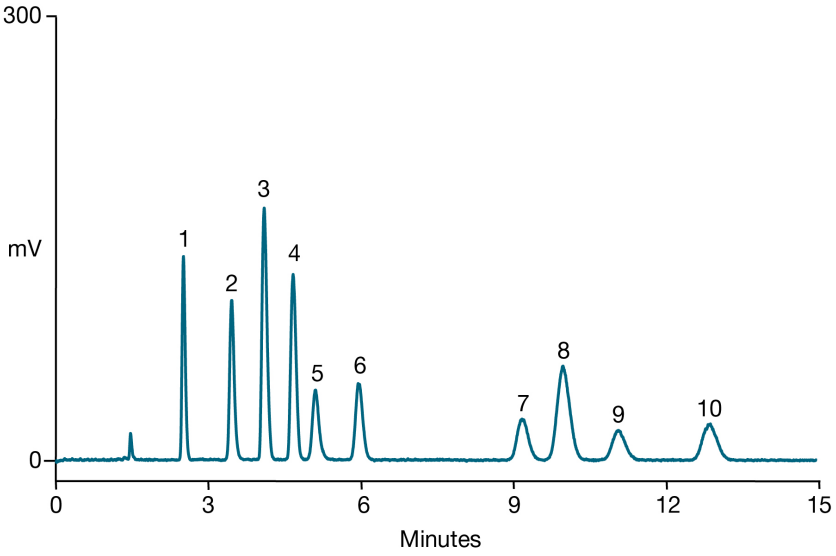


Figure 6.14 Simultaneous isocratic separation of pharmaceutical counter ions on an Acclaim Trinity P1 column, 3 µm. Column dimensions: 50 mm × 3 mm i.d.; column temperature: 30 °C; eluent: MeCN/20 mmol/L ammonium acetate, pH 5 (60:40 *v/v*); flow rate: 0.5 mL/min; detection: Corona *ultra* (neb. temp: 30 °C, gain: 100 pA, filter: medium); injection volume: 2 µL; peaks: 50–100 mg/L choline (1), tromethamine (2), sodium (3), potassium (4), meglumine (5), mesylate (6), nitrate (7), chloride (8), bromide (9), and iodide (10).

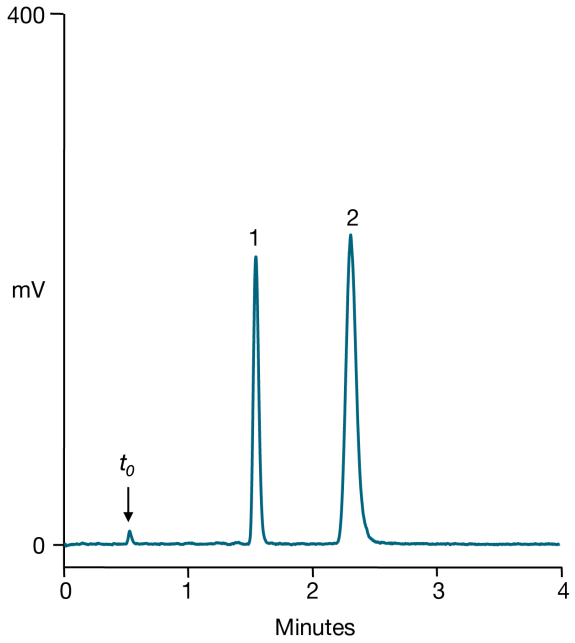


Figure 6.15 Simultaneous analysis of a hydrophilic API (penicillin G) and its counter ion (potassium) on an Acclaim Trinity P1 column, 3 µm. Column dimensions: 50 mm × 3 mm i.d.; column temperature: 30 °C; eluent: MeCN/20 mmol/L ammonium acetate, pH 5.2 (60:40 *v/v*); flow rate: 0.6 mL/min; detection: Corona *ultra* (neb. temp.: 30 °C, gain: 100 pA, filter: medium); injection volume: 2 µL; sample: 200 mg/L penicillin G, potassium salt in mobile phase; peaks: penicillin G (1) and potassium (2).

polymeric nanobeads are carboxylated. The 3 µm packing material features a specific surface area of 100 m²/g and a pore size of 300 Å. The Acclaim Trinity Q1 column is available in two different formats (50 mm × 3 mm i.d. and 50 mm × 2.1 mm i.d.) and has been specifically developed for high-resolution and high-throughput trace analysis of the herbicides paraquat and diquat by LC–MS/MS. Due to their high hydrophilicity, paraquat and diquat are hardly retained on conventional reversed-phase columns. Thus, ion-pairing reagents are typically added to the mobile phase to improve chromatographic retention. However, ion-pairing reagents are not considered to be MS-friendly due to ionization suppression effects. In addition, the reported methods were not suitable for high sample throughput.

The Acclaim Trinity P1 column has been shown to be an ideal tool for the simultaneous determination of pharmaceutical drugs and their respective counter ions. However, this column does not elute multivalent anions and cations within a reasonable time frame. Like with conventional sulfonated cation exchangers, divalent cations such as alkaline-earth metals are very strongly retained due to the sulfonated nanobeads agglomerated onto the silica substrate. It is also unsuitable for very hydrophilic neutral analytes. To maximize selectivity coverage, the Acclaim Trinity P2 column has been developed for mono- and multivalent pharmaceutical counter ion separations. While the Acclaim Trinity P1 column is a reversed-phase/weak anion-exchange/strong cation-exchange trimodal phase, the Acclaim Trinity P2 column is a trimodal phase based on HILIC/weak cation exchange/strong anion exchange and thus provides a complementary selectivity to the Acclaim Trinity P1 column. In contrast to the Acclaim Trinity P1 column, the polymeric nanobeads of the Acclaim Trinity P2 column are functionalized with quaternary ammonium groups for anion-exchange interactions, while the inner pore area of the silica substrate is modified with a covalently bonded hydrophilic layer that provides HILIC and cation-exchange retention.

7
Detection methods in ion chromatography

Detection methods applied in ion chromatography are divided into electrochemical, spectrometric, nebulization, and others. Conductometric and amperometric detection are electrochemical methods, while spectrometric methods include UV/Vis and fluorescence detection. Nebulization methods include evaporative light scattering (ELS) and charged aerosol detection (CAD). The various application forms of these detection methods are described in this chapter.

In most cases, the choice of a suitable detection mode depends on the separation method and the corresponding eluents. If detection is to be carried out by direct measurement of a physical property of the solute ion (e.g., UV absorption), the solute ion must differ substantially in this property from eluent ions that are present in much higher concentration. However, eluent and solute ions often exhibit similar properties, so direct detection is only feasible where selective detection of a limited number of solute ions is desirable.

A much broader range of applications employ detection methods that measure changes in a certain physical property of the eluent (e.g., conductance) that are caused by the elution of the solute ion. As a prerequisite, the values of this property for eluent and solute ions must differ. Most of the detection methods applied in ion chromatography are based on this technique. In the following discussion, a further subdivision into direct and indirect methods is made. Direct detection methods are those in which eluent ions exhibit a much smaller value than solute ions for the property to be measured. On the other hand, detection methods are called indirect if eluent ions exhibit a much higher value for the property to be measured than do solute ions.

7.1
Electrochemical detection methods

7.1.1
Conductivity detection

As a universal method for the detection of ionic species, conductometric detection has the highest significance in ion chromatography. For the sensitive and selective detection of ions via their electrical conductivity, a suppressor system is required. Its function is to chemically reduce the background conductivity of the electrolyte used as eluent before it enters the conductivity cell and to convert the analyte ions into a more strongly conductive form. Therefore, the suppressor system may be regarded as a part of the detection system. Higher sensitivity is the primary advantage of the suppressor technique as compared to nonsuppressed conductivity detection. In addition, the specificity of the method is also increased because chemical modification of eluent and sample in the suppressor system turns the conductivity detector from a bulk-property detector into a solute-specific detector [75]. Thus, exchanging eluent and sample counter ions with either hydronium or hydroxide ions means that only analyte ions are detected by the conductivity detector and appear in the resulting chromatogram.

The original form of a suppressor system introduced by Small et al. [1] was a column that was placed between the separator column and the conductivity cell. This suppressor column contained an ion-exchange resin: a strongly acidic cation-exchange resin in the hydrogen form to be used for anion-exchange chromatography and a strongly basic anion-exchange resin in the hydroxide form to be used in cation-exchange chromatography. The function of this suppressor column is illustrated by the analysis of chloride and bromide anions. After these anions are separated with an eluent such as sodium hydroxide on one of the anion exchangers described above, the column effluent is passed through the suppressor column prior to entering the conductivity cell, where the following reactions occur:

- Strongly conducting sodium hydroxide is converted to water by exchanging sodium ions of the eluent with hydronium ions of the cation exchanger:

$$\text{Resin}{-}\text{SO}_3\text{H} + \text{NaOH} \rightarrow \text{Resin}{-}\text{SO}_3\text{Na} + \text{H}_2\text{O} \qquad (7.1)$$

- Similarly, sodium chloride and sodium bromide are converted into their corresponding acids:

$$\text{Resin}{-}\text{SO}_3\text{H} + \text{NaCl} \rightarrow \text{Resin}{-}\text{SO}_3\text{Na} + \text{HCl} \qquad (7.2)$$

$$\text{Resin}{-}\text{SO}_3\text{H} + \text{NaBr} \rightarrow \text{Resin}{-}\text{SO}_3\text{Na} + \text{HBr} \qquad (7.3)$$

As the result of the suppressor reaction, strongly conducting mineral acids in the presence of water enter the conductivity cell and, consequently, are easily detected.

A major drawback of packed-bed suppressors is the requirement of periodic regeneration. Depending on the degree of use, the retention time of the negative water dip will change. The latter arises because the conductivity of water, as solvent for the sample, is slightly smaller than the eluent background conductivity after passing through the suppressor column. Pure deionized water injected into the ion chromatograph travels with the mobile phase through the column. The negative dip is observed at a time that is almost identical to the system void. With the water dip changing its position, analytes eluting close to the system void such as fluoride and chloride are difficult to quantitate. Also, the void volume of the suppressor column affects the quality of the separation because it determines peak broadening. The total volume of the suppressor column should be as small as possible to prevent mixing of the already separated

signals. For the resulting suppression capacity, however, the total volume of the suppressor column should be as large as possible. These two requirements are incompatible, so the dimensioning of packed-bed suppressors is always a compromise between the suppression capacity and the peak broadening caused by the void volume. For these reasons, conventional suppressor columns became technologically outdated in the early 1980s and were replaced by membrane-based suppressor systems.

Membrane-based suppressor systems In contrast to conventional suppressor columns, all membrane-based suppressor systems offered by Thermo Fisher Scientific are continuously regenerated. Continuous regeneration of the suppressor device is essential for maintaining the ion-exchange equilibrium during the suppression process, resulting in greater system stability. In general, suppressor devices can be chemically regenerated or electrolytically operated; suppressor choice very much depends on the application requirements such as type of eluent, noise level, column temperature, solvent compatibility, and the type of chromatography being practiced.

The latest version of a chemically regenerated suppressor is commercialized under the trade name Thermo Scientific™ Dionex™ CRS™ 500. It follows the concept of the micromembrane suppressor that was introduced in 1985 [76]. Its design allows the suppression capacity to be comparable to an original suppressor column but with a void volume of only 45 µL that has little effect on chromatographic separation. Figure 7.1 shows a schematic illustration of the sandwich structure of a Dionex CRS 500 suppressor. It consists of a flat, two-part enclosure in which ion-exchange screens and thin ion-exchange membranes are sandwiched together in alternating order. The two parts of the enclosure keep them together. The ion-exchange screens function as regenerant channels. The eluent screen employed in the original micromembrane suppressor were replaced with a planar bed of ion-exchange resin in the Dionex CRS 500 suppressor, improving eluent flow characteristics and increasing static capacity. Thus, peak shapes are improved making the Dionex CRS 500 suppressor compatible with the latest generation of 4 µm separator columns. As can be seen from Figure 7.1, the eluent is passed through the eluent chamber located in the center part of the suppressor while the regenerant flows countercurrent through the two regenerant chambers. The Dionex CRS 500 suppressor is available in both standard bore (5 and 4 mm) and microbore (2 and 3 mm) formats. Neutralization reactions occurring in a Dionex CRS 500 suppressor are illustrated in Figure 7.2, taking a Thermo Scientific™ Dionex™ ACRS 500 suppressor for anion analysis as an example. Hydronium ions in the sulfuric acid regenerant permeate the cation-exchange membranes and neutralize the potassium hydroxide eluent. The potassium counter ions permeate through the membranes into the regenerant chambers and combine therein with sulfate ions to maintain electroneutrality. The Dionex ACRS 500 suppressor supports hydroxide and carbonate-based eluents, while the respective version for cation analysis, the Thermo Scientific™ Dionex™ CCRS 500 suppressor, supports methanesulfonic and sulfuric acid eluents. Both suppressors are 100% compatible with water-miscible organic solvents and can be operated in three different modes: the conventional pressurized bottle mode, the displacement chemical regeneration mode, or the peristaltic pump mode.

The conventional pressurized bottle mode uses a pressurized reservoir to deliver the chemical regenerant to the micromembrane suppressor (Figure 7.3). The spent regenerant is then diverted to waste. While a sulfuric acid concentration of 10 mmol/L suffices for isocratic operation, a twofold regenerant concentration is recommended for gradient techniques. The flow rate should be adjusted to ensure sufficiently low background conductivity when the maximum eluent concentration is reached. Maintaining these conditions, one can then switch

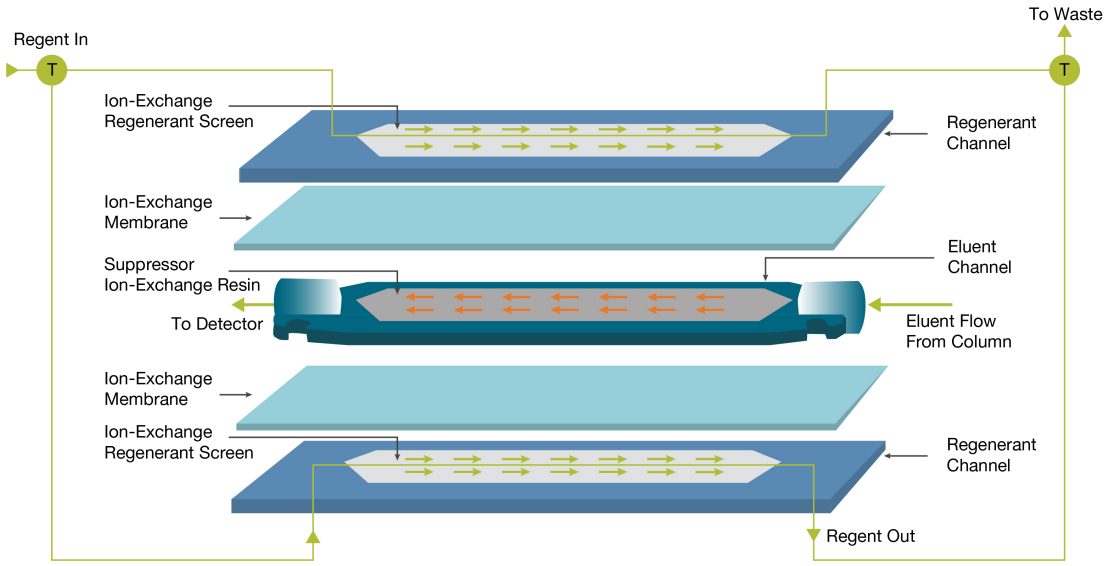


Figure 7.1 Schematic illustration of the Dionex CRS 500 suppressor.

to the initial eluent concentration. In displacement chemical regeneration mode (DCR™), the regenerant is displaced with the conductivity cell effluent, delivering the regenerant to the suppressor at a flow rate equal to the eluent flow rate (Figure 7.4). In this mode, the regenerant bottle is completely filled with regenerant upon startup. As the cell effluent is pumped into the regenerant bottle, the regenerant is forced out into the suppressor regenerant chambers. No additional pump or pressure is required. Eluent and regenerant bottles must be of equivalent volumes, and new regenerant is prepared together with a new eluent. The low regenerant flow rate minimizes waste and allows unattended operation. The new peristaltic pump mode employs a peristaltic pump to deliver the regenerant to the Dionex CRS 500 suppressor at a controlled flow rate.

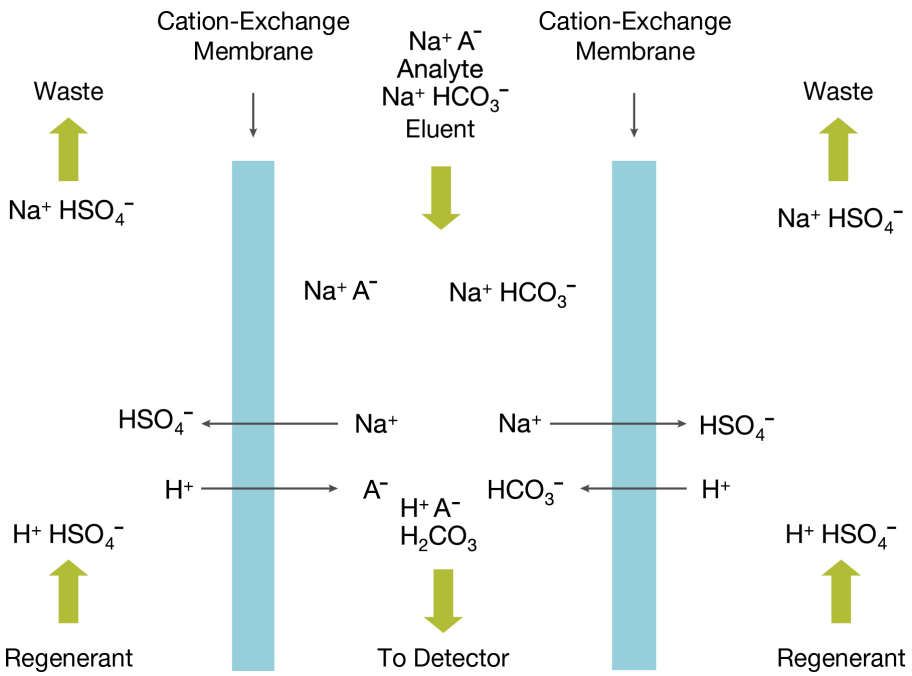


Figure 7.2 Suppression reactions inside an ACRS 500.

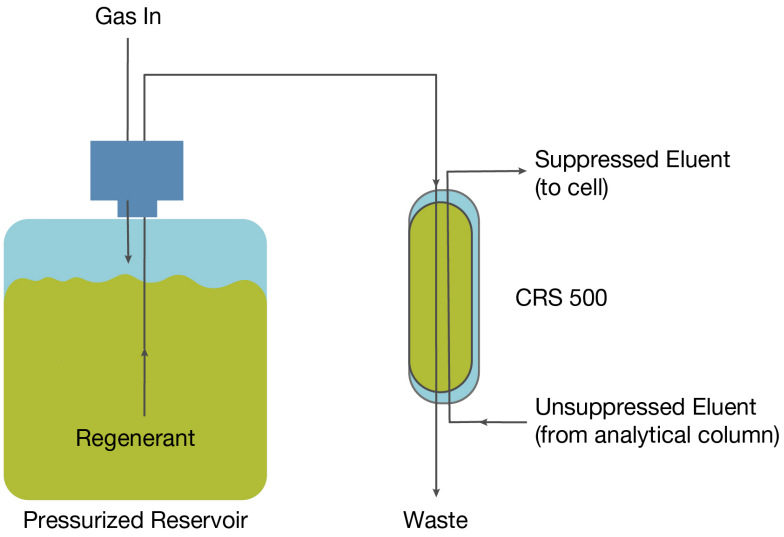


Figure 7.3 Flow diagram of a Dionex CRS 500 suppressor operated in the pressurized bottle mode.

The Thermo Scientific™ Dionex™ ACRS-ICE 500 chemically regenerated suppressor for ion-exclusion and ion-suppression separation modes been introduced. Its structure corresponds to the devices developed for anion and cation-exchange chromatography, i.e., it is a high-capacity, low void volume, membrane-based eluent suppressor. It uses chemical suppression with a tetrabutylammonium hydroxide (TBAOH) regenerant (see Table 7.1) to decrease eluent conductivity by displacing the highly conductive hydronium ions from the eluent into the regenerant chambers, followed by a neutralization step in the regenerant chambers. The resulting TBA^+ OSA^- pair has low conductance. At the same time, the weakly dissociated organic acid analytes are converted into their tetrabutylammonium salts. The result is a significant improvement in analyte detection limits. Figure 7.5 illustrates the suppression reactions inside a Dionex ACRS-ICE 500 suppressor.

In 1992, the first commercial electrolytically regenerated suppressor system was introduced: the self-regenerating suppressor (SRS) [77]. Its schematic differs from a chemically regenerated suppressor (see Figure 7.1) only in the two platinum electrodes for the electrolysis of water,

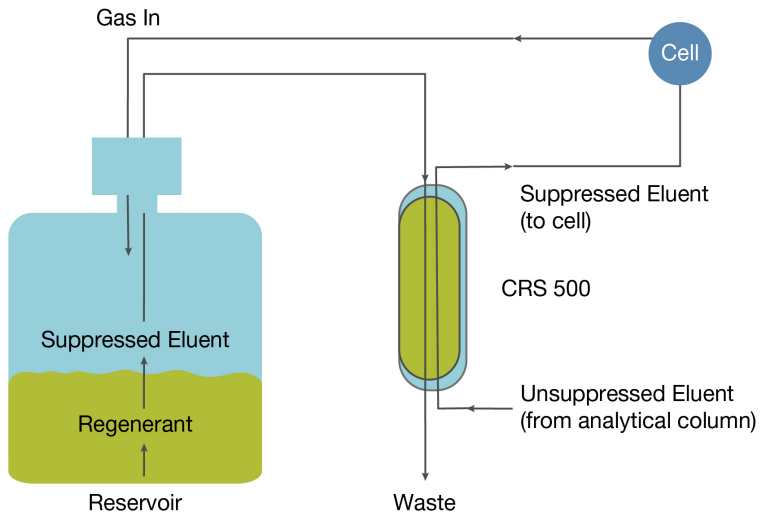


Figure 7.4 Flow diagram of a Dionex CRS 500 suppressor operated in the displacement chemical regeneration (DCR) mode.

Table 7.1 Eluents and regenerants in ion-exclusion chromatography.

Eluent	Concentration (10^3 mol/L)	Regenerant[a] (mol/L)	Background conductivity[b] (µS/cm)
HCl	0.5 ... 1		50 ... 1000
Perfluorobutyric acid	0.5 ... 1	TBAOH 0.005 ... 0.01	20 ... 45
Perfluoroheptanoic acid	0.5 ... 1		20 ... 40
Octanesulfonic acid	0.5 ... 1		25 ... 45

a) The regenerant concentration should be about 10 times as high as the eluent concentration, based on a flow rate of 2 mL/min.
b) The values indicated for the resulting background conductivity refer to an eluent flow rate of 0.8 mL/min.

which are placed at the top and at the bottom between the regenerant chamber and the enclosure. A self-regenerating suppressor comes complete with a power supply for applying an electric field for the electrolytic reactions. One advantage of this suppressor is that the water required for electrolysis can be delivered through the regenerant channels at a relatively low flow rate. Moreover, the deionized effluent of the detector cell after suppression can be used as a water source, so that an external water supply is only necessary when destructive detection methods such as ICP, MS, or CAD are used. Self-regenerating suppressors for analytical ion chromatography are currently offered in three different versions:

- Thermo Scientific™ Dionex™ DRS 600 Dynamically Regenerated Suppressor
- Thermo Scientific™ Dionex™ ERS 500e Electrolytically Regenerated Suppressor for External Water Mode
- Thermo Scientific™ Dionex™ AERS 500 Carbonate Anion Electrolytically Regenerated Suppressor for carbonate eluents.

Neutralization reactions occurring in a self-regenerating suppressor do not differ much from those in chemically regenerated suppressors. Taking an SRS for anion analysis as an example

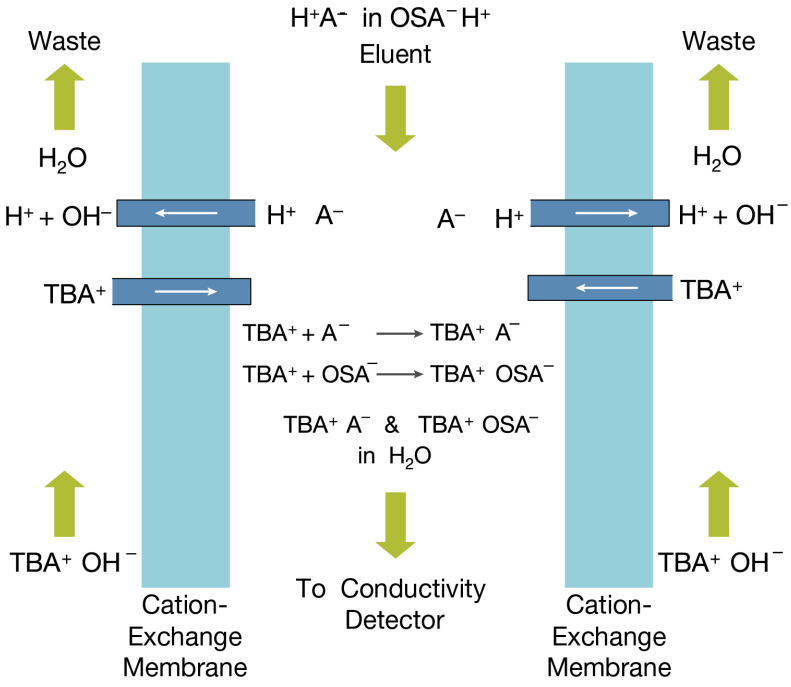


Figure 7.5 Suppression reactions inside a Dionex ACRS-ICE 500 suppressor.

(Figure 7.6), hydronium ions (after their formation at the anode) permeate the cation-exchange membrane and neutralize the sodium hydroxide eluent. The sodium counter ions are attracted by the cathode, permeate through the membrane into the cathodic regenerant chamber, and combine therein with hydroxide ions to maintain electroneutrality. Both hydrogen and oxygen gas formed at the cathode and anode, respectively, are sent to waste together with the liquid reaction products. The amount of hydrogen gas formed during electrolysis (about 21 mL/h at $I = 50$ mA) is relatively small and poses no safety problem.

In contrast to chemically regenerated suppressors, the suppressor reaction in an SRS is directed by the electrodes. In a Dionex CRS 500 suppressor, for instance, the chemical regenerant flows countercurrent through both regenerant chambers. Therefore, regenerant ions required for neutralization are provided to the eluent chamber through both membranes. Hydronium ions in a Dionex ADRS 600 suppressor are exclusively formed at the anode, so that only the ion-exchange membrane in the anodic regenerant chamber is permeable for hydronium ions. Conversely, the exchange of counter ions only occurs at the membrane in the cathodic regenerant chamber, where they associate with hydroxide ions formed therein. Thus, the anodic membrane is partly in the hydrogen form, whereas the cathodic membrane is partly in the sodium form.

A Dionex DRS 600 suppressor is typically operated in the recycle mode (AutoSuppression). In this mode, the effluent of the conductivity cell is used as the source for the required deionized water. As can be seen in the respective flow diagram in Figure 7.7, the column effluent is directed through the suppressor, where it is neutralized by exchanging counter ions for hydronium or hydroxide ions, respectively. The suppressor effluent, practically deionized (except for a few analyte ions), is then directed through the conductivity cell and back into the suppressor to be used as regenerant. Recycling of the cell effluent offers a number of advantages. In addition to the simplicity of the plumbing, the waste volume is greatly reduced because an external chemical regenerant is not needed. Therefore, operating costs are much lower, and the system is practically maintenance-free. AutoSuppression is the most widely used operating mode for a Dionex DRS 600 suppressor and can be applied in all applications with purely *aqueous* eluents below 40 °C. As an alternative to the recycle mode, AutoSuppression with external water supply can be used. In this operation mode, the deionized water needed for electrolysis is provided externally. The external water mode is predominantly chosen when employing destructive detection methods or when the application requires a small amount of

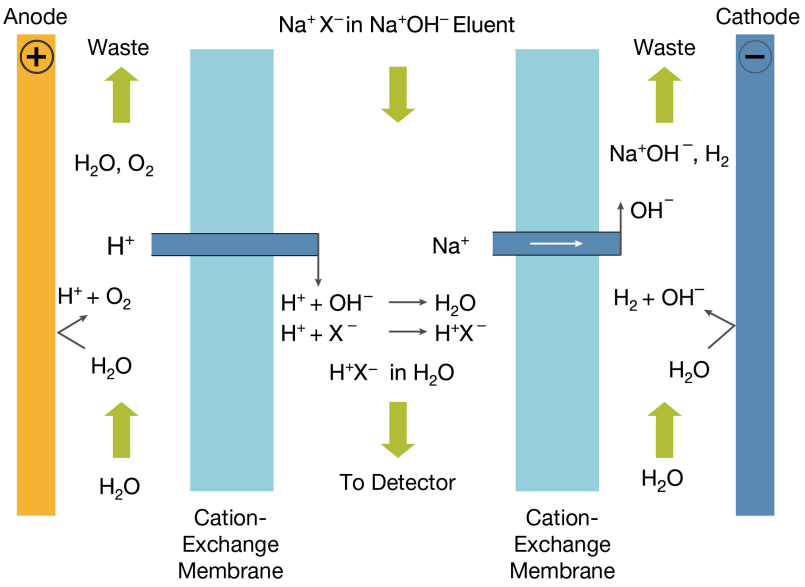


Figure 7.6 Neutralization reactions in a self-regenerating suppressor for anion analysis.

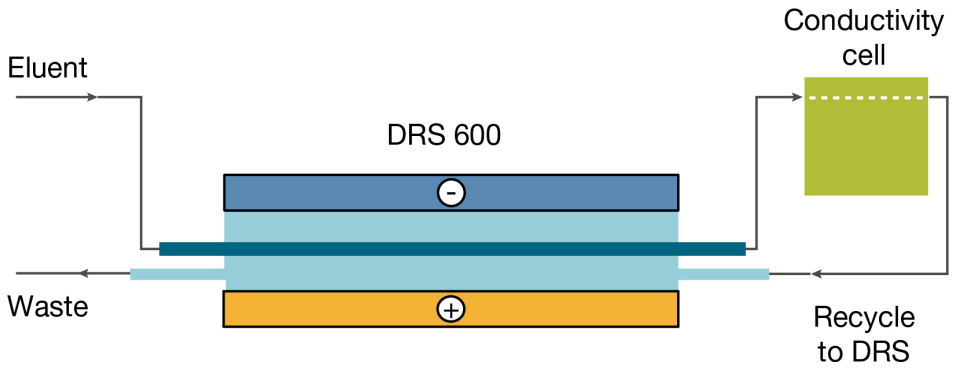


Figure 7.7 Eluent flow in the AutoSuppression recycle mode with a Dionex DRS 600 self-regenerating suppressor.

an organic solvent in the mobile phase. In the latter case, the external water mode provides lower noise and thus higher sensitivity. Suppression capacity can also be increased by applying the external water mode, since water can be delivered to the regenerant chambers at a higher flow rate, which results in a more efficient removal of eluent counter ions. This is especially important when using microbore columns, which are usually operated at a flow rate of 0.25 mL/min. This flow rate is somewhat low for AutoSuppression in the recycle mode.

The Dionex DRS 600 suppressor is the first self-regenerating suppressor that operates in the constant voltage mode rather than in the traditional constant current mode. By operating in constant voltage mode, the suppressor self-governs electrical current as long as a constant voltage is applied. When performing gradient analysis, this feature reduces the noise at lower eluent concentrations, which significantly improves the signal-to-noise ratio. It also maintains suppression efficiency when analyzing samples with high electrolyte content by accommodating sudden changes in the ion load. Figure 7.8 shows a comparison of the Dionex ADRS 600 suppressor (in constant voltage mode) with the previous generation Dionex AERS 500 suppressor (in constant current mode) under gradient conditions. As shown by the respective noise values, the Dionex ADRS 600 suppressor outperforms the Dionex AERS 500 suppressor.

The Dionex ERS 500e suppressor is designed for applications where difficult sample matrices or zwitterionic species are being analyzed. The ion-exchange screen reduces the static capacity of the suppressor, thus improving compatibility with samples and matrices that have an affinity for the ion-exchange resin found in the Dionex DRS 600 suppressor.

The Dionex AERS 500 Carbonate suppressor is designed for operation with carbonate-based eluents. It features a hardware design that significantly improves noise performance with carbonate eluents without sacrificing performance or ease-of-use. The unique hardware design delivers the lowest noise level of any electrolytically regenerated suppressor for carbonate eluents.

The internal volume of analytical membrane-based suppressor systems mentioned above is about 50 µL for the standard bore format and about 15 µL for the microbore format. These volumes are far too large to be compatible with capillary flow rates, which are typically between 5 and 30 µL/min. Scaling down from analytical to capillary scale brings many advantages to ion chromatography. The major one is that the system can be continuously operated with as little as 15 mL of mobile phase per day, translating into 5.2 L a year. Moreover, eluent generation cartridges last much longer under continuous operation mode.

Capillary ion chromatography systems are *per se* Reagent-Free IC systems, that is, the generation, purification, and suppression of the mobile phase are based on the electrolysis of high-purity deionized water. Eluent generators can be constructed with very low dead volumes and are, therefore, an ideal eluent delivery platform for both isocratic and gradient capillary separations. They are capable of providing high-fidelity gradient profiles at very low microliter/minute flow rates through precise current and flow rate control. The Dionex CES 300

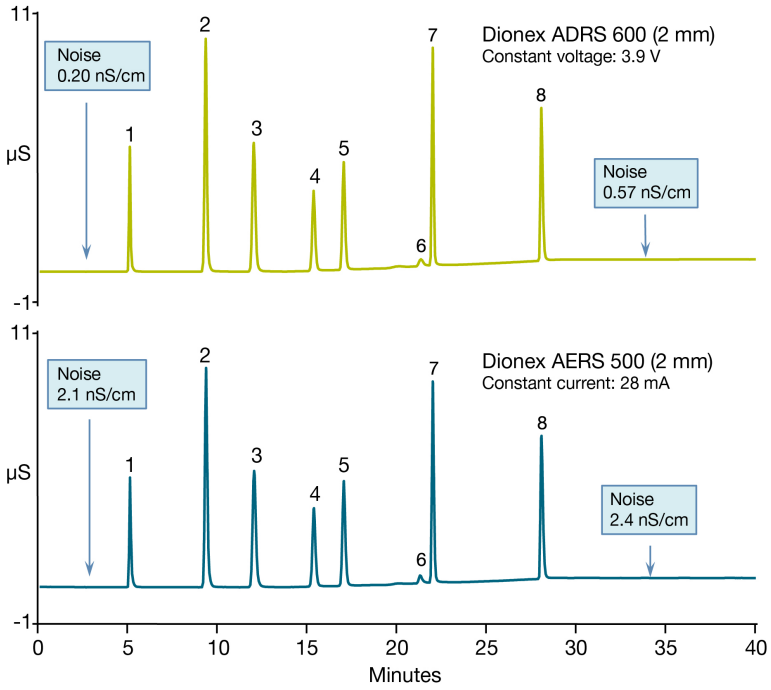


Figure 7.8 Comparison of Dionex ADRS 600 and AERS 500 supressors under gradient conditions. Separator column: Dionex IonPac AS19-4µm; column dimension: 250 × 2 mm i.d.; column temperature: 30 °C; eluent: KOH (EG); gradient: 10 mmol/L from 0 to 10 min, 10–45 mmol/L from 10 to 25 min, 45 mmol/L from 25 to 40 min; flow rate: 0.25 mL/min; injection volume: 2.5 µL; detection: suppressed conductivity; peaks: (1) fluoride, (2) chloride, (3) nitrite, (4) bromide, (5) nitrate, (6) carbonate, (7) sulfate, and (8) orthophosphate.

capillary electrolytic suppressor (Figure 7.9) is optimized for capillary eluent flow rates using a three-chamber design to minimize dead volume (<1.5 µL), while maximizing suppression capacity and reducing noise. The eluent chamber of an anion suppressor (Dionex ACES 300) consists of capillary cation-exchange tubing coiled around a spindle, which is embedded in a bed of cation-exchange resin that facilitates the efficient exchange of the eluent counter ions for regenerant ions. The two electrode chambers are separated from the eluent chamber by a pair of cation-exchange membranes. The regenerant (deionized water) first passes through the cation-exchange bed in the eluent chamber and then through the cathode and anode chambers serially. Under the influence of an electric field, regenerant ions are generated in the anode chamber and migrate into the eluent chamber, maintaining the cation-exchange bed in the eluent chamber in the regenerated form. Eluent counter ions are migrating through the cation-exchange capillary tubing into the cation-exchange resin bed and on to the cathode chamber, where they are neutralized by the hydroxide ions generated in the cathode chamber. Although capillary suppressors are electrolytically operated, the resulting background noise is not much different from that of a chemically regenerated micromembrane suppressor.

7.1.2 Amperometric detection

Amperometric detection is generally used for the analysis of solutes with p*K* values above 7, which, owing to their low dissociation, can hardly be detected or are not at all detected by suppressed conductivity. Conventional amperometric detectors employ a three-electrode detector cell consisting of a working electrode, a reference electrode, and a counter electrode.

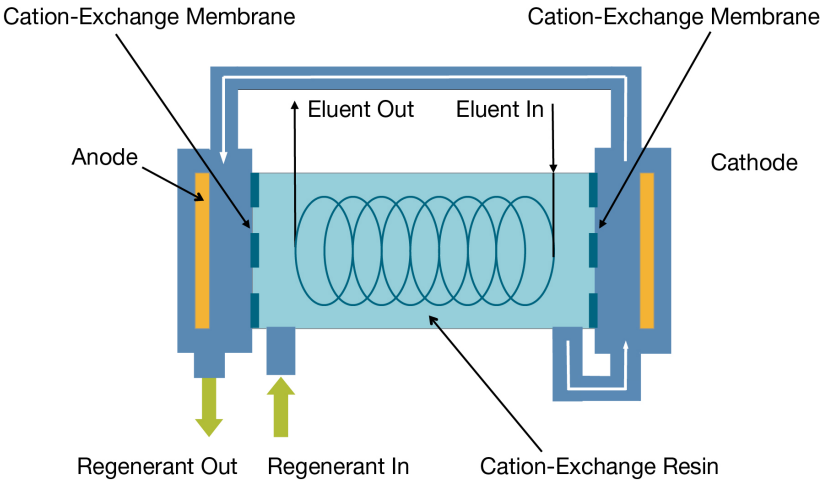


Figure 7.9 Schematics of an anion capillary electrolytic suppressor (Dionex ACES 300).

The electrochemical reaction at the working electrode is either an oxidation or a reduction. The required potential is applied to the working electrode. The Ag/AgCl electrode is utilized as a reference electrode because it is characterized by a good potential constancy at current flow. The purpose of the counter electrode, which is usually made of glassy carbon, is to maintain the potential. Furthermore, it inhibits a current flow at the reference electrode that could destroy it. When an electroactive species passes the detector cell it is partly oxidized or reduced. This reaction results in an anodic or cathodic current that is proportional to the concentration of the species over a certain range, and that may be represented as a chromatographic signal.

Such detectors are employed for analyzing a wealth of inorganic and organic ions in the µg/L range. This includes environmentally relevant anions such as sulfide and cyanide, arsenite, halide ions, oxyhalides, nitrite, thiosulfate, hydrazine, phenols, amino acids, carbohydrates, organic sulfur compounds, and many others. A survey of electrochemically active compounds and their required working electrodes and potentials is given in Table 7.2.

Amperometry with constant working potential This kind of amperometry is the most widely used electrochemical detection method in liquid chromatography. A constant DC potential is continuously applied to the electrodes of the detector cell. The working potential is chosen to be in the diffusion-controlled plateau region for the analyte ion. When several ions with different standard potentials are to be detected in the same run, the working potential must be high enough to cover the plateau regions of all ions to be analyzed. The amperometric detection with constant working potential is routinely applied to the inorganic species, phenols, and catecholamines listed in Table 7.2. Figure 7.10 shows the application of this detection method to the analysis of sulfide and cyanide. To eliminate the interfering transition metals in the matrix, the sample must be acidified, distilled according to DIN 38405 D13, and absorbed in 0.5–1 mol/L NaOH. This solution can then be injected on a high-capacity Dionex CarboPac PA1 anion exchanger using a NaOH/NaOAc eluent. DC amperometry is carried out at a silver working electrode.

Pulsed amperometry (PAD) Amperometric detection of electroactive species requires that reaction products from the oxidation or reduction of solutes do not precipitate at the electrode surface. The surface characteristics of electrodes will change if contaminated, leading to an

Table 7.2 Electroactive compounds and the required working electrodes and potentials.

Compound	Working electrode	Working potential (V)
HS^-, CN^-	Ag	0
Br^-, I^-, SCN^-, $S_2O_3^{2-}$	Ag	0.2
SO_3^{2-}	Pt	0.7
OCl^-	Pt	0.2
AsO_2^-	Pt	0.85
N_2H_4	Pt	0.5
NO_2^-	CP[a)]	1.1
ClO_2^-	CP	1.1
$S_2O_3^{2-}$	CP	1.1
Phenols	GC[b)]	1.2
Catecholamines	GC[b)]	0.8–1.2
Amino acids	Au	-1.7–1.0*
Carbohydrates	Au	0.05**
Divalent sulfur compounds	Au	0–1.1*

a) Carbon paste
b) Glassy carbon
* Integrated pulsed amperometry
** Pulsed amperometry

enhanced baseline drift, increased background noise, and a constantly changing response. This behavior is particularly pronounced in the amperometric detection of carbohydrates. Pulsed

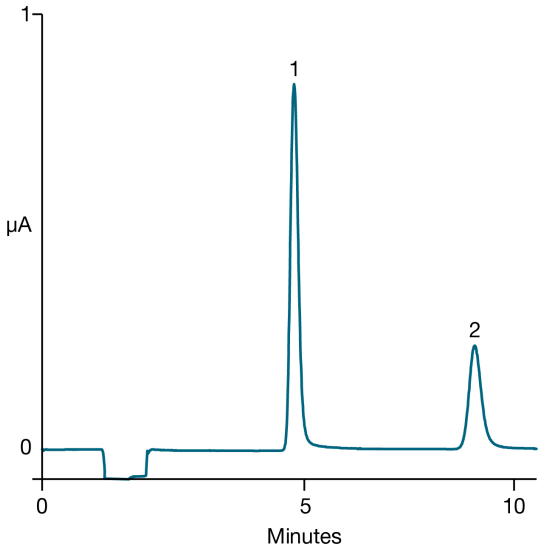


Figure 7.10 Analysis of sulfide and cyanide utilizing DC amperometry. Separator column: Dionex CarboPac PA1 with guard; eluent: 0.1 mol/L NaOH + 0.5 mol/L NaOAc + 0.5% (*v/v*) ethylenediamine; flow rate: 1 mL/min; detection: DC amperometry on a silver working electrode; oxidation potential: 0 V; injection volume: 50 µL; sample preparation: samples have been acidified, distilled acc. DIN 38405 D13, and absorbed in 0.1–1 mol/L NaOH; peaks: (1) sulfide and (2) cyanide.

amperometric detection, on the other hand, utilizes a rapidly repeating sequence of different working potentials, which are applied for different times to the gold working electrode. In contrast to conventional amperometry, the resulting current is only registered in short time intervals. By applying an additional negative cleaning potential, oxidation products may be removed from the electrode surface. The required working potentials and the pulse sequence are determined by means of cyclic voltammetry.

Based on the work of Jensen and Johnson [78] who found out that the carbohydrate oxidation products can be effectively removed by applying a strongly negative potential, Rocklin et al. [79] developed a sequence with four different potentials (also called *waveform*) for the detection of carbohydrates (see Figure 7.11) that resulted in constant response factors and excellent long-term stability. All the potentials and the pulse sequence were optimized for a maximal signal-to-noise ratio.

In 2010, a new reference electrode for use in pulsed amperometric detection was introduced. This solid-state palladium hydrogen (PdH) reference electrode has an extended lifetime in comparison to Ag/AgCl reference electrodes but does not offer the option of reporting a pH value. As can be seen from Figure 7.12, the PdH reference electrode consists of palladium and platinum electrodes immersed in an aqueous solution. With a potential applied between the two electrodes, palladium is connected as a cathode and platinum as an anode. Because of the applied potential, hydrogen gas is generated at the palladium electrode and oxygen gas at the platinum electrode. Whereas the oxygen gas is swept out of the cell in the liquid stream, a portion of the hydrogen gas is taken up by the palladium metal. An equilibrium develops between molecular hydrogen in the liquid phase and the adsorbed hydrogen in palladium. The palladium hydrogen electrode becomes a reference electrode with a half reaction of:

$$H^+ + e^- \rightarrow \tfrac{1}{2}H_2 \tag{7.4}$$

Hydrogen is supplied by the following process:

$$Pd + \tfrac{1}{2}H_2 \rightarrow PdH_{ads} \rightarrow PdH_{abs} \tag{7.5}$$

The PdH reference electrode was evaluated under a wide variety of chromatographic conditions

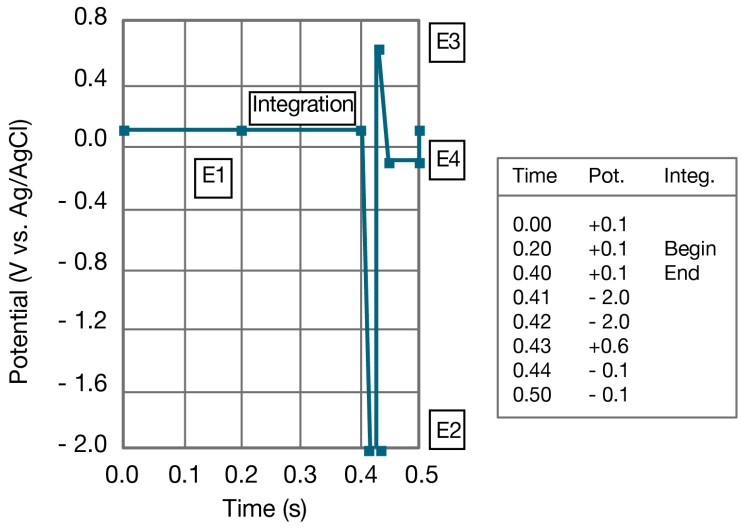


Figure 7.11 Potential sequence with four different potentials at a gold working electrode for carbohydrate detection. The detector signal is the charge (measured in Coulomb) that results from integrating the oxidation current between 0.2 and 0.4 s.

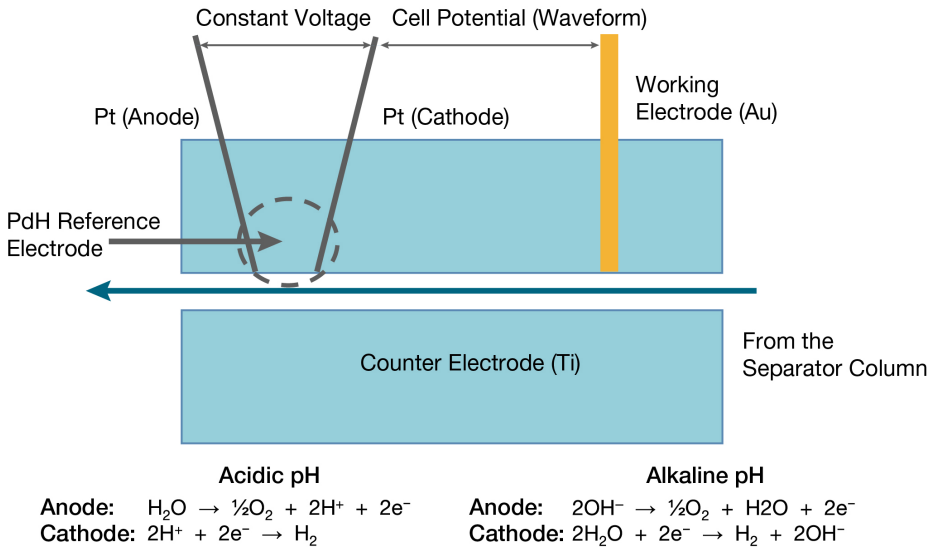


Figure 7.12 Schematics of a PdH reference electrode.

used in IC [80]. It was deployed for carbohydrate analysis performed with moderately to strongly alkaline eluents, for amino acid analysis utilizing complex gradient programs combining hydroxide and acetate-containing eluents, and for alcohol analysis using an ion-exclusion column with strong acid eluents. Under all these conditions, the PdH electrode maintains a constant and predictable reference potential.

Integrated pulsed amperometry Integrated pulsed amperometry is a variant of pulsed amperometry. It is predominantly used for the detection of amino acids, amines, and organic sulfur compounds. Their oxidation on metal electrodes is catalyzed by metal oxides. When integrated amperometry is employed, baseline disturbances caused by pH gradients, solvent gradients, ionic strength variations, and metal oxide formation are minimized. In pulsed amperometry, the resulting current from the oxidation reaction is measured at fixed oxidation potential after the application of the pulse. In integrated amperometry, the potential $E1$ is not kept constant, but alternated between a high and a low value. Thus, analyte and metal oxidation occur simultaneously at the high potential. However, the metal oxide formed at this potential is immediately afterwards reduced again at the lower potential. Because the oxidation of the electrode surface is a reversible process, while the oxidation of analytes is not, the resulting signal is mainly characterized by the contribution of the analyte oxidation. When integrating the current yield during this cycle, the net signal for the respective analyte is obtained. Positive and negative cleaning potentials are part of the pulse sequence following the integration step. A schematic example of such a sequence is illustrated in Figure 7.13.

The advantage of integrated pulsed amperometry lies in the coulometric compensation of the charges resulting from the formation and subsequent reduction of the metal oxide. Thus, baseline drifts and baseline disturbances caused by small variations in the mobile-phase composition are eliminated. Moreover, the whole system is less sensitive to variations in pH, which influences the potentials for the formation and reduction of the metal oxide.

For the detection of sulfur-containing antibiotics such as cephapirin and ampicillin, Dasenbrock and LaCourse [81] developed multicyclic pulse sequences, which result in higher sensitivity and selectivity. In this type of pulse sequence, the potential is cycled between high and low potentials during the integration period (see Figure 7.14, left illustration) leading to an

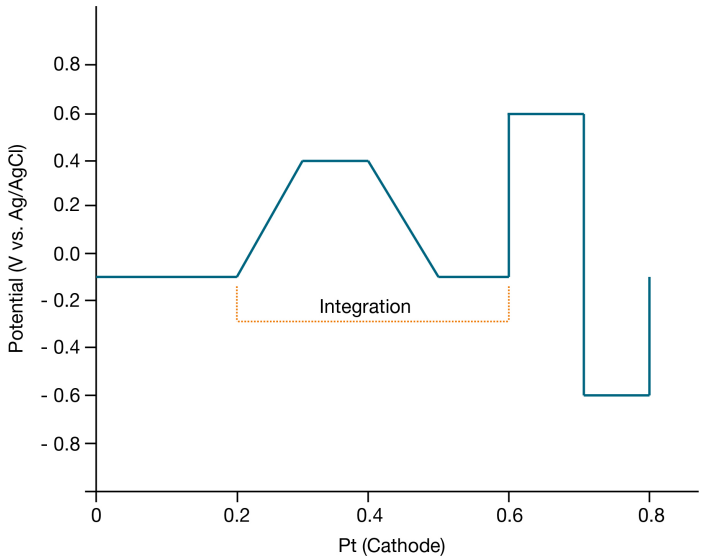


Figure 7.13 Example of a pulse sequence in integrated pulsed amperometry of amines on a gold working electrode.

improved signal-to-noise ratio. Theoretically, the signal-to-noise ratio increases with $n^{1/2}$ for n cycles. However, in practice this value is slightly lower because the frequency of the cycle is so high that only a limited number of analyte molecules can adsorb on the electrode surface between the particular cycles. The pulse sequence developed by Dasenbrock and LaCourse was modified by Hanko and Rohrer [82] who increased the number of cycles from four to ten to achieve a more stable response (see Figure 7.14, right illustration). For example, Figure 7.15 shows the separation of lincomycin on a Vydac® separator utilizing integrated amperometry with this modified pulse sequence, in comparison with UV detection at 215 nm. With the exception of the carbonyl group, lincomycin is nonchromophoric and thus UV detection is very insensitive.

Integrated amperometry is also a welcomed alternative to derivatization techniques for the detection of amino acids. Using this method, first described in 1983 by Polta and Johnson [83], amino acids are anodically oxidized in an alkaline medium. The maximal current yield in the oxidation of the amino group on a gold working electrode, however, occurs at a potential that is high enough for the oxidation of the gold surface itself. The current resulting from this

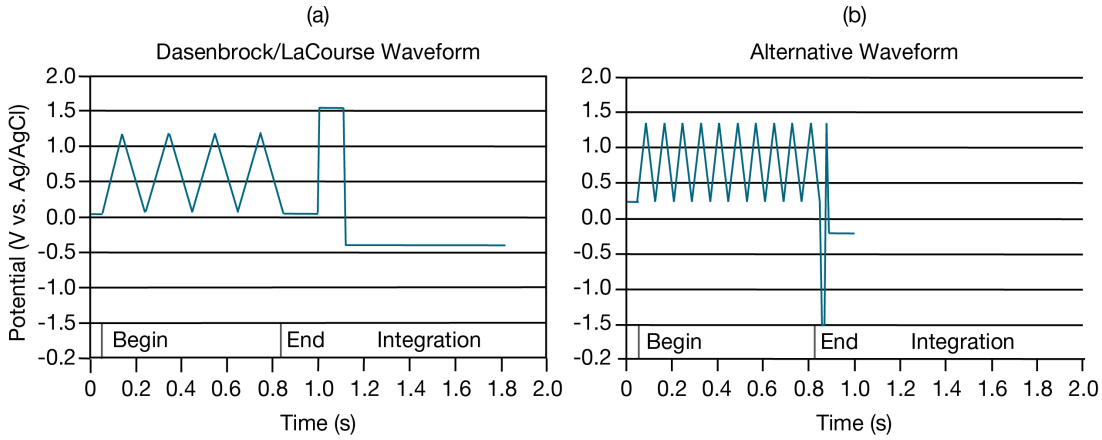


Figure 7.14 (a and b) Multicyclic pulse sequences for integrated amperometry of sulfur-containing antibiotics.

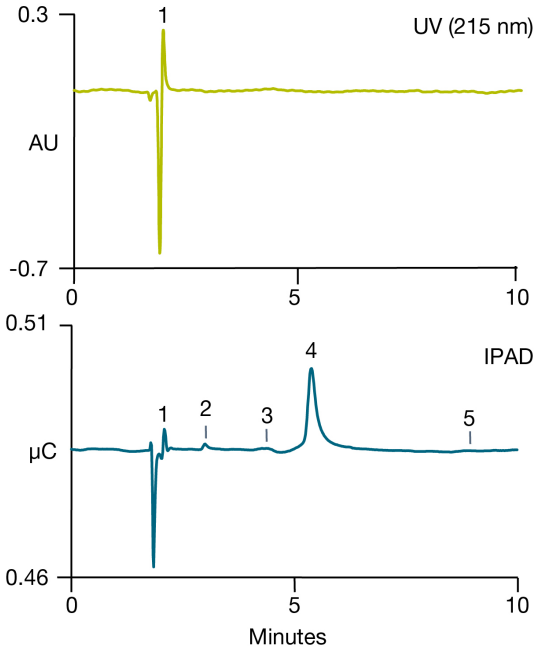


Figure 7.15 Separation of lincomycin utilizing integrated amperometry with a multicyclic pulse sequence in comparison to UV detection. Separator column: Vydac C8 (208TP5451); column dimensions: 150 mm × 4 mm i.d.; column temperature: 30 °C; eluent: 0.1 mol/L NaOAc/MeCN (91:9 *v/v*), pH 3.75; flow rate: 1 mL/min; peaks: (1)–(3), (5) unknown impurities, (4) 10 mg/L lincomycin.

process contributes to noise and to a certain baseline instability. Johnson et al. [84] solved this problem by utilizing integrated pulsed amperometry, which amplifies the signal from the amine oxidation and suppresses the signal from the gold oxidation. Figure 7.16 shows the pulse

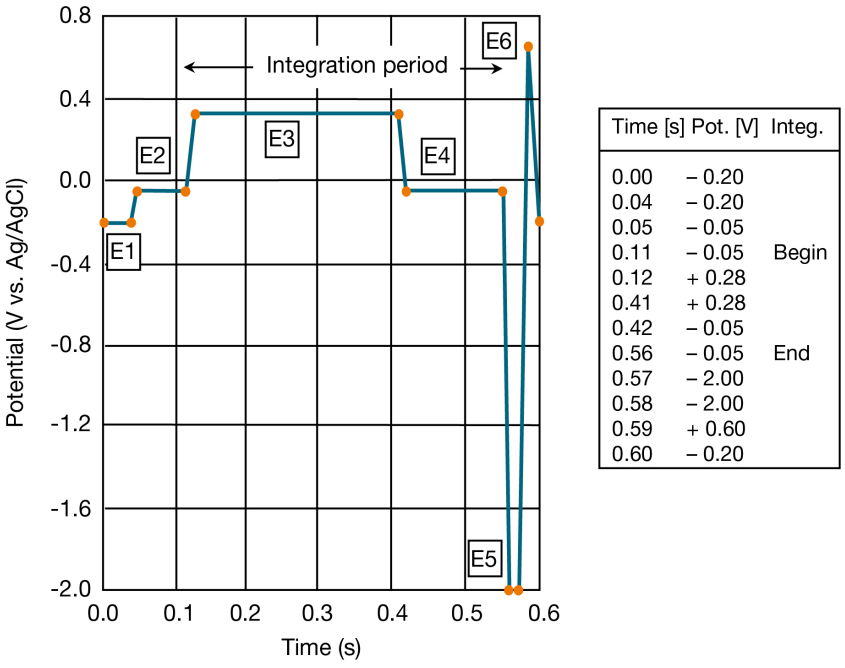


Figure 7.16 Pulse sequence for integrated amperometry of amino acids. The detector signal is the charge (measured in Coulomb) that results from the integration of the current yield from the oxidation of the amino group between 0.11 and 0.56 s.

sequence developed by Avdalovic et al. [85] for integrated amperometry of amino acids. It is very similar to the one introduced by Johnson et al. [84], but optimized for good linearity, minimal baseline drift during the gradient, and long-term stability. The pulse sequence introduced by Avdalovic et al. employs a cleaning step at negative potential which ensures a clean and active electrode surface at constant sensitivity.

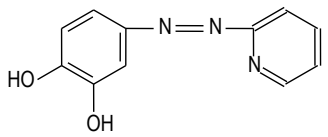
7.2
Spectrometric detection methods

7.2.1
UV/Vis detection

Direct UV detection In contrast to RPLC, UV detection is of minor importance in ion chromatography, but is considered a welcome supplement to conductometric detection. It is a disadvantage of direct UV detection that most inorganic anions do not possess an appropriate chromophore. Thus, they generally absorb at wavelengths below 220 nm.

Direct UV detection gained great significance in the determination of nitrite and nitrate [86], as well as bromide and iodide in the presence of high chloride concentrations. The optimal measuring wavelengths for the determination of those anions are listed in Table 7.3. Figure 7.17 illustrates the superiority of direct UV detection over conductivity detection with the separation of nitrite, nitrate, and bromide in the presence of a large excess of chloride on a Dionex IonPac AS9-HC column. While bromide and nitrate can be quantified with suppressed conductivity detection, the small nitrite peak is on the tail of the large chloride peak, which hampers its quantitation. Utilizing UV detection at 214 nm, all three UV-active anions can be easily determined. Determinations of this kind are typical in wastewater analysis with a large excess of chloride but may also be performed in other saline samples such as body fluids, sea water, meat products, sausages, and so on. For those applications, it is recommended to plumb the UV detector cell in series directly after the conductivity cell. It is also worth mentioning that metal–cyano and metal–chloro complexes can be detected at a wavelength of 215 nm.

UV/Vis detection in combination with derivatization techniques One of the most important applications of UV/Vis detection is photometric determination after derivatization of the column effluent. First, this includes the determination of transition metals after reaction with 4-(2-pyridylazo)-resorcinol (PAR).



PAR

The metal ions are separated on a bifunctional nanobead-agglomerated ion exchanger with anion- and cation-exchange capacity as oxalate or PDCA complexes. They are then mixed with the PAR reagent and form chelate complexes that absorb in the wavelength range between 490 nm and 530 nm. PAR is only stable in presence of an ammonia solution at pH > 9. It reacts with a number of metals, although usually only Mn, Fe(II/III), Co, Ni, Cu, Zn, Cd, Pb, and lanthanides are analyzed with PAR as a derivatization reagent. When Zn-EDTA is added to the PAR reagent, the application range of this derivatization reaction can be expanded to include alkaline-earth metals [87].

Table 7.3 Optimal UV measuring wavelengths for some selected inorganic anions.

Anion	Measuring wavelength (nm)
Bromate	200
Bromide	200
Chromate	365
Iodate	200
Iodide	227
Metal–chloro complexes	215
Metal–cyano complexes	215
Nitrate	202
Nitrite	211
Sulfide	215
Thiocyanate	215
Thiosulfate	215

A postcolumn derivatization with subsequent photometric detection has also been developed for the determination of aluminum. Using a mixture of ammonium sulfate and sulfuric acid, aluminum is separated as $AlSO_4^+$ ion on any conventional cation exchanger. It forms a stable complex with the disodium salt of 4,5-dihydroxy-1,3-benzenedisulfonic acid (Tiron) at pH 6.2 that can be detected at 313 nm.

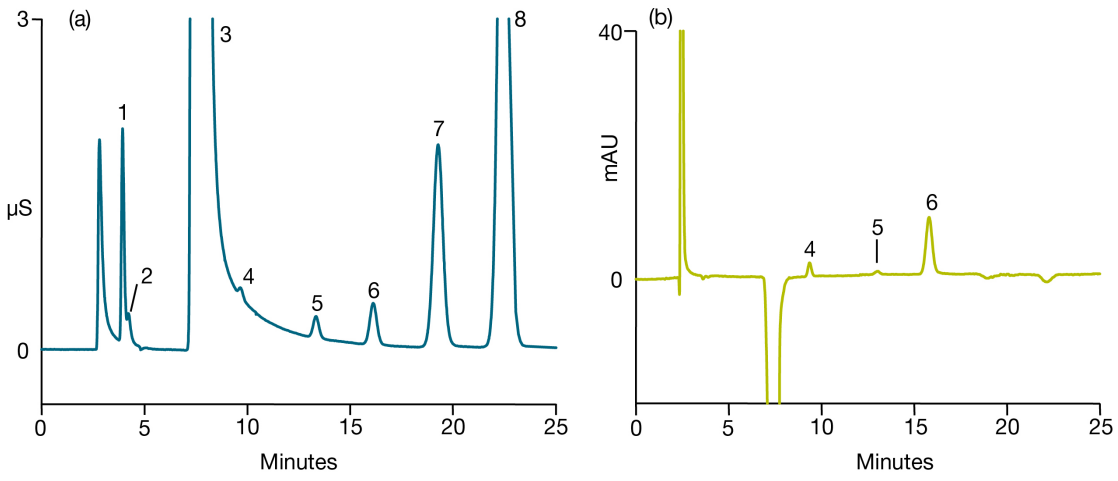
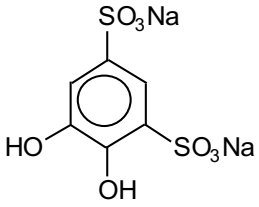


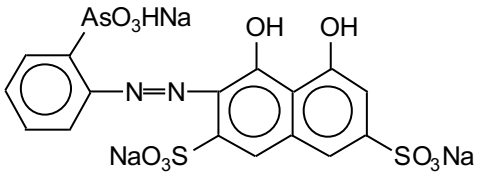
Figure 7.17 Comparison of direct UV detection and suppressed conductivity detection in the determination of nitrite, nitrate, and bromide at a large excess of chloride. Separator column: DionexIonPac AS9-HC; eluent: 9 mmol/L Na_2CO_3; flow rate: 1 mL/min; detection: (a) suppressed conductivity, (b) UV (214 nm); injection volume: 25 µL; sample: synthesized wastewater with 2 g/L chloride; peaks: (a) 1.14 mg/L fluoride (1), acetate (2), chloride (3), 0.14 mg/L nitrite (4), 0.82 mg/L bromide (5), 1.47 mg/L nitrate (6), 21.1 mg/L orthophosphate (7), and 30.3 mg/L sulfate (8), (b) 0.18 mg/L nitrite (4), 0.79 mg/L bromide (5), and 1.58 mg/L nitrate (6).



Tiron

At this pH value Tiron only reacts with aluminum and iron(III), hence this method for aluminum determination is very selective. The only disadvantage is the comparatively low extinction coefficient, $\varepsilon = 6000$ L/(mol cm), of the resulting complex at 310 nm.

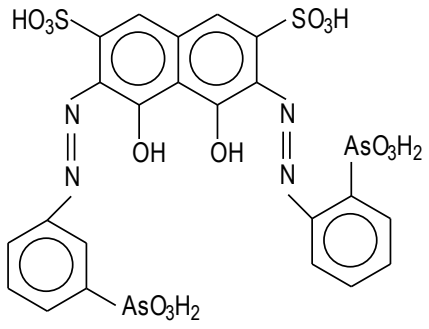
In the area of cation analysis, the extremely selective method of postcolumn derivatization of alkaline-earth metals with Arsenazo I [*o*-(1,8-dihydroxy-3,6-disulfo-2-naphthylazo)-benzenearsonic acid] is particularly suited for the determination of these elements in presence of high concentrations of alkali metals.



Arsenazo I

Characteristic applications include the determination of strontium in sea water and the determination of magnesium and calcium in ultrapure brine solutions used for the electrolysis of alkali metal chlorides. With alkaline-earth metals, Arsenazo I forms red-purple complexes that absorb at a wavelength of 570 nm.

Arsenazo III [Bis-(2-arsono-benzeneazo)-2,7-chromotropic acid] can also be used for the detection of lanthanides; it has the advantage of lower detection limits over those obtained with PAR or Arsenazo I.



Arsenazo III

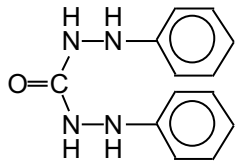
Several derivatization techniques have been developed for trace analysis of bromate in drinking water because concentrations below 2 µg/L cannot be determined by suppressed conductivity detection without preconcentration [88]. Even at the lowest microgram/liter levels, bromate has been classified by the World Health Organization (WHO) and the US EPA to be carcinogenic [89]. Lower detection limits cannot be achieved by enlarging the injection volume or by sample preconcentration because sample matrix components such as chloride, sulfate, and bicarbonate that are present at much higher concentration would overload the column and thus compromise chromatographic efficiency. With hyphenated techniques such as IC–MS via an

electrospray interface [90] and IC-ICP/MS [91] detection limits in the sub-microgram/liter range are possible, but this kind of instrumentation is not at everyone's disposal. IC-ICP/MS is highly selective due to mass-selective detection of bromate, but even this method is not free of interferences (e.g., tribromoacetic acid).

Regarding selectivity and sensitivity, the widely used alternative is the postcolumn derivatization method for the analysis of disinfection by-product anions such as bromate and chlorite that has been published as Method 326.0 by the US EPA [92]. Method 326.0 uses a postcolumn reaction that generates hydroiodic acid (HI) *in situ*, from an excess of potassium iodide that combines with bromate from the column effluent to form the tri-iodide anion, I_3^-. The reaction product can then be detected photometrically at 352 nm. Most published EPA methods specify the use of a Dionex IonPac AS9-HC column and a carbonate eluent (c = 9 mmol/L) for determining bromate and chlorite in drinking and bottled water. However, the hydroxide-selective Dionex IonPac AS19 column not only improves the selectivity for disinfection by-product anions, but also provides the typical advantages observed when using a hydroxide eluent for trace analysis, such as lower baseline noise and improved sensitivity. For example, the use of the Dionex IonPac AS19 column in combination with electrolytically generated potassium hydroxide eluent results in a bromate MDL that is approximately three times lower than with the Dionex IonPac AS9-HC column and a carbonate eluent [93].

US EPA Method 326.0 allows the combination of suppressed conductivity detection and postcolumn derivatization for UV absorbance detection, as schematically depicted in Figure 7.18. After separation and eluent suppression, standard anions and oxyhalide ions are measured using conductivity detection. The suppressed effluent from the conductivity detector is then combined with an acidic solution of potassium iodide containing a catalytic amount of ammonium molybdate tetrahydrate. The mixture is heated in the knitted reaction coil to 80 °C to facilitate complete reaction. The formed tri-iodide is measured by its UV absorption at 352 nm. Because KI is photosensitive, the KI/Mo PCR solution must be protected from light by covering it with aluminum foil and should be pressurized with helium. Because the generation of the tri-iodide ion is pH dependent, the second suppressor (Dionex CRS 500) is used to acidify the postcolumn reagent just before entering the reaction coil.

An extremely selective reagent for chromium(VI) is 1,5-diphenylcarbazide, which forms an analytically useful inner complex with dichromate under acidic conditions.



1,5-Diphenylcarbazide

In this reaction, diphenylcarbazide is oxidized to diphenylcarbazone, while at the same time chromium(VI) is reduced to chromium(III), which forms a chelate complex with diphenylcarbazide. Like a metal–PAR complex, it is red-purple colored and thus may be photometrically detected at 520 nm. As already mentioned, chromate separation is performed by means of anion-exchange chromatography.

Polyvalent anions such as polyphosphates, polyphosphonates, and sequestering agents are analyzed by anion-exchange chromatography with postcolumn derivatization, using ferric nitrate in acidic solution as a reagent [94]. The reaction of these compounds with iron(III) causes a bathochromic shift of the background absorbance up to the wavelength range between 310 and 330 nm. Because other inorganic anions such as chloride, sulfate, and so on, also cause

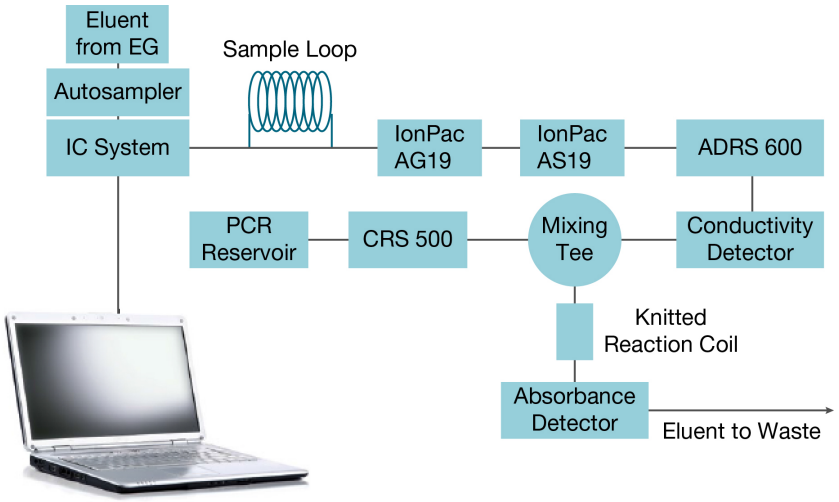


Figure 7.18 Schematic of the IC system configuration for US EPA Method 326.0.

such a shift under these conditions, the detection of complexing agents via derivatization with iron(III) is only possible in matrices with relatively low electrolyte content.

The derivatization with sodium molybdate can be utilized for the analysis of water soluble orthosilicate. Its separation can be achieved with both ion-exchange and ion-exclusion chromatography. With sodium molybdate, orthosilicate also forms the yellow heteropoly acid $H_4[Si(Mo_3O_{10})_4]$ and exhibits an absorption maximum at 410 nm.

7.2.2 Fluorescence detection

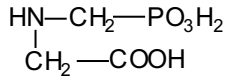
In ion chromatography, fluorescence detection is mainly utilized in combination with post-column derivatization because inorganic anions and cations, with the exception of the uranyl cation UO_2^{2+}, do not exhibit any intrinsic fluorescence. Fluorescence results from the excitation of molecules via absorption of electromagnetic radiation; it is the emission of fluorescence radiation when the excited system returns to the energetic ground level. The emitted wavelength is characteristic of the kind of molecule, while the intensity is proportional to concentration.

The best known and most widely used fluorescence method was developed by Roth and Hampai [95] for the detection of primary amino acids. It is based mainly on the reaction of α-amino acids with *o*-phthaldialdehyde (OPA) and 2-mercaptoethanol to yield an intensively blue-fluorescing complex. Even at room temperature, the reaction occurs within seconds. At an excitation wavelength of 340 nm and an emission wavelength of 455 nm, this method has a detection limit in the low picomole range. The derivatization with *o*-phthaldialdehyde is applicable to all compounds carrying a primary amino group. This includes the ammonium ion, primary amines, polyamines, and peptides.

The only drawback of the fluorescence method developed by Roth and Hampai is the relatively small stability of the *N*-substituted 1-alkylthioisoindoles that are formed from α-amino acids after reaction with OPA. Thus, the reaction product must be injected into the chromatography system at a defined time when applying OPA for the precolumn derivatization of primary amines. Much higher stabilities and partly higher fluorescence yields are obtained when naphthaline-2,3-dialdehyde (NDA) is used as a reagent, which reacts with primary amines in the presence of cyanide ions to *N*-substituted 1-cyanobenz[f]isoindoles (CBI). The excitation spectrum of these derivatives shows maxima at 246 and 420 nm; the emission is measured at a

wavelength of 490 nm. At an excitation wavelength of 246 nm, detection limits are obtained in the medium to low femtomole range.

The analysis of a secondary amine via a two-step derivatization and fluorescence detection is exemplified with an herbicide – the glyphosate [*N*-(methylphosphono)-glycine].



Glyphosate

After its separation on a cation exchanger, it is oxidized to glycine with hypochlorite at 36 °C and subsequently reacted with OPA and *N*,*N*-dimethyl-2-mercaptoethylamine-hydrochloride (Thiofluor) to yield a strongly fluorescing isoindole. In comparison with the traditionally used 2-mercaptoethanol, Thiofluor offers the advantages of being completely inodorous and more stable in solution. The instrumental setup for this postcolumn derivatization consists of two reagent pumps, two reaction coils (one must be heated), and a column oven. As seen in the corresponding chromatogram in Figure 7.19, even the main glyphosate metabolite – amino-methanesulfonic acid (AMPA) – may be detected in the same run. After AMPA is eluted, the separator column is regenerated with 5 mmol/L KOH for 2 min and then conditioned with the eluent for 7 min before the next sample can be injected. Based on an injection volume of 50 µL, the minimum detection limit for glyphosate is 1.8 µg/L. The glyphosate method is linear in the investigated concentration range between 0.05 and 10 mg/L [96].

7.3 Aerosol-based detection methods

In the past, aerosol-based detection methods such as evaporative light scattering detection (ELSD), condensation nucleation light scattering detection (CNLSD), and charged aerosol detection (CAD) were not commonly used for detecting ionic species. However, with the introduction of mixed-mode stationary phases (see Section 6.3), primarily used for pharmaceutical applications, detection methods are required that can simultaneously detect active pharmaceutical ingredients (APIs) and their counter ions. Aerosol-based detectors are mass-sensitive detectors and are claimed to provide “universal” response. According to the IUPAC Compendium of Chemical Technology [97], a universal detector is defined as one that can respond to every component in the column effluent. Clearly, detectors such as conductivity, UV/Vis, fluorescence, and electrochemical detectors do not satisfy this definition. It is widely recognized that no single HPLC detector is capable of distinguishing all possible analytes from a given chromatographic eluent, and hence the term “universal detection” is redefined as to establish a consistent relationship between the magnitudes of response and quantity injected for a range of analytes [98].

In pharmaceutical analysis, UV/Vis detection is widely used in routine assays such as formulations and batch reproducibility testing, but photometric detection requires that the analytes contain suitable chromophores. MS detection became an important tool in drug discovery, but many species are not ionizable or suffer from ion suppression. As external calibration with reference standards is required for quantitative analysis of each analyte in both UV/Vis and MS detection, quantifying unknown compounds in pharmaceutical impurity

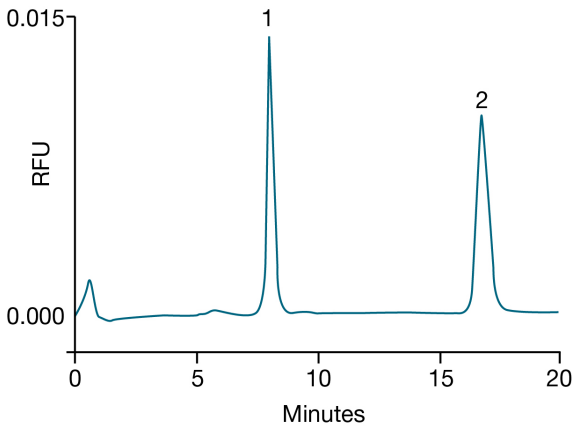


Figure 7.19 Two-step derivatization of glyphosate with subsequent fluorescence detection. Separator column: 150 mm × 4 mm i.d. glyphosate column (Pickering Laboratories); column temperature: 55 °C; eluent: 5 mmol/L potassium phosphate (pH 2); regenerant: 5 mmol/L KOH; flow rate: 0.4 mL/min; detection: fluorescence after oxidation with NaOCl and reaction with Thiofluor; reagent 1: 100 µL 5% hypochlorite solution per bottle of hypochlorite diluent (Pickering); reagent 2: 100 mg OPA + 2 g Thiofluor + 10 mL MeOH per bottle of OPA diluent (Pickering); injection volume: 100 µL; peaks: 2.5 mg/L each of aminomethanephosphonic acid (1) and glyphosate (2).

testing and mass balance assessments is often not feasible. Today, aerosol-based detectors are considered "universal detection systems" for liquid chromatography. In these detectors, the column effluent is nebulized and then dried, producing analyte particles. These dried particles are then detected optically in the case of evaporative light scattering detection and the more sensitive condensation nucleation light scattering detection, or by charge transfer in the case of charged aerosol detection. This process accommodates a large variety of different compound classes, provided they are less volatile than the mobile phase. This near-universal compatibility sets aerosol-based detectors apart from other detection methods.

7.3.1 Charged aerosol detection (CAD)

One of the more recently available near-universal detectors is the charged aerosol detector (CAD), which was commercially introduced in 2004 by ESA Biosciences (Chelmsford, MA, USA). As a first step, charged aerosol detection involves nebulization of the column effluent with nitrogen to form an aerosol. The spray is directed onto an impactor that removes larger droplets and then transported through a drying tube where the volatile components and solvents are evaporated, producing analyte particles. As illustrated in Figure 7.20, a secondary flow of nitrogen becomes positively charged as a result of having passed a high-voltage platinum corona wire. This charged nitrogen collides with the stream of analyte particles in a mixing chamber where the charge is transferred onto the particles by diffusion. After passing by a negatively charged ion trap to remove excess ionized gas, the charged particles impact with the collector where the charge is drawn off and measured by a highly sensitive electrometer. The signal generated by the electrometer is in direct proportion to the quantity of the analyte present.

The CAD detection principle has some commonality with atmospheric-pressure chemical ionization (APCI) in mass spectrometry. However, CAD operates by detecting *charged particles* that have a selected range of mobility rather than by measuring individual *gas phase*

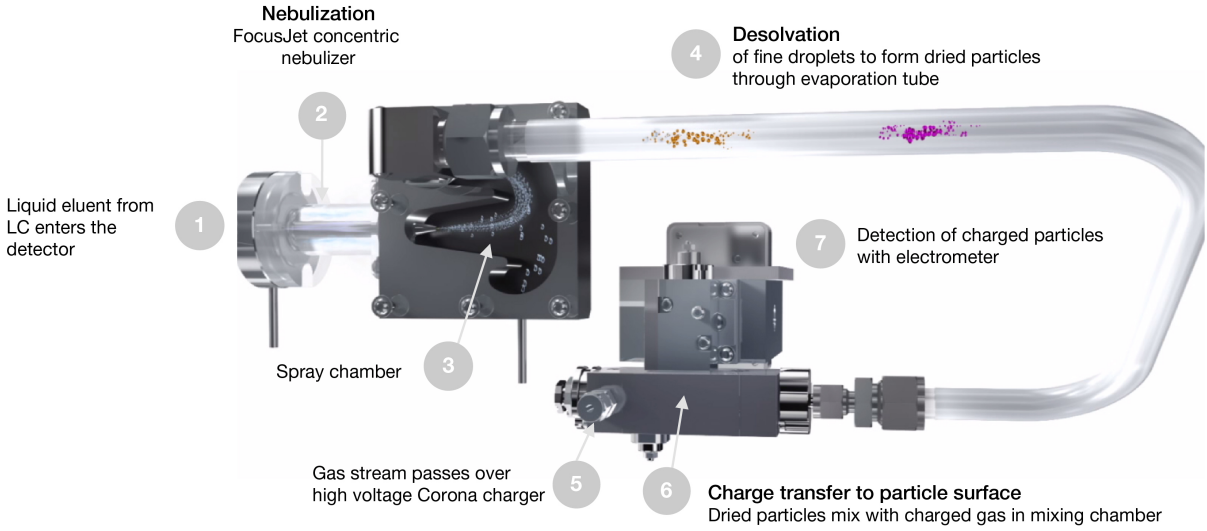


Figure 7.20 Corona charged aerosol detector (CAD) schematic.

ions that are differentiated based upon their *m/z* ratios. Thus, this more generic principle can theoretically provide more consistent interanalyte response factors and complement atmospheric pressure ionization MS such as electrospray and APCI. Like ELSD, CAD is a mass flow-dependent detector. This results in an equal response for equal mass amounts, independent of chemical structure. Only a slight variation of the response for equal amounts of compounds analyzed was observed by Gamache et al. [99] over a set of 17 chemically diverse compounds under isocratic elution conditions. This variation was approximately 8% RSD, which indicates that CAD response depends upon analyte volatility. In a more recent investigation, 10.7% RSD variation was observed when measuring the CAD response among 24 different organic compounds in a flow injection experiment. Both studies reveal a much lower response variation for CAD in comparison to ELSD for the analytes investigated.

Charged aerosol detection is nonlinear as the amount of analyte being measured depends on the charge that can be placed on the surface of the particle. This, in turn, is dependent upon the volume of the particle being formed. The relationship between particle surface area and volume is not linear. This is observed experimentally as the response curves for the Corona charged aerosol detector are typically expressed as the second-order polynomial function over a range of up to four orders of magnitude. However, over a narrow concentration range, good linearity of the CAD response is observed with sufficient accuracy. The application of power function can correct for this physical characteristic and deliver a more linear response over a larger dynamic range [100]. According to Eq. (7.6), a power function is a simple mathematic transformation where the individual data points are raised to a preset value.

$$y = \mathrm{k} \cdot c^{\mathrm{x}} \tag{7.6}$$

y	Output response signal
k	Constant
c	Concentration (for CAD it is mass)
x	Exponent (1.0–2.0)

The possible values are between 1.0 and 2.0 for the Thermo Scientific™ Dionex™ Corona™ ultra RS™ detector. The power function minimizes response factor changes with peak height,

so that the area percent value is more representative of the mass percent value. The power function value (PFV) can vary depending on the chemistry in use and the mass range under investigation. While its primary function is to increase linear range, other benefits may result from a power function, including an improved signal-to-noise ratio, reduced peak width, and better resolution [101].

A weakness of all detectors employing a nebulization process is that the droplet diameter is dependent on the composition of the mobile phase. Isocratic analyses are unaffected by this phenomenon, but in gradient elution the response factor will vary significantly with the mobile-phase composition. Higher organic content in the mobile phase leads to greater transport efficiency of the nebulizer, which results in a larger number of particles reaching the detector chamber. Gorecki et al. [102] proposed a very elegant solution to that problem by providing the detector with a constant composition of the mobile phase. In this method, a secondary stream of the mobile phase of exactly reverse composition is provided by a second pump and is added to the column effluent to ensure a constant mobile-phase composition at the detector inlet. This results in a constant response, independent of the mobile-phase composition in the separator column. This concept can be implemented with and without a delay column in the second liquid branch. Good results are obtained without such a compensation column; however, the system delay volume then must be measured. Figure 7.21 illustrates the signal increase in gradient mode with charged aerosol detection (upper chromatogram) and its compensation by applying an inverse gradient (bottom chromatogram), taking five organic compounds as an example.

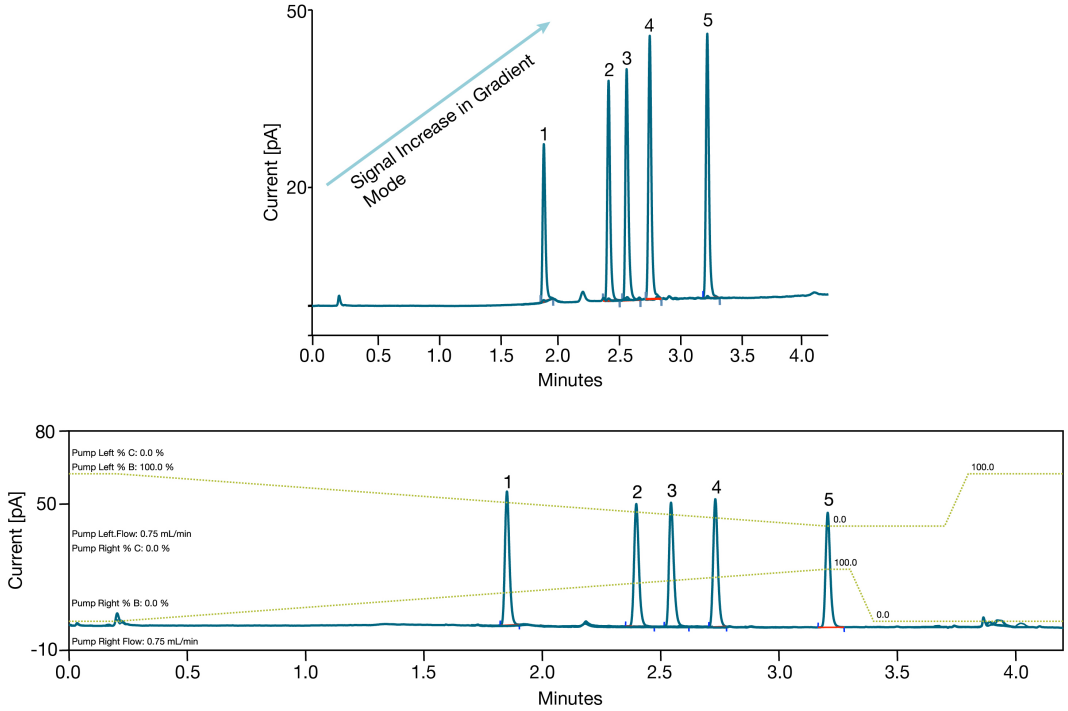


Figure 7.21 Signal increase in gradient mode with charged aerosol detection (upper chromatogram) and tandem LC results with an inverse gradient (bottom chromatogram). Separator column: Acclaim PA2, 2.2 µm; column dimensions: 50 mm × 2.1 mm i.d.; column temperature: 45 °C; eluent: (A) 98:2 (*v/v*) MeCN/20 mmol/L ammonium acetate, pH 4.5, (B) 94:4:2 (*v/v*) MeCN/IPA/20 mmol/L ammonium acetate, pH 4.5; gradient: 0–100% B in 3 min, inverse gradient profile: see bottom chromatogram; flow rate: 0.75 mL/min; detection: CAD; peaks: (1) primidone, (2) hydrocortisone, (3) ketoprofen, (4) warfarin, and (5) progesterone.

7.4
Other detection methods

7.4.1
Radioactivity monitoring

The combination of an ion chromatographic separation with a radioactivity monitor (type LB505, Berthold, Wildbad, Germany) for the analysis of radiostrontium was described by Stadlbauer et al. [103]. The objective of their study was the development of a method for the simple separation of the fission products Sr-90 and Sr-89 from other radionuclides such as barium (Ba-133), which, like Cs-137, is a companion of Sr-90/Sr-89 and is formed with a similar yield (about 6%) in a nuclear reactor. Stadlbauer et al. separated these ions on a surface-sulfonated cation exchanger that was connected to a scintillation detector cell. Figure 7.22 shows the chromatogram of a Sr-90 standard that was obtained with this setup. The peak volume in this chromatogram corresponds to 76.4 Bq of Sr-90. Although on-line detection of radio-strontium is only feasible at medium to high activity concentrations, this method provides advantages for process control and self-monitoring of nuclear medical and nuclear power plants. The fractionated collection of the column effluent makes it possible to obtain Sr-90 in pure form from samples that have a high calcium excess or a complex radioactive contamination by various nuclides. It may be determined off-line via a methane flow counter.

7.5
Hyphenated techniques

7.5.1
IC–ICP coupling

In recent years, the coupling of ion chromatography with element-specific detection methods has increasingly gained importance. Element-specific detection is carried out with atomic spectrometric techniques including atomic absorption spectrometry (AAS), atomic emission spectrometry (ICP–OES), as well as the coupling between ICP and mass spectrometry (ICP–MS). The coupling of an ICP with an ion chromatograph is relatively straightforward. It only requires a capillary that connects the separator column outlet with the nebulizer of the ICP instrument. When choosing an eluent for analyte separation, it is important to prevent a high background signal and a quick sooting of the burner by the mobile phase. Today, ICP–OES [104] is one of the most important methods for trace metal analysis; in combination with mass spectrometry [105] it is widely used for ultratrace metal analysis.

The advantage of coupling those techniques with ion chromatography includes the ability to separate and detect metals with different oxidation states. The analytical interest in chemical speciation is based on the fact that the oxidation state of an element determines toxicity, environmental behavior, and biological effects. Chromium(VI), for example, is highly toxic even in very small amounts, whereas chromium(III) compounds are essential for lipid and carbohydrate metabolism. Due to it widespread use in industrial applications such as chromium plating, dye manufacturing, and preservation of wood and leather materials, chromium concentrations in environmental samples are monitored on a routine basis. Both the United States EPA and the European Union have specified maximum admissible chromium concentrations in their respective drinking water directives. As with many other trace elements, chromium (Cr) is typically found in more than one chemical form, each of which with different chemical properties and behavior, such as bioavailability and toxicity.

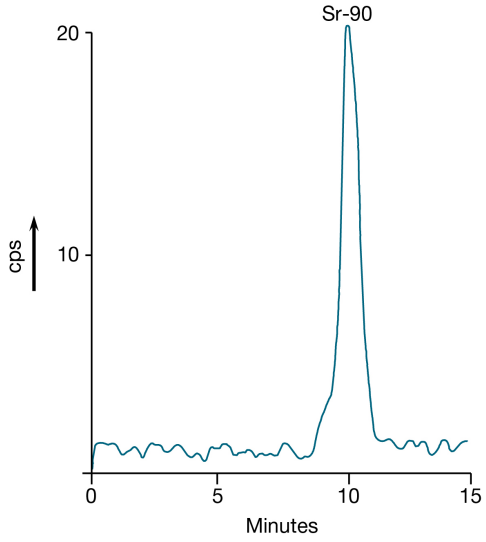


Figure 7.22 Analysis of Sr-90 utilizing a radioactivity monitor. Separator column: Dionex IonPac CS2*; eluent: 30 mol/L HCl + 2 mmol/L histidine hydrochloride; flow rate: 1.5 mL/min; detection: scintillation measurement for Sr-90; injection volume: 50 µL; peak: 76.4 Bq Sr-90 with inactive $SrCl_2$ as a carrier. (*This column is no longer commercially available, but can be replaced by a Dionex IonPac CS12A column, using a methanesulfonic acid eluent.)

The speciation analysis of chromium is a challenging task, since the stability of different chromium species is easily affected by conditions during sample collection and treatment. For example, low pH values may lead to the degradation of chromium(VI) to chromium(III) due to the increased redox potential, while high pH values may lead to the precipitation of chromium(III) as $Cr(OH)_3$. As pH increases, chromium(III) can oxidize to form chromium(VI). When designing sample preparation and analysis methods for determining the Cr(III) and Cr(VI) contents of soil, wastewater, or other samples, the biggest challenge is to ensure that the sample preparation and analysis procedures do not change the distribution of oxidation states in the sample. Because element-specific detection with a simultaneous spectrometer only requires a few seconds, the time aspect has to be considered with respect to the preceding chromatographic separation. A very fast separation of Cr(III)/Cr(VI) is obtained on a 50 mm × 4 mm i.d. nanobead-agglomerated anion exchanger, which is typically used as a guard column. However, its separation power is more than sufficient for separating these two species. Oxy metal anions are retained on this stationary phase and thus separated from metal cations, which elute as one peak in the system void. In this way, it is possible to simultaneously determine metal cations and oxy metal anions. The separation of Cr(III)/Cr(VI) in Figure 7.23 was obtained with 0.1 mol/L nitric acid as an eluent. Acidic eluents have proved to be especially suitable because the concentration ratio between Cr(III) and Cr(VI) stays constant over several days when the samples to be analyzed are acidified. Thus, dilution is the only sample preparation required when using a nitric acid eluent. In addition, the solutes are present in nitric acid after their chromatographic separation, which is of great advantage for the subsequent atomic spectrometric detection. For the separation shown in Figure 7.23 a Thermo Scientific iCAP Qc ICP–MS was used as an element-specific detection system, which was equipped with a Peltier cooled PFA chamber and a PFA-LC nebulizer. For interference-free detection of ^{52}Cr and ^{53}Cr, all measurements were carried out in a single collision cell mode, with kinetic energy discrimination, using pure helium in the collision cell. Under those conditions, detection limits (LODs) are 0.20 ng/kg for Cr(VI) and 0.38 ng/kg for Cr(III), calculated from a linear calibration between 0.75 and 15 µg/kg of each species.

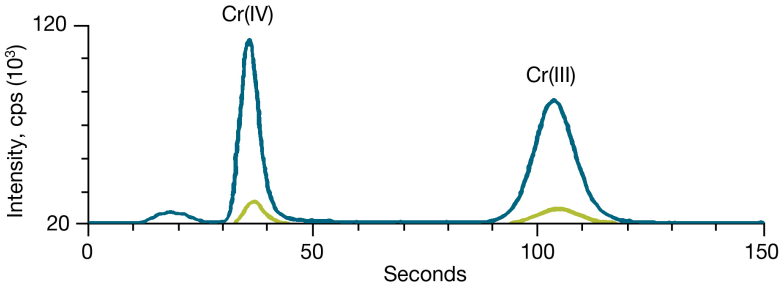


Figure 7.23 Separation of Cr(III) and Cr(VI) with element-specific detection (ICP–MS). Separator column: Dionex IonPac AG7; column dimensions: 50 mm × 2 mm i.d.; eluent: 0.4 mol/L HNO_3; flow rate: 0.4 mL/min; detection: ICP–MS (Thermo Scientific iCAP Qc ICP–MS); injection volume: 20 µL; peaks: 5 µg/kg each of Cr(III) and Cr(VI).

Another important example for the benefit of IC–ICP/OES or IC–ICP/MS is the speciation of arsenic [106]. Many arsenic compounds are present in environmental and biological systems. Today, it is widely accepted that the determination of the total arsenic concentration alone is insufficient because the toxicity of arsenic is dependent on the chemical species being present. While the inorganic forms of arsenic—As(III) and As(V)—are highly toxic, the organic forms are not considered to be toxic. Because the human body metabolizes inorganic arsenic by reductive methylation, all the reaction products must be analyzed. Body fluids such as urine have a high chloride content; therefore, interferences are observed due to the formation of the argon chloride ion with identical mass to that of elemental As. The polyatomic species $^{40}Ar^{35}Cl^+$ and $^{38}Ar^{37}Cl^+$, both having a nominal *m/z* 75, can interfere with the detection of monoisotopic $^{75}As^+$ using ICP as an element-specific detection technique. This interference can be eliminated when coupling ICP with a high-capacity anion exchanger, using an eluent system capable of separating chloride from the arsenic compounds of interest such as ammonium carbonate [107]. Ammonium salts are especially useful as eluents for this application as they have shown very little drift over a long period of time. Figure 7.24a shows the analysis of a human urine sample containing a relatively high concentration of 400 µg/L arsenate, which the patient incorporated orally by drinking contaminated well water. Despite the high chloride concentration, neither overloading effects nor other interferences are observed. Figure 7.24b reveals that after 1 week the amount of excreted arsenic has decreased to 1/50 of the original value. ICP–MS is usually the only detection method that can achieve such detection limits (low microgram/liter range) for methylarsonic acid, dimethylarsinic acid, and arsenobetain in body fluids.

IC–ICP/MS can also be applied for the speciation of iodine. The source of iodine for humans is mainly food and drinking water. Investigations on the speciation of iodine in food are mainly focused on milk and seafood (fish and seaweed) because of the importance of milk iodine to humans, especially to infants, and the high concentration of iodine in these types of foods. In addition, lack of iodine supplementation to newborns can result in slow brain development, which makes iodine speciation in human milk an important task. The observation of iodine toxicity mainly focuses on iodide or iodate, which is present in iodized table salt used in many countries as an iodine supplement. The bioavailability and toxicity of iodine, like other essential elements, is species dependent. Inorganic iodide and iodate are less toxic than molecular iodine and some organically bound forms of the element. A representative chromatogram illustrating the separation of iodate and iodide with ICP–MS detection in approximately 10 min is shown in Figure 7.25. This separation has been carried out on an ExcelPak ICS-A23 anion exchanger (Yokogawa, Japan) with an ammonium carbonate eluent. Alternatively, A Dionex IonPac AS16

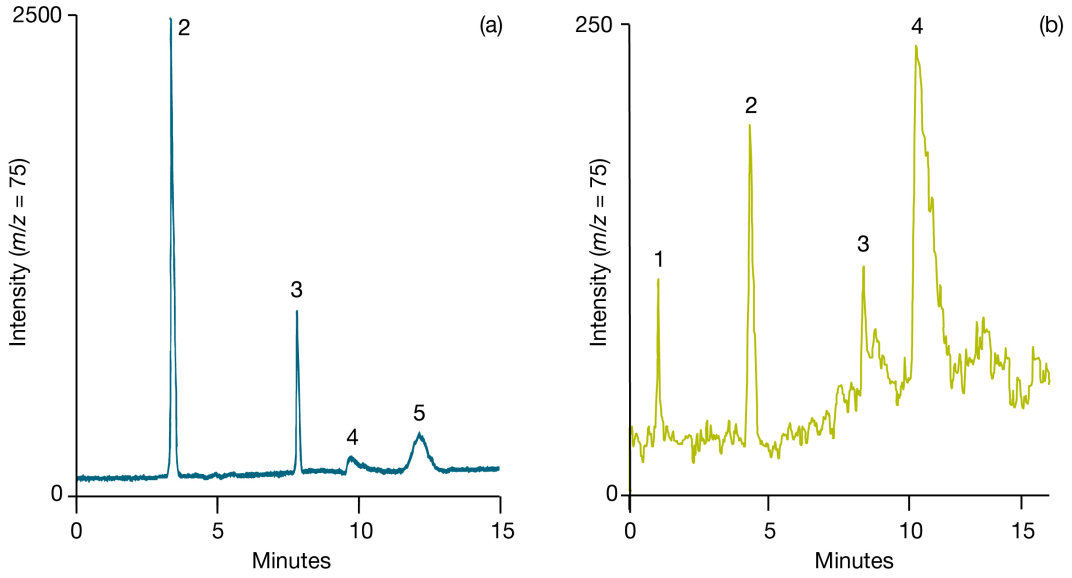


Figure 7.24 Simultaneous determination of various arsenic species in human urine with element-specific detection. Separator column: Dionex IonPac AS7; eluent: NaOH/Na_2CO_3 gradient; flow rate: 1 mL/min; injection volume: 100 µL; suppressor: AMMS-II; regenerant: 12.5 mmol/L H_2SO_4; detection: ICP–OES (measuring wavelength: 193.7 nm); peaks: (1) arsenobetain, (2) methylarsonic acid, (3) dimethylarsinic acid, (4) chloride, and (5) arsenate; (a) after oral incorporation of a contaminated well water, (b) after 1 week; (see Ref. [108]).

column can be used for this separation, which was especially designed for the rapid elution of polarizable anions such as iodide. However, eluent conditions must be modified in this case. Because iodide is unstable in both deionized water and the eluent, 0.01% KOH must be used as the storage medium for field sampling. Under the chromatographic conditions used in Figure 7.25 the linear range for both species is more than four orders of magnitude from 5 nmol/L to 50 µmol/L.

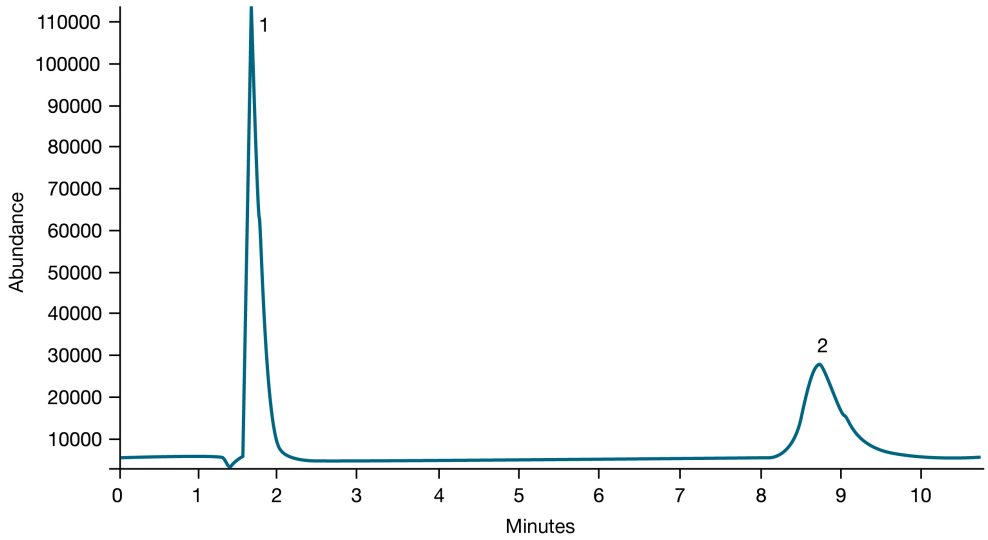


Figure 7.25 Separation of iodate and iodide with element-specific detection. Separator column: ExcelPak ICS-A23; eluent: 30 mmol/L ammonium carbonate, pH 9.4; flow rate: 1.5 mL/min; detection: ICP–MS; peaks: 50 nmol/L iodate (1) and iodide (2) (courtesy of Agilent Technologies).

7.5.2
IC–MS coupling

The second important hyphenation is the coupling of an ion chromatograph to a mass spectrometer; this combination provides the analyst with information on analyte structure and molecular weight. While mass-selective detection in gas chromatography became routine many years ago, the coupling of a liquid chromatograph to a mass spectrometer used to be problematic because the relatively large amounts of liquid mobile phase were not compatible with the high vacuum in the ion source of a mass spectrometer. This incompatibility problem has been solved with various types of LC–MS interfaces; the electrospray interface is used exclusively today. The most important characteristic of an LC–MS interface is the transfer of analyte molecules from the separator column into the high vacuum of a mass spectrometer. This means that an analyte molecule dissolved in the mobile phase must make the transition to an analyte molecule isolated in the gas phase. To achieve high sensitivity, as many analyte molecules as possible have to be transferred while the mobile-phase constituents have to be largely removed. The maximum amount of liquid that can be introduced into the high vacuum of a mass spectrometer without increasing pressure in the ion source depends solely on the capacity of the vacuum pumps. Split techniques as well as microbore columns with a small inner diameter of 2 mm and low flow rates of 0.25 mL/min help to significantly decrease the amount of liquid entering the ion source of a mass spectrometer.

An additional problem is the mobile phase that is used for separating ionic species on ion exchangers. Such separations cannot be performed without electrolytes. Therefore, either volatile eluents such as ammonium sulfate, which can be pumped out of the ion source, or continuously regenerated suppressor systems must be used, with which eluents can be converted to water prior to entering the MS interface. Meanwhile, many ion exchangers for anion and cation chromatography are available that allow anions and cations to be eluted efficiently and with high resolution using potassium hydroxide or methanesulfonic acid eluents. As early as 1990, Simson et al. [109] successfully employed a suppressor system for desalting eluents. They placed it between an anion exchanger and the MS interface and determined a residual sodium concentration of 13–30 µmol/L when pumping an eluent concentration of 10–100 mmol/L.

Electrospray interface Electrospray ionization (ESI) [110] is an atmospheric-pressure ionization technique (API) that allows the transfer of ions from solution to the gas phase, from which the ions can be subjected to mass spectrometric analysis. The transfer of ions from solution to the gas phase is a strong endothermic process because in solution the ion is interacting with solvent molecules that form a solvation sphere around the ion. The energy required to transfer an ion from an aqueous solution to the gas phase is larger than the energy required to break a carbon C–C bond. If that energy is supplied at once over a short period of time, the act of freeing an ion from the solvent can lead to fragmentation. The softness of the ion-transfer method can be defined as the degree to which fragmentation of the ions is avoided. From that standpoint, ESI–MS is the softest technique available.

There are three major steps in the production of gas-phase ions from electrolytic ions in solution by electrospray:

- Production of charged droplets at the electrospray capillary tip.
- Shrinkage of the charged droplets by solvent evaporation and repeated droplet dis-integrations, ultimately leading to very small, highly charged droplets capable of producing gas-phase ions.
- The actual mechanism by which gas-phase ions are produced from the very small and highly charged droplets.

As shown in the schematic representation in Figure 7.26, a voltage of 2–3 kV is applied to the metal spray capillary (needle), which typically has an inner diameter of 0.1 mm and is located 1–3 cm from the counter electrode. In ESI–MS, this counter electrode may be a plate with an orifice leading to the mass spectrometric sampling system. Because the capillary tip is very thin, the electric field in the air at the tip is very high ($E_c \approx 10^6$ V/m). Considering a typical solution present in the capillary with methanol as the solvent and a low concentration (10^{-5}–10^{-3} mol/L) of an organic base as the salt, the field E_c, when turned on, will penetrate the solution at the capillary tip. The positive and negative electrolyte ions in the solution will move under the influence of the electric field until a charge distribution results that counteracts the imposed field and leads to essentially field-free conditions inside the solution. When the capillary is the positive electrode, positive ions will drift toward the meniscus of the liquid, and negative ions will drift away from the surface. The mutual repulsion of the positive ions at the surface overcomes the surface tension of the liquid and the surface begins to expand, allowing the positive charges and liquid to move downfield. A so-called Taylor cone is formed, and if the applied field is sufficiently high, a fine jet emerges from the cone tip, which breaks up into small charged droplets. The charged droplets produced by the spray then shrink, owing to the solvent evaporation while the charge remains constant. The energy required for solvent evaporation is provided by the thermal energy of the ambient air. The charge of the droplet is expected to remain constant because the emission of ions from the solution to the gas phase is highly endoergic. The decrease of the droplet radius R at constant droplet charge q leads to an increase of the electrostatic repulsion of the charges at the surface until the droplets reach the Rayleigh stability limit:

$$q_{Ry} = 8\,\pi\,(\varepsilon_0\,\gamma\,R^3)^{1/2} \tag{7.7}$$

The Rayleigh equation describes the condition at which the electrostatic repulsion becomes equal to the force due to the surface tension γ, which holds the droplet together. The charged droplet becomes unstable when its radius R and charge q satisfy Eq. (7.7). In a very complex process that is not yet completely understood, droplets undergo fission when they are close to the Rayleigh limit. The fragmentation is generally referred to as coulombic fission. More detailed information on the mechanism of ESI–MS has been published by Kebarle and Ho [111].

The position of the spray capillary relative to the ion sampling orifice within the sampling plate is of significant importance. By avoiding directly spraying at the sampling plate orifice, the instance of charged droplets being sampled (as opposed to gas-phase ions) is lowered, hence

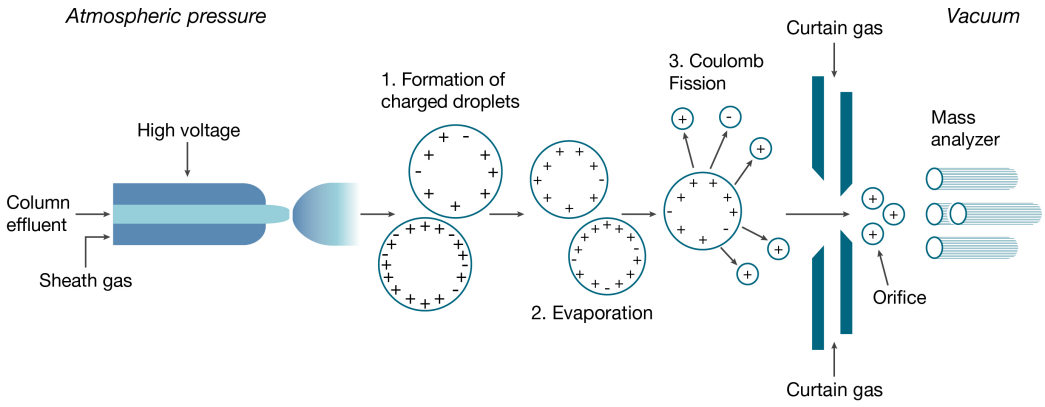


Figure 7.26 Schematic diagram of the ionization process in electrospray ionization ("IonSpray", Perkin-Elmer Sciex).

increasing instrument response. In most modern MS instruments the sprayer position may be adjusted in one, two, or three axes that will allow optimization relative to the sampling orifice. By moving to an off-axis position, the ion signal observed by the mass spectrometer is more stable and at least as high as in the on-axis position. It is desirable to take ions, but no droplets into the mass analyzer. Charged droplets transported into the mass spectrometer may impinge on the ion optics or on the mass analyzer, creating spikes in the mass spectra. Off-axis positioning can be taken a step further by placing the sprayer in a diagonal position inside the source. The spray is not aimed at the sampling orifice, but at a position beyond, in order to reduce the chance of shooting droplets into the mass spectrometer. Diagonal positioning is most effective for pneumatically assisted electrospray. Optimization of the sprayer position and the capillary voltage are interrelated and should be optimized empirically together. The most important practical advance in sprayer positioning has come with the introduction of orthogonal source designs, where the sprayer is positioned orthogonally to the sampling orifice. This design has several advantages, including sampling of fewer charged droplets relative to ions and the ability to tolerate higher flow rates.

Collision induced dissociation Classical API spectra tend to show very little fragmentation due to the "soft" nature of the ionization processes, i.e., during the formation of ions, the analyte ions do not receive enough energy to break the intramolecular bonds. However, some fragmentation may easily be induced in one of the higher-pressure regions, so that some structural information may be gathered. Acceleration of ions between the orifice and the skimmer, or between the skimmer and the RF-only multipole, results in collision of ions with the background gas. This process is known by various names depending upon the instrument manufacturer and includes "in-source collision-induced dissociation" (CID), "nozzle-skimmer fragmentation", "cone-voltage fragmentation", and so on. By increasing the potential difference between the skimmer and the quadrupole, or between the nozzle and the skimmer, the energy imparted to the analyte molecule through increasing frequency and collision energy can be high enough to cause intra-molecular bonds to be broken and for fragmentation to occur. In the case of larger molecules such as peptides and proteins, the excess energy can often be absorbed in several vibrational modes, and high potential differences are required to fragment these kinds of molecules. The major advantage of this technique for the production of spectra containing a greater amount of structural information is its simplicity. One voltage needs to be adjusted and there is no need for switching or adjustment of collision gases, nor any retuning of ion optics. In negative ion mode, for instance, the degree of collision-induced dissociation (CID) can be estimated using the drug naproxen that normally only shows the $[M–H]^-$ ion at *m/z* 229. With small nozzle–skimmer potential differences, the naproxen molecule readily loses CO_2, giving rise to a peak at *m/z* 189.

Collision-induced dissociation can also be used to improve baseline noise and increase signal-to-noise ratios in LC–MS. When ions pass through the sampling orifice into the vacuum region, the background density of neutral gas ions falls rapidly. If ions moving in a low-density gas are accelerated by the nozzle–skimmer region, mild CID may be affected, which will be enough to cleave the hydrogen bonds inside the ion–water or ion–neutral gas clusters. In addition, heating of the ion clusters, which occurs during collisions, will also aid desolvation of the cluster ions. Moderate acceleration of clusters is effective and widely used to decluster ions.

Considerations in IC–ESI/MS instrumentation A typical IC–MS system consists of an ion chromatographic system, a mass spectrometer, and a delivery system for the addition of a desolvation solution (usually an AXP-MS pump and a static mixing tee). Figure 7.27 shows the

configuration of a preferred IC–MS system setup. A standard microbore IC system is recommended for routine MS detection because it has a flow rate range from 0.1 to 0.5 mL/min that is within the preferred flow characteristics of most electrospray ionization sources. In principle, higher flow rates (used for 4 mm columns) are feasible, but the excessive flow ends up diverted to waste, with no benefit of sensitivity enhancement. Therefore, 2 mm columns and consumables are recommended for MS applications. Reagent-free ion chromatography (RFIC) systems are the preferred systems for the delivery of a highly reproducible mobile phase that is electrolytically generated from an eluent generator. An electrolytically operated suppressor is used to continuously convert the high-ionic strength eluent to deionized water prior to entering the conductivity and MS detectors by exchanging the eluent counter ions for hydronium or hydroxide ions. Because the eluent is not recoverable in MS, the suppressor must be operated in external water mode, with deionized water as the regenerant delivered by an auxiliary pump. To enhance desolvation, the effluent from the conductivity cell is combined with an organic desolvation solvent and delivered by another pump through a static mixing tee and a grounded union prior to entering the electrospray ionization source of the MS detector. The grounding union between the conductivity cell and the high-voltage potential prevents possible current backstreaming in case the connection tubing becomes filled with a conductive solution. An additional back pressure of 30–40 psi is recommended for the proper functioning of the suppressor and conductivity cell to prevent eluent in the cell from outgassing due to abrupt volume changes. However, excessive back pressure may cause adverse effects such as peak tailing or peak broadening and a decrease in resolution due to the expanded flow path. It also may cause irreversible damage to the suppressor hardware. The current Dionex DRS 600 suppressors should be operated at back pressures <100 psi (0.69 MPa) for IC–MS applications; back pressures >450 psi (3.10 MPa) are not recommended.

Because ion-exchange separations are essentially carried out in 100% aqueous media, they require a higher nebulizer gas volume and higher temperatures for desolvation. Therefore, an organic solvent is usually added for IC–MS applications to the column effluent prior to entering the ion source of the MS detector. Different solvents may be used for this purpose, including acetonitrile, methanol, or 2-propanol. The best signal improvements are always observed with

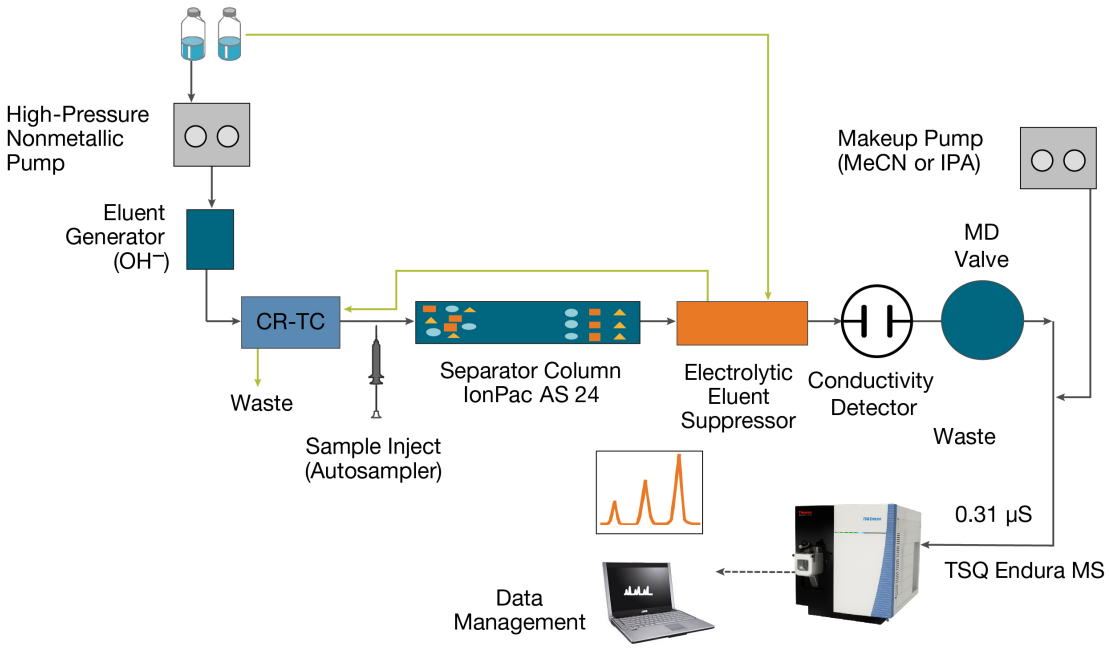


Figure 7.27 Schematic diagram of a Reagent-Free IC–MS system.

100% organic solvent. While acetonitrile demonstrates the best improvements for inorganic anions and organic acids, 2-propanol is very well suited for cation applications. As shown with the MS spectra of lithium in Figure 7.28, 2-propanol as the desolvation solvent provides the cleanest adduct MS spectrum with the best intensity for the main adduct. Its use improves the desolvation/ionization process and the detection sensitivity, forming adducts that facilitate the detection of analyte ions with an extremely low nominal mass. Lithium ions, for instance, are singly charged species with *m/z* 7, which is below the lowest calibrated mass of any MS detector. With the addition of 2-propanol (IPA), $[Li+nIPA]^+$ clusters are observed, that is, $[Li+2IPA]^+$, $[Li+3IPA]^+$, and $[Li+5IPA]^+$, with *m/z* 127, 187, and 307, respectively, which are within the calibrated mass range.

Another consideration that is important for IC–MS configurations is the dimension of the inner diameter and length of the connecting tubing between the ion chromatograph and the MS detector, which must be minimized to reduce the extra-column volume. However, the total additional back pressure should not exceed 300 psi (2.07 MPa).

8
Applications

The analytical method known as ion chromatography is suited for the analysis of a variety of inorganic and organic anions and cations. It is characterized by a high selectivity and sensitivity. The selected applications portrayed below exemplify the versatility of this method.

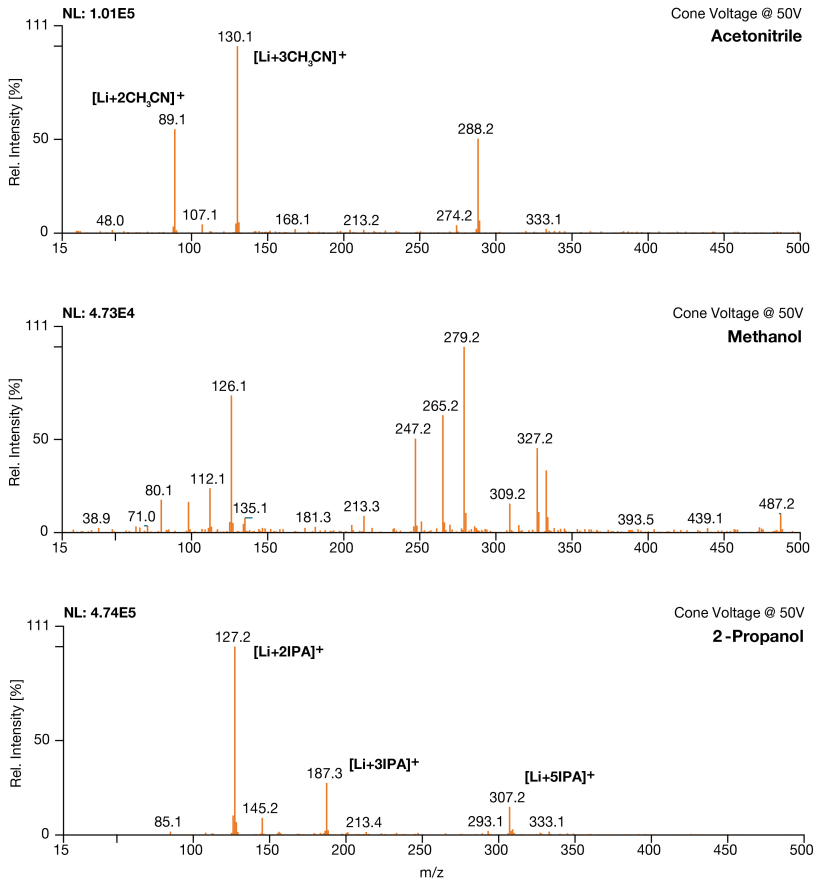


Figure 7.28 MS spectra of Li with different organic desolvation solvents.

Ion chromatography has become an indispensable tool for the analytical chemist, especially in the area of anion analysis. In many cases, this method has replaced conventional wet chemical methods such as titration, photometry, gravimetry, turbidimetry, and colorimetry, all of which are labor-intensive, time-consuming, and occasionally susceptible to interferences. Numerous publications have shown that results obtained by ion chromatographic methods are comparable to those of conventional analytical methods, thus completely dissolving the skepticism with which ion chromatography was initially met. In the field of cation analysis, it is attractive because of its simultaneous detection of inorganic and organic cations, its sensitivity, and its applicability for speciation analysis. It provides a welcome complement to atomic spectrometric methods such as AAS and ICP.

The emphasis of ion chromatographic applications is mostly on the following areas:

- Environmental analysis
- Power plant chemistry
- Semiconductor industry
- Household products and detergents
- Food and beverages
- Electroplating
- Metal processing
- Pharmaceuticals
- Biotechnology
- Mining
- Agriculture
- Pulp and paper industry

The separation and detection methods employed in ion chromatography, as well as a selection of ionic species that may be analyzed with this method, are summarized in Tables 8.1 and 8.2.

8.1 Environmental applications

Conventional water analysis The main focus of applications in environmental analytical chemistry is the qualitative and quantitative analysis of anions and cations in all kinds of water. Using ion chromatography, standard anions or cations can all be separated and determined today in less than 10 min. The high sensitivity of this method (detection limit with a direct injection of 25 µL of sample: ~2–5 µg/L) and the potential for automation were two features that helped ion chromatography rapidly become a widely used analytical tool.

The determination of common inorganic anions in *drinking water* is one of the most important applications of ion chromatography worldwide. IC has been approved for compliance monitoring of inorganic anions in US drinking water since the mid-1980s, as described in US EPA Method 300.0 [4]. In addition, other standards organizations, including the American Society for Testing and Materials (ASTM) [112], American Water Works Association (AWWA) [113], and International Organization for Standardization (ISO) [114] have validated IC methods for the determination of inorganic anions in drinking water. In the original EPA Methods 300.0 and 300.1 (Part A), the Dionex IonPac AS4A-SC and Dionex IonPac AS9-HC anion exchangers were specified. As advancements in column technology continued, new carbonate- and hydroxide-selective columns were proposed, such as the carbonate-selective Dionex IonPac AS22 or the hydroxide-selective Dionex IonPac AS18 anion exchangers.

Inorganic anion analysis in water samples can be performed in two different ways:

- Traditionally, by employing carbonate/bicarbonate mobile phases on carbonate-selective stationary phases.
- By employing electrolytically generated hydroxide eluents (Reagent-Free Ion Chromatography) on hydroxide-selective stationary phases.

Table 8.1 Survey of separation techniques used in ion chromatography.

Separation technique	Mechanism	Resin functionality	Recommended Eluents	Species to be analyzed
HPIC anions	Ion exchange	$–NR_3^+$	Na_2CO_3/$NaHCO_3$ KOH	$F^–$, $Cl^–$, $Br^–$, $I^–$, $SCN^–$, $CN^–$ $H_2PO_2^–$, $HPO_3^{2–}$, $HPO_4^{2–}$, $P_2O_7^{4–}$, $P_3O_{10}^{5–}$, $PO_3F^{2–}$, $CN^–$, $OCN^–$, $NO_2^–$, $NO_3^–$, $N_2O_2^{2–}$, $N_2O_3^{2–}$, $N_3^–$, $S^{2–}$, $SO_3^{2–}$, $SO_4^{2–}$, $S_2O_3^{2–}$, $SCN^–$, $S_2O_6^{2–}$, $S_2O_8^{2–}$, $OCl^–$, $ClO_2^–$, $ClO_3^–$, $ClO_4^–$, $BrO_4^–$, $IO_3^–$, $SiO_3^{2–}$, $SiF_6^{2–}$, $SeO_3^{2–}$, $SeO_4^{2–}$, $AsO_2^–$, $HAsO_3^{2–}$, $WO_4^{2–}$, $MoO_4^{2–}$, $CrO_4^{2–}$, carbohydrates, peptides, proteins, and so on
HPIC cations	Ion exchange	$–SO_3^–$	MSA[a)] H_2SO_4	Li^+, Na^+, NH_4^+, K^+, Rb^+, Cs^+, Mg^{2+}, Ca^{2+}, Sr^{2+}, Ba^{2+}, small aliphatic amines
		$–SO_3^–$ / $–NR_3^+$	PDCA[b)] Oxalic acid	Fe^{2+}, Fe^{3+}, Cu^{2+}, Ni^{2+}, Zn^{2+}, Co^{2+}, Pb^{2+}, Mn^{2+}, Cd^{2+}, Al^{3+}, Ga^{3+}, V^{4+}, V^{5+}, UO_2^{2+}, lanthanides
HPICE	Ion exclusion	$–SO_3^–$	HCl Octanesulfonic acid Perfluorobutyric acid	Aliphatic carboxylic acids, borate, orthosilicate, carbonate, alcohols, glycols, aldehydes
MPIC anions	Ion-pair formation	neutral	NH_4OH TMAOH[c)] TPAOH[d)] TBAOH[e)]	In addition to the anions listed under HPIC: anionic surfactants, metal–cyanide complexes, aromatic carboxylic acids
MPIC cations	Ion-pair formation	neutral	HCl Hexanesulfonic acid Octanesulfonic acid	In addition to the cations listed under HPIC: aliphatic and aromatic amines, quaternary ammonium compounds, cationic surfactants, sulfonium compounds, phosphonium compounds, hydrazinium compounds

a) MSA Methanesulfonic acid
b) PDCA Pyridine-2,6-dicarboxylic acid
c) TMAOH Tetramethylammonium hydroxide
d) TPAOH Tetrapropylammonium hydroxide
e) TBAOH Tetrabutylammonium hydroxide

Carbonate/bicarbonate mobile phases are recommended when an isocratic separation of the analyte anions is sufficient. In case a gradient separation – or the option for it – is required, hydroxide eluents are by far better suited. Moreover, hydroxide eluents can be used for isocratic *and* gradient elution, but optimal performance (high reproducibility and low baseline drift) can only be achieved by using an eluent generator (see Section 3.3). Figure 8.1a shows a typical example of anionic constituents in the municipal drinking water of the city of San Jose, CA, USA, at ambient temperature on the hyperbranched Dionex IonPac AS22 column. Under isocratic conditions, all seven anions were separated and eluted within 13 min. If a faster separation is required, a Dionex IonPac AS22-Fast-4µm column can be used, which has been

Table 8.2 Survey of the detection methods used in ion chromatography.

Detection method	Principle	Applications
Conductivity	Electrical conductivity	Anions and cations with pK_a or pK_b < 7
Amperometry	Oxidation or reduction on Ag-/Pt-/Au-/Glassy Carbon- and Carbon Paste electrodes	Anions and cations with pK_a or pK_b > 7
Charge	Response to ionic species by drawing a current to a fixed potential	Weakly dissociated anions and cations
UV/Vis detection with or without postcolumn derivatization	UV/Vis light absorption	UV-active anions and cations, transition metals after reaction with PAR, aluminum after reaction with Tiron, lanthanides after reaction with Arsenazo I, polyvalent anions after reaction with iron(III), orthosilicate and orthophosphate after reaction with molybdate
Fluorescence in combination with postcolumn derivatization	Excitation and emission	Ammonium, amino acids, and primary amines after reaction with OPA
Refractive index	Change in refractive index	Anions and cations at higher concentrations
Evaporative light scattering	Measurement of light scattered by particles	All nonvolatile species, simultaneous detection of anions and cations
Charged aerosol detection	Measurement of charge transferred from N_2 to particles	All nonvolatile species, simultaneous detection of anions and cations
Radioactivity	Scintillation measurement	Radiostrontium analysis
ICP–OES, ICP–MS	Atomic emission	Hyphenation technique for selective and sensitive transition metal analysis
MS	Electrospray ionization	Hyphenation technique for structural elucidation of inorganic and organic anions and cations

designed for fast separations using a carbonate/bicarbonate eluent. The corresponding chromatographic result of another municipal water sample illustrated in Figure 8.1b demonstrates the separation of milligram/liter concentrations of the anionic constituents in less than 5 min, which reduces the cycle time, increases the sample throughput, and minimizes the overall cost.

Fast separations of common inorganic anions in drinking water are also possible with the Dionex IonPac AS18-4µm column, which offers the benefit of increased peak efficiencies and better resolution in comparison with the standard 7.5 µm Dionex IonPac AS18 column, even in a short column format and at higher flow rates. The Dionex IonPac AS18-4µm column is ideal for use with hydroxide eluents under isocratic conditions, but its high anion-exchange capacity also allows the use of hydroxide gradients. Figure 8.2 shows the excellent resolution of the common inorganic anions in a drinking water sample in less than 5 min using the maximum flow rate of 0.38 mL/min.

The simultaneous analysis of alkali and alkaline-earth metals is another important ion chromatographic application in the field of drinking water analysis. The ASTM [115] and the ISO [116] have validated IC methods for the determination of alkali and alkaline-earth metals in drinking water and wastewater. A corresponding chromatogram in Figure 8.3 shows the

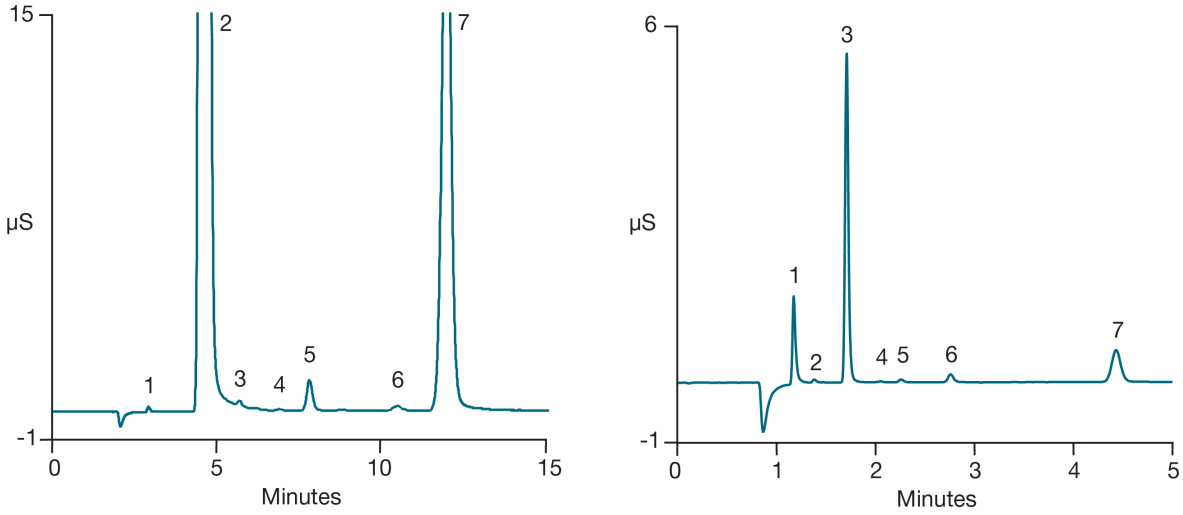


Figure 8.1 Conventional and fast isocratic separation of anions in municipal drinking water. (a) Separator column: Dionex IonPac AS22; column dimensions: 250 mm × 4 mm i.d.; column temperature: ambient; eluent: 4.5 mmol/L Na_2CO_3 + 1.4 mmol/L $NaHCO_3$; flow rate: 1.2 mL/min; detection: suppressed conductivity; injection volume: 25 µL; peaks: 0.19 mg/L fluoride (1), 98.1 mg/L chloride (2), 0.54 mg/L nitrite (3), 1.22 mg/L bromide (4), 2.43 mg/L nitrate (5), 3.12 mg/L orthophosphate (6), and 48.2 mg/L sulfate (7), (b) separator column: Dionex IonPac AS22-Fast-4µm; column dimensions: 250 mm × 4 mm i.d.; column temperature: 30 °C; eluent: 4.5 mmol/L Na_2CO_3 + 1.4 mmol/L $NaHCO_3$; flow rate: 2 mL/min; detection: suppressed conductivity; injection volume: 40 µL; peaks: 0.87 mg/L fluoride (1), formate (2), 3.35 mg/L chloride (3), nitrite (4), 0.14 mg/L chlorate (5), 0.34 mg/L nitrate (6), and 1.42 mg/L sulfate (7).

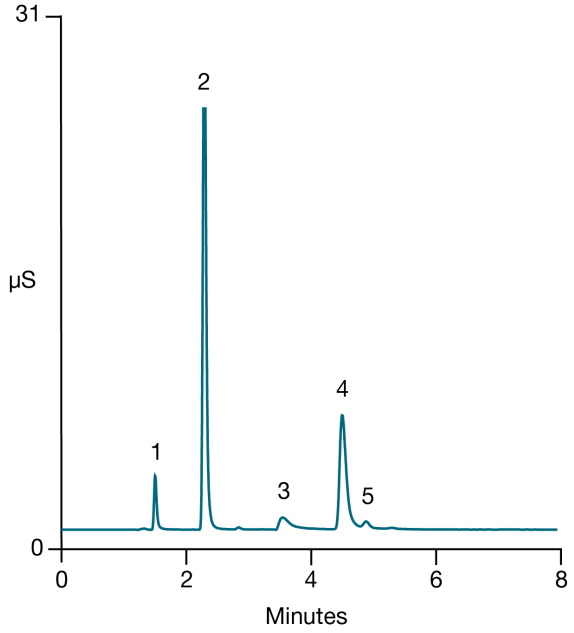


Figure 8.2 Fast IC of anions in municipal drinking water using RFIC. Separator column: Dionex IonPac AS18-4µm; column dimensions: 150 mm × 2 mm i.d.; column temperature: 30 °C; eluent: 23 mmol/L KOH (EG); flow rate: 0.38 mL/min; detection: suppressed conductivity; injection volume: 5 µL; sample: municipal drinking water; peaks: 0.86 mg/L fluoride (1), 18 mg/L chloride (2), carbonate (3), 12 mg/L sulfate (4), and 0.57 mg/L nitrate (5).

separation of sodium, ammonium, potassium, magnesium, and calcium in a municipal drinking water sample on a capillary Dionex IonPac CS12A weak acid cation exchanger using gradient elution and suppressed conductivity detection. All cations of interest were eluted within 12 min. If disparate concentration ratios of sodium and ammonium are to be determined, a high-capacity cation exchanger such as the Dionex IonPac CS16 must be used. The selectivity of this column is optimized for an elevated operating temperature of 40 °C for maximum peak efficiencies. Under isocratic conditions, common inorganic cations and ammonium can be eluted in 20 min. The required acid concentration in the mobile phase, however, requires the use of a suppressor device. Figure 8.4 shows the separation of small amounts of ammonium in the presence of alkali and alkaline-earth metals on a high-capacity Dionex IonPac CS16 analytical column.

The analysis of anions and cations in snow and ice core samples, as well as in other weakly contaminated matrices such as *rainwater*, *groundwater*, and *swimming pool water*, is equally simple. However, the very low electrolyte content in snow and ice core samples sometimes requires the injection of larger sample volumes (up to 200 µL). Apart from the obligatory membrane filtration (0.45 µm), the only sample preparation in the analysis of rainwater, groundwater, and swimming pool water is a dilution with deionized water.

Surface waters are mostly analyzed using ion exchangers with a slightly higher ion-exchange capacity that compensates for the occasionally high electrolyte concentration in such samples. High concentration differences between major and minor components sometimes also require the capability to inject such samples undiluted without overloading the separator column.

The term *wastewater* includes any water that has been adversely affected in quality by anthropogenic influence. Depending on its origin, it comprises a wide range of potential contaminants and concentrations. Wastewater can come from many different sources such as sewage treatment plant discharge, industrial drainage, agricultural drainage, and many others. In some areas, treated wastewater is recycled for irrigation purposes and even as drinking water. This reuse of water is gaining closer scrutiny as demand increases for water resources. Therefore, wastewater discharge monitoring requires careful analysis of analytes such as inorganic anions. Figure 8.5 shows the separation of common inorganic anions in a typical treated wastewater sample that has been injected directly after filtration down to 0.45 µm.

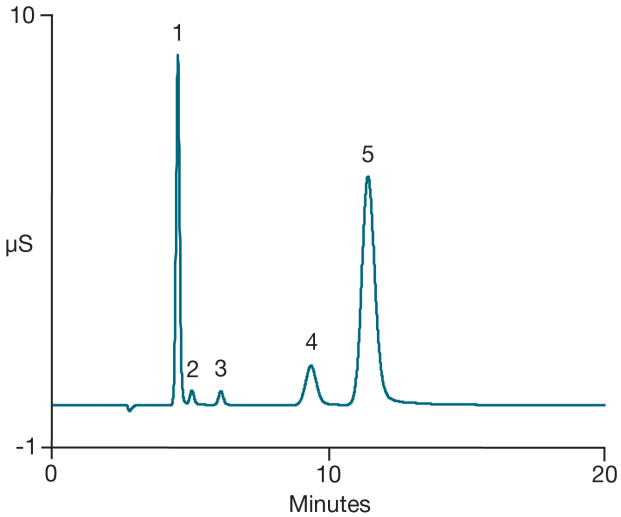


Figure 8.3 Simultaneous analysis of alkali and alkaline-earth metals in drinking water using capillary IC. Separator column: Dionex IonPac CS12A; column dimensions: 250 mm × 0.4 mm i.d.; column temperature: 40 °C; eluent: MSA (EG); gradient: 6–65 mmol/L from 0 to 30 min; flow rate: 10 µL/min; detection: suppressed conductivity; injection volume: 0.4 µL; sample: municipal drinking water (undiluted); peaks: 3.3 mg/L sodium (1), 0.11 mg/L ammonium (2), 0.25 mg/L potassium (3), 0.37 mg/L magnesium (4), and 2.9 mg/L calcium (5).

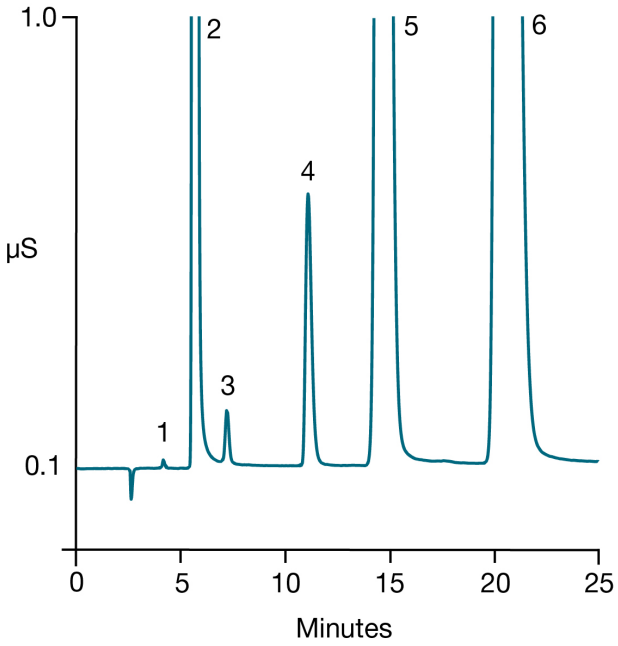


Figure 8.4 Separation of small amounts of ammonium in presence of alkali and alkaline-earth metals in drinking water on a high-capacity weak acid cation exchanger. Separator column: Dionex IonPac CS16; column dimensions: 250 mm × 5 mm i.d.; column temperature: 40 °C; eluent: 30 mmol/L MSA (EG); flow rate: 1 mL/min; detection: suppressed conductivity; injection volume: 25 µL; sample: municipal drinking water (undiluted); peaks: 0.01 mg/L lithium (1), 19.73 mg/L sodium (2), 0.07 mg/L ammonium (3), 0.99 mg/L potassium (4), 7.21 mg/L magnesium (5), and 18.54 mg/L calcium (6).

This application was performed by capillary reagent-free IC using a high-capacity capillary Dionex IonPac AS19 column. Baseline resolution of all analytes of interest was achieved by

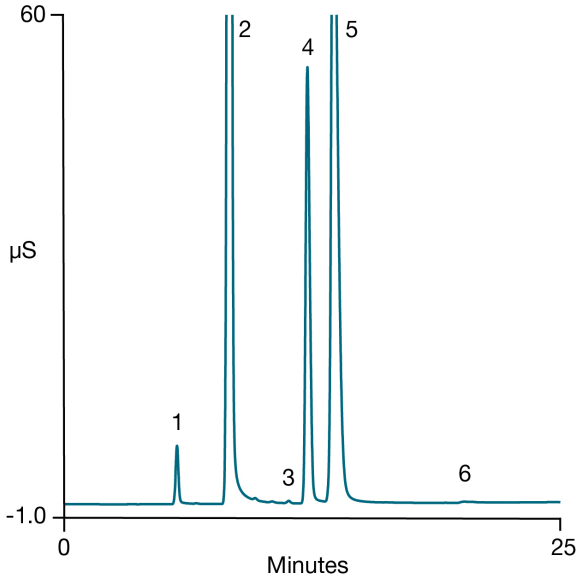


Figure 8.5 Separation of common inorganic anions in a treated wastewater by capillary ion chromatography. Separator column: Dionex IonPac AS19; column dimensions: 250 mm × 0.4 mm i.d.; column temperature: 30 °C; eluent: KOH (EG); gradient: 10–25 mmol/L linearly in 10 min, then to 70 mmol/L in 10 min; flow rate: 10 µL/min; detection: suppressed conductivity; injection volume: 0.4 µL; peaks: 1.8 mg/L fluoride (1), 180.2 mg/L chloride (2), 0.4 mg/L bromide (3), 11.8 mg/L nitrate (4), 96.9 mg/L sulfate (5), and 0.9 mg/L ortho-phosphate (6).

applying a KOH gradient electrolytically generated from deionized water. Analytes were detected by suppressed conductivity with an electrolytically operated suppressor in the capillary format.

The determination of the three nitrogen compounds – nitrite, nitrate, and ammonium – is of special interest in the field of wastewater analysis. Because wastewater samples usually contain a high amount of chloride, nitrite, which elutes immediately after chloride, can only be determined unequivocally by means of UV detection (see Figure 7.17). In case nitrite and nitrate are the only analytes of interest, high-throughput analysis is possible by employing a conventional anion exchanger with NaCl as the eluent. Due to the high elution power of the chloride anion, nitrite and nitrate are separated and eluted within 3 min and detected via their UV absorbance at 220 nm. The corresponding chromatogram is illustrated in Figure 8.6.

Analysis of water disinfection by-products In the past, drinking water disinfection was carried out by chlorination. However, in the 1970s it was found that carcinogenic compounds such as trihalomethanes are formed during this process. Therefore, people searched for alternative disinfection techniques to minimize the amount of potentially harmful compounds. The most promising alternative to chlorination is ozonation. However, if the raw water being processed for drinking water contains bromide, the latter will be oxidized to bromate by ozone. The concentration of the bromate formed depends on the following parameters [117]:

- Bromide concentration in the raw water
- Ozone dose
- Ozonation time
- Water temperature
- pH of the water

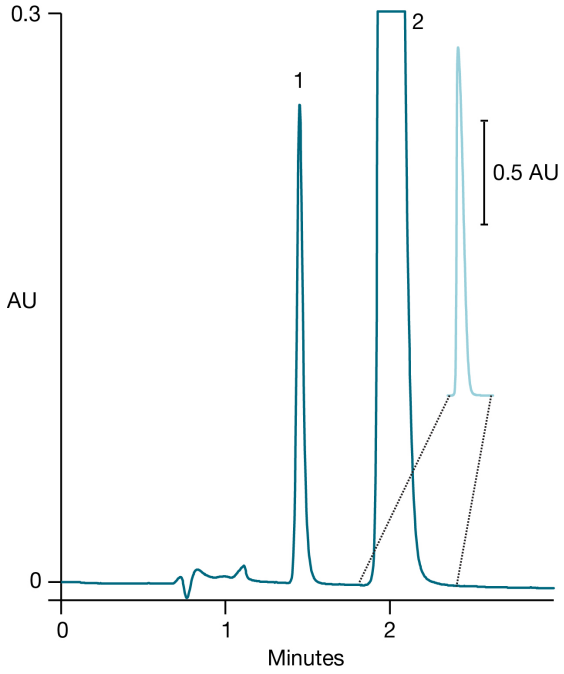


Figure 8.6 Analysis of nitrite and nitrate in in a sewage plant effluent using UV detection. Separator column: Dionex IonPac AS4A-SC; column dimensions: 250 mm × 2 mm i.d.; eluent: 30 mmol/L NaCl; flow rate: 0.5 mL/min; detection: UV (220 nm); injection volume: 25 µL; peaks: 0.3 mg/L nitrite (1) and 19.9 mg/L nitrate (2).

Toxicological studies indicate that bromate is suspected to be carcinogenic even in the low microgram/liter range. Various health and environmental protection agencies have been discussing concentration limits for bromate in drinking water for quite some time. The WHO, for example, recommends in their directive a concentration limit of 25 µg/L, while the US EPA and the European Union currently endorse a limit of 10 µg/L [118]. In connection to this value, a minimum detection limit of 2.5 µg/L is recommended for bromate analyses.

A number of different ion chromatographic techniques exist for bromate analyses and are summarized in Table 8.3.

The Dionex IonPac AS23 column is specifically designed to be used with a carbonate/ bicarbonate eluent for the determination of chlorite, bromate, and chlorate, together with common inorganic anions, including bromide (precursor to bromate), in drinking water. It allows the direct injection of 250 µL drinking water to easily meet the 10 µg/L regulatory requirements.

The combination of an optimized selectivity for disinfection by-product anions, high anion-exchange capacity and improved selectivity of carbonate from inorganic anions and oxyhalides makes this column the currently most advanced carbonate-selective anion exchanger for trace-bromate analysis and thus an ideal replacement for the Dionex IonPac AS9-HC column used in the past. Using the Dionex IonPac AS23 column in combination with a carbonate removal device (Dionex CRD 300) to remove carbonic acid (carbon dioxide dissolved in water) after suppression provides almost hydroxide-like performance and detection sensitivity. The Dionex CRD 300 is plumbed between the membrane suppressor and the conductivity cell. The regenerant channel that encloses the Dionex CRD 300 membrane is flushed with a solution of c = 0.2 mol/L NaOH, which aids the removal of carbon dioxide as carbonate. The net result is a significant reduction of background conductivity below 3 µS/cm, resulting in a noticeable improvement in analyte sensitivity. Under these chromatographic conditions, bromate is well separated from the neighboring chlorite and chloride, and can be detected down to about 2 µg/L. As can be seen from the corresponding chromatogram in Figure 8.7, the injection dip is greatly reduced in size, which improves the quantitation of early eluting peaks such as fluoride.

Low microgram/liter concentrations of bromate can also be analyzed in municipal drinking water and bottled water using a hydroxide eluent and suppressed conductivity detection. The US EPA Office of Water has determined that the use of hydroxide eluents in EPA Method 300.1

Table 8.3 Summary of US EPA Methods for bromate analysis based on ion chromatography techniques.

Technique	**EPA method**	**Dionex separator column**	**Eluent**	**MDL (µg/L)**
IC – suppressed conductivity	300.1 (B)	IonPac AS9-HC or AS23 IonPac AS19	Carbonate Hydroxide	5.0, 1.63 0.32
IC – suppressed conductivity	300.1 (B)	IonPac AS9-HC or AS23 IonPac AS19	Carbonate Hydroxide	5.0, 1.63 0.32
IC × IC – suppressed conductivity	302.0	IonPac AS19, 4 mm to AS24, 2 mm IonPac AS19, 4 mm to AS20, 0.4 mm	Hydroxide Hydroxide	0.036 ~ 0.004
IC – suppressed conductivity + postcolumn ODS	317.0	IonPac AS9-HC IonPac AS19	Carbonate Hydroxide	**UV/Vis** 0.14
IC – suppressed conductivity + postcolumn acidified KI	326.1	IonPac AS9-HC IonPac AS19	Carbonate Hydroxide	0.17
IC–ICP/MS	321.8	CarboPac PA100	Nitric acid + ammonium nitrate	**MS** 0.01

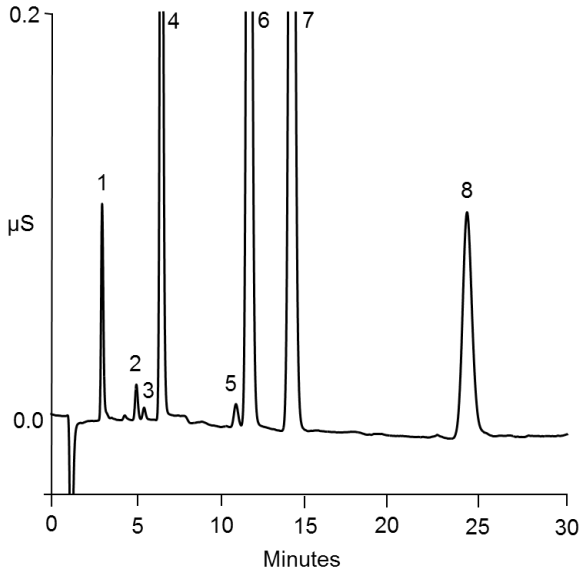


Figure 8.7 Trace analysis of bromate on a high-capacity carbonate-selective anion exchanger using the Dionex CRD 300 carbonate removal device. Separator column: Dionex IonPac AS23 with guard; column temperature: 30 °C; eluent: 4.5 mmol/L Na_2CO_3 + 1 mmol/L $NaHCO_3$; flow rate: 1 mL/min; detection: suppressed conductivity; injection volume: 100 µL; peaks: 10 µg/L fluoride (1), 10 µg/L chlorite (2), 5 µg/L bromate (3), 100 µg/L chloride (4), 10 µg/L chlorate (5), 500 µg/L bromide (6), 500 µg/L nitrate (7), and 100 µg/L sulfate (8).

is acceptable for compliance monitoring under the Clean Water Act and Safe Drinking Water Act. The most suitable column for this approach is the high-capacity Dionex IonPac AS19 column that also allows large-volume injections [119]. Figure 8.8 shows a chromatogram of a 250 µL injection of a Sunnyvale, CA, USA drinking water sample spiked with oxyhalides and bromide. As shown, bromate is very well resolved from chloride with bromate recovered at nearly 100%. Larger injection volumes are theoretically possible but would cause some column overloading and, therefore, a lower bromate recovery.

All the above-mentioned IC methods for the analysis of bromate and other oxyhalides at trace levels are based on suppressed conductivity detection. No matter whether a carbonate- or hydroxide-selective stationary phase with the respective eluent is used, the minimum detection limit for bromate based on a large-loop injection is between 1 and 2 µg/L. Lower detection limits for bromate can only be achieved with either derivatization techniques, heartcutting techniques (IC × IC), or hyphenated techniques such as IC–ICP/MS or IC–MS. At present, the most widely used derivatization methods for the trace analysis of bromate is EPA Method 326.0 [92]. The biggest advantage of this technique originally developed by Sahli and von Gunten is the ability to combine it with suppressed conductivity detection for determining the major constituents such as chloride, nitrate, and sulfate.

The determination of bromate at trace level in samples with a high electrolyte content using suppressed conductivity detection is subject to potential interferences and loss of sensitivity. Although the postcolumn derivatization methods described above do not generally suffer from interferences by common inorganic anions, column overloading with high-ionic strength samples can still cause peak broadening and an associated loss of response. Recently, a heart-cutting technique (IC × IC) based on a dual configuration ion chromatography system has been introduced for this application [120]. In an IC × IC setup, a 4 mm high-capacity Dionex IonPac AS19 column in the first channel is used to resolve bromate from matrix anions. While the matrix anions are diverted to waste, approximately 2 mL of the suppressed eluent containing the bromate (cut volume) is diverted from the first channel column to a concentrator column

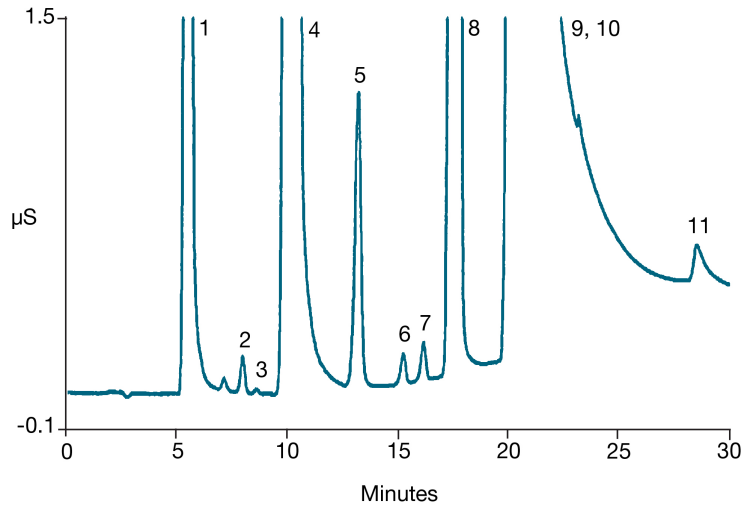


Figure 8.8 Gradient separation of bromate at trace level in a simulated drinking water using a high-capacity hydroxide-selective anion exchanger. Separator column: Dionex IonPac AS19 with guard; column dimensions: 250 mm × 4 mm i.d.; column temperature: 30 °C; eluent: KOH (EG); gradient: 10 mmol/L from 0 to 10 min, then linearly to 45 mmol/L in 15 min; flow rate: 1 mL/min; detection: suppressed conductivity; injection volume: 250 µL; peaks: 1 mg/L fluoride (1), 0.02 mg/L chlorite (2), 0.005 mg/L bromate (3), 50 mg/L chloride (4), 0.1 mg/L nitrite (5), 0.02 mg/L chlorate (6), 0.02 mg/L bromide (7), 10 mg/L nitrate (8), 100 mg/L carbonate (9), 50 mg/L sulfate (10), and 0.1 mg/L orthophosphate (11).

used in place of the sample loop in the second channel switching valve. The concentrator column has a very low back pressure but sufficient anion-exchange capacity to quantitatively trap the bromate anions in the suppressed eluent. In this way, bromate is separated from the matrix ions and concentrated on a trap column. The heart-cut portion of the first channel chromatogram is then eluted off the concentrator column and onto a smaller diameter (2 mm) analytical column (Ion Pac AS24) that has a different selectivity in comparison with the Dionex IonPac AS19 column in the first channel. Known today as EPA Method 302.0 [121], this method can be fully automated using a dual-channel reagent-free IC (RFIC) system. Bromate detection limits equivalent to or better than postcolumn derivatization methods can be achieved.

For a long time, the presence of other disinfection by-products such as haloacetic acids (HAAs) was somewhat ignored. Haloacetic acid can be formed during disinfection of water with chlorine dioxide, which effectively controls infectious microbial contaminants in raw water. However, during this process, hypochlorous acid is formed, which can react with naturally occurring organic matter such as humic acids and fulvic acids that naturally occur in raw water to form haloacetic acids. In recent years, the adverse effects of HAAs on human health have been increasingly recognized. There are a total of nine haloacetic acids that contain chlorine or bromine: monochloroacetic acid (MCAA), monobromoacetic acid (MBAA), dichloroacetic acid (DCAA), monobromomonochloroacetic acid (BCAA), dibromoacetic acid (DBAA), trichloroacetic acid (TCAA), monobromodichloroacetic acid (MBDCA), monochlorodibromoacetic acid (CDBAA), and tribromoacetic acid (TBAA). Among the nine HAAs, di- and trichloroacetic acids are animal carcinogens. Due to the potential adverse effects of these compounds on human health, the US EPA has introduced guidelines for the maximum contamination levels (MCLs) of haloacetic acids [122].

The determination of haloacetic acids in waters destined for human consumption can be carried out with a variety of different analytical techniques, which have been summarized by Bruzzoniti et al. [123]. Much work has been performed on hydroxide-selective columns using gradient elution. In 2007, the high-capacity Dionex IonPac AS24 anion exchanger was

introduced and was specifically designed for the analysis of haloacetic acids, allowing the separation of all nine HAAs under hydroxide gradient conditions. In combination with MS/MS techniques this method has been published as EPA Method 557; it is recommended for high-ionic strength matrices without sample pretreatment [124]. The fact that IC–MS has also been evaluated for the trace analysis of bromate indicates a clear trend toward mass-selective detection in ion chromatographic trace analysis. The simplicity of ion chromatography as a separation tool for HAAs combined with the selectivity and sensitivity of ESI–MS detection makes IC–MS an ideal approach to the determination of HAAs in drinking water at trace levels.

Figure 8.9 shows the separation of all nine haloacetic acids with MS/MS detection in the form of an MRM channel overlay. The grey boxes indicate the matrix diversion windows where the analytical flow is diverted to waste during elution of the matrix ions (see Figure 7.27). The hydroxide gradient is illustrated above the chromatogram. In this method, four isotope-labeled internal standards have been used for all analytes due to availability issues. The chosen internal standards elute in each of the three sections of the gradient method because the composition of the background changes over the course of the run. Period 1 of the gradient uses 7 mmol/L KOH as the eluent; during this time, MCAA and MBAA elute. Chloride elutes at the end of this region; that is, a matrix diversion window separates this first section of the gradient from the second one. Brominated acetic acids, in particular MBAA, are known to be susceptible to decomposition at higher temperature and pH. Thus, MBAA-1-^{13}C is used for accurate determination of MBAA. MCAA-1-^{13}C is used as an internal standard in the first section for

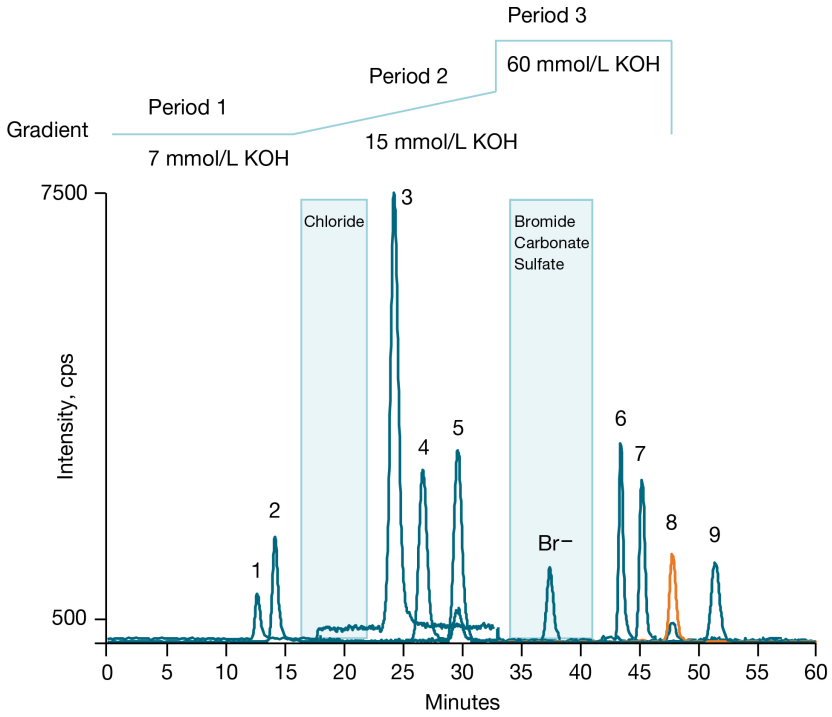


Figure 8.9 Separation of nine haloacetic acids (HAAs) with ESI–MS/MS detection. Separator column: Dionex IonPac AS24; column dimensions: 250 mm × 2 mm i.d.; column temperature: 15 °C; eluent: KOH (EG); gradient: 7 mmol/L from 0–18 min, linearly to 18 mmol/L in 18.5 min, then to 60 mmol/L in 0.3 min; flow rate: 0.3 mL/min; detection: ESI–MS/MS in the negative MRM mode; desolvation solvent: MeCN at 0.3 mL/min; injection volume: 100 µL; peaks: 1 µg/L each of monochloroacetic acid (1), monobromoacetic acid (2), dichloroacetic acid (3), monobromomonochloroacetic acid (4), dibromoacetic acid (5), trichloroacetic acid (6), monochlorodibromoacetic acid (7), monobromodichloroacetic acid (8), and tribromoacetic acid (9).

the quantification of MCAA. In period 2, dihaloacetic acids and dalapon are eluting by ramping up the concentration of KOH to 18 mmol/L. The internal standard for this period is DCAA-2-^{13}C. The second section ends with the diversion of sulfate, nitrate, and bicarbonate to waste. In period 3, the concentration of KOH is ramped up to 60 mmol/L to elute the trihaloacetic acids, using TCAA-2-^{13}C as the internal standard (not commercially available) for this section. For method simplicity, 15 °C was chosen as the column temperature because the recovery of seven of the haloacetic acids is better than 90% (relative to a column temperature of 10 °C) at this temperature level. Only MBAA and monochlorodibromoacetic acid (CDBAA) show losses at a column temperature of 15 °C.

Despite the high capacity of the Dionex IonPac AS24 anion exchanger, overloading with sample matrix may cause peak broadening and significant shifts in retention times. However, the ion-exchange capacity of 140 µequiv for the 2 mm column format is high enough to tolerate a simulated matrix consisting of 250 mg/L sulfate and chloride, 20 mg/L nitrate, and 100 mg/L ammonium chloride. Table 8.4 shows stable peak areas and retention times, even in a high-ionic strength matrix.

Table 8.5 provides linearity in deionized water and high-ionic strength matrix for this method at optimized MRM conditions for maximum sensitivity. Standards in matrix were used to calculate worst-case minimum detection limits against the deionized water calibration. The high-ionic strength matrix comprised 350 mg/L chloride, 250 mg/L sulfate, 150 mg/L bicarbonate, and 30 mg/L nitrate. This chloride content includes 250 mg/L sodium chloride and 100 mg/L ammonium chloride that is typically added for sample preservation. The transition for monobromodichloroacetic acid (BDCAA) is 287/81, but the intensity for this transition is low resulting in high % RSD and MDL. Better quantification is observed by monitoring the 79/79 signal for this analyte. To overcome the disadvantage of long analysis times with the Dionex IonPac AS24 column, the Dionex IonPac AS31 column has been introduced for faster separation of haloacetic acids (HAAs), dalapon, and bromate in drinking water.

Perchlorate analysis Significant amounts of ammonium perchlorate, an important constituent of solid rocket propellants, have been found in drinking water wells in areas where rocket fuel, ammunition, or pyrotechnic articles are developed, tested, or manufactured. Sources for the

Table 8.4 Retention time stabilities and recoveries for the nine haloacetic acids in deionized water and simulated matrix[a)].

Analyte	Concentration (µg/L)	Peak area ×10^4 DI water *n* = 7	Peak area ×10^4 matrix *n* = 7	% Recovery	Retention time (min) DI water *n* = 7	Retention time (min) matrix *n* = 7	Shift (min)
MCAA	3	11.1	11.6	104	10.56	10.48	-0.08
MBAA	2	16.0	17.2	107	11.86	11.80	-0.06
DCAA	3	126	132	105	19.26	19.28	0.02
BCAA	2	19.3	20.0	103	20.72	20.72	0.00
DBAA	1	11.6	12.0	102	23.08	23.10	0.02
TCAA	1	9.15	9.22	100	37.16	36.70	-0.46
BDCAA	2	8.96	9.13	101	40.18	40.10	-0.08
CDBAA	5	14.8	15.3	103	43.34	43.34	0.00
TBAA	10	14.8	15.5	104	47.00	47.02	0.02

Table 8.5 Transitions, linearity, detection limits, and accuracy for the nine haloacetic acids.

Analyte	Transition	ISTD 3 or 5 µg/L	r^2 0.25–20 µg/L DIW/matrix	r^2 0.25–5 µg/L matrix with NH_4Cl	MDL (µg/L/% RSD) (n = 7, 1 µg/L in matrix)	Accuracy (%) (at 500 ng/L) DIW/HI matrix with NH_4Cl
MCAA	92.9/34.9	MCAA-1-^{13}C	0.0007/0.9989	0.9962	0.440/14.7	87.5/81.6
MBAA	137/78.8	MBAA-1-^{13}C	0.9999/0.9990	0.9981	0.126/4.2	102/74
DCAA	127/82.9	DCAA-2-^{13}C	0.9999/0.9991	0.9924	0.095/3.3	96.7/73.3
BCAA	170.8/78.8	DCAA-2-^{13}C	0.9999/0.9992	0.9964	0.100/0.8	93.5/88.8
DBAA	214.7/170.7	DCAA-2-^{13}C	0.9999/0.9993	0.9957	0.325/10.8	107.0/79.9
TCAA	161/116.9	TCAA-2-^{13}C	0.9999/0.9993	0.9970	0.091/0.3	113.0/87.3
BDCAA	207/81 79/79	TCAA-2-^{13}C	0.9991/0.9991	0.9963	0.637/18.9	105/89.0
CDBAA	207/78.8	TCAA-2-^{13}C	0.9992/0.9994	0.9972	0.521/16.4	128/108.0
TBAA	250.75/78.8	TCAA-2-^{13}C	0.9994/0.9998	0.9954	0.360/9.9	102/95.6

contamination also include chemical fertilizers and air bag inflators. Even in low concentrations, perchlorate represents a health risk for human beings because it interferes with the ability of the thyroid gland to produce thyroid hormones. In 2002, the US EPA recommended a maximum containment level (MCL) for perchlorate at 1 µg/L. The California Department of Health Services (CDHS) has adopted an action level for perchlorate in drinking water of 6 µg/L. So far, perchlorate has been detected in more than 100 Californian drinking water wells; many of them had to be closed because they exceeded the above-mentioned limit.

The determination of perchlorate at trace levels is a difficult analytical task. Ion chromatography probably represents the only viable means for the quantification of such low perchlorate concentrations. A number of different ion chromatographic techniques exist for perchlorate analyses and are summarized in Table 8.6.

The conventional ion chromatography method with suppressed conductivity detection (EPA Method 314.0) [125] for the determination of perchlorate allows its quantitation down to 2 µg/L using the 4 mm Dionex IonPac AS16 column and large-loop injection. However, this is only applicable for weakly contaminated samples. If the samples are rich in electrolytes, perchlorate must be preconcentrated on a Dionex IonPac Cryptand C1 concentrator column, followed by a matrix rinse according to US EPA Method 314.1 [126]. In analogy to US EPA Method 302.0 for trace bromate analysis, lower detection limits for perchlorate can be achieved with a heart-cutting technique (IC × IC) based on a dual-configuration ion chromatography system that has been introduced for this application [127]. For this purpose, a 4 mm high-capacity Dionex IonPac AS20 column in the first channel is used to resolve perchlorate from matrix anions using a KOH gradient at a flow rate of 1 mL/min. The preconcentrated heart-cut portion of the first channel chromatogram is then eluted onto a smaller diameter (2 mm) Dionex IonPac AS16 analytical column that has a different selectivity in comparison to the Dionex IonPac AS20 column in the first channel. Perchlorate is eluted off the Dionex IonPac AS16 column with KOH under isocratic conditions and quantitated using the external standard method. Known today as EPA Method 314.2 [128], this method can be fully automated using a dual-channel RFIC system.

As an alternative to IC × IC, mass spectrometry can also provide lower detection limits than suppressed conductivity detection, especially when analyzing samples with high-ionic strength matrices. The IC–MS method for perchlorate analysis (EPA Method 332.0 [129]) uses the same instrument configuration with a diverter valve as shown in Figure 7.27. It may be used with a

Table 8.6 Summary of US EPA Methods for perchlorate analysis based on ion chromatography techniques.

Technique	EPA method	Dionex column	Eluent	MDL in water (µg/L)
IC – Suppressed Conductivity	314.0	IonPac AS16 or AS20	Hydroxide	1.0
IC – Suppressed Conductivity Matrix Rinse Elimination Primary and Confirmatory Columns	314.1	IonPac AS16 + AS20 IonPac AS19	Hydroxide	0.03
IC × IC – Suppressed Conductivity	314.2	IonPac AS16, 4 mm to AS20, 2 mm IonPac AS20, 2 mm to AS16, 0.4 mm	Hydroxide Hydroxide	0.06 0.005
IC–MS SIM *m/z* 99 and 101	332.0	IonPac AS16 or AS20	Hydroxide	0.01
IC–MS/MS SRM *m/z* 99/83 and 101/85	332.0	IonPac AS16 or AS20	Hydroxide	0.005
LC–MS/MS SRM *m/z* 99/83 and 101/85	331.0	IonPac AS21	Methyl amine	0.005

single quadrupole MS detector or with a tandem mass spectrometer as outlined in EPA Method 331.0 [130]. Perchlorate is separated from constituent cations and anions in the sample using a potassium hydroxide mobile phase [131]. The mass spectrometer is a more selective detector than suppressed conductivity in that it monitors the mass-to-charge ratio (*m/z*) of the analyte. The *m/z* ratio provides peak identification information for perchlorate at both *m/z* 99 and 101 due to the relative isotopic abundance of ^{35}Cl and ^{37}Cl. The high selectivity and sensitivity of MS detection is demonstrated in Figure 8.10 with the separation of perchlorate in a groundwater sample using EPA Method 332.0. While perchlorate is not detectable in the conductivity trace, both SIM channels (*m/z* 99 and 101) clearly show perchlorate at a concentration level of about 7–8 µg/L. This capability gives IC–MS the unique ability to be inherently a confirmatory method for perchlorate.

Seawater analysis Seawater as well as subsurface brines, geothermal brines, and high salinity groundwaters represent the most difficult environmental water matrices in which the concentration of ions extends over several orders of magnitude. The analysis of major components such as chloride, sulfate, and sodium, which are present at extremely high concentrations, is relatively straightforward, only requiring a dilution before direct injection of very small sample volumes (~1 µL) into the ion chromatograph. However, the analysis of trace ionic constituents in samples of high salinity represents a special challenge for ion chromatography and can only be carried out by carefully selecting chromatographic conditions. The use of ion chromatography for the analysis of ions in environmental water samples of high salt concentration has been reviewed by Gros [132]. In addition to the use of conventional IC with suppressed conductivity detection, other approaches include the use of chloride eluents, heart-cutting techniques in combination with matrix elimination, and more selective detection methods such as UV/Vis, DC amperometry, postcolumn derivatization, and ICP–MS.

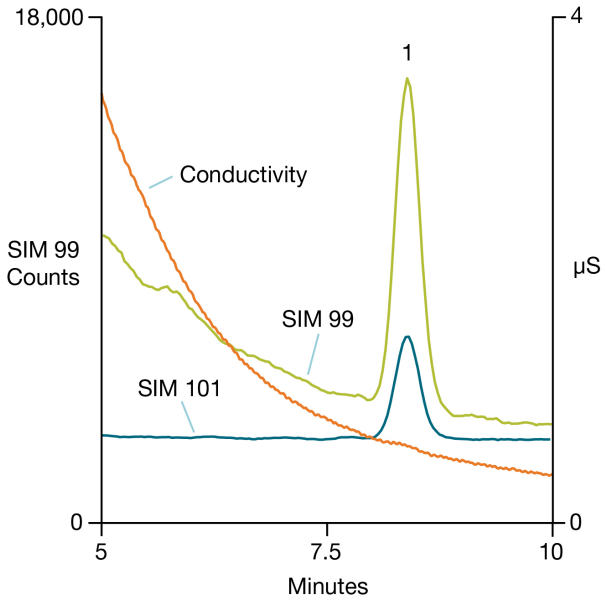


Figure 8.10 IC–MS of perchlorate in a groundwater sample according to EPA Method 332.0. Separator column: Dionex IonPac AS16 with guard; column dimensions: 250 mm × 2 mm i.d.; eluent: 65 mmol/L KOH (EG); flow rate: 0.3 mL/min; detection: suppressed conductivity and MS (SIM channels *m/z* 99 and 101), negative ion mode, cone voltage: 70 V, needle temperature: 350 °C ; injection volume: 250 µL; sample: California groundwater, 1:10 diluted; peak: 7–8 µg/L perchlorate (1).

The use of sodium or potassium chloride as an eluent combined with low-wavelength UV detection allows the determination of UV-absorbing anions such as bromide, nitrate, and iodide in samples of high salinity without sacrificing chromatographic performance [133]. Seawater is a large natural source of iodide, typically containing 50–60 µg/L iodide with a wide range of observed concentrations. For example, iodide amounts can range from less than 1 µg/L to greater than 60 µg/L. Detection of iodide in seawater and synthetic sea salt by suppressed conductivity is challenging due to the high concentrations of chloride, sulfate, and bicarbonate in the sample. However, when using UV detection, reagent-free IC with electrolytic eluent generation allows the determination of iodide in such matrices. Iodide can be separated from other matrix anions on the high-capacity Dionex IonPac AS20 column, which has been designed for the rapid separation of polarizable anions. The unique selectivity of this column is ideal for separating iodide in saline matrices. Due to its high capacity, sample preparation other than membrane filtration is not required. Furthermore, by detecting iodide via its UV absorption, matrix anions such as chloride, sulfate, and bicarbonate are not detected and thus do not interfere with iodide determination. Figure 8.11 shows the separation of iodide spiked into natural seawater collected in Half Moon Bay, CA, USA. As shown, iodide is easily determined by UV detection with a recovery of 96%.

In the field of cation analysis in samples of high salinity, Hodge et al. [134] successfully used a Dionex IonPac CS12A column with a sulfuric acid eluent to separate sodium, potassium, magnesium, and calcium in brine samples under isocratic conditions with suppressed conductivity detection. Trace ammonium, however, is difficult to be quantified under those conditions because sodium and ammonium elute in close proximity to each other on weak acid cation exchangers.

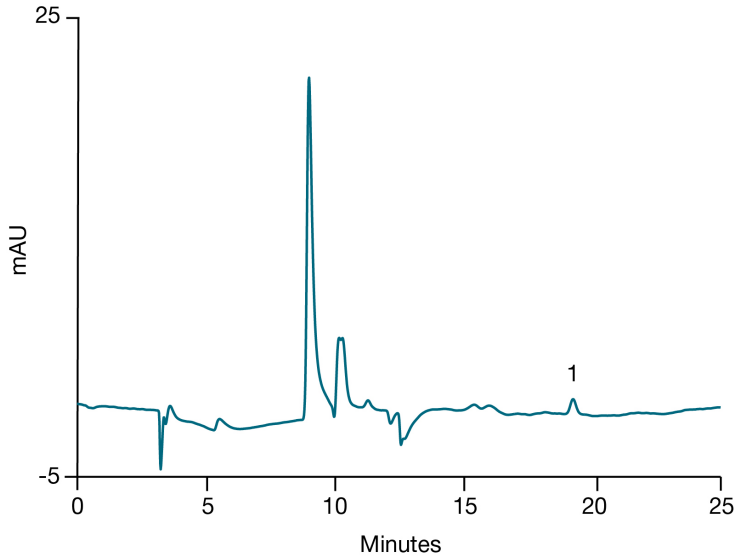


Figure 8.11 Separation of iodide in a natural seawater sample by RFIC using UV detection. Separator column: Dionex IonPac AS20 with guard; column dimensions: 250 mm × 2 mm i.d.; eluent: KOH (EG); gradient: 13 mmol/L for 0 to 10 min, then 13–45 mmol/L for 10 to 15 min, 45 mmol/L for 15 to 25 min; flow rate: 0.25 mL/min; detection: UV (223 nm); injection volume: 10 µL; sample: seawater collected from Half Moon Bay, CA, USA, spiked with 73.2 µg/L iodide; peak: iodide (1).

Soil analysis The analysis of inorganic ions in soils was amongst the earliest applications of ion chromatography [135, 136]. The ability to quantify different metal oxidation states and stable metal complexes in soil extracts is beneficial in determining the fate, transport, and toxicity of metals in soil. Also, the analysis of total nitrogen, phosphorus, and sulfur, and their corresponding oxide anions, for example, nitrite, nitrate, orthophosphate, and sulfate, is of importance in assessing soil conditions and fertility.

Sample preparation is extremely important in the field of soil analysis. Ideally, solutions injected for IC analysis should be low in organic materials, strong acids, and soluble salts; hence, many of the traditional methods used for the extraction or digestion of soils are not compatible with IC [137]. However, when the sample is a filtrate of a neutral sludge, it may be diluted with deionized water due to the high electrolyte content. Therefore, the quality of the resulting chromatogram hardly differs from that of a drinking water chromatogram. For cation analysis in a sewage sludge, the sample is usually digested with nitric acid, diluted, and then injected through a cartridge containing an anion-exchange resin for neutralization.

In contrast, soil samples are often extracted with a 10% potassium chloride solution. Due to the chloride matrix, the nitrogen parameters of interest, nitrite and nitrate, cannot be analyzed using a separation system with a carbonate/bicarbonate eluent. This problem is easily solved, however, by employing potassium chloride as an eluent and determining the analyte ions nitrite and nitrate photometrically. When a stationary phase with both anion and cation exchange capacities (e.g., Dionex IonPac CS5A) is used, the ammonium ion is also retained, which may be detected very sensitively by derivatization with *o*-phthaldialdehyde and subsequent fluorescence detection. By combining both detection methods, all three nitrogen parameters can be determined in one run.

Air analysis For several decades, the emission of particulate matter (PM) and gaseous pollutants has continuously increased due to growing population, industrialization, and human activities. Air pollution is the term for a mixture of solid particles, gases, and liquid droplets found in the air. These particles and gases can include dirt, soot, aerosols, dust, and exhaust.

They are made up of many different chemicals, some of which can be identified as either inorganic (nitrate, sulfate, particle ammonium) or organic (carbon, semivolatile) compounds.

Particulate matter is a complex mixture of tiny particles found in air that comes in a wide range of sizes. Particles with a diameter of less than 10 µm (PM 10) are small enough to enter the lungs unnoticed, potentially causing serious health problems including heart attacks, asthma, and respiratory symptoms such as difficult breathing. Particles less than 2.5 µm in diameter (PM 2.5) are called "fine" particles, which are found in smoke and haze caused by the emissions of power plants, motor vehicles, industrial processes, residential wood burning, forest fires, and agricultural burning.

Air pollution, and especially particulate matter, must be managed for health and environmental reasons. Studying and monitoring the chemical composition and size distributions of particulate matter can help us understand the dynamics of air pollution. Particulate pollution can damage sensitive ecosystems by acidifying lakes and streams, changing the nutrient balance in coastal waters and river basins, depleting nutrients in soil, and damaging forests and farm crops. Particulate pollution also affects the energy balance of our planet by scattering solar radiation and reduces visibility through the formation of haze.

Ion chromatographic applications in the area of air hygiene include the analysis of inorganic anions and cations in fly ash [138] and atmospheric aerosols [139], and the analysis of formaldehyde and acetaldehyde after appropriate sampling [140]. Gases such as cyanic acid, sulfur dioxide, and nitrogen oxides can also be determined ion chromatographically. For the determination of sulfur dioxide, for example, Velásquez et al. [141] used a Graseby/Anderson apparatus (Atlanta, GA, USA), with which the air to be analyzed is directed through 50 mL of an absorber solution with a flow rate of 185 mL/min for 24 h. The absorber solution, which consists of a dilute H_2O_2 solution, is prepared by mixing 20 mL of 30% H_2O_2 solution with 100 µL HCl (c = 0.6 mol/L) in a 1 L volumetric flask, which is then filled up to the mark with deionized water. To keep the air flow free of particles, the authors used a 5 µm PTFE filter. The sulfate formed via oxidation of sulfur dioxide can then be determined by means of anion-exchange chromatography. The minimum detection limit for sulfate, expressed as threefold standard deviation of the blank value (5.5 µg/m³), is 0.044 µg/m³ with this method.

The classical approach today for the determination of ionic components in particulate matter is the collection of PM on filter media, followed by extraction and IC analysis. Gases are usually collected with denuders, as already described by Forrest et al. [142] in 1982 with the analysis of nitrate and nitric acid in the atmosphere. A denuder is a cylindrical or annular tube internally coated with a reagent that selectively reacts with a stable flow of gas drawn through the tube [143]. In a liquid diffusion denuder, the high diffusivity of gases causes them to migrate to the denuder wall surface where they are removed from the sample stream, as schematically depicted in Figure 8.12. The denuder solution is typically a hydrogen peroxide solution (c ~ 5 mmol/L) to increase the solubility of SO_2 by rapid conversion of SO_2 to sulfate. Such denuders are integrated components of air sampling devices such as the URG 9000 Series Ambient Ion Monitor (AIM) (URG, Chapel Hill, NC, USA), which is an advanced air sampling instrument that simultaneously measures both fine particle components (PM 2.5) and precursor gases. AIM samplers provide time-resolved direct measurements of ionic components in PM 2.5 including anions (nitrate, sulfate, nitrite, orthophosphate, and chloride) and cations (ammonium, sodium, calcium, potassium, and magnesium). Furthermore, the AIM provides time-resolved direct measurements of anions (hydrogen chloride, nitric acid, nitrous acid, and sulfur dioxide) and cations (ammonia) in gases. The analysis method for the AIM is ion chromatography. AIM samplers can be configured with one or two ion chromatography systems that separate, identify, and quantify the ionic pollutants. These ion chromatography systems include dual-piston pumps and conductivity detectors with chemically or electrolytically operated suppressors; they can be configured for manually prepared or electrolytically generated eluents.

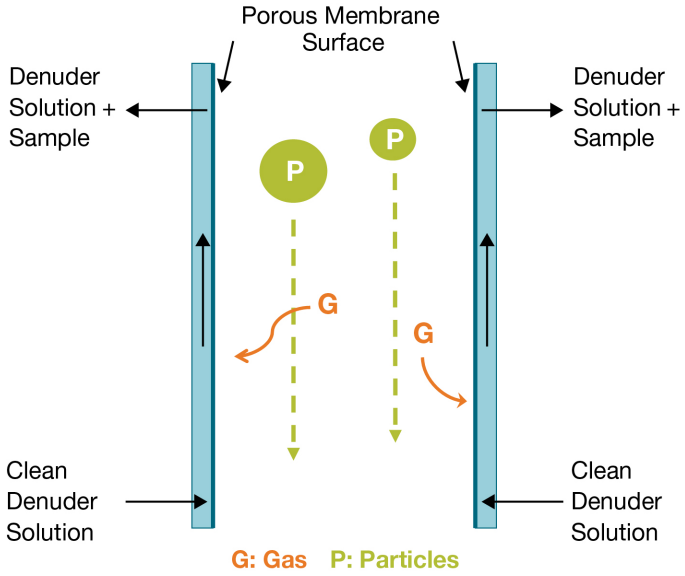


Figure 8.12 Schematic depiction of the operation of a liquid diffusion denuder.

Other environmental applications Ion chromatography is also employed as a detection method in the sum detection of organically bonded halogen and sulfur compounds (AOX and AOS) [144]. In these methods, the water sample is treated with nitrate solution and nitric acid to displace interfering chloride and sulfate ions. An activated carbon low in chlorine, bromine, and sulfur and almost ash-free is added, at which the organically bonded halogen and sulfur compounds are adsorbed. After this concentration step the loaded activated carbon is filtered off through a polycarbonate filter and incinerated together with the filter at 1000 °C in an oxygen stream. In the past, the resulting reaction gases were introduced into the titration cell of a microcoulometer, where chloride, bromide, and iodide are precipitated with silver ions present in the electrolyte. The silver ion concentration is determined potentiometrically. The silver ion concentration is readjusted to its initial value via anodic oxidation of a silver electrode. The amount of charge measured during the electrolysis is equivalent to the amount of chloride, bromide, and iodide that entered the cell. In this type of analysis, chlorine, bromine, and iodine are detected as molar sum; it is impossible to differentiate the species. For the AOS determination, the reaction gases are passed into an aqueous solution and are analyzed using an appropriate detection method.

However, if ion chromatography is used as a detection method, the AOX and AOS parameters may be determined in one working step. Moreover, it is possible to differentiate between organically bonded chlorine, bromine, and iodine. A basic buffer solution made of sodium bicarbonate and sodium carbonate used for ion chromatographic analysis is applied as absorption solution for the acidic reaction gases. A small amount of hydrogen peroxide is added to achieve complete oxidation of sulfite to sulfate. Typical chromatograms obtained in the simultaneous determination of AOX and AOS show signals for nitrite and nitrate in addition to halides and sulfate. Nitrite and nitrate are present due to the application of nitrate, which is added to prevent chloride and sulfate adsorption.

8.2
Ion chromatography in power plant chemistry

The evaluation of the water, steam, and condensate quality is one of the most important applications of ion chromatography in power plant technology. In the past, water quality was monitored via registration of the electrical conductance. Routine plant control by this method allows leaks in condensers to be identified and the functioning of water purification devices to be checked. However, conductivity detection is unspecific; an increase in signal does not provide information about the type of contaminant. In addition, sodium chloride or sodium sulfate impurities resulting from inflowing cooling water or insufficiently regenerated condensate purification filters cannot be reliably detected in the sub-microgram/liter range.

High-purity water analysis Shortly after its introduction in 1975, ion chromatography was adopted by the power generating industry to assay high-purity water for anionic and cationic impurities. Table 8.7 summarizes the IC detection methods for the analysis of mineral acids, orthosilicate, borate, alkali and alkaline-earth metals, and transition metals in high-purity water. While the analysis of orthosilicate and transition metals requires postcolumn derivatizations with subsequent photometric detection, all other analytes can be determined using suppressed conductivity detection.

In order to analyze high-purity water free of contamination, the instrument should be directly connected to the sampling line. If the direct connection of the instrument to the sampling line is not possible, samples must be transported in FEP-Teflon (Nalgene™) containers, as only this material has proven to exhibit no wall adsorption effects. To determine ions at trace level, a preconcentration technique has typically been utilized [145]. With this method, the analytes are preconcentrated on a small concentrator column improving detection limits down to the single-digit nanogram/liter levels. The introduction of high-capacity ion exchangers and gradient elution techniques in the late 1980s allowed large volumes of sample (1000–2000 µL) to be injected directly on column [146], resulting in detection limits in the midnanogram/liter level. The most modern form of analyte preconcentration is RFIC-ESP™ (reagent-free ion chromatography with electrolytic sample preparation), which is a new concept for fully automated in-line sample preconcentration and in-line calibration down to single-digit nanogram/liter levels using an electrolytic water purifier (EWP). In this approach, the column effluent is passed through the EWP and is used to transfer samples and calibration standards from the respective loops to the concentrator column. In this way, only one analytical pump is

Table 8.7 IC methods for monitoring impurities in high-purity water.

Analyte	IC detection method
Anions (e.g., fluoride, chloride, sulfate)	Suppressed conductivity
Boron (as $B(OH)_4^-$)	Suppressed conductivity
Silica (as dissolved orthosilicate)	Postcolumn derivatization with subsequent UV/Vis detection
Cations (e.g., alkali and alkaline-earth metals)	Suppressed conductivity
Transition metals	Postcolumn derivatization with subsequent Vis detection

needed in the system, which simplifies trace analysis. The various forms of analyte enrichment techniques for analyzing high-purity water derived from conventional and nuclear power plants are summarized by Weiss [147].

Conventional preconcentration techniques make use of a so-called concentrator column, which is a short column containing a stationary phase that is similar or even identical to the separator column being used. The function of the concentrator column is to strip the analyte ions from a measured volume of a high-purity water sample. In the simplest configuration, the concentrator column is connected to the injection valve in the same position as the sample loop. When switching the concentrator column into the eluent stream, the analyte ions are eluted from the concentrator onto the separator column where they are separated either isocratically or by gradient elution. To concentrate analyte ions on a concentrator column, the analytes must be present in a matrix with a relatively low ionic strength. Otherwise, the sample acts as an eluent and prevents analyte retention on the concentrator column. Because ion-exchange concentrator columns have a finite capacity, analyte ions are not quantitatively retained when the capacity is exceeded. The situation is more complicated if the sample contains analytes of widely different affinities. An analyte ion may act as an eluent if its affinity for the ion-exchange resin is much larger than the affinity of the ions already associated with the resin. An early breakthrough is possible in this case. To ensure the quantitative retention of analyte ions on the concentrator column, the breakthrough volume must be determined for a simulated sample matrix. The breakthrough volume is defined as the volume of sample that causes an analyte ion to be eluted from the concentrator column.

The biggest problem with conventional preconcentration techniques is the calibration. Because external calibration methods are used, it is critical that calibration standard solutions are correctly prepared and stored. Working calibration standards at sub-microgram/liter levels, however, cannot be prepared by diluting higher concentrated stock standards due to the likelihood of contamination. Therefore, a second valve plumbed in line with the injection valve as shown in Figure 8.13. Connecting an injection loop to this second valve, a small volume of a higher concentrated standard can be added to the preconcentrated volume of sample. Standard and sample loading is carried out with a loading pump, which is a potential source of contamination.

Large-volume direct injection techniques No matter how advanced preconcentration techniques are today, additional hardware is required. This includes a concentrator column used to preconcentrate the ions of interest and a second pump to load the sample. In addition, extra time is required for the preconcentration step, and loading efficiency can be compromised when additives are present in the sample. Today, sensitivities in the submicrogram/liter range can also be obtained by direct injection of large sample volumes up to 2000 µL [148]. Thus, an additional sampling pump is not required in this case. However, large-volume injections require special separator columns. For anion analysis, high-capacity, hydroxide-selective anion exchangers have proved to be ideal. Anions are focused at the column head at low eluent concentration and subsequently eluted by increasing the ionic strength in the mobile phase. Typically, microbore columns with 2 mm internal diameter are used because they exhibit a fourfold increase in mass sensitivity over the 4 mm format. The column of choice is the Dionex IonPac AS28-Fast-4µm column (the successor product of the Dionex IonPac AS15 column), separating common inorganic anions and low-molecular weight organic acids under gradient conditions. Electrolytic eluent generation has significantly improved the technique of large-volume injection. As outlined above, it prevents carbonate contamination and thus minimizes baseline drift during the gradient run. The Dionex IonPac AS28-Fast-4µm column exhibits high selectivity for glycolate, formate, and acetate. The detection of these acids at trace level is of

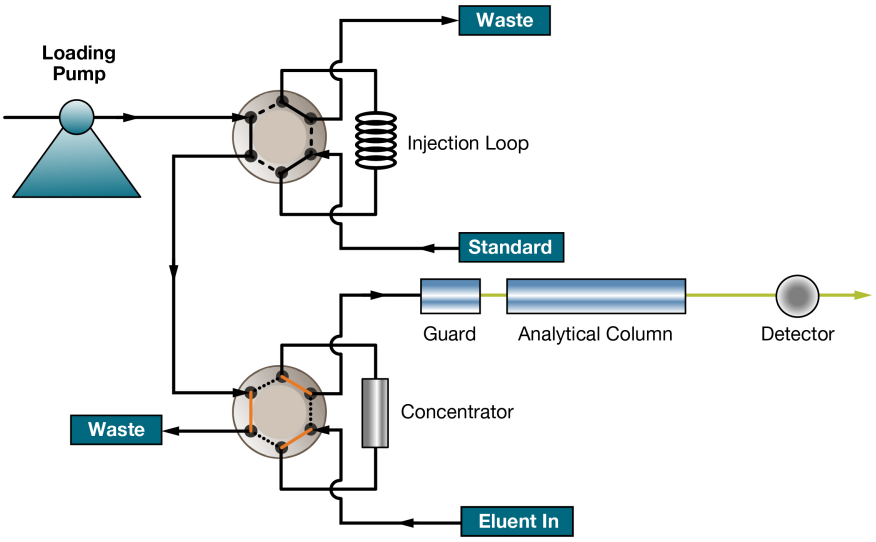


Figure 8.13 Preconcentration configuration for a concentrator column and an injection loop.

great interest because they negatively affect the operation of a power station by increasing the conductivity of the steam and lowering the pH value.

Even though the Dionex IonPac AS28-Fast-4µm column separates fluoride from glycolate, acetate, and formate, several analytes are not very well resolved under the respective chromatographic conditions: acrylate and chloride as well as carbonate, benzoate, and methacrylate. However, these anions can be separated very well using the Dionex IonPac AS17-C column. A typical chromatogram for a low-level standard is shown in Figure 8.14. The presence of chloride and sulfate is the major cause for corrosion in steam generators, reactor vessels, heat exchangers, turbines, and pipes. In conventional power stations, therefore, the concentrations of these anions in the feed water of steam generators are monitored in the range below 1 µg/L; in some nuclear power stations, concentrations below 0.2 µg/L are monitored. Large injection volumes can also be applied for cation analysis to obtain detection limits in the lowest microgram/liter range by direct injection. Because a suppressor system is utilized, background conductivity is almost zero, so that early eluting cations are not interfered with by a large water dip.

RFIC-ESP Reagent-free ion chromatography with electrolytic sample preparation is a new concept for fully automated in-line sample preconcentration and in-line calibration down to single-digit nanogram/liter levels. It is the latest extension of RFIC technologies to simplify trace analysis because only one analytical pump is needed in the system. In addition to a conventional RFIC system, an electrolytic water purifier (EWP) and a 10-port valve are used to perform trace analysis of ions. The EWP is an electrodeionization (EDI) device [149], which produces low flow-rate streams of water of exceptional purity. This ultrapure water can be used to load samples or standard solutions onto concentrator columns, thus eliminating the need for a loading pump. In the past, loading pumps were identified to be a source of contamination, reducing the ability of a system to perform trace analysis. The closed loop RFIC-ESP system significantly reduces contamination.

An additional feature of RFIC-ESP is the use of a small loop (10 µL)/large loop (10 mL) scheme for calibration. Both loops are mounted at the 10-port valve. This allows the system to be calibrated with standards at a concentration level that is 1000-fold higher than that of the sample. The ultrapure water from the EWP is used to transport the large volume of sample or the small volume of standard to the concentrator column on the injection valve. Rinsing both loops with this water prevents sample carryover and results in very low blanks. This approach

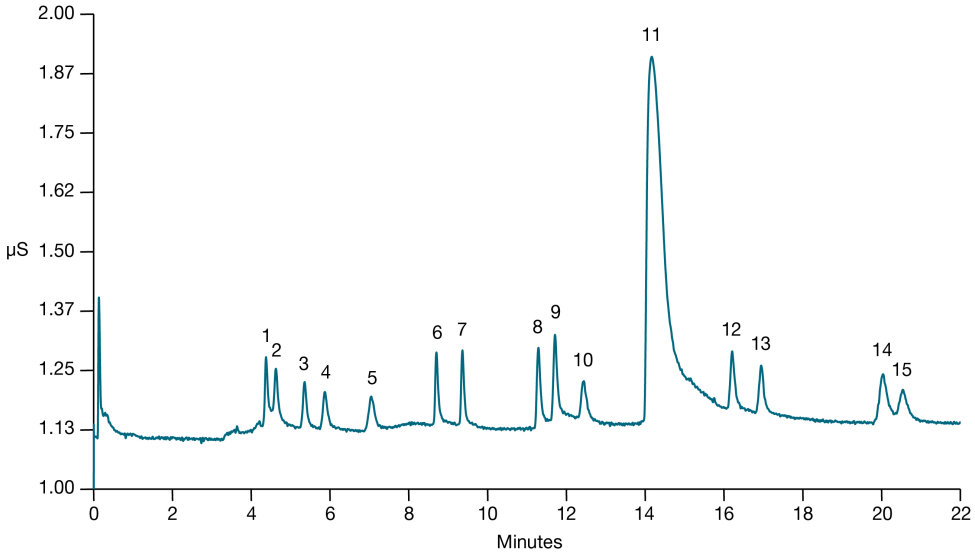


Figure 8.14 Separation of anions at trace level by direct injection. Separator column: Dionex IonPac AS17-C; column dimensions: 250 mm × 2 mm i.d.; column temperature: 30 °C; eluent: KOH; gradient: 1 mmol/L for 3 min isocratic, then linearly to 12 mmol/L in 7 min, then linearly to 20 mmol/L in 4 min; flow rate: 0.5 mL/min; detection: suppressed conductivity; injection volume: 1000 µL; peaks: 0.6 µg/L fluoride (1), 7 µg/L acetate (2), 1.5 µg/L formate (3), 3.7 µg/L acrylate (4), 4.2 µg/L methacrylate (5), 1.2 µg/L chloride (6), 2 µg/L nitrite (7), 3.1 µg/L bromide (8), 3.8 µg/L nitrate (9), 7 µg/L benzoate (10), carbonate (11), 2.1 µg/L sulfate (12), 1.6 µg/L oxalate (13), 10.8 µg/L phthalate (14), and 3.8 µg/L orthophosphate (15).

of purifying the waste stream and using the purified waste stream for calibration and sample processing is referred to as *AutoPrep*. The diagram in Figure 8.15 shows the basic configuration of such an IC system for trace analysis utilizing an EWP and a 10-port valve with two loops. With the addition of a large-volume autosampler (e.g., Thermo Scientific Dionex AS-HV), calibration and sample analysis are completely automated with this configuration. Multiple

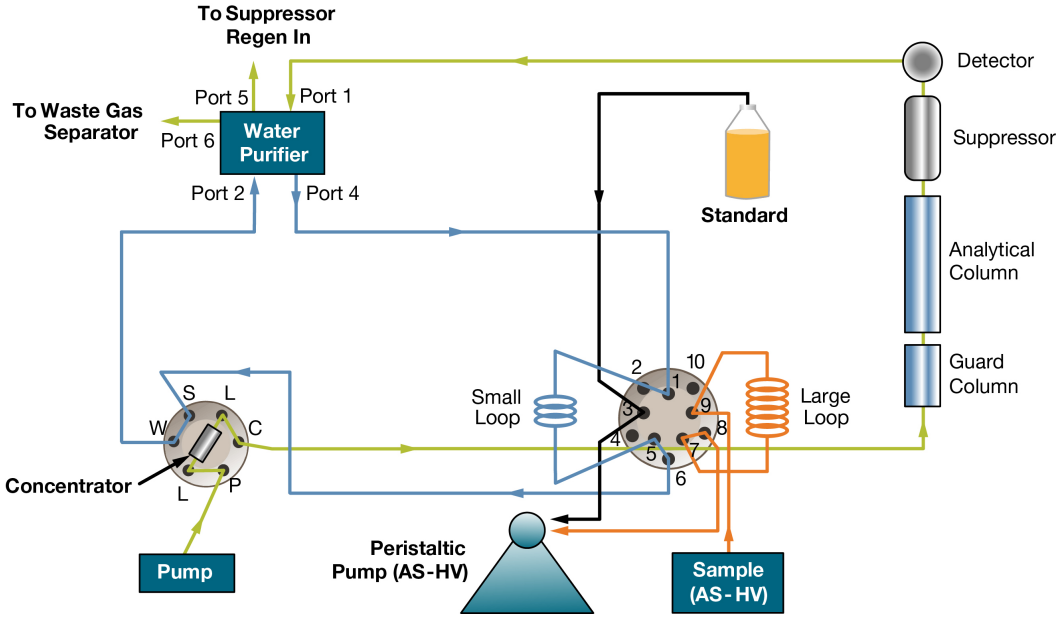


Figure 8.15 Schematic flow diagram of a system for trace ion analysis utilizing an electrolytic water purifier.

calibration levels are simulated by repeatedly filling and dispensing the volume of the small 10 µL loop to the Dionex IonPac UTAC-LP1concentrator column. Loading the concentrator column with 1, 2, 4, and 10 loop volumes produces standards of 1, 2, 4, and 10 times the concentration of the stock solution. The respective chromatogram with the overlaid standard levels is shown in Figure 8.16.

Analysis of conditioned waters In addition to high-purity water analysis, ion chromatography is the primary method used for analyzing conditioned waters, which is a lot more difficult because the concentrations of the chemicals used for conditioning exceed the analyte concentrations by many orders of magnitude. Typical conditioning agents include amines such as ammonium, ethanolamines, morpholine, and others with concentrations in the lower milligram/liter range. In some cases, mixtures of various amines are used for corrosion inhibition, which renders the analysis of corrosive ions more difficult. Two different strategies can be followed to increase resolution between matrix components and analytes with their disparate concentration levels. On the one hand, special stationary phases such as the crown ether modified Dionex IonPac CS15 column or the high-capacity cation exchanger Dionex IonPac CS16 column can be used, with which a significantly better resolution between sodium and ammonium is obtained. A characteristic example for trace analysis of sodium in the presence of monoethanolamine on a Dionex IonPac CS15 column is illustrated in Figure 8.17. In the given example, the monoethanolamine concentration is so high that ammonium (as an impurity) merely elutes as a shoulder peak on the slope of the monoethanolamine peak. If monoethanolamine and ammonium would be present in comparable concentrations, both compounds can be separated to baseline.

For the conditioning of feed water, ammonia is often used in combination with morpholine; morpholine acts as corrosion inhibitor and ammonia is used for adjusting pH (Figure 8.18). Both compounds can be separated from common inorganic cations on a Dionex IonPac CS14 weak acid cation exchanger. The tailing of the morpholine peak is reduced by adding 5% (*v/v*) acetonitrile to the mobile phase.

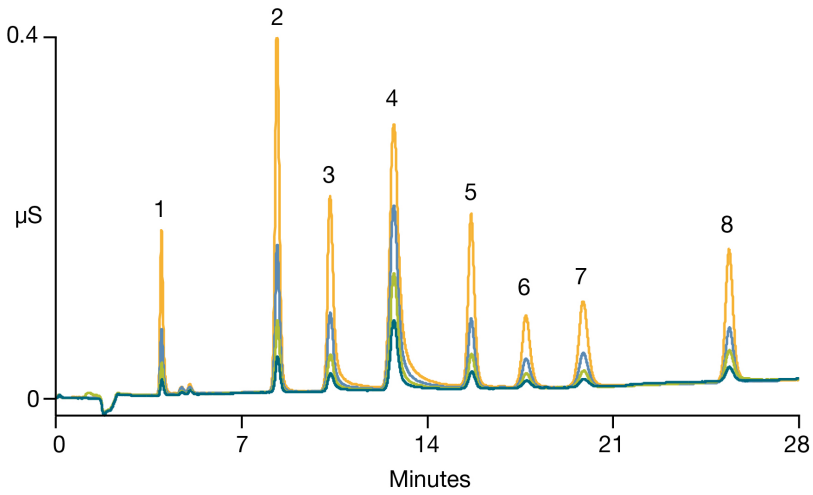


Figure 8.16 Overlay of four calibration levels in trace anion analysis by RFIC-ESP. Separator column: Dionex IonPac AS15; column dimensions: 250 mm × 4 mm i.d.; column temperature: 35 °C; eluent: KOH (EG); gradient: 22–44 mmol/L in 18 min, then 44–65 mmol/L in 10 min; flow rate: 1.2 mL/min; detection: suppressed conductivity; peaks: (1) fluoride, (2) chloride, (3) nitrite, (4) carbonate, (5) sulfate, (6) bromide, (7) nitrate, and (8) orthophosphate.

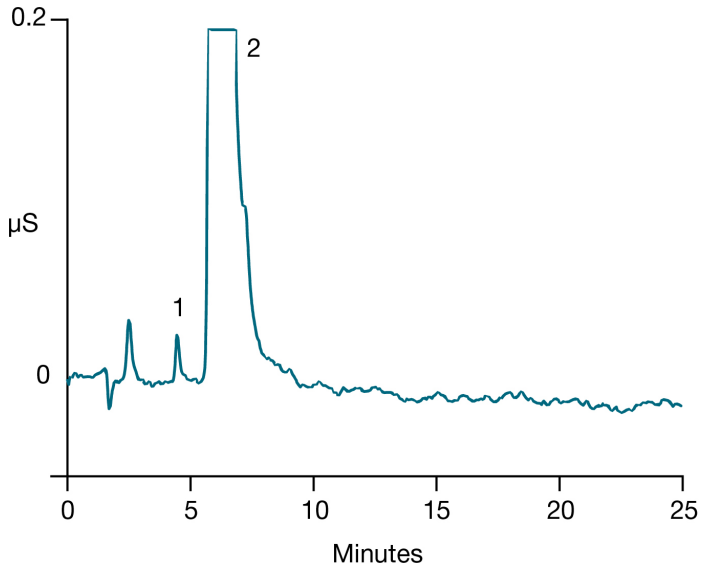


Figure 8.17 Trace analysis of sodium in presence of a high excess of monoethanolamine on a crown ether-modified stationary phase. Separator column: Dionex IonPac CS15; column dimensions: 250 mm × 2 mm i.d.; column temperature: 40 °C; eluent: 5 mmol/L H_2SO_4/MeCN (91:9 *v/v*); flow rate: 0.3 mL/min; detection: suppressed conductivity; injection volume: 25 µL; peaks: 10 µg/L sodium (1) and 200 mg/L monoethanolamine (2).

Cooling water analysis A very challenging application is the determination of submicrogram/liter concentrations of chloride and sulfate in borate-treated water used in nuclear power plants to minimize or inhibit corrosion. There are two common types of nuclear reactors: the

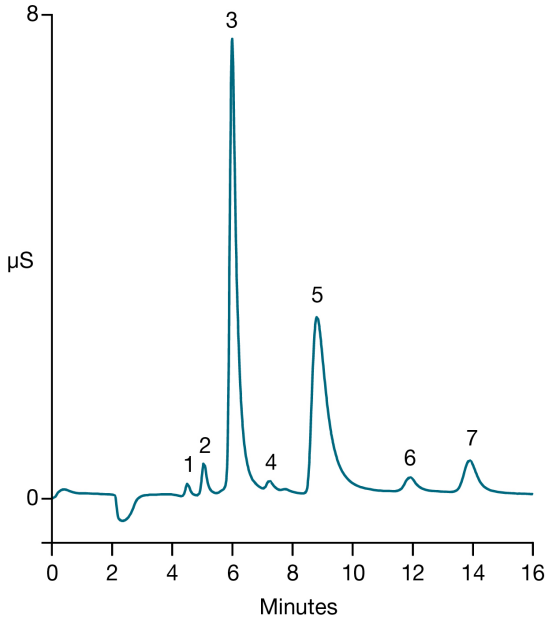


Figure 8.18 Trace analysis of cations in presence of a large excess of ammonium and morpholine. Separator column: Dionex IonPac CS14; column dimensions: 250 mm × 2 mm i.d.; eluent: 8 mmol/L methane-sulfonic acid/MeCN (95:5 *v/v*); flow rate: 0.25 mL/min; detection: suppressed conductivity; injection volume: 1 mL preconcentrated on Dionex IonPac CG14 (2 mm); peaks: 0.5 µg/L lithium (1), 2 µg/L sodium (2), 150 µg/L ammonium (3), 2 µg/L potassium (4), 2000 µg/L morpholine (5), 2 µg/L magnesium (6), and 10 µg/L calcium (7).

most popular one is the pressurized water reactor (PWR). Its design is distinguished by having a primary cooling circuit that flows through the core of the reactor at very high pressure, and a secondary circuit where steam is generated to drive the turbine. The next most common reactor type is known as a boiling water reactor (BWR). Its design is similar to the PWR, except only a single circuit exists where the water is maintained at a lower pressure to allow the water to boil at approximately 285 °C. Pressurized water reactors (PWRs) use boric acid as a water-soluble neutron absorber to control the nuclear reaction. At the beginning of the fuel cycle, its concentration is relatively high (1000–2500 mg/L), gradually decreasing as the fuel is spent. Lithium hydroxide is added to set the pH at 6.9 or higher. Typical LiOH concentrations are between 1.8 and 4 mg/L. However, trace impurities from the reagents or the water source can accumulate over time, serving as catalysts for corrosion. Traditionally, a matrix elimination technique in combination with analyte preconcentration is used that can perform this analysis with high precision and accuracy. After the preconcentration step, the concentrator column is rinsed with high-purity water to remove boric acid from the interstitial volume of the concentrator column. Only then can the concentrator column be placed in line with a high-capacity analytical separator column. The column switching for this application is depicted schematically in Figure 8.19. It is extremely important to use deionized water of the highest possible quality for rinsing the concentrator column. For further purification, the water is directed through an anion trap column (ATC) to exclude contaminations as much as possible. Despite matrix elimination, the concentration of boric acid that gets onto the analytical separator column is still very high, so that a high-capacity anion exchanger is required as the stationary phase. Optimum selectivity for this type of analysis is provided by a Dionex IonPac AS10 column, on which borate and chloride can be separated to baseline. The respective chromatogram of a high-purity water sample containing approximately 2% boric acid is shown in Figure 8.20. Fluoride, chloride, and sulfate were detected at the lowest microgram/liter level. Without matrix elimination, the boric acid peak preceding chloride would make it impossible to quantitate the chloride signal.

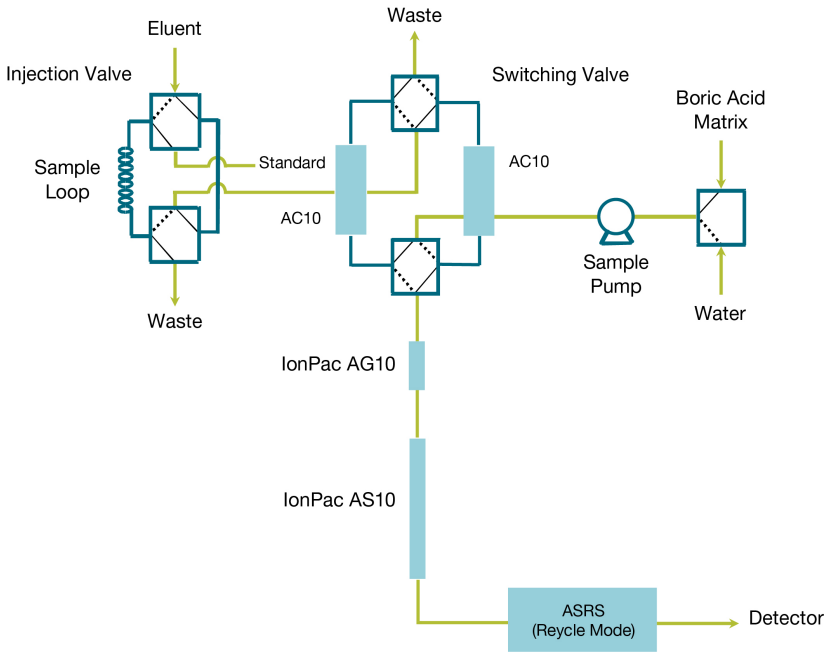


Figure 8.19 Column switching for trace analysis of anions in presence of high boric acid concentrations.

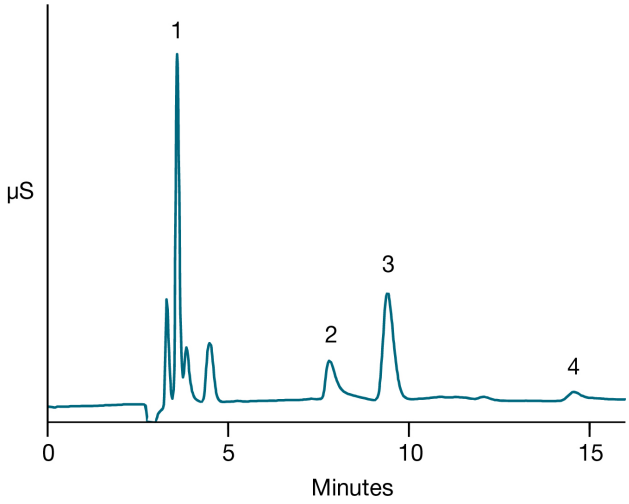


Figure 8.20 Trace analysis of anions in the primary cooling circuit of a nuclear power plant after matrix elimination. Separator column: Dionex IonPac AS10; eluent: 85 mmol/L NaOH; flow rate: 1 mL/min; detection: suppressed conductivity; concentrator column: Dionex IonPac AC10; preconcentrated volume: 12 mL; peaks: fluoride (1), boric acid (2), 5 µg/L chloride (3), and sulfate (4).

The latest development for this type of application combines the key benefits of electrolytic eluent generation and matrix elimination to facilitate the determination of trace anionic impurities in borated water [150]. The required system configuration is also based on two valves: one 10-port valve with a large sample loop (2000 µL) and a small loop (10 µL) for introducing standards, and one 6-port valve with a low-pressure concentrator column. The system also includes two analytical pumps, one to deliver deionized water for the eluent generator, the other to deliver deionized water – which is further purified with a continuously regenerated anion trap column (CR-ATC) – as the transfer solution. The CR-ATC between the two valves removes LiOH from the samples prior to the preconcentration step. The separator column for this approach used to be a Dionex IonPac AS15 column in the 2 mm format, which is now replaced by a Dionex IonPac AS28-Fast-4µm column. The method is applicable for the determination of trace anions in water containing 1000 mg/L boron and 1.8 mg/L lithium.

Flue gas scrubber solutions Nowadays, a predominant number of fossil fuel power plants are equipped with a multistage flue gas scrubber system consisting of denitrification, dedusting, and desulfurization. A large number of the desulfurization plants operate according to the lime or limestone washing technique. The final product is gypsum, which in many cases is directed to a material utilization. Routine monitoring of a flue gas desulfurization is more or less limited to the control of some chemical process parameters such as pH value, concentration of solids, and sulfite content. As in the monitoring of the water–steam cycle, the control of a few key parameters is sufficient to monitor flue gas desulfurization. In addition, product monitoring (e.g., the gypsum quality) and chemical monitoring of the wastewater processing systems that exist in many power plants have been introduced. Today, the chemical process of wet flue gas desulfurization with lime or limestone is matured. The washing liquids contain a number of ionic components, which can be analyzed by means of ion chromatography. In flue gas desulfurization based on gypsum, sulfite is oxidized to sulfate, which can be determined together with mineral acids in the same run.

Sulfur compounds other than inorganic sulfate may be detected in scrubber solutions from flue gas desulfurization. For example, dithionate, $S_2O_6^{2-}$, is formed via recombination of two sulfite radicals and can also be separated by ion chromatography [151]. Since dithionate

exhibits a strong affinity toward the stationary phase of an anion exchanger, ion-pair chromatography was commonly employed for this application. Figure 8.21 shows an example chromatogram for the determination of dithionate in a flue gas scrubber sample. As dithionate is a surface-inactive compound, tetrabutylammonium hydroxide was used as an ion-pair reagent, while organic solvents such as acetonitrile or methanol served to adjust the polarity of the mobile phase. Suppressed conductivity detection is the only viable detection method because dithionate is UV transparent. With the introduction of the Dionex IonPac AS32-Fast-4µm column, dithionate can be analyzed by anion-exchange chromatography. However, for the analysis of polythionates, $S_nO_6^{2-}$ ($n > 2$), ion-pair chromatography is still indispensable. The same is true for the analysis of sulfur–nitrogen compounds [152], which includes compounds such as amidosulfonic acid, iminodisulfonic acid, hydroxylaminedisulfonic acid, hydroxylamine-trisulfonic acid, and nitrilotrisulfonic acid, all of which were clearly identified in the scrubber solution from flue gas desulfurization. While amidosulfonic acid elutes close to chloride, nitrilotrisulfonic acid as a trivalent species exhibits a much higher retention than dithionate. The appearance of these compounds is not only of interest for the analytical chemist but also of practical relevance for the desulfurization process.

8.3 Ion chromatography in the semiconductor industry

Ion chromatography offers an efficient method for analyzing ionic species for the production of semiconductor components and printed circuit boards. Conventional methods based on the SEMI (*S*emiconductor *E*quipment and *M*aterials *I*nstitute, Inc.) regulations for identifying and quantifying ionic species responsible for corrosion are labor-intensive and time-consuming. However, it is possible to obtain complete anion and cation profiles within 10 min or less using ion chromatography. The various application areas and typical analytical examples are summarized in Table 8.8. In the following section, the most important applications for ion chromatography in the field of microelectronics are illustrated by characteristic examples.

High-purity water analysis The quality of the ultrapure water required in high quantities in the manufacture of printed circuit boards is of great importance. As in the field of power plant chemistry, ion chromatographic methods for determining mineral acids, orthosilicate, borate, alkali metals and transition metals have been used in the semiconductor industry for years to ensure this quality. Today, these methods are fully automated. The necessary instrument hardware may be configured for both laboratory and on-line operation. On-line operation allows continuous monitoring of the ultrapure water quality at several sampling locations that are connected to the chromatograph via appropriately dimensioned tubing. A personal computer with the appropriate software serves as the control unit of such an on-line ion chromatograph. With the software, individual sample streams can be selected in any sequence and frequency, and subsequently analyzed. The calibration of the system can also be fully automated. In this case, the required standards are prepared by the programmable dilution of concentrated standard solutions in a specially designed module and then delivered to the chromatograph as a separate stream. The analysis report is usually obtained not in form of actual chromatograms, but rather as numeric printouts of the determined solute concentrations or graphic depictions of trending diagrams.

With an increase in integration density of semiconductor components on the printed circuit boards, the demands on the purity of water and other chemicals also increase rapidly. While at present the specifications for ionic contaminations in high-purity water are in the lowest nano-gram/liter range, the picogram/liter level is under consideration as the quality profile in the

Table 8.8 Application areas and typical analytical examples in the semiconductor industry.

Application area	Analytical example
Water analysis	Determination of mineral acids in high-purity water, process liquors, and rinsing and wastewater
Etching solutions	Determination of main components and impurities in HF/HNO_3^- and $HF/HAc/HNO_3$ mixtures
Solvents	Determination of ionic impurities (directly or after matrix elimination)
Acids	Determination of anionic impurities such as chloride, nitrate, and sulfate in orthophosphoric acid (electronic grade)
Hydroxides	Determination of sodium in 46% KOH
Polymers	Determination of anionic and cationic impurities in encapsulation plastics

coming years. Thus, large-volume injection with detection limits in the mid-nanogram/liter range as described in Section 8.2 cannot be applied in the semiconductor industry for lack of sensitivity. Therefore, sample preconcentration techniques using concentrator columns are invaluable in this type of industry. Corresponding examples were presented in Section 8.2.

The production of ultrahigh-purity water used in the manufacture of semiconductors and other modern electronic components includes deionization to remove corrosive strong acid anions such as chloride and sulfate. However, deionization cartridges exhaust their capacity over time and require replacement or regeneration. When these deionization cartridges begin to fail, one of the first ions to break through the cartridge and contaminate the water is orthosilicate, while the water still yields an acceptable resistivity reading of 18 MΩ·cm. Therefore, orthosilicate is a very good early indicator of cartridge depletion. Monitoring orthosilicate can help prevent manufacturing failure of expensive electronic components due to the presence of the more corrosive chloride and sulfate from cartridge exhaustion. While orthosilicate can be separated by anion-exchange chromatography at high pH, it cannot be detected sensitively by suppressed conductivity. However, orthosilicate reacts with molybdate to form a complex that can be detected with high sensitivity at 410 nm. To ensure the early recognition of cartridge depletion, orthosilicate must be detected at concentrations lower than 1 µg/L.

Detection limits for orthosilicate in the sub-microgram/liter range can only be achieved by preconcentrating ultrahigh-purity water on a Dionex IonPac AG4A-SC concentrator column, then separating the preconcentrated sample using a hydroxide-selective anion exchanger [153]. For best reproducibility and highest sensitivity, it is recommended to prepare the hydroxide eluent electrolytically (reagent-free IC) and to use an AutoPrep configuration to prepare the low-level standards for the calibration and the automatic generation of the calibration curve. Figure 8.21 shows the schematic of the configuration for this application. The ion chromatograph needs to be equipped with one 10-port valve, one 6-port valve, and one low-pressure valve. Also, two peristaltic pumps (Trovion, Singapore) are needed for this application; each one has two channels allowing it to sample from two different sources. The first peristaltic pump is used for the external chemical mode of the CR-ATC and Dionex ADRS 600 suppressor, while the second one is used for loading either the standard or the sample for the analysis.

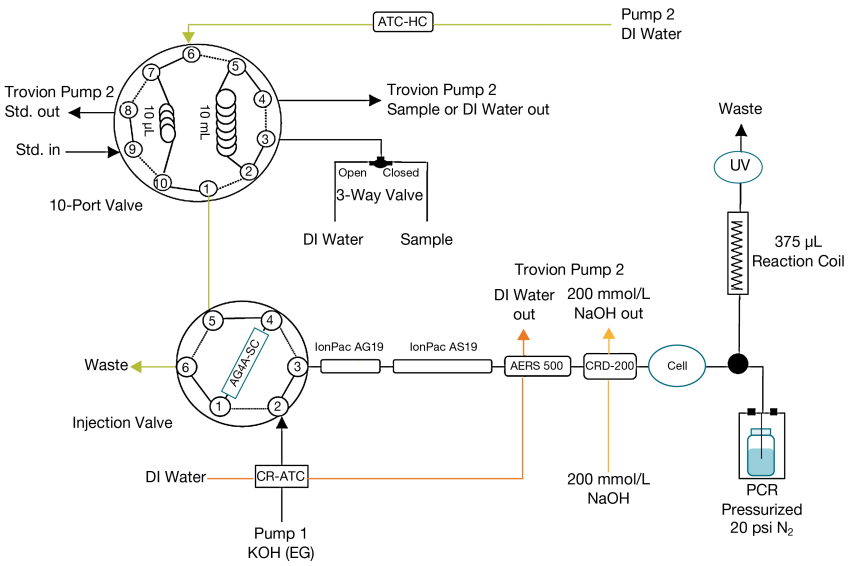


Figure 8.21 Schematic of the system configuration for the simultaneous determination of standard inorganic anions and orthosilicate based on the AutoPrep concept.

The AutoPrep system uses a series of automated steps to prepare samples and standards on-line. It has been developed to facilitate trace analysis by combining large-volume sampling with automated sample preparation. Calibration typically requires dilution of standards to establish a calibration curve. With the AutoPrep system, dilution is no longer required. A calibration curve is automatically generated using accurately timed valve toggles to load standards from a single stock standard solution. Through precise software control and accurate timing, the AutoPrep system can create calibration curves with coefficients of determination typically better than 99.9%. The analysis of samples is accomplished through the large loop on the 10-port valve. Samples are drawn into the 10 mL loop and diverted to the injection valve, where they are concentrated and loaded onto the analytical column. The autosampler should use aspiration to deliver the sample to the loop. In this way, the sample is never exposed to pump seals or other contaminants and thus detection limits are greatly improved.

The original application note developed by Rohrer et al. described the use of a low-capacity Dionex IonPac AS17 anion exchanger in the 4 mm format. However, in the latest revision of this method the separator column has been changed to a high-capacity Dionex IonPac AS19 in the microbore format. Although this column change prolongs the analysis time, the peak shape for orthosilicate is improved, and the reduction in column diameter reduces the amount of eluent used and waste generated. To accurately determine low levels of orthosilicate, it is important that the water used for the AutoPrep application is orthosilicate-free. Rohrer et al. [153] used the CIRA water polishing device from Trovion to create orthosilicate-free water. This device can be used to prepare water from a pressurized bottle of deionized water or on-line with a second pump taking the water that exits the regenerant line of the CR-ATC and directs this to the CIRA device, which delivers the purified water to the large loop when required. Alternatively, deionized water can be pumped through a high-capacity anion trap column (ATC-HC) for further purification, as illustrated in Figure 8.21. The respective chromatography illustrated in Figure 8.22 shows good linear peak area response over the concentration range chosen for all eight anions.

Surface contaminations It is well known that ionic contaminations on the surface of electronic components impair their functioning. Corrosion of aluminum after etching, for example, is regarded to be one of the major problems in the dry, chlorine gas-based plasma etching of aluminum. Traces of chlorine that remain at the surface of the silicon wafers after the etching process, when exposed to ambient air, are the major origin of corrosion phenomena. This typically leads to bubble-type outgrowths on aluminum conductors. With an increased copper content in aluminum, this problem is aggravated because this system then acts like a galvanic cell, which is characterized by HCl-catalyzed chemical reactions. Current procedures for decreasing corrosion phenomena include intensive rinsing after the etching processes and heating of the silicon wafers at high temperatures. In the past, extractions with relatively large volumes (25 mL) limited the applicability of ion chromatography to identify and quantify ionic contaminants. Thus, contaminations on the silicon wafer surface could only be investigated on relatively large sections. However, if small extraction volumes are applied, corrosion phenomena can be investigated on single integrated circuits on the silicon wafer. To do this,

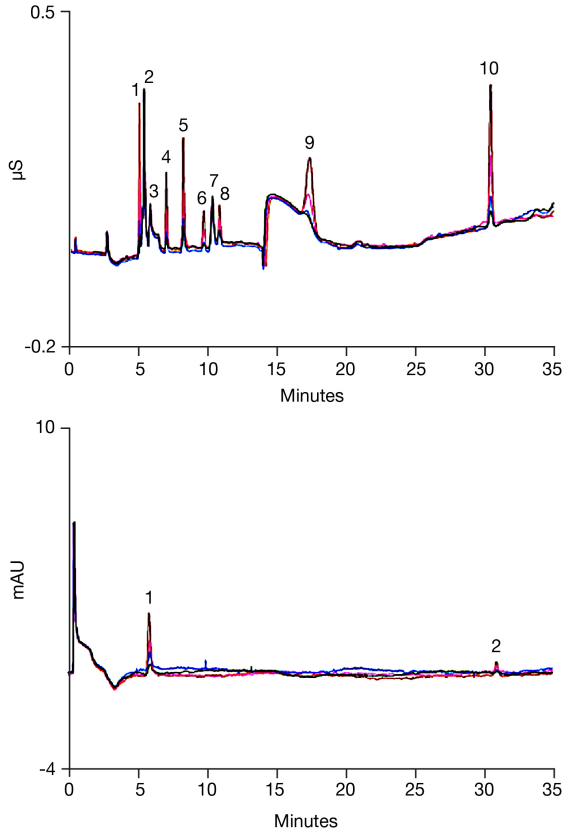


Figure 8.22 Chromatograms of calibration standards of common inorganic anions and orthosilicate using RFIC and the AutoPrep concept. Separator column: Dionex IonPac AS19; column dimensions: 250 mm × 2 mm i.d.; column temperature: 35 °C; eluent: KOH (EG); gradient: 15 mmol/L from 0 to 12 min, then linearly to 50 mmol/L in 23 min; flow rate: 0.3 mL/min; detection: (a) suppressed conductivity, (b) UV/Vis at 410 nm after postcolumn derivatization; postcolumn reagent: 20 mmol/L sodium molybdate + 0.2 mol/L HNO_3 + 6 mmol/L sodium lauryl sulfate; reaction coil: 375 µL; PCR flow rate: 0.15 mL/min; sample: calibration standards (see Table 8.9); preconcentrated volume: 10 mL.

Table 8.9 Calibration standards of common inorganic anions and orthosilicate using RFIC and AutoPrep.

	Concentration (ng/L)			
Analyte	**Level 1 (1×)**	**Level 2 (2×)**	**Level 3 (4×)**	**Level 4 (8×)**
Fluoride	10	20	40	80
Orthosilicate	500	1000	2000	4000
Chloride	10	20	40	80
Nitrite	20	40	80	160
Bromide	15	30	60	120
Nitrate	10	20	40	80
Sulfate	60	120	240	480
Orthophosphate	100	200	400	800

100 µL of high-purity water, for example, is pipetted with an Eppendorf® pipette onto the silicon wafer surface. Then the area of the drop is measured for subsequent calculations. After 2 min, the volume is picked up and transferred into a thoroughly rinsed polyethylene vessel. With the same pipette tip, 100 µL of high-purity water is charged twice and added to the sample, which is then diluted with high-purity water to a final volume of 1300 µL. This volume can then be directly injected into the ion chromatograph using the large-volume injection technique described by Kaiser et al. [154] for trace analysis without preconcentration. In this way, anionic contaminants down to the 20 ng/L range can be localized on the silicon wafer surface and thus single process steps can be optimized in terms of corrosion.

In cooperation with the Read-Rite Company Ltd. in Thailand, Heberling et al. [155] developed a microextraction technique that is especially suited for corrosion studies on hard disk reading heads. According to this technique, the reading head to be investigated, which has an area of 0.097 cm² (nano slider) or 0.038 cm² (pico slider), is placed into the microextraction cell (see Figure 8.23) comprising a cell body and a screw made of PEEK. Using a pair of tweezers, the reading head is positioned in an O-ring, which tightens the cell at the same time. After closing the cell, high-purity water with a flow rate of 0.8 mL/min is directed through the cell for 3 min. The extractable ions are trapped on a concentrator column and subsequently separated on an analytical column. The microextraction technique allows corrosion studies on single components, through which the manufacturing process can be optimized. The valve switching that is necessary in the coupling of microextraction and ion chromatography is divided into three substeps: loading, extraction, and chromatography. The anion chromatogram of a microextract is depicted in Figure 8.24. Usually, fluoride, chloride, orthophosphate, nitrate, and sulfate are found as anionic contaminants on a reading head. A Dionex IonPac AS12A column in the microbore format has proved to be a suitable stationary phase for separating these anions, which are eluted with a carbonate/bicarbonate eluent mixture. The minimum detection limits for a single reading head obtained with this technique are in the single-digit ng/cm² range.

Analysis of solvents In the semiconductor industry, a number of organic solvents such as isopropanol, acetone, and *N*-methyl-2-pyrrolidone (NMP) are used for cleaning purposes. Because these solvents come into contact with microelectronic circuits, they also have to be investigated for ions that can cause corrosion. In the past, a large sample volume was

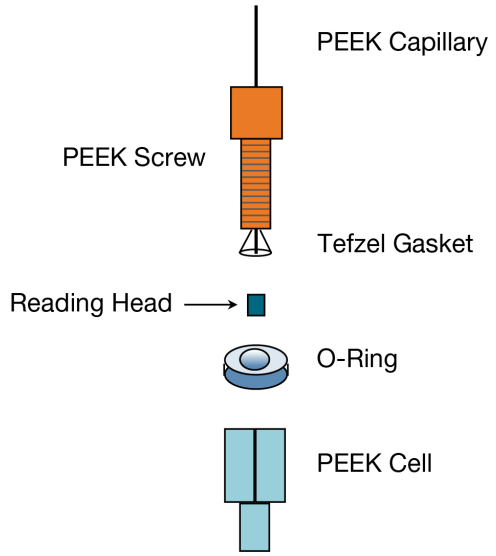


Figure 8.23 Schematic representation of a cell for microextraction of magnetic reading heads.

evaporated and the anions were separately determined by colorimetry and turbidimetry. This is not only time-consuming and labor-intensive but also very insensitive. Specifications in the microgram/liter range that are required today cannot be achieved with this method. As early as 1994, Kaiser and Wojtusik [156] demonstrated the applicability of ion chromatography for the determination of anions in isopropanol. Although modern anion exchangers are 100% solvent compatible, direct determination by injecting a large volume of solvent is not recommended for lack of reproducibility. Also, calibration via standard addition is very cumbersome.

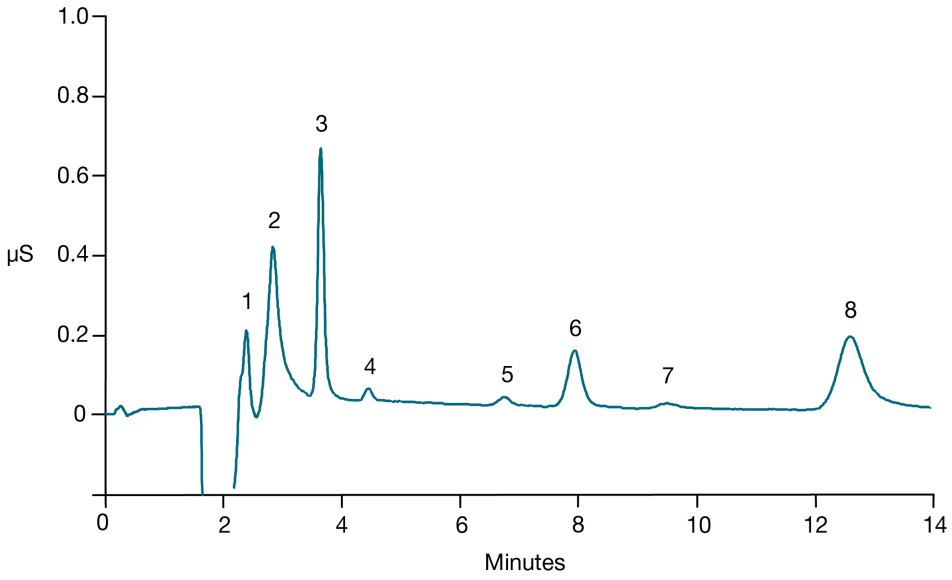


Figure 8.24 Anion chromatogram of a microextract of a single reading head. Separator column: Dionex IonPac AS12A; column dimensions: 250 mm × 2 mm i.d.; eluent: 2.7 mmol/L Na_2CO_3 + 0.3 mmol/L $NaHCO_3$; flow rate: 1 mL/min; concentrator column: 15 mm × 2 mm i.d. AMC-1; preconcentrated volume: 2.4 mL; detection: suppressed conductivity; peaks: (1) fluoride, (2) carbonate, (3) chloride, (4) unknown component, (5) unknown component, (6) nitrate, (7) orthophosphate, and (8) sulfate.

For this reason, Kaiser and Rohrer [157] utilized the technique of matrix elimination comprising four working steps:

1. Filling of the injection loop with sample
2. Loading of the concentrator column
3. Elimination of the solvent matrix
4. Separation of the preconcentrated ions

Figure 8.25 illustrates the required valve switching for this technique. In the first step (Figure 8.25a), the injection loop (5 mL) is filled with the solvent sample. Sample delivery is carried out with a simple isocratic pump out of a reservoir with a small head pressure, so that the loop is rinsed several times with sample. After loading the loop, the sample is transferred with ultrahigh-purity water to the concentrator column, in which the anions or cations are retained (Figure 8.25b). The remaining solvent is rinsed off with ultrahigh-purity water. Afterwards, the concentrator column is put in line with the analytical separator column. The preconcentrated ions are then eluted from the concentrator column in opposite directions and separated on the analytical separator column (Figure 8.25c). The ultrapure water used for the sample transfer is directed through a trap column prior to entering the switching valve. In this trap column, anionic or cationic impurities are retained. This is necessary to avoid contamination of the concentrator column during the rinse step. The great advantage of matrix elimination is the ability to prepare standards in deionized water and to calibrate externally. Kaiser et al. obtained detection limits between 0.2 and 1 µg/L for anions such as chloride, sulfate, orthophosphate, and nitrate. A representative chromatogram of an isopropanol sample spiked with 10 µg/L each of the anions of interest is shown in Figure 8.26. The Dionex IonPac AS10 high-capacity anion exchanger used as a stationary phase was operated with a NaOH eluent.

Cation analysis in organic solvents such as methyl-*n*-amylketone (MAK), *n*-butylacetate (NBA), and propyleneglycol-monomethylether acetate (PMA) was described by Sanders [158]. Because these solvents are not water-miscible, the cations must be extracted prior to IC analysis. To simplify sample preparation as much as possible, Sanders carried out the extraction in an autosampler vial, in which he pipetted the sample and an internal standard solution. The vial was then sealed, shaken for 10 min, and placed in the autosampler. The autosampler needle was adjusted in the way that it charged the aqueous extract short of the bottom of the vial. For the separation of mono- and divalent cations Sanders used a Dionex IonPac CS12A cation exchanger with a dilute sulfuric acid eluent. An elevated column temperature of 50 °C was applied to improve the peak efficiencies of magnesium and calcium, which did not have any negative effect on the column lifetime but shortened total analysis time to less than 8 min. Figure 8.27 shows the chromatogram of a 5 µg/L cation standard under the given chromatographic conditions.

Analysis of acids, bases, and etching agents Concentrated acids also play an important role in the manufacturing of semiconductor devices. Hydrofluoric acid, for example, is used to remove oxide layers from wafer surfaces; glycolic acid is a major component of nonaqueous soldering agents; and concentrated orthophosphoric acid is used for etching silicon nitride on wafer surfaces.

The extremely large excess of matrix ions renders the analysis of anionic impurities very difficult. Concentration differences between matrix and analyte ions of 10^6:1 are not unusual (e.g., chloride contamination in the range of 100 µg/L in 24.5% (*v/v*) HF). Although the matrix problem can be solved by diluting the acids, the sample then falls below the detection limits for the analyte ions. Siriraks et al. [159], for example, obtained detection limits between 0.04 and 0.08 mg/L for chloride, bromide, orthophosphate, and sulfate in 0.25% HF by direct injection.

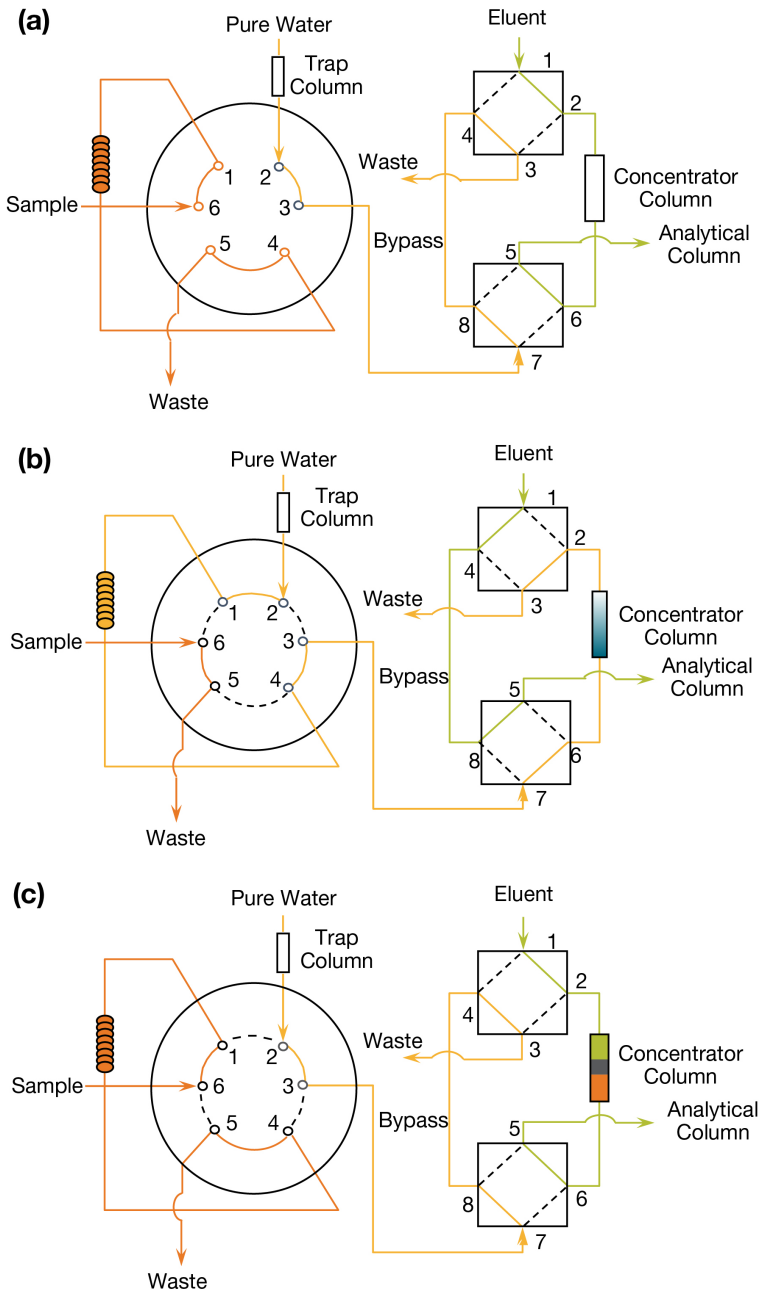


Figure 8.25 Configuration of an IC system for matrix elimination. (a) Filling of the injection loop, (b) loading of the concentrator column and elimination of the matrix, and (c) separation of the retained ions.

However, this sensitivity is by far insufficient to meet today's demands. On the other hand, 5% HF is the maximum concentration that can be injected directly into conventional ion exchangers. To improve this, Watanabe and Ishizaki [160] developed a two-dimensional technique with a preseparation between weak and strong acids on an ion-exclusion column. In a second step, the fraction with the anionic contaminants of interest was separated on an anion exchanger. With this technique, detection limits of 30 µg/L in 25% HF were achieved for the first time. Chen and Wu [161] optimized the experimental conditions of this technique and applied it to the determination of anionic contaminants in orthophosphoric acid. Finally, Kaiser et al. [162] modified this technique by using an anion exchanger in the microbore format, which increased sensitivity even further. The required instrument configuration with two switching valves is very similar to the one illustrated in Figure 8.25. The only difference is the ion-exclusion column for the preseparation mounted between the two valves. Loading of the

injection loop is carried out pneumatically from a pressurized (helium) reservoir. The anionic contaminants are then preseparated from the acid on an ion-exclusion column. After the first part of the column effluent is discarded, the concentrator column is put in line with the ion-exclusion column, so that the fraction with the anionic contaminants can be preconcentrated.

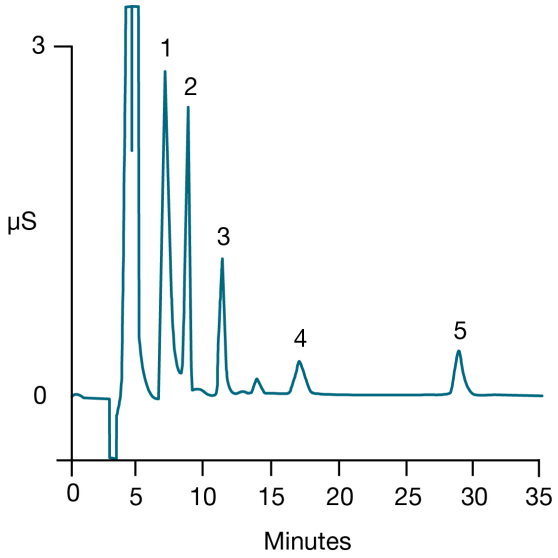


Figure 8.26 Separation of anions in a spiked isopropanol sample after matrix elimination. Separator column: Dionex IonPac AS10 with guard column; eluent: 0.1 mol/L NaOH; flow rate: 1 mL/min; detection: suppressed conductivity; preconcentrated volume: 5 mL; concentrator column: Dionex IonPac AC10; sample: isopropanol (electronic grade); peaks: carbonate (1) and 10 µg/L each of chloride (2), sulfate (3), orthophosphate (4), and nitrate (5).

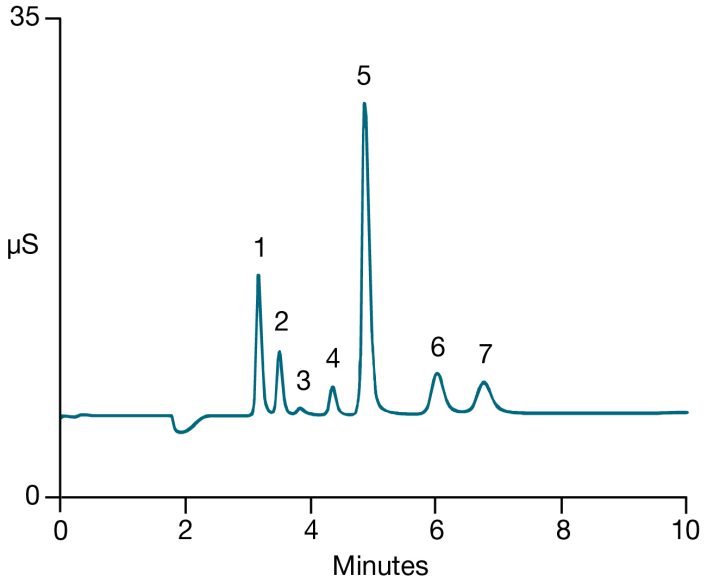


Figure 8.27 Trace analysis of a cation standard after matrix elimination. Separator column: Dionex IonPac CS12A; eluent: 11 mmol/L H_2SO_4; flow rate: 1 mL/min; detection: suppressed conductivity; preconcentrated volume: 2 mL; concentrator column: Dionex IonPac CG12A; peaks: 5 µg/L each of lithium (1), sodium (2), ammonium (3), potassium (4), magnesium (6), and calcium (7), 140 µg/L rubidium (5) as an internal standard (reproduced with permission from Ref. [158]. Copyright 1998, Elsevier Science B.V.).

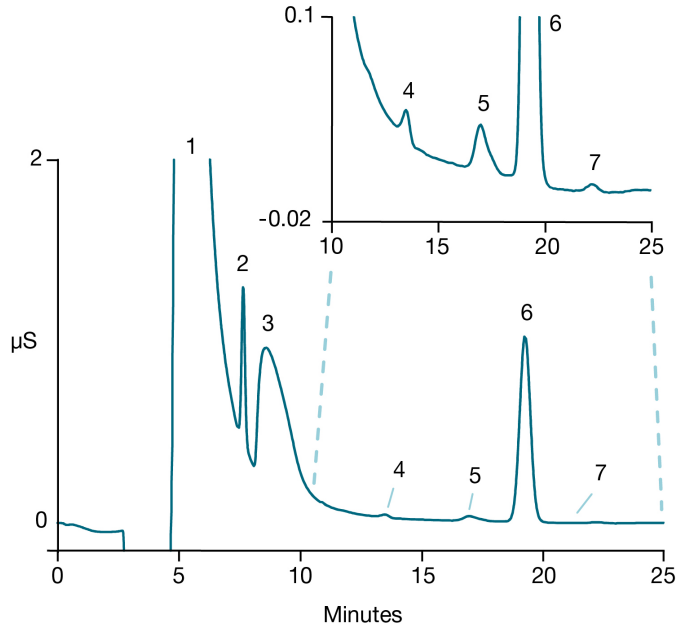


Figure 8.28 Separation of chloride, nitrate, and sulfate in 24.5% hydrofluoric acid (*w/w*) after preseparation on a Dionex IonPac ICE-AS6. Eluent for the preseparation: deionized water; flow rate: 0.55 mL/min; concentrator column: IonPac AG9-HC (4 mm); separator column: Dionex IonPac AS9-HC (2 mm); eluent: 8 mmol/L Na_2CO_3 + 1.5 mmol/L NaOH; flow rate: 0.25 mL/min; injection volume: 750 µL; peaks: fluoride (1), 7.9 µg/L chloride (2), carbonate (3), 0.9 µg/L nitrate (4), unknown (5), 10.1 µg/L sulfate (6), and 2.4 µg/L orthophosphate (7).

In the last step, the concentrator column is put in line with the anion exchanger and the preconcentrated anions are eluted. As an example, Figure 8.28 shows the separation of inorganic anions in 24.5% HF (*w/w*) on an IonPac AS9-HC following a preseparation on an IonPac ICE-AS6. The concentrated hydrofluoric acid must be diluted with ultrapure deionized water because repeated injections of a 49% HF (*w/w*) lead to column bleeding. As can be seen from Figure 8.28, the fluoride matrix is so much impoverished after the preseparation on an ICE column that the analysis of the anionic impurities can be done without interferences. A satisfactory separation between chloride and carbonate, which elute immediately after fluoride, is also ensured under these chromatographic conditions. The method of standard addition is recommended for calibration. The relative standard deviation is less than 10% in the concentration range between 10 and 50 µg/L.

For the determination of cationic impurities in concentrated acids, the AutoNeutralization technique must be used. The direct injection of the acids is not possible for lack of sensitivity. Moreover, if a weak acid cation exchanger was used for the simultaneous analysis of mono- and divalent cations, the high acid concentration would lead to a complete protonation of the ion-exchange groups which, in turn, would result in a loss of separation power. Figure 8.29 shows the cation analysis in 12% hydrofluoric acid, which is representative of all the other acids mentioned above. Separation was carried out on an IonPac CS12 cation exchanger with methanesulfonic acid as an eluent. In addition to alkali and alkaline-earth metals, ammonium and methylamine could be detected. The latter were not quantitated because they are partly released from the suppressor membrane used for AutoNeutralization; this is attributed to the high acid strength in the original sample.

AutoNeutralization also has to be applied for trace analysis of anions in bases such as sodium hydroxide, ammonium hydroxide, tetramethylammonium hydroxide, and tetrabutylammonium hydroxide because the high concentration of hydroxide in the matrix would in practice act as an eluent and thus, render impossible the preconcentration of anions in a concentrator column.

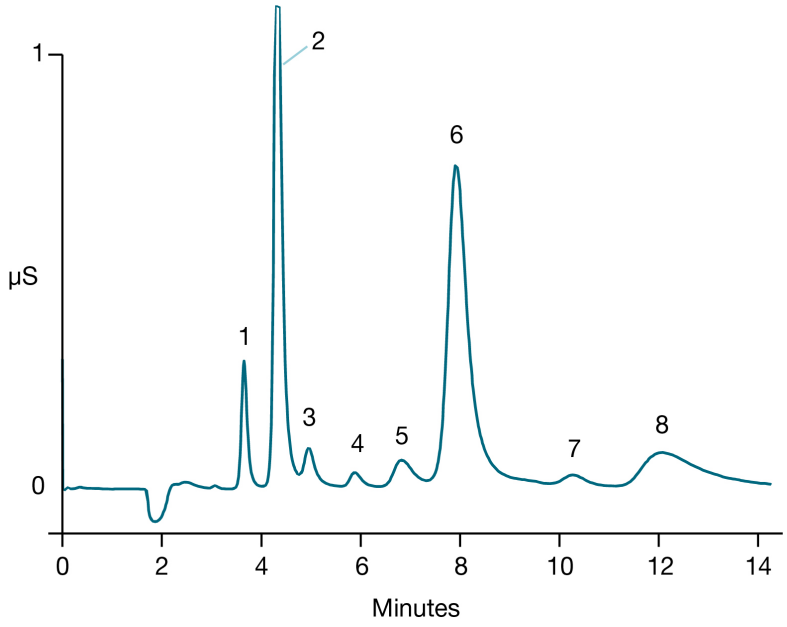


Figure 8.29 Separation of cations in 12% hydrofluoric acid after AutoNeutralization. Separator column: Dionex IonPac CS12; eluent: 19 mmol/L methane-sulfonic acid; flow rate: 1 mL/min; detection: suppressed conductivity; injection volume: 100 µL; peaks: 8 µg/L sodium (1), ammonium (2), monomethylamine (3), 5 µg/L potassium (4), dimethylamine (5), 69 µg/L magnesium (6), 4.5 µg/L calcium (7), and trimethylamine (8).

In the semiconductor industry, quaternary ammonium bases serve as developers for light-sensitive paints and, therefore, also must be investigated for anions responsible for corrosion.

One of the most important chemicals in the semiconductor industry is hydrogen peroxide, which is used in a number of etching and cleaning processes. Due to the high demands for the purity of this chemical, it also must be investigated for ionic impurities. While hydrogen peroxide solutions with content below 3% can be analyzed by ion chromatography without any sample preparation, a significant decrease in the lifetime of conventional ion exchangers is observed at higher H_2O_2 concentrations. In the case of anion analysis, the high eluent pH also supports the formation of peroxohydroxide anions, HO_2^-, and thus causes oxidation of the resin material. Because dilution is not an option due to the required detection limits, the matrix must be eliminated in some way. In the past, the sample was transferred into a platinum crucible, in which hydrogen peroxide is broken down to water and oxygen under the catalytic effect of platinum. However, this kind of sample preparation is very time-consuming and prone to contamination. If the hydrogen peroxide to be analyzed contains a stabilizer, there is a high risk that this stabilizer will be degraded by the heat generated during the platinum treatment. Today, on-line matrix elimination is the sample preparation method of choice, as described above for anion analysis in organic solvents. Since inorganic anions and traces of organic acids are to be expected as anionic contaminants, Kerth and Jensen [163] applied a hydroxide gradient elution technique on a Dionex IonPac AS11 anion exchanger. Because the hydrogen peroxide used in the semiconductor industry does not contain stabilizers such as pyrophosphate, analysis time is on the order of 15 min. Figure 8.30 shows the chromatogram of a 35% hydrogen peroxide solution (electronic grade), which was obtained after preconcentration of a 750 µL sample. Besides the very low concentrations of chloride, nitrate, and sulfate in the lowest microgram/liter range, the sample shows a relatively complex pattern of organic acids in the retention range of sulfate. Under the given chromatographic conditions, sulfate is separated from these components and can be quantitated without difficulty.

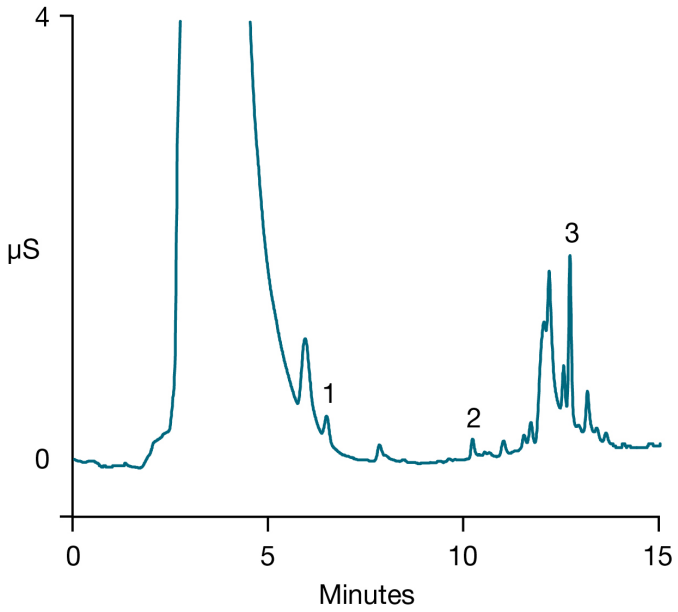


Figure 8.30 Trace analysis of anions in 35% hydrogen peroxide solution after matrix elimination. Separator column: Dionex IonPac AS11; eluent: (A) 50 mmol/L NaOH, (B) water; gradient: 3% A for 3 min isocratic, then linearly to 80% A in 13 min; flow rate: 1 mL/min; detection: suppressed conductivity; injection volume: 750 µL; concentrator column: Dionex IonPac AG11; sample: 35% hydrogen peroxide (electronic grade); peaks: 2.5 µg/L chloride (1), 1.4 µg/L nitrate (2), and 2.9 µg/L sulfate (3) (reproduced with permission from Ref. [163]. Copyright 1995, Elsevier B.V.).

Another application area for ion chromatography is the analysis of etching solutions (i.e., mixtures of different acids), with which metal oxides and other impurities can be removed from metal surfaces. The choice of acids depends on the type of materials to be etched. While a mixture of hydrofluoric acid, nitric acid, and acetic acid etches silicon dioxide without affecting elemental silicon itself, mixtures of orthophosphoric acid, acetic acid, and nitric acid are used for etching aluminum. The efficiency of the etching process depends on the temperature of the etching solution and on the concentration of the acids. Because the depth of penetration in the etching process must be thoroughly controlled, the concentration of the individual acids must be determined with an absolute tolerance between 0.5 and 1%. In the past, titration and spectrophotometric techniques were applied, which were very time-consuming due to extensive sample preparation. Figure 8.31 shows the chromatogram of a HF/HNO_3/HOAc etching mixture diluted 1:500 with deionized water, which was obtained by ion-exclusion chromatography with suppressed conductivity detection. It must be noted that with ion chromatography, only the total fluoride concentration and not the concentration of the free hydrofluoric acid is detected because metal–fluoride complexes (for example FeF^{2+}) are formed during the etching process. At alkaline pH, these complexes decompose to form metal hydroxides.

Solar cell manufacturing Energy generation based on renewable energy sources, including biomass, biogas, biofuels, water, wind, and solar energy, becomes more and more important. Special interest applies to the sheer inexhaustible solar energy. Solar cells used in photovoltaic sites convert the energy of light directly into electricity. Solar cells are made from multicrystalline silicon wafers. Prior to the doping process with boron and phosphorus, the wafer surfaces are pretreated with etching solutions (texturization baths). The dissolution of silicon in a solution of hydrofluoric acid (HF) and nitric acid (HNO_3) removes the saw damage from the wafer surface and creates a surface structure that improves the light coupling into the solar cell and, therefore, the cell efficiency. Both the etching rate and the surface morphology

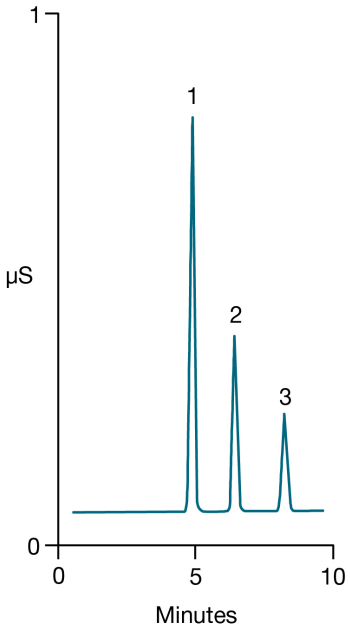


Figure 8.31 Analysis of a HF/HNO_3/HOAc etching mixture. Separator column: Dionex IonPac ICE-AS1; eluent: 1 mmol/L octanesulfonic acid/2-propanol (95:5 *v/v*); flow rate: 1 mL/min; detection: suppressed conductivity; injection volume: 10 µL; sample: etching mixture (1:500 diluted); peaks: 200 mg/L HNO_3 (1), 200 mg/L HF (2), and 800 mg/L HOAc (3).

mainly depend on the chemical composition of the texturization bath. Hence, maintaining a constant process requires a fast and reliable analytical method for determining the main bath components. In addition to titrimetric methods, Acker and Henßke [164] described an ion chromatographic method for the analysis of acid texturization baths. While the nitric acid amount is directly measured as nitrate, the concentrations of HF and H_2SiF_6 are both calculated from the measured fluoride concentrations. Therefore, a precipitation step is needed to remove hexafluorosilicate from the solution. The HF concentration can then be determined in the filtrate; the H_2SiF_6 concentration is calculated from the fluoride concentration of the redissolved and hydrolyzed precipitate. Although this approach is highly accurate, the high expenditure of time does not allow an implementation as an on-line process control method. A much faster solution for this analytical problem was reported by Zimmer et al. [165], combining suppressed conductivity detection for the determination of fluoride and nitrate with postcolumn derivatization of the present orthosilicate with sodium molybdate. Orthosilicate is transformed from hexafluorosilicate in the alkaline eluent according to the following equation:

$$Na_2SiF_6 + 4NaOH \rightarrow Si(OH)_4 + 6NaF \qquad (8.1)$$

Thus, injection of hexafluorosilicate results in a fluoride peak in suppressed conductivity detection and an orthosilicate peak in UV/Vis detection following postcolumn derivatization. If there are no other sources of fluoride or orthosilicate present, a mass balance can be derived from the respective peak areas, which confirms that the hexafluorosilicate concentration can be calculated stoichiometrically from the determined fluoride and orthosilicate concentrations. The determination of free hydrofluoric acid is then calculated as the difference between the total fluoride and the hexafluorosilicate concentration. This method not only enables the simultaneous measurement of all components in acid texturization baths, but can also be employed as a fully automated on-line method with a cycle time of 8 min. For this application, Zimmer et al. used a high-capacity Dionex IonPac AS11-HC hydroxide-selective anion exchanger with an eluent of 30 mmol/L KOH and a slightly elevated flow rate of 1.3 mL/min.

8.4
Ion chromatography in the detergent and household product industry

Detergents Detergents for household and industrial use consist of many very dissimilar individual components. As a result of technical developments, and in response to ecological and economical restrictions, these products are constantly changing. Thus, it is not surprising that in this field analytical methods are continually under further development and that product-related analytical methods come of age fairly rapidly.

Ionic detergent components like the following play a key role in the washing process:

- Surfactants
- Builders
- Bleaching agents
- Fillers and finishing materials

The determination of these compounds represents a classic analytical problem for the detergent industry. Conventional wet-chemical and instrumental methods for analyzing ionic detergent components such as gravimetry, potentiometry, complexometry, and photometry are labor-intensive and time-consuming. Therefore, they were replaced by much faster and more sensitive ion chromatographic techniques [166, 167].

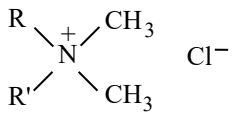
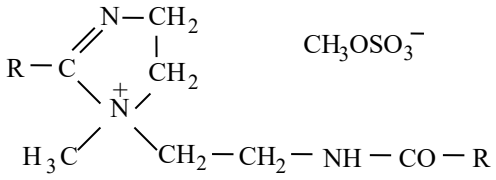
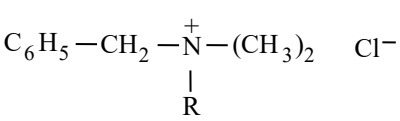
Surfactants are the most important group of detergent components; they are constituents of all detergents. Generally, they are water-soluble, surface-active compounds that carry both a hydrophilic functional group and a long alkyl chain as a hydrophobic rest. These substances are generally categorized into the following surfactant groups:

- Anionic surfactants
- Cationic surfactants
- Nonionic surfactants
- Amphoteric surfactants

Surfactants that can presently be analyzed by means of ion chromatography are summarized in Table 8.10. A chromatographic determination of the compounds can be achieved using either ion-pair chromatography in combination with conductivity or UV detection or, more recently, with mixed-mode liquid chromatography in combination with conductivity, UV, or charged aerosol detection. For an unequivocal identification of surfactants in detergent products, however, a wet-chemical preseparation into the individual surfactant types is necessary because detergent formulations often contain a combination of surfactants such as anionic and nonionic surfactants. The carbon-chain distribution can then be determined by ion-pair chromatography. If a crude material consists of several compound classes, a comparison of the chromatographic "fingerprints" can provide valuable information for raw material control.

The *builders* include complexing agents such as sodium tripolyphosphate, ion exchangers such as Zeolite A, and washing alkalines such as sodium carbonate and sodium orthosilicate. Inorganic and organic complexing agents are of central importance in the course of the washing process because magnesium and calcium ions stemming from water, dirt, or textiles are complexed with their help. In many countries, sodium tripolyphosphate is no longer used for environmental reasons, as high consumption contributed to eutrophication of aquatic systems. In the so-called phosphorus-free detergents, sodium tripolyphosphate has been replaced by Zeolith A and polyphosphonic acids. Both sodium tripolyphosphate and polyphosphonic acids are analyzed with a Dionex IonPac AS7 nanobead-agglomerated anion exchanger in combination with a specific postcolumn derivatization [168, 169]. The elution of condensed phosphates in finished products requires the use of 0.07 mol/L nitric acid. Sulfate, which is added to the washing powder as a formulating agent, can also be detected under these

Table 8.10 Summary of surfactants that can be analyzed by ion chromatography.

Formula		Chemical name
$R–CH_2–COONa$	$R = C_{10}$ to C_{16}	Soap
$R–C_6H_4–SO_3Na$	$R = C_{11}$ to C_{13}	Alkylbenzene sulfonate
$R–CH(R')–SO_3Na$	$R, R' = C_{10}$ to C_{17}	Alkane sulfonate
$R–CH_2–CH{=}CH–(CH_2)_n–CH_2–SO_3Na$	$R = C_{10}$ to C_{14} $n = 1$ to 2	α-Olefin sulfonate
$R–CH_2–CH(OH)–(CH_2)_n–CH_2–SO_3Na$	$R = C_9$ to C_{13} $n = 1$ to 2	Hydroxyalkane sulfonate
$R–CH(SO_3Na)–COOCH_3$	$R = C_{14}$ to C_{16}	α-Sulfofatty acid methylester
$R–CH_2–O–SO_3Na$	$R = C_{11}$ to C_{17}	Alkyl sulfate
$R–CH(R')–O–(C_2H_4O)_2–SO_3Na$	a) $R' = H$ $R = C_{10}$ to C_{13} b) $R, R' = C_{10}$ to C_{14}	Alkylether sulfate a) Fatty alcohol ethersulfate b) *sec.* Alkyl ether sulfate
	$R = C_{16}$ to C_{18}	Dialkyl-dimethylammonium chloride
	$R = C_{16}$ to C_{18}	Imidazolinium salts
	$R = C_8$ to C_{18}	Alkyl-dimethyl-benzyl-ammonium chloride

chromatographic conditions. Calibration for the quantitation of tripolyphosphate is difficult because commercial tripolyphosphate also contains noticeable amounts of pyrophosphate. In the absence of a high-purity tripolyphosphate, one can use the phosphorus-specific detection method developed by Vaeth et al. [170], in which pyrophosphate and tripolyphosphate are hydrolyzed to orthophosphate. The latter is then detected photometrically after reaction with sodium molybdate. This method is extremely well suited for determining the degree of phosphate conservation. Today, condensed phosphates can be separated together with mineral acids on hydroxide-selective stationary phases with a hydroxide gradient and detected by suppressed conductivity.

The *bleaching process* is based on sodium peroxoborate, which hydrolyzes in aqueous solution to form hydrogen peroxide. To obtain a good bleaching process at temperatures below 60 °C, so-called bleaching activators such as tetraacetylethylenediamine (TAED) are used. Together with hydrogen peroxide they form organic peracid intermediates at pH values between 9 and 12. When stored for some time, TAED decomposes, as acetic acid is lost. As illustrated in Figure 8.32, perborate and acetic acid can be easily and rapidly determined via ion chromatography in the finished detergent product. Carbonate analysis in the same run is possible, too.

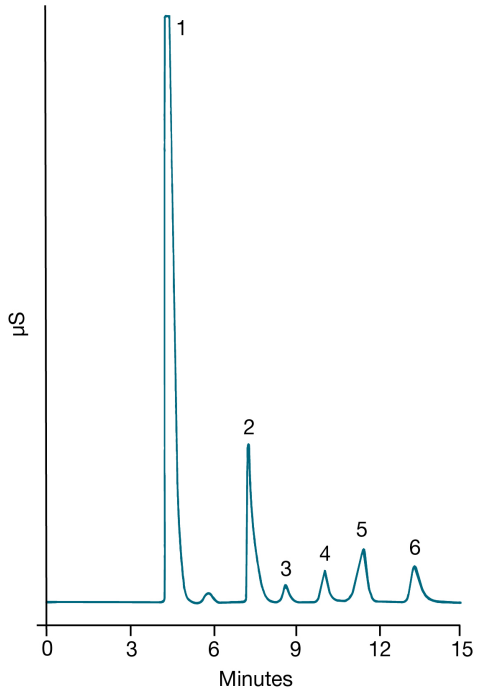


Figure 8.32 Analysis of sodium perborate by ion-exclusion chromatography. Separator column: Dionex IonPac ICE-AS1; eluent: 1.1 mmol/L octanesulfonic acid; flow rate: 1 mL/min; detection: suppressed conductivity; injection volume: 50 µL; sample: 0.05% detergent solution; peaks: (1) sulfate, (2) perborate, (3) H_2O_2 interference, (4) acetate, (5) NTA, and (6) carbonate.

Sodium sulfate is generally used as a *filler* in powdered detergents. Sulfate can be analyzed by anion-exchange chromatography in a single run with chloride and orthophosphate. Orthophosphate, via the pyrophosphate anion, represents the degradation product of tripolyphosphate. In comparison with wet-chemical methods, ion chromatography requires significantly less time for each sulfate determination. Furthermore, ion chromatography has the advantage that short-chain alkyl sulfonates and alkyl sulfates do not interfere with the inorganic sulfate determination, and chloride and orthophosphate can be detected in the same run. The finishing of liquid detergents and cleansers typically employs short-chain alkylbenzene sulfonates such as toluene sulfonate or cumene sulfonate which, because of their hydrotropic properties, ensure the solubility of other detergent components in an aqueous environment. Aryl sulfonates can be separated by mixed-mode liquid chromatography using a silica-based column that provides both reversed-phase and anion-exchange retention mechanisms. As illustrated in Figure 8.33, the Acclaim Surfactant Plus column provides adequate retention of xylene sulfonate, which, under the same chromatographic conditions, would elute close to the void on a conventional C18 column. The mobile phase consists of ammonium acetate and acetonitrile and is compatible with a variety of detection methods, including UV, CAD, ELSD, and MS.

Household products As an example of a household product, Figure 8.34 shows the separation of inorganic constituents in toothpaste by anion-exchange chromatography. Besides monofluorophosphate (MFP), which is included in many formulations, orthophosphate as its degradation product as well as chloride and sulfate can be analyzed simultaneously. In principle, such an analysis can be carried out under isocratic conditions on any conventional anion exchanger using a carbonate-based eluent; however, shorter analysis times, higher sensitivity, and better separation between the inorganic constituents and the various organic

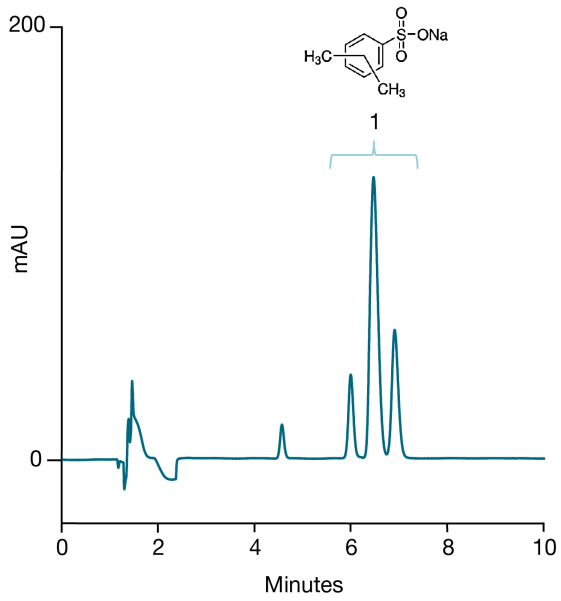


Figure 8.33 Separation of xylene sulfonate using mixed-mode liquid chromatography. Separator column: Acclaim Surfactant Plus, 3 µm; column dimensions: 150 mm × 3 mm i.d.; column temperature: 30 °C; eluent: MeCN/20 mmol/L NH_4OAc, pH 5 (30:70 *v/v*); flow rate: 0.6 mL/min; detection: UV (225 nm); injection volume: 2 µL; peak: 1 mg/mL xylene sulfonate (1).

acids present in the sample are obtained with a gradient elution technique using a low-capacity Dionex IonPac AS17 anion exchanger with a hydroxide concentration gradient. To prepare the sample, the weighed amount is suspended in deionized water and the extract is passed through a Thermo Scientific™ Dionex™ OnGuard™ cartridge prior to injection to remove nonionic organics and especially the titanium dioxide colloid.

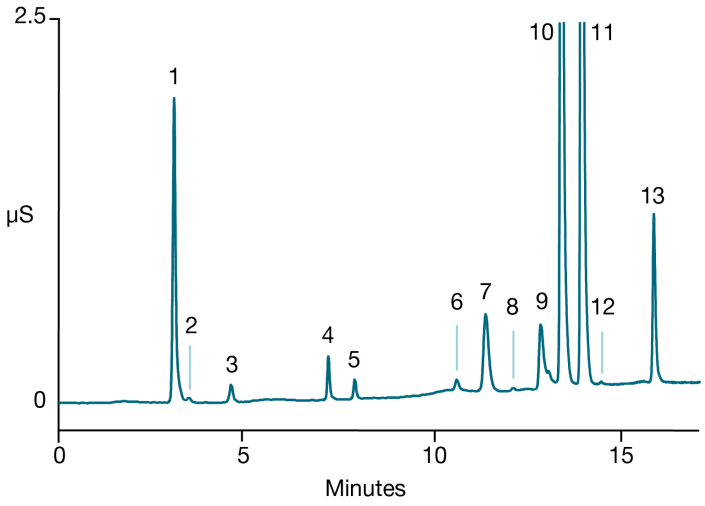


Figure 8.34 Analysis of anionic toothpaste constituents using a gradient elution technique. Separator column: Dionex IonPac AS17; column temperature: 30 °C; eluent: KOH (EG); gradient: 1 mmol/L isocratic for 3 min, then linear to 12 mmol/L in 7 min, then to 35 mmol/L in 4 min; flow rate: 1 mL/min; detection: suppressed conductivity; injection volume: 10 µL; sample: 0.5% toothpaste solution; peaks: (1) fluoride, (2) acetate, (3) formate, (4) chloride, (5) nitrite, (6) nitrate, (7) benzoate, (8) unknown, (9) carbonate, (10) monofluorophosphate, (11) sulfate, (12) oxalate, and (13) orthophosphate.

A characteristic example in the field of cosmetics is the separation and determination of surfactants in shampoos, which contain various combinations of anionic, cationic, nonionic, and amphoteric surfactants. The surfactant combination is designed to influence the essential characteristics of the shampoo: cleansing, foam, conditioning, and viscosity. Laurylether sulfates and alkylbenzene sulfonates are common anionic surfactants used in shampoos to provide lather and cleansing. The only required sample preparation step is the dilution of the product to be analyzed in deionized water and subsequent membrane filtration (0.45 µm) prior to injection. Anionic surfactants in a commercial shampoo can be analyzed by ion-pair chromatography with simultaneous nonsuppressed conductivity and UV detection. While the peak pattern in the UV trace would indicate the presence of alkylbenzene sulfonate (ABS), the characteristic peak profile for alkylether sulfates is evident in the conductivity trace after visual subtraction of the ABS signal. Therefore, ion chromatography provides a way to unequivocally identify surfactant raw materials in finished cosmetic products without time-consuming sample preparation. The individual oligomers of laurylether sulfate in a shampoo sample can also be separated by mixed-mode liquid chromatography on Acclaim Surfactant Plus employing charged aerosol detection (Figure 8.35).

Cationic surfactants are widely used as fabric softeners and antimicrobial agents. The most popular cationic surfactants include alkyl quaternary ammonium, alkylbenzyl ammonium, alkyl pyridinium, and alkyl imidazolium compounds. When separating cationic surfactants on reversed-phase columns, it is often difficult to obtain sharp, symmetrical peaks because of the undesired interactions between the free silanol groups at the stationary phase surface and the quaternary ammonium analytes. This problem can be solved by using either polymer-based reversed-phase materials in the ion-pair mode or mixed-mode liquid chromatography on Acclaim Surfactant Plus. The surface chemistry of this separator column effectively deactivates the surface silanol activity, so that cationic surfactants elute as symmetrical and efficient peaks compared to those obtained on silica- and polymer-based reversed-phase columns. Figure 8.36 demonstrates the separation of a wide variety of cationic surfactants with excellent resolution and peak symmetry.

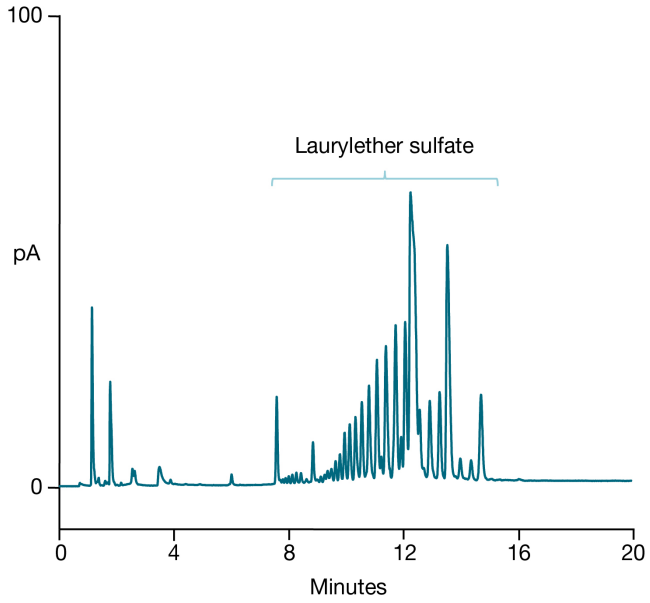


Figure 8.35 Gradient elution of anionic surfactants in a shampoo using mixed-mode liquid chromatography on a silica-based column. Separator column: Acclaim Surfactant Plus, 3 µm; column dimensions: 150 mm × 3 mm i.d.; column temperature: 30 °C; eluent: (A) MeCN, (B) 0.02 mol/L NH_4OAc, pH 5.2; gradient: linear, 31–80% A in 10 min; flow rate: 0.6 mL/min; detection: Corona CAD (gain: 100 pA, filter: med., neb. temp.: 20 °C); injection volume: 2 µL; sample: shampoo solution (1:40 diluted with deionized water).

8.5
Ion chromatography in the food and beverage industry

Ion chromatography has been adopted by many test and research laboratories in the food and beverage industry because minimal sample preparation is required for analyses utilizing this method. Often, sample extraction with deionized water and membrane filtration (0.45 µm) are completely sufficient for sample preparation. The reason ion chromatography easily deals with complex matrices lies not only in the stability and resistance to fouling of the stationary phases used, but also in the sensitivity and selectivity of the detection methods employed. A survey of the applicability of ion chromatography in the food and beverage industry is given in Table 8.11.

Beverage analysis One of the key applications of ion chromatography in the food and beverage industry is the analysis of inorganic and organic anions and carbohydrates in beverages of all kinds. This includes predominantly the investigation of wine, beer, fruit juices, various refreshers, coffee, and tea. In addition to inorganic anions, all these beverages contain various organic acids whose retention behavior is very similar to that of inorganic anions. Thus, modern anion exchangers of any type do not allow a baseline-resolved separation of all inorganic and organic anions under any chromatographic conditions. In special cases, inorganic

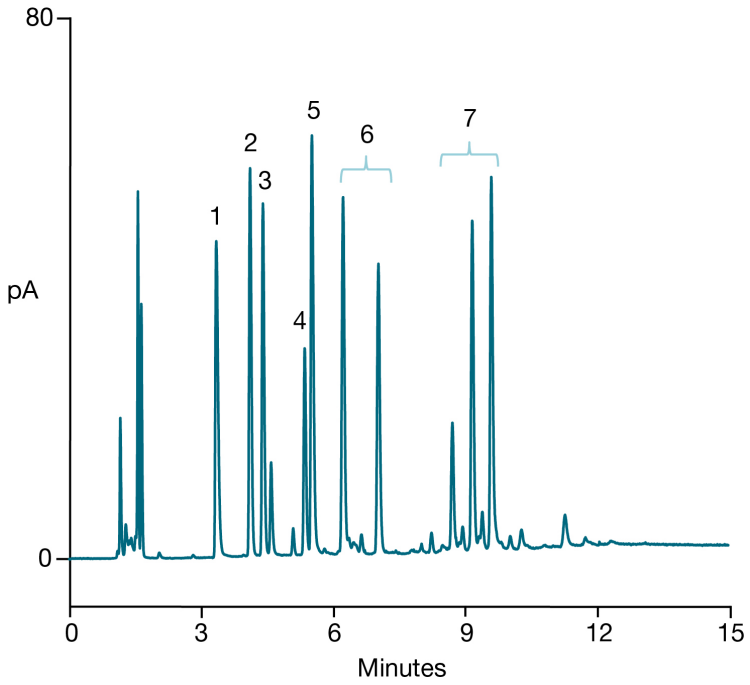


Figure 8.36 Separation of common cationic surfactants using mixed-mode liquid chromatography and charged aerosol detection. Separator column: Acclaim Surfactant Plus, 3 µm; column dimensions: 150 mm × 3 mm i.d.; column temperature: 30 °C; eluent: (A) MeCN, (B) 0.1 mol/L NH_4OAc, pH 5; gradient: 35–85% A (*v/v*) in 8 min; flow rate: 0.6 mL/min; detection: Dionex Corona CAD (gain: 100 pA, filter: med., neb. temp.: 20 °C); injection volume: 5 µL; peaks: 200–400 µg/mL each of lauryl pyridinium (1), lauryl-dimethylbenzyl ammonium (2), octyl-phenoxy-ethoxyethyl-dimethylbenzyl ammonium (3), cetyltrimethyl ammonium (4), cetyl pyridinium (5), diethyl-heptadecyl imidazolium (6), and dimethyl-dihydrogenated tallow ammonium (2M2HT) (7).

Table 8.11 Application areas and typical analytical examples in the food and beverage industry.

Application area	Analytical example
Milk products	Determination of iodide in whole milk; chloride and/or sodium in butter; lactate, pyruvate, and citrate in cheese
Meat processing	Determination of the nitrite/nitrate ratio in meat products; nitrate in the water being used
Beverages	Determination of inorganic anions and cations in the water being used, in sweeteners and flavours and in the finished products; organic acids and carbohydrates in beer, wine, and juice
Canned food	Determination of chloride, nitrate, sodium, organic acids, and transition metals in canned fruit and canned vegetables, spices, vinegar, and fish
Baby food	Determination of iodide, choline, and transition metals
Cereal products	Determination of bromate and propionate in bakery products; iron(II)/iron(III) ratio
Fats, oils, carbohydrates, and flavours	Determination of fatty acids and carbohydrates in corn syrup

anions such as chloride, nitrate, orthophosphate, and sulfate together with dicarboxylic acids such as malic acid and tartaric acid may be separated to baseline if two anion exchangers with different selectivities are combined. Detection is performed by suppressed conductivity utilizing a continuously regenerated membrane suppressor. However, this method is not applicable to the analysis of citrus juices because the citric acid that is present in high concentrations in these products is strongly retained. After repeated injections, this results in a marked reduction of the ion-exchange capacity. Therefore, a gradient technique with a hydroxide eluent on suitable stationary phases must be used for analyzing such samples.

The introduction of hydroxide-selective anion exchangers greatly simplified gradient elution of organic acids. Out of all the hydroxide-selective anion exchangers available today, the Dionex IonPac AS11-HC column is the most suitable for fruit juice analysis. For the first time, the above-mentioned organic acids could be separated together with the most important mineral acids using this column and a purely aqueous hydroxide gradient [172]. Meanwhile, this column is also available with 4 µm supermacroporous resin beads providing higher efficiency peaks and better resolution. The high ion-exchange capacity of this column allows the injection of more concentrated samples without overloading and peak broadening. A wide range of inorganic and organic acids can be separated in approximately 40 min using a hydroxide gradient at a controlled temperature of 30 °C. Figure 8.37 illustrates the separation of inorganic and organic acids in orange juice on a Dionex IonPac AS11-HC-4µm column in the capillary format. Malic acid and succinic can be resolved with this column under the given chromatographic conditions, even at very different concentrations, which was very difficult if not impossible with the Dionex IonPac AS11-HC column.

An important application area of ion chromatography in the characterization of fruit juices is the analysis of carbohydrates. Traditionally, sorbitol and the main sugars such as glucose, fructose, and sucrose were determined with enzymatic methods, which are usually very sensitive and specific. Interferences caused by the matrix are rare; however, they are possible when determining sucrose. Using ion chromatography, small amounts of sucrose in the presence of large amounts of glucose can be determined without any problem. Interferences in enzymatic analysis are also observed with colored samples. Enzymatic determination of sorbitol is especially interfered with by the fruit-owned anthocyanins in colored juices. These interferences do not occur in anion-exchange chromatography with pulsed amperometric detection. Due to the high sensitivity of this detection method, the samples to be analyzed can be strongly diluted, so matrix interferences are usually not observed. For example, the

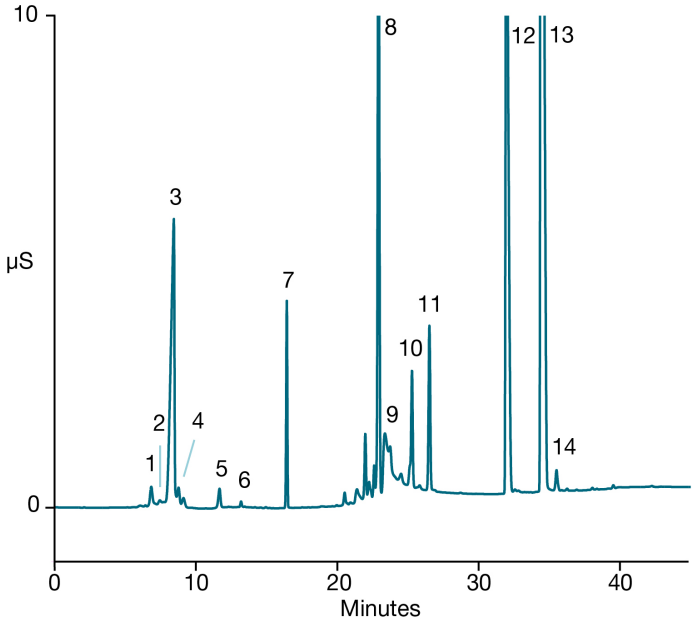


Figure 8.37 Gradient elution of inorganic and organic anions in orange juice on a high-capacity, nanobead-agglomerated capillary anion exchanger with 4 µm support particles. Separator column: Dionex IonPac AS11-HC-4µm; column dimensions: 250 mm × 0.4 mm i.d.; column temperature: 30 °C; eluent: KOH (EG); gradient: 1 mmol/L for 8 min isocratically, then linearly to 15 mmol/L in 10 min, then to 30 mmol/L in 10 min; flow rate: 15 µL/min; detection: suppressed conductivity; injection volume: 0.4 µL; sample: orange juice (1:50 diluted); peaks: (1) quinate, (2) fluoride, (3) lactate, (4) acetate, (5) formate, (6) pyruvate, (7) chloride, (8) malate, (9) carbonate, (10) sulfate, (11) oxalate, (12) ortho-phosphate, (13) citrate, and (14) isocitrate.

determination of sorbitol in apple juice was carried out by direct injection of the sample diluted 1:500 with deionized water (Figure 8.38).

In some countries, ion chromatography is used for characterizing beer and for monitoring the brewing process [173]. The analysis of organic acids, carbohydrates, sulfite, ascorbic acid, and ethanol is of primary interest. Monitoring the water used in the brewing process for inorganic anions and cations is also an important application. The determination of alcohols such as ethanol and glycerol is usually performed with an ion-exclusion phase and refractive index detection. A typical example is the determination of ethanol in an alcohol-free beer on an Aminex® HPX 87H column, which is illustrated in Figure 8.39. While sugars eluting close to the system void are not separated or are incompletely separated, lactic acid, glycerol, and ethanol can be detected in this matrix without any problem. For this purpose, the sample to be analyzed is degassed and diluted 1:10 with the eluent. The analysis of organic acids in beer can only be achieved by anion-exchange chromatography. Using the new high-capacity Dionex IonPac AS11-HC-4µm column, samples high in ionic strength can be injected directly without causing overloading problems or poor peak shapes, especially for weakly retained monovalent organic acids such as lactate, acetate, propionate, formate, and butyrate. The solvent compatibility of the Dionex IonPac AS11-HC-4µm column permits the use of water-miscible HPLC solvents in the eluent to modify selectivity.

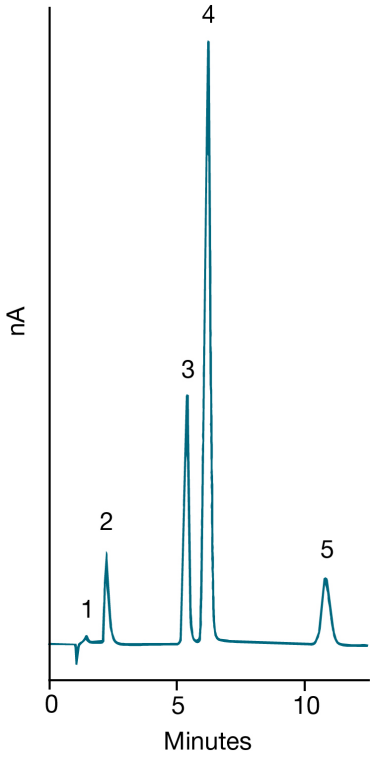


Figure 8.38 Analysis of carbohydrates in an apple juice. Separator column: Dionex CarboPac PA1; eluent: 0.1 mol/L NaOH; flow rate: 1 mL/min; detection: pulsed amperometry on a gold working electrode; injection volume: 50 µL; sample: apple juice (diluted 1:500); peaks: (1) inositol, (2) sorbitol, (3) glucose, (4) fructose, and (5) sucrose (reproduced with permission from Ref. [172]).

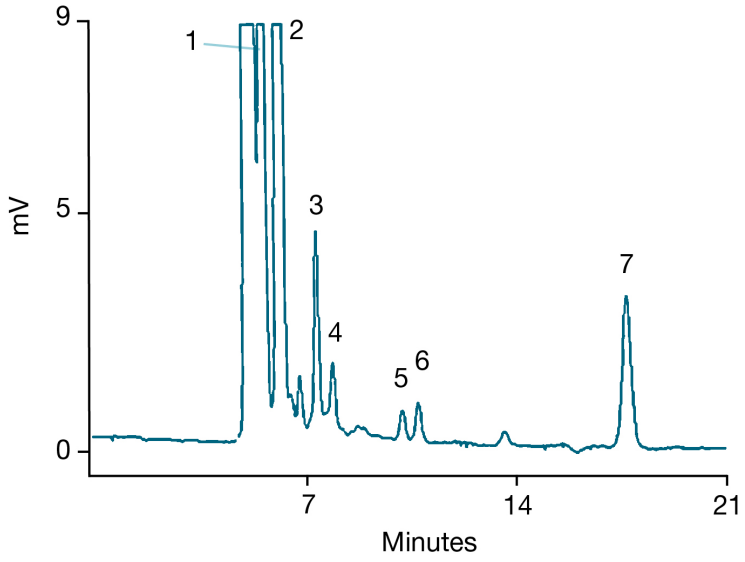


Figure 8.39 Determination of ethanol in an alcohol-free beer by ion-exclusion chromatography. Separator column: Aminex HPX 87H; column dimensions: 300 mm × 7.8 mm i.d.; column temperature: 65 °C; eluent: 5 mmol/L H_2SO_4; flow rate: 0.6 mL/min; detection: RI; sample preparation: dilution 1:10 with eluent and degassing; peaks: (1) maltotriose, (2) maltose + sucrose, (3) glucose, (4) fructose, (5) lactic acid, (6) glycerol, and (7) ethanol (reproduced with permission from Ref. [173]. Copyright 1993, Elsevier B.V.).

In the field of wine analysis, wet-chemical and enzymatic techniques still play an important role. However, the liquid chromatographic determination of the main sugars such as glucose, fructose, and sucrose together with glycerol and ethanol gains in importance due to its potential for automation. Separation is performed on a calcium-loaded ion-exclusion phase with pure deionized water as an eluent; refractive index detection is applied. A potential problem of such separations is the interference of organic acids, which—depending on the type of column—must be removed via strong basic anion exchangers. Ohs [174] and Baumgärtner [172] made important investigations using the HPAE-PAD method into the analysis of carbohydrates in wine, in which small quantities of sorbitol, rhamnose, arabinose and, to some extent, trehalose are present in addition to the main components glucose and fructose. All wines that have been investigated by Baumgärtner have a common characteristic peak pattern in the oligosaccharide region.

The spectrum of organic acids in wine is extremely complex and represents a challenge for the ion chromatographic analysis due in part to large concentration differences. Thus, isocratic elution on an anion exchanger with a carbonate/bicarbonate eluent is inappropriate because peak purity of inorganic and organic acids is not ensured. Even conventional low-capacity, hydroxide-selective anion exchangers suitable for gradient elution do not possess the required ion-exchange capacity to obtain acceptable resolution among early eluting monocarboxylic acids. However, this problem can be solved with the Dionex IonPac AS11-HC anion exchanger. Figure 8.40 shows an example chromatogram obtained with this stationary phase. Optimum separation is obtained—especially in the retention range of tartaric acid—by increasing and decreasing the content of methanol during the gradient run. The only disadvantage is the long analysis time of about 40 min, which is typical for hydroxide-selective, high-capacity anion exchangers. Even higher resolution among the early eluting monocarboxylic acids such as lactate and acetate can be achieved with this column in the 4 µm particle diameter format.

Primary fermentation with yeasts as well as malolactic fermentation, which is standard for most red wine production and for some white varieties such as chardonnay, have been associated with the occurrence of biogenic amines such as tyramine, putrescine, cadaverine, histamine, and phenylethylamine. Histamine can produce headaches and hypotension, whereas some aromatic amines such as tyramine and phenylethylamine can cause migraines and hypertension. The concentration and content of biogenic amines in wines are variable and depend on the storage time and conditions, quality of raw materials, and possible microbial contamination during the wine-making process. The determination of biogenic amines presents a challenging analytical problem because they are usually hydrophobic, nonchromophoric, and often occur in low concentrations in complex matrices. Reversed-phase liquid chromatography combined with either pre- or postcolumn derivatization and UV or fluorescence detection is the most common analytical method to determine biogenic amines in alcoholic beverages. Ion chromatography has not been widely used for the determination of biogenic amines, which is at least partially due to the strong hydrophobic interactions between the protonated amines and the stationary phase, resulting in long retention times and poor peak shapes. This problem could be overcome with the development of the Dionex IonPac CS18 column, a weak carboxylic acid cation-exchange column that reduces the interactions of hydrophobic analytes. The combination of this separator column with suppressed conductivity detection was successfully applied to the determination of biogenic amines in various matrices. A newer cation-exchange column, the Dionex IonPac CS19 column, was specifically designed for the determination of small polar amines. Although suppressed conductivity is a very common and simple detection technique, dopamine, tyramine, and serotonin cannot be detected in this way because they are uncharged following suppression. However, integrated pulsed amperometric detection (IPAD) after postcolumn addition of base is suitable for the determination of all underivatized biogenic amines [175]. As an example, Figure 8.41 shows the chromatograms of a California red wine sample that were obtained using both IPAD and UV detection [176].

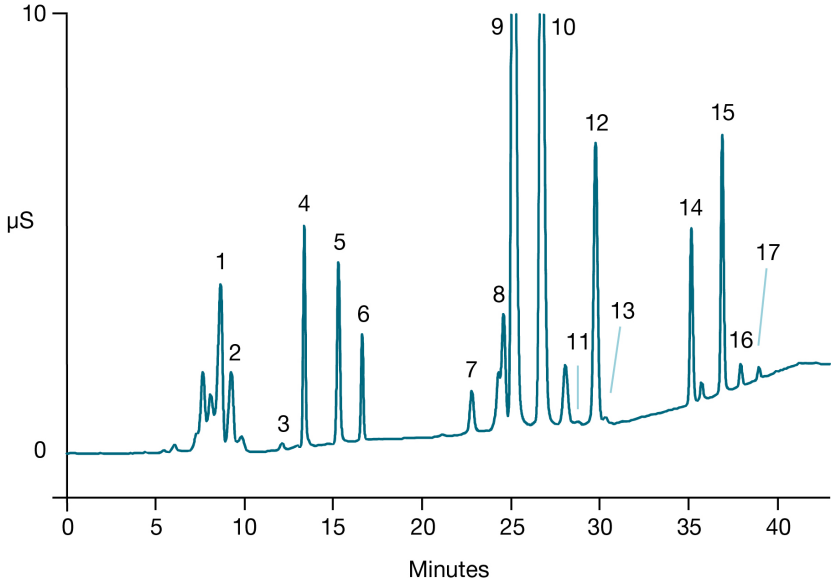


Figure 8.40 Gradient elution of inorganic anions and organic acids in wine. Separator column: Dionex IonPac AS11-HC with guard; column temperature: 30° C; eluent: NaOH/MeOH; gradient: 1 mmol/L NaOH isocratically for 8 min, then linearly to 30 mmol/L in 20 min, then to 60 mmol/L in 10 min, 0–20% MeOH from 8 to 18 min, 20% from 18 to 28 min, 20–0% from 28 to 38 min; flow rate: 1.5 mL/min; injection volume: 10 µL; detection: suppressed conductivity; sample: wine diluted 1: 0 with deionized water, sample preparation via Dionex OnGuard RP; peaks: (1) lactate, (2) acetate, (3) formate, (4) pyruvate, (5) galacturonate, (6) chloride, (7) nitrate, (8) succinate (9) malate, (10) tartrate, (11) fumarate, (12) sulfate, (13) oxalate, (14) orthophosphate, (15) citrate, (16) isocitrate, and (17) *cis*-aconitate.

In comparison to fruit juices, beer, and wine, the matrix of soft drinks is not as problematic for their ion chromatographic characterization. In almost all cases, sample preparation is restricted to dilution with deionized water, degassing (for carbonated soft drinks), and filtration (0.45 µm). A fast analysis of orthophosphoric acid in cola soft drinks [177] is possible with the Dionex IonPac Fast Anion IIIA column, which was developed for this type of sample without applying any other type of sample preparation than degassing and filtration. The hydrophilic nature of the resin repels the brownish caramel matrix of cola soft drinks, so that a large number of samples (~5000) can be injected without further pretreatment other than degassing and filtration. The orthophosphoric acid concentration is monitored to maintain product quality as well as minimize production cost. Citric acid is used for the same purposes and may be used in combination with orthophosphoric acid, mainly in diet soft drinks. The Dionex IonPac Fast Anion IIIA column provides rapid analysis of orthophosphoric and citric acids in cola soft drinks or cola syrup in less than 7 min using an electrolytically generated potassium hydroxide eluent under isocratic conditions and suppressed conductivity detection. Figure 8.42 illustrates the determination of orthophosphoric acid and citric acid in a cola soft drink under these conditions. To ensure the highest possible precision of this determination, the injection volume is lowered to 1.3 µL to avoid a dilution of the sample. In this way, peak area precision for the orthophosphoric acid peak has been determined to be 0.2% RSD, based on 160 consecutive injections of undiluted cola soft drinks.

In many cases, organic additives are added to carbonated soft drinks. These additives include caffeine, sweeteners such as saccharin, acesulfame K, or aspartame, preservatives such as benzoic acid, and flavors such as citric acid. All these compounds can be analyzed in one run using a mixed-mode stationary phase with anion-exchange and reversed-phase properties, utilizing UV detection. As an example, Figure 8.43 shows the chromatograms of two diet cola drinks.

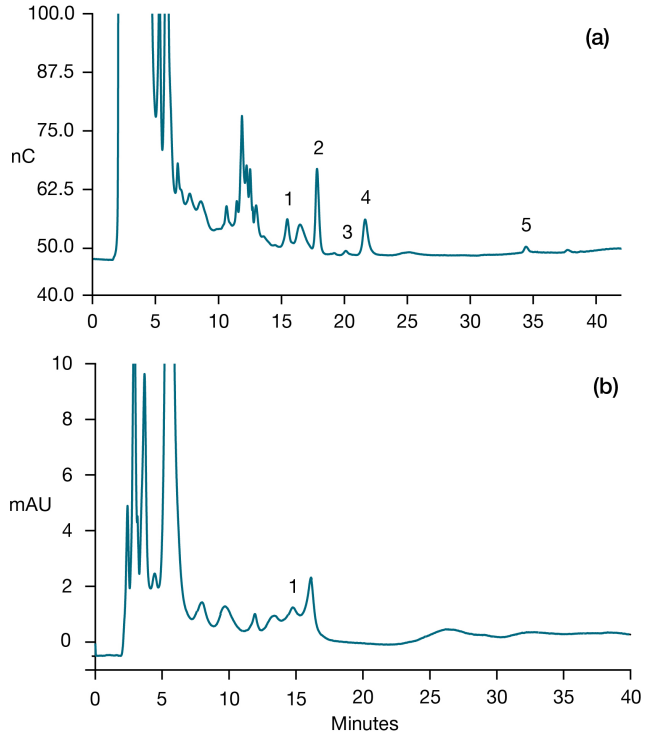


Figure 8.41 Determination of biogenic amines in a California red wine. Separator column: Dionex IonPac CS18; column dimensions: 250 mm × 2 mm i.d.; column temperature: 40 °C; eluent: MSA (EG); gradient: 3 mmol/L from 0 to 6 min, 3–10 mmol/L from 6 to 10 min, 10–15 mmol/L from 10 to 22 min, 15 mmol/L from 22 to 28 min, 15–30 mmol/L from 28 to 35 min, 30–45 mmol/L from 35 to 45 min; flow rate: 0.3 mL/min; detection: (a) IPAD after postcolumn addition of 0.1 mol/L NaOH, (b) UV (276 nm); postcolumn flow rate: 0.24 mL/min; injection volume: 5 µL; samples: BV Cabernet Sauvignon (1:5 diluted); peaks: 2.6 mg/L tyramine (1), 16.1 mg/L putrescine (2), 0.35 mg/L cadaverine (3), 4.9 mg/L histamine (4), and 1.7 mg/L spermidine (5).

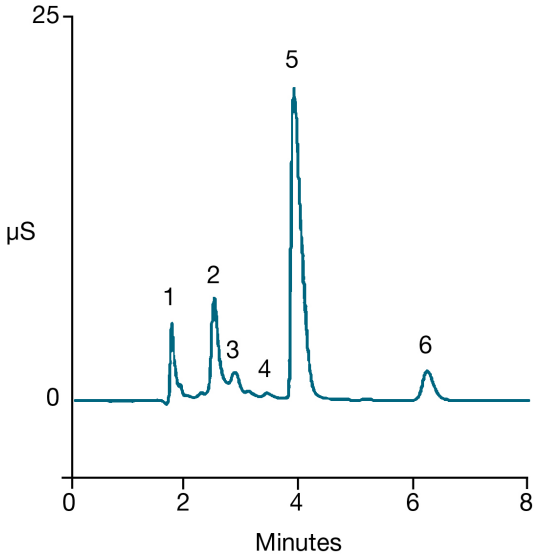


Figure 8.42 Separation of orthophosphoric acid and citric acid in a cola soft drink. Separator column: Dionex IonPac Fast Anion IIIA; column dimensions: 250 mm × 3 mm i.d.; column temperature: 30 °C; eluent: 22 mmol/L KOH (EG); flow rate: 1 mL/min; detection: suppressed conductivity; injection volume: 1.3 µL; sample: cola soft drink (undiluted); peaks: (1)–(4) unknown, 435 mg/L orthophosphate (5), and 53 mg/L citrate (6).

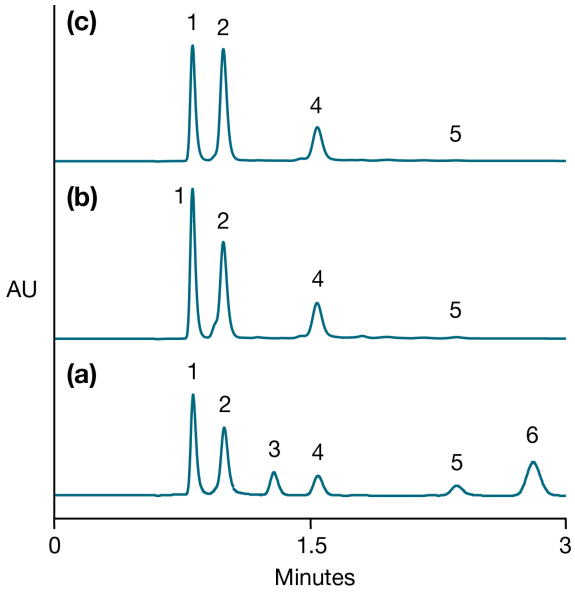


Figure 8.43 Mixed-mode separation of typical organic additives in cola soft drinks. Separator column: Acclaim Mixed-Mode WAX-1, 5 µm; column dimensions: 150 mm × 4.6 mm i.d.; column temperature: 30 °C; eluent: MeCN/0.12 mol/L phosphate buffer, pH 2.9 (57:43 *v/v*); flow rate: 1 mL/min; detection: UV (210 nm); injection volume: 2.5 µL; sample: (a) standard, (b) Diet Coke®, and (c) Diet Pepsi®; peaks: caffeine (1), aspartame (2), sorbate (3), benzoate (4), citric acid (5), and acesulfame K (6).

Dairy products For health purposes, the determination of inorganic anions such as nitrite, nitrate, and iodide in milk and milk products is of great importance. Together with secondary amines, nitrite, for example, can form carcinogenic nitrosamines; an excess of iodide can provoke thyroid malfunction. To prolong the lifetime of the stationary phase being used, it is recommended that milk proteins be removed from the sample. For the analysis of iodide in milk or whey, the milk proteins are precipitated with acetic acid and/or nitric acid. For this purpose, 50 mL of sample are put into a 100 mL measuring flask, 4 mL of a 3% acetic acid and 1 mL of concentrated nitric acid are added, and the flask is filled up to the meniscus with deionized water. This solution is then passed through a Whatman®-2V filter, a Centriflo filter, and then through a Dionex OnGuard RP reversed-phase extraction cartridge.

In addition to anion-exchange chromatography, which is the most commonly used method for determining iodide, mixed-mode liquid chromatography can be applied. The Acclaim Mixed-Mode WAX-1 column, for instance, is suitable for the determination of iodide and iodate, using a mobile phase consisting of a phosphate buffer and acetonitrile. Because anion exchangers are now available on which iodide can be rapidly eluted with purely aqueous eluents and without any tailing, anion-exchange chromatography is the preferred technique. In general, iodide can be detected by suppressed conductivity, UV, or DC and pulsed amperometry. To detect very low iodide concentrations in milk (100–300 µg/L) with sufficient precision, either DC or pulsed amperometric detection on a silver or platinum working electrode is used. While silver electrodes require a low oxidation potential of 0.05 V and can be used directly, platinum electrodes must be conditioned with a saturated potassium iodide solution prior to their use and require a significantly higher oxidation potential of 0.8 V. As an example, Figure 8.44 shows the separation of iodide in milk on a Dionex IonPac AS11 anion exchanger with a dilute nitric acid eluent. The detection limit for iodide with this method is approximately 3 µg/L.

A number of years ago, the food industry began adding fructanes to dairy products and other foods for nutritional and physiological reasons. These are carbohydrates such as inulin and fructooligosaccharides (FOS) that are not hydrolyzed by enzymes in the human body. Inulins

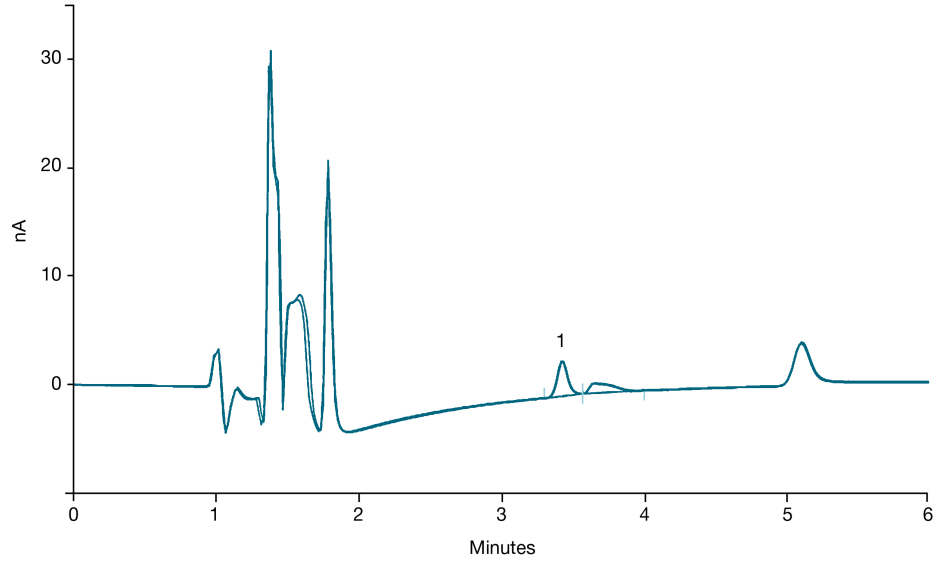


Figure 8.44 Separation of iodide in milk powder using anion-exchange chromatography and pulsed amperometric detection. Separator column: Dionex IonPac AS11; column temperature: 30 °C; eluent: 50 mmol/L HNO_3; flow rate: 1.5 mL/min; detection: pulsed amperometry on a silver working electrode; waveform: see Table 8.12; injection volume: 50 µL; sample: 1.014 g of milk powder dissolved in 10 mL of deionized water; peak: 1.06 µg/g iodide (1).

Table 8.12 Waveform for pulsed amperometry of iodide in milk powder (see Figure 8.44).

Time (s)	Potential (V)	Integration
0.00	+0.10	
0.20	+0.10	On
0.90	+0.10	Off
0.91	–0.60	
0.93	–0.30	
1.00	–0.30	

exhibit a degree of polymerization from DP3 to DP60 with an average molecular weight of about 6000 Da. The lower-molecular weight fraction (DP3 to DP20) is termed fructo-oligosaccharides (FOS). Due to their intensive commercialization in foods, fructo-oligosaccharides must be declared and thus characterized and quantitated. For this purpose, Durgnat and Martinez [178] developed an HPAE-PAD technique, which is less cumbersome than gas chromatography. Because individual fructo-oligosaccharides are not available as reference compounds, the quantitation of FOS sugars can be carried out via commercial FOS mixtures such as Actilight-95®[1] with known contents of 1-kestose (38.6%), nystose (46.8%), and fructosylnystose (8.6%). Therefore, sugars such as 1-kestose and nystose can be used as tracer for quantitation of Actilight-95. A typical example is the chromatogram of a strawberry yoghurt shown in Figure 8.45. Sample preparation in this case was carried out in a traditional way by

[1] Actilight-95® is distributed by Béghin-Meiji Industries in France and manufactured enzymatically from sugar cane with fructosyl transferase.

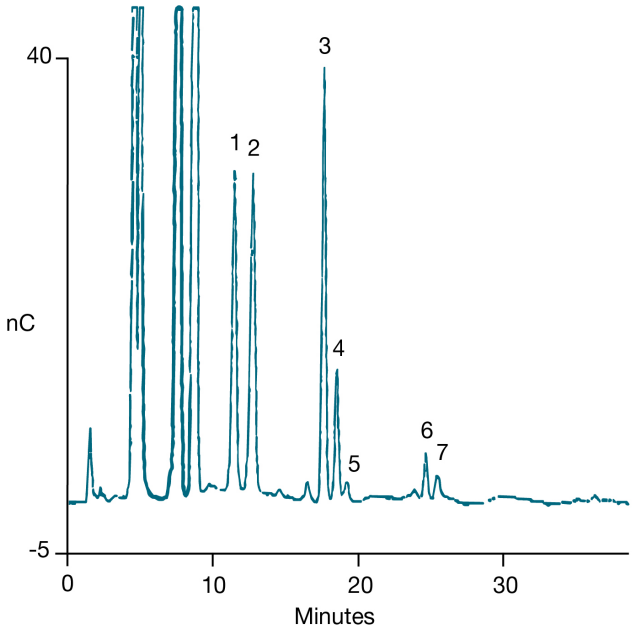


Figure 8.45 HPAE-PAD chromatogram of a strawberry yoghurt containing fructo-oligo-saccharides. Separator column: Dionex CarboPac PA1; eluent: NaOH/NaOAc gradient; flow rate: 1 mL/min; detection: pulsed amperometry on a gold working electrode; injection volume: 20 µL; sample: strawberry yogurt no. 1; sample preparation: Carrez I and II; peaks: (1) 1-kestose, (2) maltose, (3) nystose, (4) maltotriose, (5) inulotriose, (6) fructosylnystose, and (7) maltotetraose (reproduced with permission from Ref. [178]).

precipitating the proteins with Carrez I and II. The detection limit for this technique was determined by Durgnat and Martinez to be 80 ng for nystose; the recovery rates were between 95 and 108%.

Last but not least, milk caseins can also be separated on a suitable anion exchanger. The separation of caseins from bovine milk in Figure 8.46 on ProPac SAX column with a mixture of urea, 2-mercaptoethanol, imidazole, and NaCl as an eluent is clearly superior to a conventional separation on a wide-pore reversed-phase column, whether an acetonitrile or an isopropanol gradient is used.

Another typical application example in the field of milk products is the determination of polyphosphates in cheese, which serve as emulsion stabilizers. Commercial polyphosphates are mixtures of polyphosphates of different chain lengths. With ion chromatography, those chain length distributions can be easily determined. Baluyot and Hartford [179] used a hydroxide-selective anion exchanger in the microbore format with an aqueous hydroxide gradient and subsequent conductivity detection. As can be seen from the chromatograms of two different hexamethaphosphate solutions in Figure 8.47, fingerprint chromatograms are obtained in 40 min. In this case, both solutions were prepared from the same dry substance, however, the cheese products showed completely different characteristics. The comparison of the two resulting chromatograms in Figure 8.47 indicates a different degree of hydrolysis during processing which, thereafter, could be optimized.

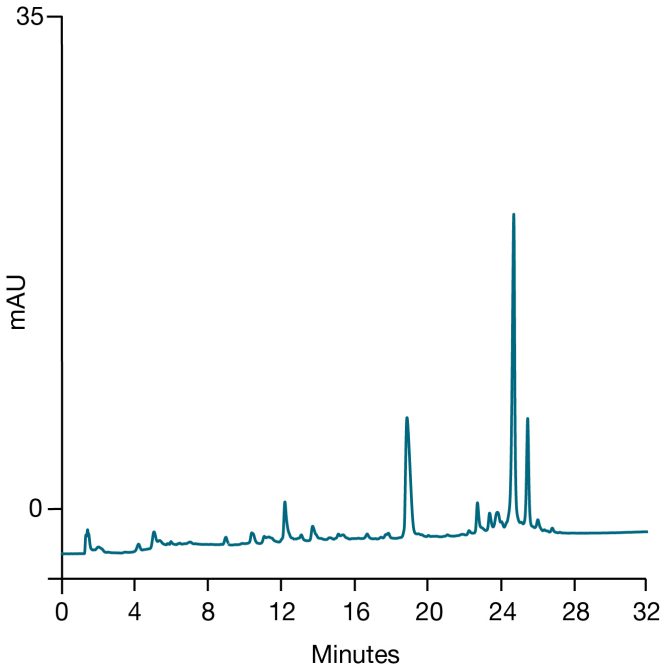


Figure 8.46 Separation of caseins from bovine milk on an anion exchanger. Separator column: ProPac SAX; eluent: (A) 4 mol/L urea + 0.1 mol/L 2-mercaptoethanol + 10 mmol/L imidazole, pH 7, (B) 1 mol/L NaCL in A; gradient: 100% A isocratic for 3 min, then from 5% B to 30% B in 27 min; flow rate: 1 mL/min; detection: UV (280 nm); sample: 1 µg/mL caseins from bovine milk obtained by precipitation at pH 4.5 of whole bovine milk.

Meat processing A key problem in the investigation of meat and sausage products is the determination of nitrite and nitrate, whose tolerable concentrations are between 100 and 600 mg/kg depending on the product group. This regulation protects consumers and requires frequent control of the traded goods. Ion chromatographic methods for nitrite/nitrate analyses were developed at an early stage to replace classical methods such as the cadmium reduction or enzymatic methods used so far. This does not include sample preparation, which is regulated in many countries, no matter which analytical method is applied. Sample preparation [180] includes homogenization of the sample (10 g to 100 mL of deionized water), which is then heated to 70–80 °C for 15 min. After cooling down to room temperature, the sample is centrifuged at 6000 rpm for 10 min. The supernatant solution is then passed through a 1.2 µm Whatman No. 2, GF/A and a 0.2 µm Acrodisc® filter. The filtrate can be injected directly into the ion chromatograph. Figure 8.48 shows the chromatogram of a ham extract with a clear separation of nitrite and nitrate. To validate the signals, the sample was spiked with both components and chromatographed again. Nitrite and nitrate were clearly identified in this way with good recovery of the spiked amounts. The contents of sodium nitrite and sodium nitrate were 11.6 and 5.37 mg/kg, respectively. Both values are clearly below the threshold of 100 mg/kg. Spiking experiments yielded recovery rates of 103% for nitrite and 92% for nitrate. The negative peak observed ahead of nitrite is chloride, which lowers eluent background absorption for a short time due to its high concentration. At the measuring wavelength of 225 nm the size of this negative peak in relation to the nitrite signal is the lowest.

Cutter and reddening agents used in meat processing may also be assayed using ion chromatography for their active constituents, which include inorganic anions such as chloride and pyrophosphate, ascorbic acid, and carbohydrates such as glucose, sucrose, and maltose. While the easiest way to analyze ascorbic acid is via ion-exclusion chromatography on a totally sulfonated cation exchanger, anion-exchange chromatography with either suppressed conductivity or photometric detection after derivatization with iron(III) is used to analyze

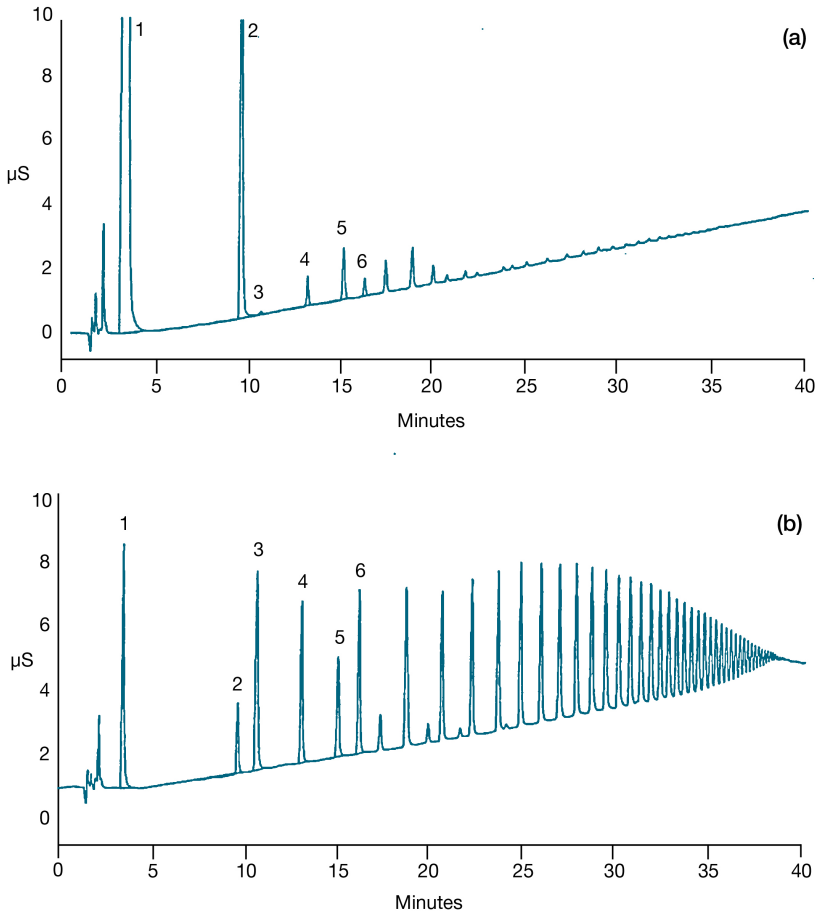


Figure 8.47 "Fingerprint" analysis of polyphosphates in cheese products. Separator column: Dionex IonPac AS11; column dimensions: 250 mm × 2 mm i.d.; eluent: NaOH gradient; flow rate: 0.3 mL/min; detection: suppressed conductivity; injection volume: 10 µL; samples: (a) bad charge, (b) good charge; peaks: (1) orthophosphate, (2) pyrophosphate, (3) trimetaphosphate, (4) tripolyphosphate, (5) tetrametaphosphate and (6) tetrapolyphosphate (reproduced with permission from Ref. [179]. Copyright 1996, Elsevier B.V.).

pyrophosphate. Selectivity and sensitivity of both methods are so high that an extensive sample preparation is not necessary. The analyte samples are only dissolved in deionized water and membrane-filtered (0.45 µm) prior to injection.

Baby food The determination of choline, a water-soluble micronutrient, is an important application [181]. Choline plays an important role in cardiovascular and liver health; it is essential to proper metabolism and critical for the function and synthesis of the neurotransmitter acetylcholine. Therefore, it is often added to vitamin formulations, animal feeds, and infant formulas. The separation of choline and acetylcholine, together with alkali and alkaline-earth metals, can easily be carried out on a weak acid cation exchanger utilizing suppressed conductivity as a detection method [182]. Free choline is water-soluble and can easily be extracted from foods. However, total choline is usually determined by hydrolysis of esters to release the bound choline to its free form. The most recent hydrolysis protocol [182] describes the use of hydrochloric acid to extract choline from infant formula samples. After acid digestion, the hydrolysate is filtered through a 0.2 µm PES syringe filter. PES filters do not bind choline and are, therefore, suitable for efficient filtration of such samples. Phospholipase D may be added to the filtrate to release choline that may still be present as

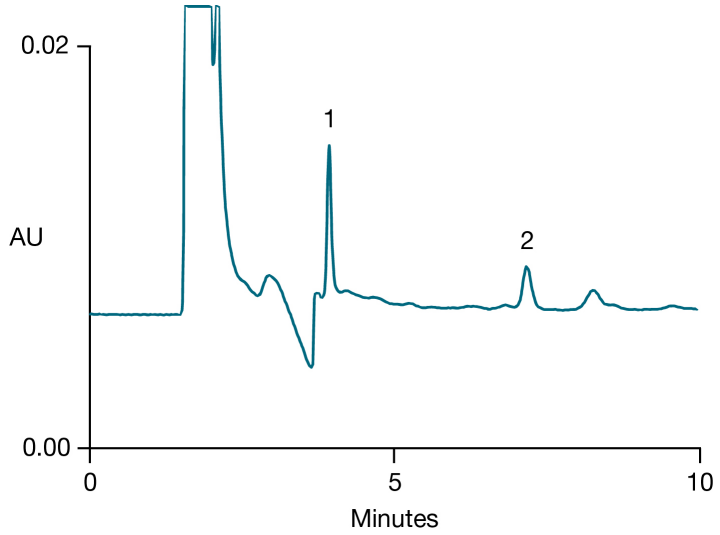


Figure 8.48 Analysis of nitrite and nitrate in a ham extract. Separator column: Dionex IonPac AS11; eluent: 5 mol/L NaOH; flow rate: 1 mL/min; detection: UV (225 nm); injection volume: 25 µL; sample: ham extract (weighted sample: 10 g to 100 mL water); peaks: 1.16 mg/L nitrite (1) and 0.54 mg/L nitrate (2) (reproduced with permission from Ref. [180]. Copyright 1998, Elsevier B.V.).

phosphatidylcholine. Figure 8.49 shows an isocratic separation of choline in an infant formula sample using the Dionex IonPac CS19 column, which has an optimized selectivity for small hydrophilic amines. The choline peak is well resolved from other cations.

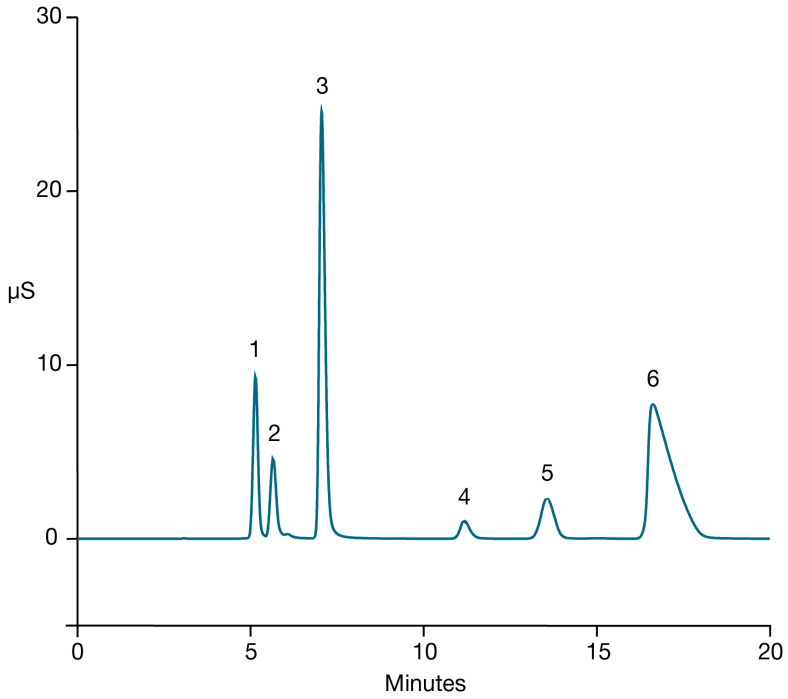


Figure 8.49 Separation of choline in an infant formula sample using cation-exchange chromatography and suppressed conductivity detection. Separator column: Dionex IonPac CS19 with guard; column dimensions: 250 mm × 2 mm i.d.; eluent: 6.4 mmol/L methanesulfonic acid; flow rate: 0.25 mL/min; detection: suppressed conductivity; injection volume: 5 µL; sample: infant formula hydrolysate (1:200 diluted), peaks: (1) sodium, (2) ammonium, (3) potassium, (4) 1.77 µg/g choline, (5) magnesium, and (6) calcium.

In recent years, the determination of phytic acid (*myo*-inositol hexaphosphate) has become important. Phytic acid is believed to have an effect on the bioavailability of mineral substances. To elucidate the nutritive scientific relevance of phytic acid, Fitchett and Woodruff [5] used the postcolumn derivatization technique with iron(III) that was originally developed for determining polyphosphates in detergents. Meanwhile, the Dionex IonPac AS7 separator column used by Fitchett and Woodruff is somewhat outdated. Today, the highest efficiency separation of phytic acid is achieved with the hydroxide-selective Dionex IonPac AS11 column, which had been designed for rapid elution of strongly retained anions [183]. Using an electrolytically generated potassium hydroxide eluent of a relatively high concentration (c = 65 mmol/L) under isocratic conditions, phytate is eluted in 7 min. Under these conditions, common inorganic anions elute early and do not interfere with phytate determinations.

Kaine and Wolnik [184] successfully applied the method of HPAE-PAD, which has already been mentioned above, to characterize various milk- and soy-based baby foods by determining the carbohydrate profiles. Although the carbohydrate portion itself does not differ much, the type of carbohydrates does vary depending on the product. Among the most important constituents are lactose, sucrose, maltodextrins, and starch, which are worked into the formulations in varying proportions. Because all constituents must be declared, these products can be monitored in the same run by anion-exchange chromatography in a fast and efficient way. The key parameter is clearly lactose, which is not contained in soy formulations. Thus, adulterations and mistakes can be identified, for example, by the presence of lactose. This is extremely important because a mix-up of milk and soy products can have severe consequences for health.

The determination of inositol in infant formula is important due to its biochemical relevance, its universal occurrence in phospholipids, and its presence at high level in human milk. Inositol levels are supplemented in some commercially available infant formulas and nutritional products. Tagliaferri et al. [185] were among the first to use the fully functionalized supermacroporous Dionex CarboPac MA1 column for inositol analysis. Because of its very high anion-exchange capacity, this separator column requires a much larger hydroxide concentration (c > 0.4 mol/L) in the eluent, so that the effect of carbonate impurities in manually prepared eluents is negligible. While sample preparation for the determination of supplemental or free inositol only involves a simple aqueous extraction, the determination of "total" inositol in infant formulas requires hydrolysis. However, the complete hydrolysis of phosphorylated forms of inositol to free inositol is rather difficult. Since the liberation of inositol in alkaline hydrolysis with 3 mol/L KOH is incomplete after 1 h, a second step involving an enzymatic dephosphorylation with a nonspecific phosphatase is necessary. Under these conditions, inositol monophosphate is completely hydrolyzed after 30 min.

Quantitative analysis of free carbohydrates in human milk has also been carried out using HPAE-PAD. Besides 7% lactose, human milk contains approximately 1% neutral oligosaccharides and about 0.1% acidic oligosaccharides. Thus, oligosaccharides make up a large part of human milk composition, similar to the level of glycoproteins. The biological function of these oligosaccharides is not yet fully understood. However, there is evidence that human milk oligosaccharides (HMOS) are important for the prebiotic effect as well as the anti-infective and allergy-preventive properties of human milk. Roughly 130 different neutral and acidic oligosaccharides have been characterized so far. It can be assumed that the oligosaccharide fraction is extremely complex, consisting of at least 1000 different components, with most of them occurring in minor quantities.

Sample preparation for the HPAE-PAD analysis of individual oligosaccharide compounds from human milk was described by Thurl et al. [186]. Briefly, human milk samples are heated for 30 min at 70 °C as a preventative measure against virus contamination. After adding stachyose and galacturonic acid as internal standards, the samples are centrifuged at 2000 *g* and ultrafiltered using the Centrifree® system (Merck-Millipore, Darmstadt, Germany). The

protein- and lipid-reduced samples are then fractionated by gel permeation chromatography on a Toyopearl® HW 40 (S) column (80 cm × 1.6 cm i.d.) (Tosoh Bioscience, Stuttgart, Germany) because the high abundant lactose has to be removed that would otherwise prevent the exact determination of HMOS by HPAE-PAD. The carbohydrate fractions are eluted with 0.02% aqueous sodium azide at 1 mL/min flow rate and monitored by refractive index detection. Chromatography on the Toyopearl column also allows a complete separation of neutral and acidic oligosaccharides, which principally can be analyzed in a single run. Figure 8.50 shows the HPAE-PAD profile of the neutral oligosaccharide fraction from human milk of a Lewis (a–b+) donor. Due to the complex mixture of especially the neutral fucosylated oligosaccharides the peaks at the beginning of the chromatogram are not completely resolved. Nevertheless, all dominating oligosaccharides are separated in approximately 50 min. Peak L represents residual lactose that was not completely separated from the oligosaccharide fraction. The structures of lactose and 14 other oligosaccharides that could be quantified are listed in Table 8.13.

In recent years, there were reported cases of food adulteration associated with melamine. In September 2008, for instance, several companies in China were implicated in a scandal involving milk and infant formula that had been adulterated with melamine. Melamine, a common component of plastic manufacturing, and cyanuric acid, a compound often used for swimming pool chlorination, are closely related chemicals that are nontoxic by themselves. However, melamine combines with cyanuric acid to form melamine cyanurate, a toxic insoluble crystal that causes kidney stones and can lead to renal failures. Because melamine has high nitrogen content, it has been added to animal feed and milk products to cause the protein content in food to appear higher than the true value. Meanwhile, a number of authorities including the US FDA have issued methods for the analysis of melamine and cyanuric acid. A detailed overview on the various analytical techniques for melamine and cyanuric acid analysis has been published by Sun et al. [187]. An HPLC–UV method [188] is presently a popular choice for most laboratories. Both melamine and cyanuric acid are hydrophilic compounds that are not sufficiently retained on a conventional C18 reversed-phase column. With an ion-pair reagent in the mobile phase, e.g., octane sulfate, melamine is well retained, but this method is not compatible with MS detection. Therefore, hydrophilic interaction (HILIC) or mixed-mode liquid chromatography in the reversed-phase or HILIC mode are typically employed for simultaneously separating both melamine and cyanuric acid.

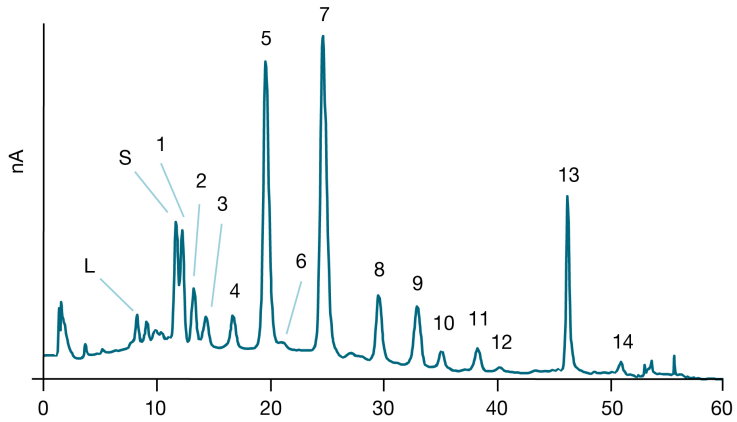


Figure 8.50 HPAE-PAD profile of the neutral oligosaccharide fraction from human milk of a Lewis (a–b+) donor. Separator column: Dionex CarboPac PA100; column dimensions: 250 mm × 4 mm i.d.; column temperature: ambient; eluent: NaOH/NaOAc; gradient: 30 mmol/L NaOH isocratically for 20 min, then linearly to 100 mmol/L NaOH in 14 min, linearly to 28 mmol/L NaOAc/0.1 mol/L NaOH in 14 min, linearly to 0.2 mol/L NaOAc/0.1 mol/L NaOH in 7 min; flow rate: 1 mL/min; detection: PAD on a gold working electrode; injection volume: 25 µL; sample: human milk; peaks: see Table 8.13 (reproduced with permission from Ref. [186]. Copyright 1996, Academic Press).

Table 8.13 Structures and relative retention times of neutral oligosaccharides from human milk (see Figure 8.50) (reproduced with permission from Ref. [186]. Copyright 1996, Academic Press).

Peak	Trivial name	Structure	Relative retention
S	Stachyose	Gal(α1–6) Gal(α1–6) Glc(α1–2) βFru	1
L	Lactose	Gal(β1–4) Glc	0.59
1	2‘-FL	Fuc(α1–2) Gal(β1–4) Glc	0.74
2	3-FL	Gal(β1–4) Glc Fuc(α1–3)/	0.37
3	LDFT	Fuc(α1–2) Gal(β1–4) Glc Fuc(α1–3)/	0.51
4	LNT	Gal(β1–3) GlcNAc(β1–3) Gal(β1–4) Glc	1.41
5	LNnT	Gal(β1–4) GlcNAc(β1–3) Gal(β1–4) Glc	1.07
6	LNFP I	Fuc(α1–2) Gal(β1–3) GlcNAc(β1–3) Gal(β1–4) Glc	0.90
7	LNFP II	Gal(β1–3) GlcNAc(β1–3) Gal(β1–4) Glc Fuc(α1–4)/	0.44
8	LNFP III	Gal(β1–4) GlcNAc(β1–3) Gal(β1–4) Glc Fuc(α1–3)/	0.41
9	LNDFH I	Fuc(α1–2) Gal(β1–3) GlcNAc(β1–3) Gal(β1–4) Glc Fuc(α1–4)/	0.36
10	LNDFH II	Gal(β1–3) GlcNAc(β1–3) Gal(β1–4) Glc Fuc(α1–4)/ Fuc(α1–3)/	0.26
11	LNH	Gal(β1–4) GlcNAc(β1–6)\ Gal(β1–4) Glc Gal(β1–3) GlcNAc(β1–3)/	1.56
12	2’-F-LNH	Gal(β1–4) GlcNAc(β1–6)\ Gal(β1–4) Glc Fuc(α1–2) Gal(β1–3) GlcNAc(β1–3)/	1.33
13	3’-F-LNH	Fuc(α1–3)\ Gal(β1–4) GlcNAc(β1–6)\ Gal(β1–4) Glc Gal(β1–3) GlcNAc(β1–3)/	1.18
14	2’,3’-DF-LNH	Fuc(α1–3)\ Gal(β1–4) GlcNAc(β1–6)\ Gal(β1–4) Glc Fuc(α1–2) Gal(β1–3) GlcNAc(β1–3)/	0.65

The cationic melamine can be separated on Acclaim Mixed-Mode WCX-1 using an ammonium acetate buffer as the eluent. This column demonstrates great potential for separating samples that contain a mixture of ionic and neutral compounds. The common procedure for preparing milk and milk powder samples for melamine analysis only requires precipitation with dilute acetic acid, subsequent centrifugation and filtration, and is suitable for both liquid milk and milk powder products. However, the complex matrix of milk products may sometimes yield a false positive for melamine. HILIC in combination with charged aerosol detection is an alternative method for the analysis of polar compounds such as melamine and cyanuric acid. Both compounds can be separated on the 3 µm Acclaim HILIC-10 column, which is a general-

purpose, silica-based HILIC column based on high-purity, spherical porous silica that is covalently modified with a proprietary hydrophilic layer. Using an ammonium acetate/acetonitrile eluent, both compounds are eluted in less than 5 min under isocratic conditions. Similar separation results are obtained with a Thermo Scientific™ Accucore™ HILIC column that uses core-enhanced technology to facilitate fast and high efficiency separations. The 2.6 µm diameter particles are not totally porous, but rather have a solid core and a porous outer layer, resulting in much lower back pressures than typically seen with sub-2 µm materials. The corresponding chromatogram in Figure 8.51 has been obtained with MS detection, switching the ionization mode during the chromatographic run. Using the parent ion transitions *m/z* 128→85 and 128→42 for cyanuric acid (–ESI) and the *m/z* 127→85 and 128→68 transitions for melamine (+ESI) both compounds can be detected at 1 and 50 ng/mL, respectively.

Groceries and luxuries The use of an eluent generator is essential for analyzing carbohydrates in soluble coffee (ISO 11292, AOAC 995.13) [189] by means of HPAE-PAD. Coffee is one of the most popular beverages in the world because of its refreshing and stimulating properties. Sugars relevant for coffee include mannitol, fucose, arabinose, rhamnose, galactose, glucose, sucrose, xylose, mannose, fructose, and ribose. Free carbohydrates in soluble coffee can be analyzed after a simple dissolution of the product in water and subsequent cleanup of the solution through a C18 SPE cartridge and membrane filtration (0.2 µm). The total carbohydrate profile is usually measured after hydrolysis of the product with 1 mol/L HCl. Subsequent neutralization is performed by filtration of the hydrolysate through a SPE cartridge filled with a cation exchanger in the silver form, and finally passed through a membrane filter (0.2 µm). The sample can then be injected into the chromatograph. All important mono-

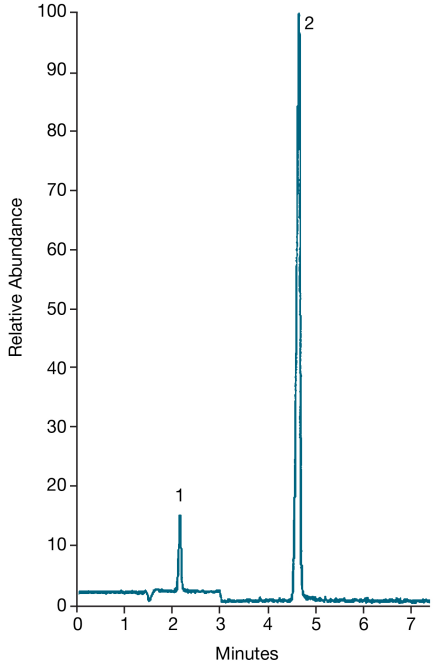


Figure 8.51 HILIC separation of melamine and cyanuric acid with MS detection. Separator column: Accucore HILIC, 2.6 µm; column dimensions: 150 mm × 4.6 mm i.d.; eluent: MeCN/50 mmol/L NH_4OAc, pH 5 (90:10 *v/v*); flow rate: 1 mL/min; detection: MS, negative mode from 0 to 3 min, positive mode from 3 to 10 min; injection volume: 5 µL; peaks: 70 µg/mL each of cyanuric acid, *m/z* 128.1 (1) and melamine, *m/z* 127.1 (2).

saccharides and some sugar alcohols can be completely separated by HPAE-PAD on a Dionex CarboPac PA10 column with a very dilute, electrolytically generated carbonate-free hydroxide eluent. After the elution of ribose, eluent concentration is raised to 100 mmol/L for 5 min as a rinsing step before the column is equilibrated again with the initial KOH concentration. The robustness of this modified AOAC method is remarkable; the relative standard deviations for the retention times of the individual sugars are between 0.5 and 0.8% based on nonstop operation over more than 100 h. Figure 8.52 shows the chromatograms of a soluble coffee sample and a corresponding standard.

Sweeteners More and more frequently, food is sweetened with sugar substitutes instead of sugar or glucose syrup. Because of the diversity of products containing artificial sweeteners, the matrices that need to be investigated are very complex. In addition, sweeteners may be present in these products individually or in combination.

The best-known sugar substitutes include saccharin, sodium cyclamate, and acesulfame K. As these compounds exist as anions in an alkaline medium, anion-exchange chromatography with subsequent conductivity detection provides a welcome alternative to RPLC with UV detection. All three compounds can be separated in the same run when employing a hydroxide concentration gradient.

Another sugar substitute is palatinitol. The preparation of palatinitol uses sucrose as a starting material. After enzymatic rearrangement into palatinose (isomaltulose) and hydration, palatinitol is formed, representing an equimolar mixture of the isomers α-D-glucopyranosido-1,6-mannitol and α-D-glucopyranosido-1,6-sorbitol. Like all carbohydrates, palatinitol may be separated in an alkaline medium on the Dionex CarboPac PA1 strong basic anion exchanger and may be detected via pulsed amperometry. Both isomers can be separated together with sorbitol, mannitol, and isomaltose (as impurities) in the same run.

In recent years, diterpene glycosides of *Stevia rebaudiana* Bertoni have gained importance as noncaloric sweeteners. The stevia plant and its extracts have long been used as sweeteners in Asia and Latin America. Steviol glycosides are compounds of up to 300-times the sweetness of sucrose, which makes them an attractive sugar substitute for the food industry. There are over 32 steviol glycosides identified in *S. rebaudiana* Bertoni leaves. Two steviol glycosides, stevioside and rebaudioside A, are largely responsible for the sweet flavor.

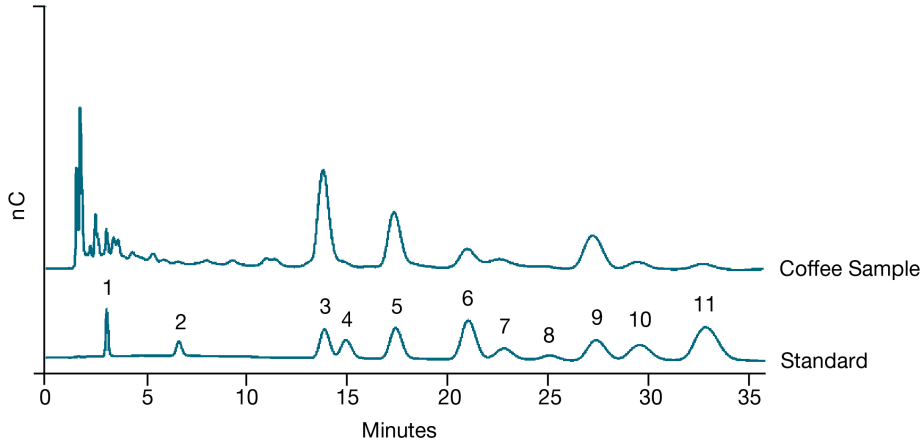


Figure 8.52 Separation of carbohydrates in an instant coffee sample. Separator column: Dionex CarboPac PA10; eluent: 2.3 mmol/L KOH (EG), rinsing step with 0.1 mol/L for 5 min; flow rate: 1 mL/min; detection: PAD, Au working electrode; peaks: (1) mannitol, (2) fucose, (3) arabinose, (4) rhamnose, (5) galactose, (6) glucose, (7) sucrose, (8) xylose, (9) mannose, (10) fructose, and (11) ribose.

Rebaudioside A has the greatest sensorial taste without the bitter aftertaste. All relevant compounds are glycosides of steviol, a diterpene compound. Other diterpene glycosides present at higher levels are steviolbioside, rebaudioside B, C, D, F, dulcoside A, and rubososide. The structures of these nine compounds are shown in Table 8.14.

Due to comparable chemical structures and their weak UV absorbance, the analytical determination of steviol glycosides in sweeteners by liquid chromatography is challenging. Because steviol glycosides do not have a strong UV absorption, other detection methods such as charged aerosol detection can be employed to improve steviol glycoside quantification. Mixed-mode liquid chromatography on the 3 µm Acclaim Trinity column in the HILIC mode currently provides the best separation of steviol glycosides. As shown in Figure 8.53, a separation with baseline resolution of eleven steviol glycosides is achieved in less than 20 min with an optimized column length, column temperature, and mobile phase composition.

8.6 Ion chromatography in the pharmaceutical industry

Over the last two decades, a significant number of IC methods have been successfully developed and validated for the characterization, lot-release, and stability studies of pharmaceutical and biological products. IC has been applied to the analysis of raw materials, bulk active ingredients, counter ions, impurities and degradation products, excipients and diluents at different stages of the production process and predominantly in the early stages of research. With the increasing demand for new therapeutic products, a growing interest in the application of IC in the pharmaceutical industry is observed. This is due to the ability to analyze different counter ions that improve stability and solubility properties of active pharmaceutical ingredients (APIs), the availability of mixed-mode stationary phases, and the use of novel

Table 8.14 Structures and molecular weights of the nine most abundant steviol glycosides.

Compound	R1	R2	MW
Stevioside	Glc(β)-	Glc(β1–2) Glc(β)-	804.9
Rebaudioside A	Glc(β)-	Glc(β1–2) Glc(β)- Glc(β1–3)/	967.0
Rebaudioside B	H-	Glc(β1–2) Glc(β)- Glc(β1–3)/	804.9
Rebaudioside C	Glc(β)-	Rha(α1–2) Glc(β)- Glc(β1–3)/	951.0
Rebaudioside D	Glc(β1–2) Glc(β)-	Glc(β1–2) Glc(β)- Glc(β1–3)/	1129.2
Rebaudioside F	Glc(β)-	Xyl(β1–2) Glc(β)- Glc(β1–3)/	937.0
Steviolbioside	H-	Glc(β1–2) Glc(β)-	642.7
Dulcoside A	Glc(β)-	Rha(α1–2) Glc(β)-	788.9
Rubososide	Glc(β)-	Glc(β)-	642.7

R1: substituted hydrogen on the carboxyl group of steviol
R2: substituted hydrogen on the hydroxide group of steviol

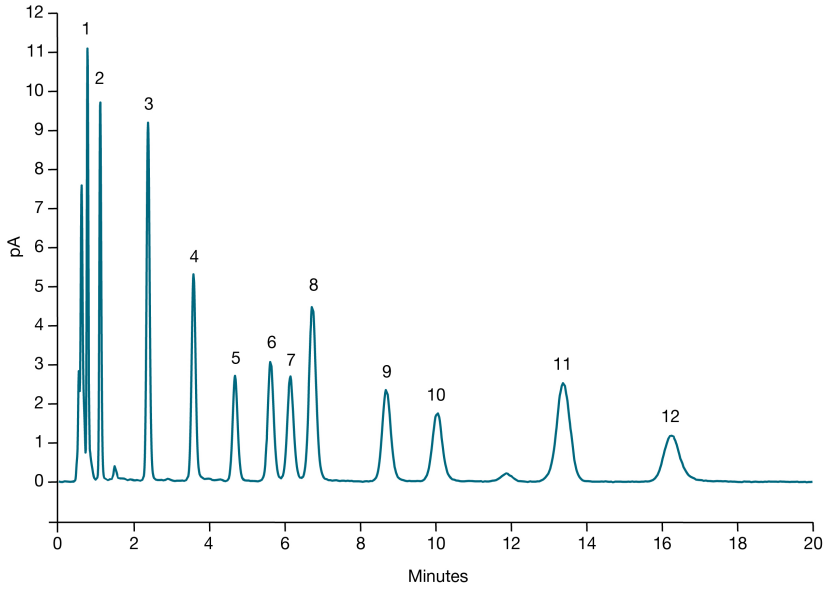


Figure 8.53 Separation of eleven steviol glycosides by mixed-mode liquid chromatography. Separator column: Acclaim Trinity, 3 µm; column dimensions: 150 mm × 2.1 mm i.d.; column temperature: 30 °C; eluent: MeCN/ammonium formate, pH 3.1 (88:12 *v/v*); flow rate: 0.8 mL/min; detection: Corona Veo RS charged aerosol detection (evap. temp.: 35 °C, 2 Hz, filter: 5 s, PF 1.0); injection volume: 2 µL; peaks: 90 mg/L each of (1) isosteviol, (2) steviol, (3) rubusoside, (4) dulcoside A, (5) stevioside, (6) steviolbioside, (7) rebaudioside C, (8) rebaudioside F, (9) rebaudioside B, (10) rebaudioside A, (11) sodium, and (12) rebaudioside D.

detection systems. Advances in technology have permitted IC to be hyphenated with mass spectrometry, which is a critical milestone for a wider interest in IC as a product characterization tool. Ion-exchange chromatography can be the method of choice for ionic, ionizable, and highly polar compounds, inorganic ions and compounds that do not have suitable chromophores for detection by absorption measurements. Over the last decade, the number of USP monographs has increased significantly, illustrating the growing acceptance of IC as one of the methods of choice in the pharmaceutical industry.

Counter ion analysis Salt formation is a critical step during the drug development process. Various salt forms are used to improve the biological and physicochemical properties of active pharmaceutical ingredients (APIs). At present, about 56% of all drug molecules used in medicinal therapy are administered as salts. Chloride and sodium counter ions remain the dominant salt forms for the manufacture of basic and acidic drugs, respectively. These counter ions have advantages over other salt forms due to their low molecular weight, higher solubility in water, and low toxicity. Other common anionic counter ions include sulfate and bromide or simple organic acids such as mesylate, maleate, citrate, tartrate, and acetate. Somewhat rare anionic counter ions include methyl sulfate, methanesulfonate, and trifluoroacetate. Typical cationic counter ions are ammonium, potassium, magnesium, and calcium.

The precise quantitation of counter ion concentrations in pharmaceutical drug formulations is of great importance to establish stoichiometry as well as to determine completeness of salt formation and mass balance. Because many of these ions are nonchromophoric, traditional reversed-phase liquid chromatography with UV detection cannot be employed. In contrast, ion-exchange chromatography with suppressed conductivity detection is the preferred technique for pharmaceutical counter ion analysis because of its applicability to a wide range of chromophoric and nonchromophoric anions and cations [190].

Traditionally, carbonate/bicarbonate eluents have been used for the determination of common inorganic anions in samples of pharmaceutical origin. Carbonate-based eluents offer the advantage of being easy to prepare from eluent concentrates. However, carbonate-based eluents are not suitable for gradient elution due to the significant increase in background conductance with increasing eluent concentration, which limits the number of analytes that can be separated in a single run. Deviations from a linear calibration curve are observed due to the formation of the weakly ionized carbonic acid in the suppressor. Figure 8.54 shows a typical example for counter ion analysis with a carbonate-based eluent. An over-the-counter multisymptom cold/flu medication was selected as a representative pharmaceutical product. This formulation contains pseudoephedrine and dextromethorphan that are administered as chloride and bromide salts, respectively. Both anions can be separated with a carbonate/bicarbonate eluent on a Dionex IonPac AS14 column in less than 10 min. Besides the labeled content of the pharmaceutical product, other anionic impurities may also be present that may arise from raw materials used in manufacturing. Sulfate could be detected in the multisymptom cold/flu formulation at very low level. Although this method offers the benefit of a relatively short run time, it can be shortened even more by using the shorter 5 µm Dionex IonPac AS14A (150 mm × 3 mm i.d.) column.

Hydroxide eluents are of particular interest for the separation of anions in pharmaceutical formulations because they provide a number of advantages over traditional carbonate-based eluents. The primary advantage of a hydroxide eluent is its use for gradient applications because increasing the hydroxide concentration during gradient elution does not significantly influences the background signal. Moreover, conversion of hydroxide to water in the suppressor results in strictly linear calibration curves. However, undesirable baseline shifts and irreproducible retention times can only be avoided by employing an eluent generator that produces high-purity hydroxide eluents by means of electrolysis. Hydroxide gradients are predominantly used for screening analysis of pharmaceutical formulations. An example chromatogram using a hydroxide gradient is shown in Figure 8.55 for Humatin, which is a commercially available paromomycin sulfate drug product. Paromomycin is a well-known aminoglycoside antibiotic prepared in the sulfate form that is widely used in the treatment of various bacterial infections. It is not uncommon for these compounds to contain up to 30% sulfate by weight. Anion-exchange chromatography with suppressed conductivity detection can be used to rapidly determine the sulfate concentration with good precision and accuracy [191]. As shown in Figure 8.55, it can also be used to determine trace concentrations of undesirable impurities that can originate from the raw materials used in the manufacturing process. Screening analyses are best performed using the Dionex IonPac AS11-HC column, a hydroxide-selective, high-capacity anion exchanger that provides high-resolution separations of inorganic and low-molecular weight organic acids.

A primary consideration in the development of a suitable IC method for pharmaceuticals is the solubility of the API in water. Many drugs and intermediates are insoluble in aqueous solutions that are typically used in IC systems. This poses a potential analytical challenge as it could lead to precipitation of the API in the chromatography system and, therefore, cause excess column back pressure and column contamination. The best solution to overcome this challenge is the replacement of the regular guard column by a Dionex IonPac NG1 (50 mm × 4 mm i.d.) column, which contains a nonfunctionalized divinylbenzene resin. Hydrophobic APIs get adsorbed on this resin due to strong interaction with the aromatic polymer substrate. These columns can be used continuously for quite some time and flushed thereafter with an organic solvent to remove the APIs. To avoid a manual exchange of columns, two of these guard columns can be mounted on an additional 10-port valve as shown in Figure 8.56. Once the capacity of the first Dionex IonPac NG1 column is expended, the 10-port valve is switched to put the second column in line, while the other one can automatically be backflushed with an organic solvent using a Thermo Scientific™ Dionex™ AXP auxiliary pump.

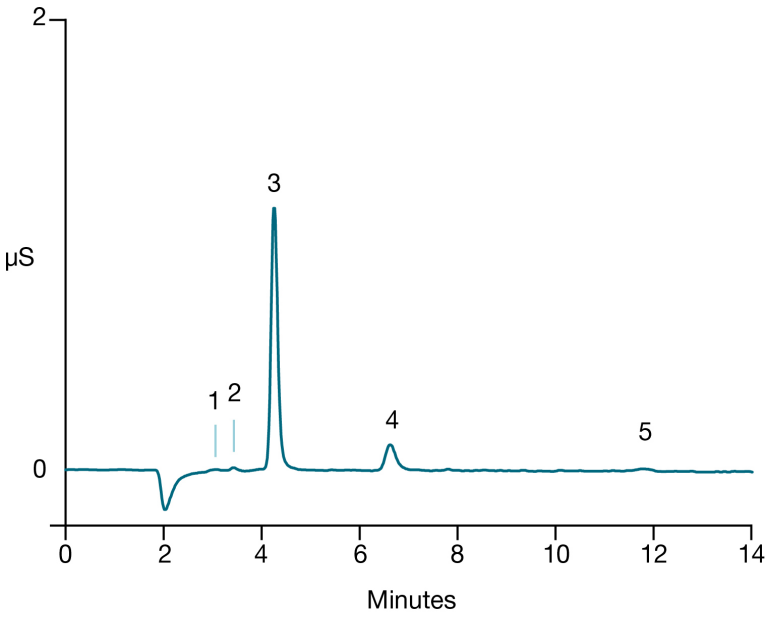


Figure 8.54 Separation of chloride and bromide counter ions in a multisymptom cold/flu medication. Separator column: Dionex IonPac AS14; column dimensions: 250 mm × 4 mm i.d.; eluent: 3.5 mmol/L Na_2CO_3 + 0.8 mmol/L $NaHCO_3$; flow rate: 1.2 mL/min; detection: suppressed conductivity; injection volume: 10 µL; sample: medication diluted 1:100 with deionized water; peaks: (1) and (2) unidentified, 3 mg/L chloride (3), 1 mg/L bromide (4), and 0.08 mg/L sulfate (5).

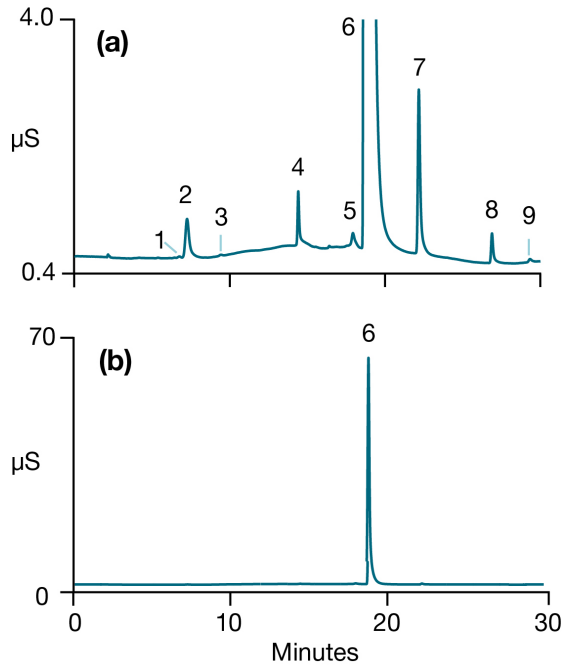


Figure 8.55 Separation of sulfate counter ion and anionic impurities in Humatin by gradient anion-exchange chromatography. Separator column: Dionex IonPac AS11-HC + guard; column dimensions: 250 mm × 2 mm i.d.; column temperature: 30 °C; eluent: KOH (EG); gradient: 1 mmol/L for 0 to 5 min, 1–5 mmol/L for 5 to 9 min, 5–38 mmol/L for 9 to 20 min, 38–60 mmol/L for 20 to 25 min, 60 mmol/L for 25 to 30 min; flow rate: 0.38 mL/min; detection: suppressed conductivity; injection volume: 5 µL; sample: (a) 2.5 mg/mL paromomycin, (b) 1:10 dilution of sample (a); peaks: (a) unknown (1), 0.08% acetate (2), unknown (3), 0.03% chloride (4), carbonate (5), sulfate (n. d.) (6), 0.23% ortho-phosphate (7), 0.04% pyrophosphate (8), and unknown (9), (b) 24.7% sulfate (6).

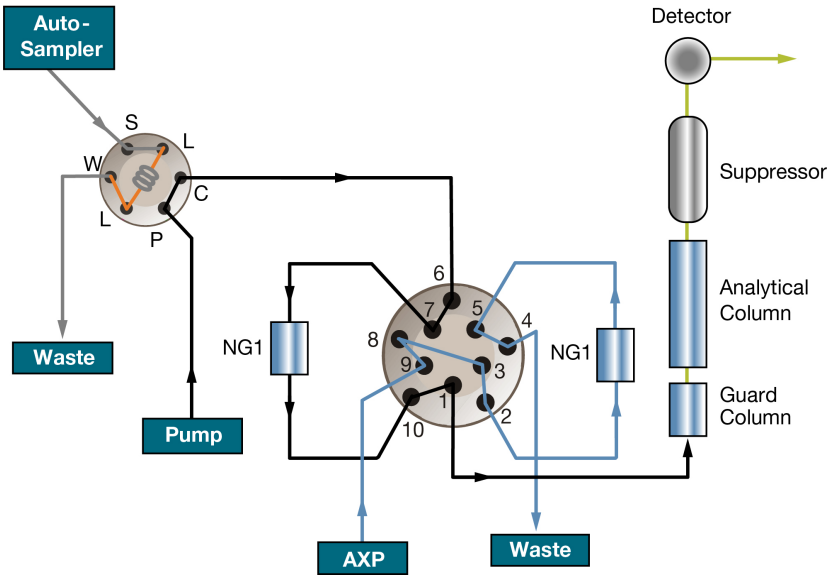


Figure 8.56 Schematic of a matrix elimination technique with an additional 10-port valve and two Dionex IonPac NG1 columns to remove water-insoluble APIs.

Pharmaceutical counter anions can also be separated by mixed-mode liquid chromatography using an Acclaim Mixed-Mode WAX-1 column (see Section 6.3). The column packing material features a stationary phase that incorporates both hydrophobic and weak anion-exchange properties. Pharmaceutically relevant anions can be separated on this stationary phase with an eluent mixture of acetonitrile and a phosphate buffer under isocratic conditions. The only drawback is that this eluent is not compatible with suppressed conductivity detection, so that such separation is limited to UV-absorbing anions. Alternatively, charged aerosol detection can be employed when using a volatile buffer such as ammonium acetate as the eluent.

In case anionic and cationic counter ions must be separated in the same chromatographic run, mixed-mode liquid chromatography with nonspecific detection must be employed. The fastest separations are obtained using the trimodal Acclaim Trinity P1 column (see Section 6.3). Figure 8.57 demonstrates that this column provides excellent selectivity for separating counter ions (both anions and cations) using a mobile phase of acetonitrile and ammonium acetate. Baseline separation of five cations and five anions is achieved in less than 15 min. Column selectivity is designed such that cations elute before anions. The mobile phase used is compatible with a number of detection methods, including charged aerosol (CAD), UV/Vis, and MS detections. However, the Corona CAD detector is the preferred detector for this application because it detects any nonvolatile analyte and offers high sensitivity and gradient compatibility.

Analysis of amines A major problem in pharmaceutical analysis is the analysis of aliphatic amines. Because they are nonchromophoric compounds, UV detection is only possible at very low wavelengths (<200 nm) that are prone to interferences by matrix and eluent components. Moreover, amines undergo interactions with free silanol groups if chemically bonded silica is used as a stationary phase. Thus, conventional HPLC methods are not very well suited for the analysis of amines. In contrast, primary, secondary, and tertiary amines can easily be separated by cation-exchange chromatography on suitable stationary phases and detected via suppressed conductivity or integrated pulsed amperometry [192] on a gold working electrode.

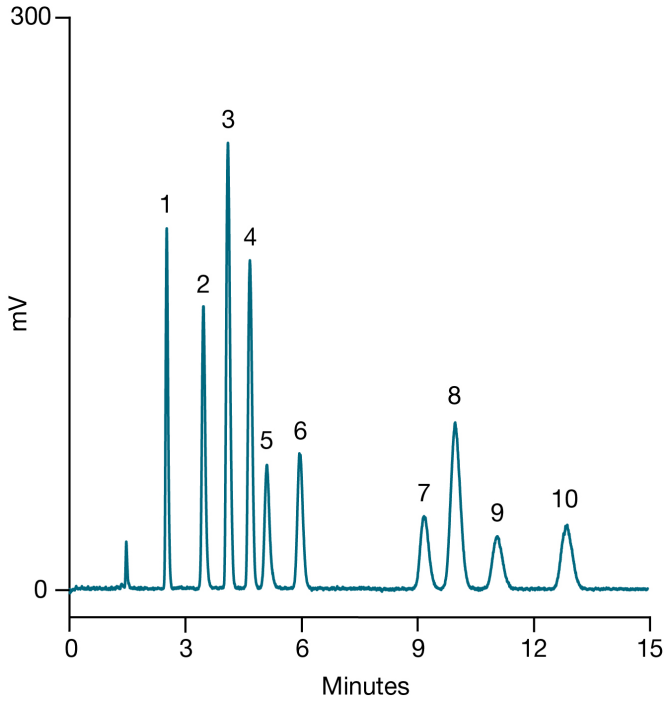


Figure 8.57 Isocratic separation of pharmaceutically relevant inorganic and organic anions and cations by mixed-mode liquid chromatography. Separator column: Acclaim Trinity P1, 3 µm; column dimensions: 100 mm × 3 mm i.d.; column temperature: 30 °C; eluent: 60:40 (*v/v*) MeCN/ 20 mmol/L (total) NH_4OAc, pH 5; flow rate: 0.5 mL/min; detection: Corona CAD, gain: 100 pA, filter: med, neb. temp.: 30 °C; injection volume: 2 µL; peaks: 50–100 mg/L each of choline (1), tromethamine (2), sodium (3), potassium (4), meglumine (5), mesylate (6), nitrate (7), chloride (8), bromide (9), and iodide (10).

Alkanolamines such as *N*,*N*-dimethylethanolamine, monoisopropanolamine, and *N*-methylethanolamine are used in the synthesis of antihistamines among other pharmaceutical products, and *N*-methyldiethanolamine is an intermediate in the production of analgesics. Hydroxylamines and *N*-alkylhydroxylamines are used as reducing agents in pharmaceutical processes and can be separated as cations after protonation in acidic eluents. Hydroxylamine itself cannot be detected by suppressed conductivity because the suppression product is not dissociated. It is also not UV active and does not react with *o*-phthaldialdehyde (OPA) to form a fluorescent product like other primary amines. However, hydroxylamine is electroactive; together with its *N*-alkyl derivatives it can be oxidized on a gold working electrode at high pH [193], which is much more selective and sensitive in comparison with nonsuppressed conductivity detection. A corresponding chromatogram is shown in Figure 8.58. Because the separation of hydroxylamines on a cation exchanger requires an acid eluent, a sodium hydroxide concentrate must be added postcolumn to convert eluent pH into a strongly alkaline one to apply integrated pulsed amperometry.

Cation-exchange chromatography with suppressed conductivity detection can also be applied for the analysis of aliphatic quaternary ammonium compounds. Typical examples are carbachol (2-[(aminocarbonyl)oxy]-*N*,*N*,*N*-trimethylethanaminium chloride) and bethanechol (2-[(aminocarbonyl)oxy]-*N*,*N*,*N*-trimethylpropan-1-aminium chloride). Both compounds are classified as chlolinergic agonists. Carbachol is primarily used for ophthalmic applications such as solutions used for glaucoma treatment or ophthalmic surgery. It constricts the iris and ciliary body resulting in a reduction of intraocular pressure in patients with glaucoma. Analytical

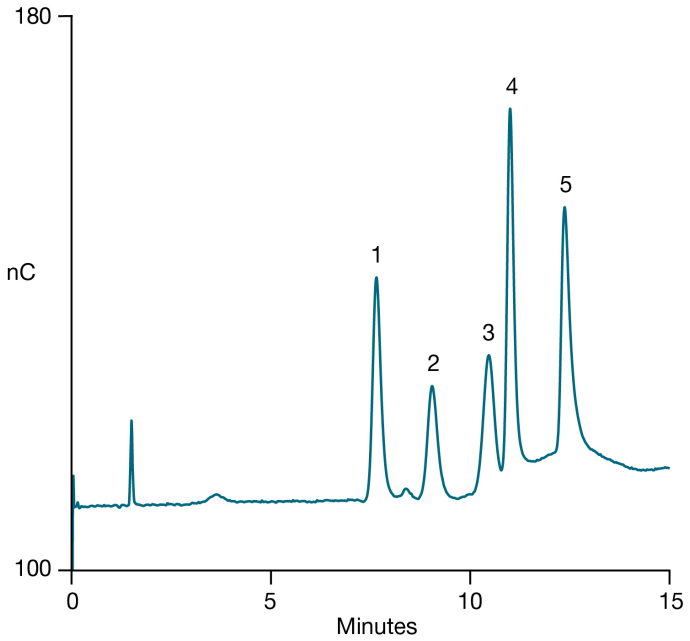


Figure 8.58 Isocratic separation of hydroxylamine and *N*-alkylhydroxylamines by cation-exchange chromatography with integrated pulsed amperometric detection. Separator column: Dionex IonPac CS17; column dimensions: 250 mm × 4 mm i.d.; column temperature: 30 °C; eluent: MSA (EG); gradient: 1.8 mmol/L isocratically for 8 min, then linearly to 16 mmol/L in 3 min, isocratically for 4 min; flow rate: 1 mL/min; detection: IPAD, Au working electrode after postcolumn addition of 1 mol/L NaOH at 1 mL/min, AAA waveform; injection volume: 25 µL; peaks: 1 mg/L hydroxylamine (1), 5 mg/L methylhydroxylamine (2), 40 mg/L ethylhydroxylamine (3), 5 mg/L dimethylhydroxylamine (4), and 8.5 mg/L diethylhydroxylamine (5) (reproduced with permission from Ref. [193]. Copyright 2012, John Wiley & Sons).

methods are needed to ensure that the concentration in these solutions remain at therapeutically active level. Bethanechol is administered for the treatment of urinary retention. The ion chromatography assay allows the determination of bethanechol and its degradation product, 2-hydroxypropyltrimethylammonium chloride (2-HPTA) [194]. This assay separates bethanechol from other inorganic cations by using a Dionex IonPac CS14 column and a methanesulfonic acid eluent. Bethanechol is detected directly by suppressed conductivity. In alkaline solutions, carbachol degrades to choline, a member of the vitamin B group that is also known for its activity as neurotransmitter. Therefore, a method is required that selectively separates and detects carbachol and choline. Using a Dionex IonPac CS17 weak acid cation exchanger and a methane-sulfonic acid eluent, carbachol, bethanechol, and choline can be separated simultaneously in less than 30 min under isocratic conditions [195].

Benzalkonium chloride, a typical aromatic quaternary ammonium compound, is often used as an antiseptic. Usually, it is a mixture with alkyl chains R that vary in length and have even-numbered members between C_{12} and C_{18}. The C_{12} and C_{14} homologs are the major species in a benzalkonium chloride preparation. In the past, separation of benzalkonium chloride was performed by ion-pair chromatography using hydrochloric acid as ion-pair reagent. Because benzalkonium chloride is an aromatic compound, it can be detected very sensitively with UV detection at 215 nm. With the introduction of mixed-mode stationary phases, ion-pair chromatography is no longer the method of choice for the analysis of benzalkonium chloride. Mixed-mode columns such as the Acclaim Surfactant Plus column feature reversed-phase, anion-exchange, and hydrogen bonding mechanisms, which allow the column to be used differently than a conventional reversed-phase column. Thus, the retention behavior of benzalkonium chloride on an Acclaim Surfactant Plus column can be varied by changing ionic

strength, eluent pH, and the type and amount of organic solvent in the mobile phase. An example chromatogram of a nasal spray shown in Figure 8.59 was obtained with an acetonitrile/ammonium acetate gradient. This eluent mixture is compatible with charged aerosol detection used in this example, which is a welcome alternative to UV detection.

Analysis of organic acids Organic acids can be analyzed by a number of liquid chromatography methods such as:

- Ion-exclusion chromatography
- Anion-exchange chromatography
- Ion-pair chromatography
- Reversed-phase chromatography in the ion-suppression mode
- HILIC
- Mixed-mode liquid chromatography

As always in analytical chemistry, the choice of method depends on the type and number of organic acids to be analyzed, the required sensitivity, and the type and concentration of matrix components that have to be separated from the analytes. Due to the huge number of organic acids (e.g., aliphatic, aromatic, carboxylic acids, sulfonic acids, phosphonic acids, etc.) none of the above-mentioned LC methods is universally applicable. While ion-exclusion chromatography separates weakly dissociated organic acids from totally dissociated acids that elute as one peak in the void volume, anion-exchange chromatography offers the advantage of separating inorganic and organic acids in the same chromatographic run. In most of the cases, ion-pair chromatography can be replaced by modern anion-exchange or mixed-mode chromatography today and thus does not have the significance anymore for organic acid analysis. Reversed-phase liquid chromatography in the ion-suppression mode on conventional

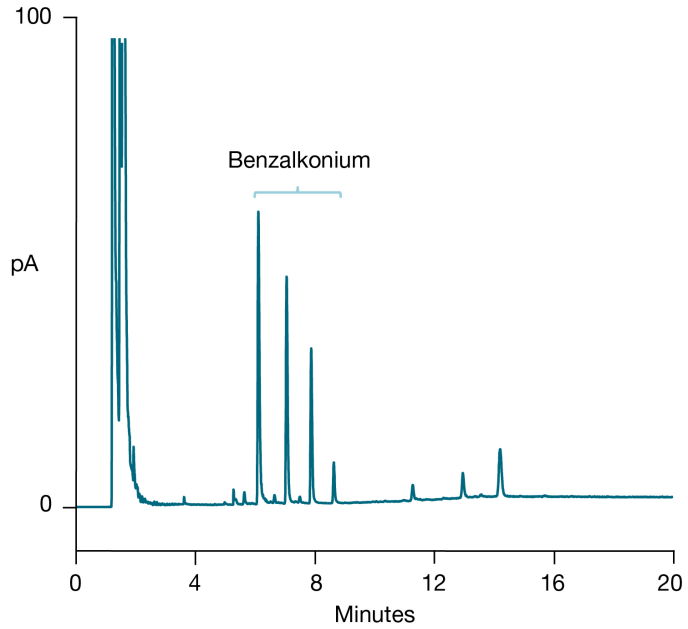


Figure 8.59 Analysis of benzalkonium chloride in a nasal spray by mixed-mode liquid chromatography. Separator column: Acclaim Surfactant Plus, 3 µm; column dimensions: 150 mm × 3 mm i.d.; column temperature: 30 °C; eluent: 25:75 (*v/v*) MeCN/ 0.1 mol/L ammonium acetate, pH 5.2 to 80:20 (*v/v*) MeCN/0.1 mol/L ammonium acetate, pH 5.2 in 10 min; flow rate: 0.6 mL/min; detection: Corona CAD (gain: 100 pA, filter: med, neb. temp.: 20 °C); injection volume: 5 µL; sample: nasal spray (direct injection after filtration).

C18 phases is predominantly used for the analysis of long-chain fatty acids, while aromatic carboxylic acids are very well separated on polar-embedded stationary phases. Mixed-mode liquid chromatography is a powerful method employing both reversed-phase and anion-exchange mechanisms for the analysis of small-molecular weight organic acids such as quinic, lactic, acetic, formic, and ascorbic acids that are very difficult to separate by anion-exchange chromatography.

As a typical example for an anion-exchange separation of an organic acid under isocratic conditions is the analysis of tartaric acid in tolterodine tartrate drug substance. Tolterodine is a quaternary ammonium compound used to treat urinary incontinence. The counter ion assay can serve as a check of the amount of API in the sample. In the past, reversed-phase liquid chromatography with UV detection has been employed for this application, but tartaric acid has a very low response at the measuring wavelength of 210 nm. A much better choice for tartrate analysis is anion-exchange chromatography with suppressed conductivity detection. The fastest method with superior reproducibility has been described by Tukkeeree et al. [196] using a Dionex IonPac AS20 anion exchanger and an electrolytically generated hydroxide eluent. Figure 8.60a shows the determination of tartrate in a capsule drug product containing tolterodine tartrate. The organic acid is resolved from other anions in the sample using 20 mmol/L potassium hydroxide as the mobile phase. Figure 8.60b shows the same sample to which an additional 4 mg/L tartrate was added. For a set of five samples there was a 100% recovery of the added tartrate. The same unfortified samples had 98.4% of the labeled amount of drug substance as measured by tartrate concentration. Both results indicate good method accuracy.

A significantly larger hydroxide eluent concentration is required for the analysis of benzenesulfonic acid which is the counter ion in amlodipine besylate. This potent API is a

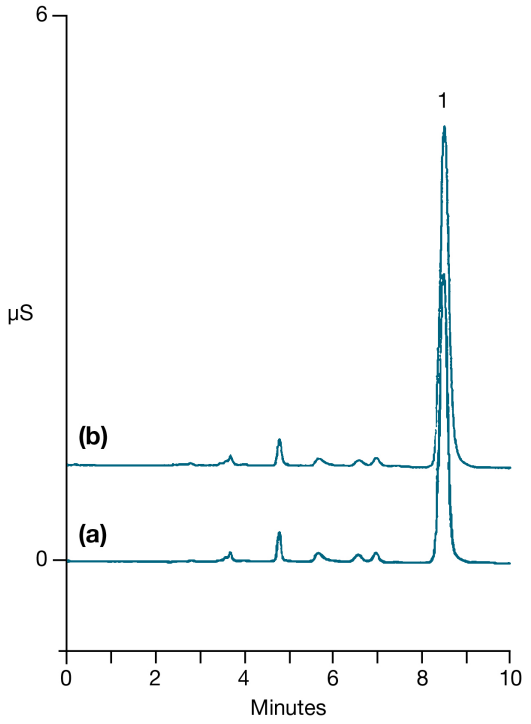


Figure 8.60 Analysis of tartrate in tolterodine tartrate by anion-exchange chromatography. Separator column: IonPac AS20; column dimensions: 250 mm × 4 mm i.d.; column temperature: 30 °C; eluent: 20 mmol/L KOH (EG); flow rate: 1 mL/min; detection: suppressed conductivity; injection volume: 10 µL; sample: 184 mg of tolterodine tartrate ad 100 mL deionized water; peaks: (a) sample with 12.4 mg/L tartrate (1), (b) spiked sample with 16.4 mg/L tartrate (1).

calcium channel blocker used for the treatment of hypertension and angina. While a US Pharmacopeia (USP) monograph describes the RPLC separation of the API, there is currently no USP method for the analysis of benzenesulfonic acid. However, benzenesulfonic acid is a strong acid that is completely dissociated in aqueous solution and thus can be separated by anion-exchange chromatography followed by suppressed conductivity detection [197]. To separate benzenesulfonic acid in amlodipine besylate, a high-capacity, hydroxide-selective Dionex IonPac AS18 anion exchanger can be used with an electrolytically generated 60 mmol/L KOH eluent, followed by suppressed conductivity detection. Under these chromatographic conditions, benzenesulfonic acid has a retention time of approximately 12 min. Sample throughput can be increased by using a Dionex IonPac AS18-4µm column. The smaller particle size provides higher peak efficiencies and improved resolution so that higher flow rates can used, which, in turn, produce faster analysis times. Figure 8.61 demonstrates the separation of benzenesulfonic acid in approximately 5 min on the 4 µm column. The higher flow rate and shorter column length decrease the retention time of the target analyte by 60%.

Antibiotics Penicillins were among the first drugs to be effective against bacterial infections caused by *staphylococci* and *streptococci*. All penicillins are *β*-lactam antibiotics that are used in the treatment of bacterial infections by gram-positive organisms. The term penicillin is often generically used to refer to benzylpenicillin (penicillin G), procaine benzylpenicillin (procaine penicillin), benzathine benzylpenicillin (benzathine penicillin), and phenoxymethyl-penicillin (penicillin V). Penicillin V is more acid-stable than penicillin G, which allows it to be administered orally. It is usually only used for the treatment of mild to moderate infections. Before the introduction of ion-exchange chromatography the precursor of penicillin V, phenoxyacetate, and degradation products such as acetate were determined enzymatically in order to monitor the manufacturing process. When looking at the penicillin V structure, it is not surprising that this compound can also be eluted from an anion exchanger because the carboxyl group located at the thiazolidine ring is fully dissociated under alkaline conditions. Thus, all three compounds may be determined together with sulfate in the same run, a very impressive example of the time saving capability of ion chromatography, replacing three different methods by one IC method for characterizing penicillin V.

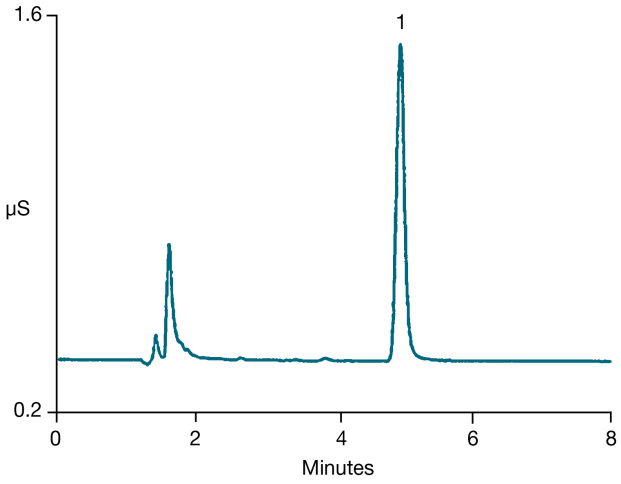
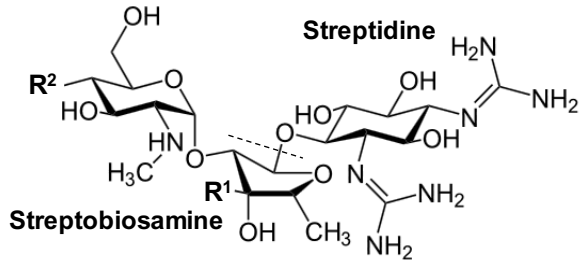


Figure 8.61 Analysis of benzenesulfonic acid in amlodipine besylate by anion-exchange chromatography. Separator column: Dionex IonPac AS18-4µm with guard; column dimensions: 150 mm × 2 mm i.d.; eluent: 60 mmol/L KOH (EG); flow rate: 0.38 mL/min; detection: suppressed conductivity; injection volume: 5 µL; sample: 10 µg/mL of amlodipine besylate; peak: 2.8 µg/mL benzene-sulfonic acid (1).

Aminoglycoside antibiotics belong to another class of antibiotics that can be analyzed by ion chromatography. Examples of aminoglycoside antibiotics include tobramycin, streptomycin, neomycin, kanamycin, amikacin, gentamicin, and netilmicin. Only a few aminoglycosides are assayed by liquid chromatography because most aminoglycosides have a weak chromophore and are, therefore, poorly detected by UV absorbance. Statler was among the first to show that HPAE-PAD could be used to assay tobramycin [198]. Though most aminoglycosides are not charged at neutral pH, they can be ionized and separated at high pH. However, aminoglycosides are very weakly retained on anion exchangers, so that weak hydroxide eluents (c < 5 mmol/L) must be used. This, in turn, requires the postcolumn addition of a sodium hydroxide concentrate to achieve the necessary detection sensitivity. The original HPAE-PAD method developed by Statler had a number of limitations which, over the years, could be overcome by introducing additional separator columns with differences in selectivity and capacity, new waveforms to detect both carbohydrate and amino acids on a gold working electrode in highly basic eluents, and the introduction of electrolytic eluent generation that is capable of producing high-purity hydroxide eluents at low concentrations. As a consequence of these improvements, USP monographs have been developed for assaying streptomycin (USP L46) and kanamycin (USP L47).

The best separator column to retain streptomycin is the Dionex CarboPac PA1 column. Streptomycin is purified from the fermentation of *Streptomyces griseus*. The current US Pharmacopeia method for streptomycin sulfate measures streptomycin A as the primary antibiotic [199], but less abundant forms such as streptomycin B could also be detected in *S. griseus* fermentation broths.



Streptomycin	R_1	R_2
A	–CHO	–OH
B	–CHO	–Mannose
Dihydro-	$–CH_2OH$	–OH

Figure 8.62 shows the separation of USP grade streptomycin A and dihydrostreptomycin using a Dionex CarboPac PA1 column and a sodium hydroxide eluent under isocratic conditions [200]. The separation of streptomycin and its impurities is highly dependent on eluent concentration. The greatest effect on retention is observed between 50 and 77 mmol/L NaOH, where very minor changes in hydroxide concentration produce large changes in retention. Reducing eluent concentration by 10% improves the separation of the earlier eluting impurities, but increases the retention time and peak tailing of streptomycin A.

Kanamycin and amikacin are broad-spectrum aminoglycoside antibiotics that are closely related. Kanamycin is used to treat a wide variety of serious gram-negative bacterial infections and is purified from fermentation of *Streptomyces kanamyceticus*. The main component of kanamycin is kanamycin A with minor structurally related constituents such as kanamycins B, C, and D.

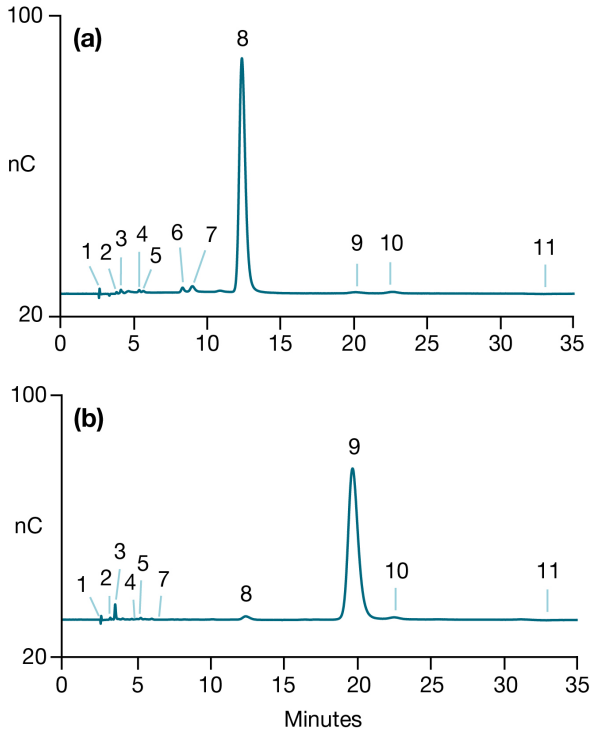
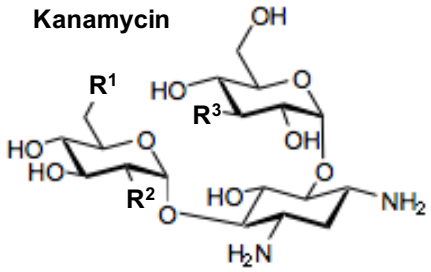
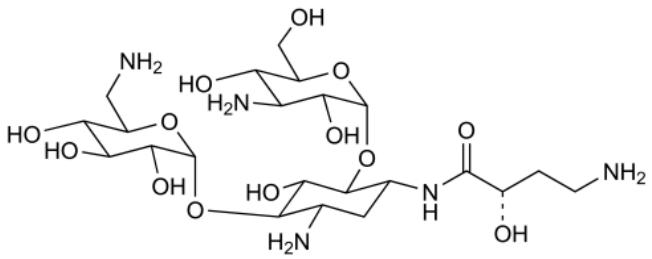


Figure 8.62 Separation of streptomycin A and dihydrostreptomycin with integrated pulsed amperometric detection. Separator column: Dionex CarboPac PA1; column dimensions: 250 mm × 4 mm i.d.; column temperature: 30 °C; eluent: 70 mmol/L NaOH; flow rate: 0.5 mL/min; detection: IPAD; injection volume: 20 µL; sample: 30 µg/mL each of streptomycin sulfate (a) and dihydrostreptomycin sulfate (b); peaks: (1) void, (2)–(7) impurities, (8) streptomycin A, (9) dihydrostreptomycin, (10) impurity, and (11) oxygen dip.



Kanamicin	R_1	R_2	R_3
A	$–NH_2$	–OH	$–NH_2$
B	$–NH_2$	$–NH_2$	$–NH_2$
C	–OH	$–NH_2$	$–NH_2$
D	$–NH_2$	–OH	–OH

Amicacin is also used to treat gram-negative infections resistant to kanamycin, gentamicin, or tobramycin. It is synthesized by acylation of the amino group of kanamycin A.



Amikacin

The purity of antibiotics must be determined and must meet specific criteria before clinical use. The US Pharmacopeia monographs for kanamycin and amikacin describe a revised HPAE-PAD method using a Dionex CarboPac MA1 column and a disposable working electrode [201, 202]. The use of disposable electrodes provides the benefits of shorter equilibration time and greater electrode-to-electrode reproducibility. Figure 8.63 shows the separation of kanamycin and amikicin on a Dionex CarboPac MA1 column with a total analysis time of 10 min [203]. A small baseline dip is seen at ~6 min that coelutes with kanamycin. However, at relevant kanamycin concentrations, the contribution of the dip to kanamycin peak area is insignificant (<3%).

HPAE-PAD has also been applied for the analysis of gentamicin sulfate. Gentamicin sulfate is an aminoglycoside antibiotic purified from fermentation of *Micromonospora purpurea*. It is a mixture of four major compounds: gentamicins C1, C1a, C2, and C2a. In addition, gentamicins C2b as well as related compounds such as sisomicin, garamine, and gentamicin B1 are commonly formed in small amounts during fermentation. Gentamicin congeners are structurally very similar, but their antimicrobial potencies and toxicities are different.

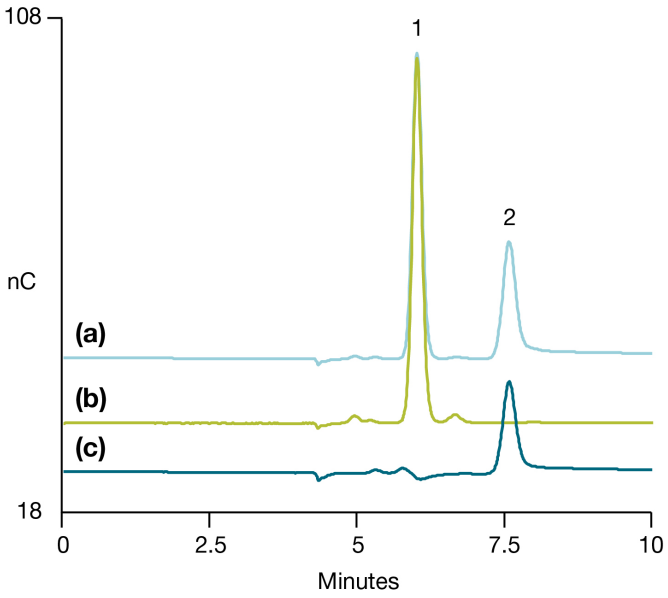
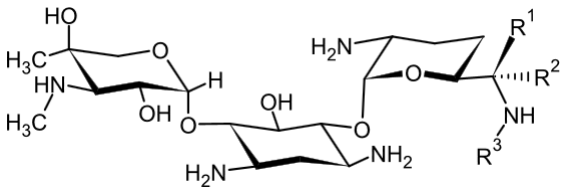


Figure 8.63 Separation of kanamycin A and amikacin with integrated pulsed amperomtric detection. Separator column: Dionex CarboPac MA1; column dimensions: 250 mm × 4 mm i.d.; column temperature: 30 °C; eluent: 115 mmol/L NaOH; flow rate: 0.5 mL/min; detection: IPAD; injection volume: 20 µL; sample: (a) 8 µg/mL of kanamycin and 20 µg/mL amikacin, (b) commercial kanamycin A sulfate sample, and (c) commercial amikacin sample; peaks: (1) kanamycin and (2) amikacin.



Gentamicin	R_1	R_2	R_3
C1	$–CH_3$	$–CH_3$	–H
C1a	–H	–H	–H
C2	–H	$–CH_3$	–H
C2a	$–CH_3$	–H	–H
C2b	–H	–H	$–CH_3$

Aminoglycoside antibiotic assays, including those for gentamicin sulfate, are typically microbial assays. These assays measure activity but cannot quantify impurities or determine the content of specific compounds in a commercial product. For this reason, chromatographic techniques are favored for improved specificity and the ability to differentiate impurities that have the potential for both antibiotic activity and unintended side effects. However, the structural similarities of gentamicin C congeners that differ by methylation at three potential sites make their separation difficult. Separation of gentamicin C congeners by reversed-phase liquid chromatography is possible but requires strong ion-pair reagents in the mobile phase to assist in accentuating their small differences in hydrophobicity. In addition to separation challenges, gentamicin C congeners are nonchromophoric compounds, making UV detection insensitive. To compensate for this lack of a chromophore, the USP gentamicin sulfate monograph, for instance, specifies content determination after gentamicin derivatization with *o*-phthaldialdehyde (OPA) and subsequent separation on a C18 column followed by UV detection [204]. The lowest TFA concentration that results in consistent resolution of gentamicin congeners is 0.3% when using an Acclaim PolarAdvantage II (PA2) column [205]. This column also allows good separation of the primary components of gentamicin as well as sisomicin, which elutes just before gentamicin C1a. A small concentration of heptafluoro-butyric acid (HFBA) is included in the gradient to improve retention of gentamicin degradation products such as garamine. Figure 8.64 shows the separation of USP grade gentamicin sulfate on this column with a HFBA/TFA gradient. Column temperature is set to 15 °C, which allows better separation between gentamicin C2b and gentamicin C2.

Ionic drugs Among the many pharmaceutical drugs, some can be analyzed directly by ion chromatography. A known and important example is the group of bis-phosphonates that are used to treat bone disorders including osteoporosis, Paget's disease, and hypercalcaemia. Clodronate, the disodium salt of dichloromethylenebisphosphonic acid, is one of the most investigated compounds in the bisphosphonate class of APIs, which is registered for use in the effective management of hypercalcaemia and bone pain associated with skeletal metastases in patients with multiple myeloma or carcinoma of the breast. Clodronate has been in clinical use for more than 20 years. It is synthesized in a two-step reaction from tetraisopropyl-methylenebisphosphonic acid, which is first reacted with hypochlorite to the chloro-substituted ester followed by deesterification with refluxing hydrochloric acid. Possible impurities from

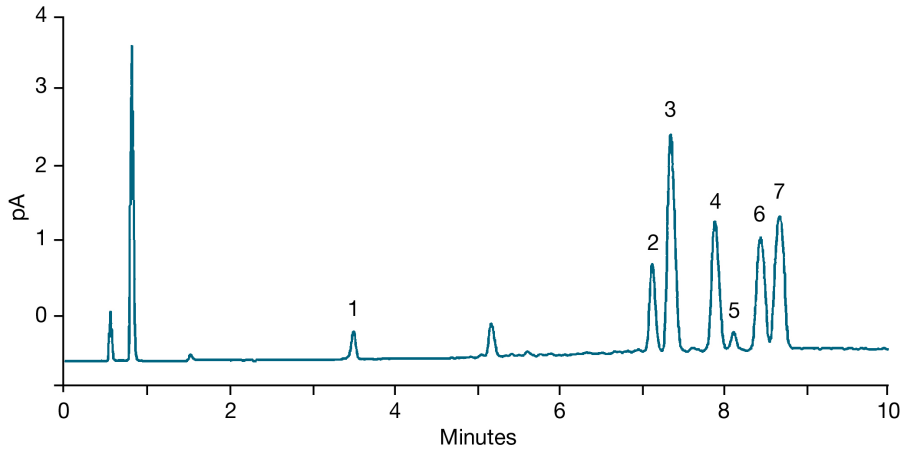


Figure 8.64 Separation of gentamicin sulfate and related substances by RPIPC with charged aerosol detection. Separator column: Acclaim PA2, 2.2 µm; column dimensions: 100 mm × 2.1 mm i.d.; column temperature: 15 °C; eluent: (A) HFBA/ MeCN/water (0.025:5:95 *v/v/v*), (B) TFA/MeCN/ water (0.3:5:95 *v/v/v*); gradient: 1–10% B from 0 to 1 min, 10–100% B from 3 to 8 min; flow rate: 0.45 mL/min; detection: CAD (neb. temp.: 15 °C); injection volume: 1 µL; sample: 0.25 mg/mL USP grade gentamicin sulfate; peaks: (1) garamine-like component, (2) sisomicin, (3) gentamicin C1a, (4) gentamicin C2, (5) gentamicin C2b, (6) gentamicin C2a, and (7) gentamicin C1.

this synthesis include, among others, chloride, chloro-substituted partial esters, methylene-bisphosphonic acid, and monochloromethylenebisphosphonic acid. Under alkaline conditions, orthophosphate and carbonylphosphonate can also occur as degradation products. The reported analytical methodologies for bisphosphonates are summarized and compared in a review article published by Zacharis and Tzanavaras in 2008 [206].

Taylor [207] developed an ion chromatographic procedure with which the drug component itself and possible impurities can be separated on an anion exchanger by applying a hydroxide gradient and suppressed conductivity detection. This procedure was based on the idea that the components to be analyzed are multivalent anions that elute under alkaline conditions in the order of increasing valency. Considering that the separation of bisphosphonates not only depends on their charge but also on their hydrophobic interactions with the stationary phase, Taylor tested a series of hydroxide-selective anion exchangers for their suitability for this separation problem; the Dionex IonPac AS5 column delivered the best results at that time. As can be seen from the chromatogram of a model solution in Figure 8.65, all components of interest can be separated to baseline using a purely aqueous hydroxide gradient. The model solution comprised the following components: 830 µg/mL monotributylammonium clodronate containing 2.7% monochloromethylenebisphosphonic acid, 6 µg/mL methylenebisphosphonic acid, 167 µg/mL monotributylammonium clodronate degraded under alkaline conditions, and 167 µg/mL of a mixture of partially esterified components. The concentrations of inorganic anions were 5 mg/L each.

The most modern quantitative method for the direct analysis of bisphosphonates and excipients in pharmaceutical formulations is capillary ion chromatography with suppressed conductivity and mass spectrometric detections [208]. Capillary IC offers improved sensitivity with injection of the same amount when compared to analytical IC, and better sensitivity when coupled with a capillary ESI interface to a mass spectrometric detector. A hydroxide-selective Dionex IonPac AS18-Fast capillary column can be used for such separation because it offers baseline resolution of targeted bisphosphonates (clodronate, etidronate, and tiludronate) and excipients (citrate, benzoate, and *p*-hydroxybenzoate), as well as commonly seen inorganic anions. The IonPac AS18-Fast capillary column is only 150 mm long, thus improving method

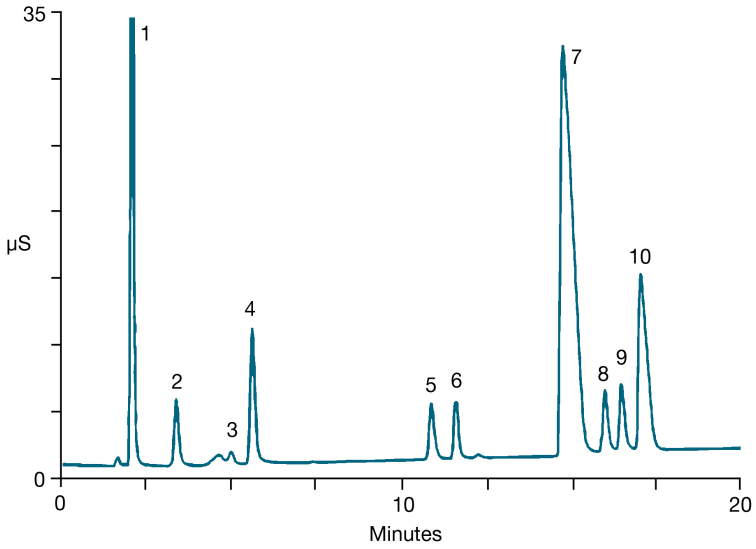


Figure 8.65 Separation of clodronate and potential impurities on a hydroxide-selective anion exchanger. Separator column: Dionex IonPac AS5; column temperature: 45 °C; eluent: NaOH; gradient: linear, 20–100 mmol/L in 20 min; flow rate: 1 mL/min; detection: suppressed conductivity; peaks: (1) chloride, (2) nitrate, (3) diester of dichloromethylene-bisphosphonic acid, (4) sulfate, (5) orthophosphate, (6) monoester of dichloromethylenebisphosphonic acid, (7) clodronate, (8) monochloromethylenebis-phosphonic acid, (9) methylenebisphosphonic acid, and (10) carbonylbisphosphonic acid (reproduced with permission from Ref. [207]. Copyright 1997, Elsevier B.V.).

throughput while still offering sufficient chromatographic resolution. The optimized separation is shown in Figure 8.66. All bisphosphonates and excipients are well separated from the early eluting inorganic anions and from each other in less than 15 min.

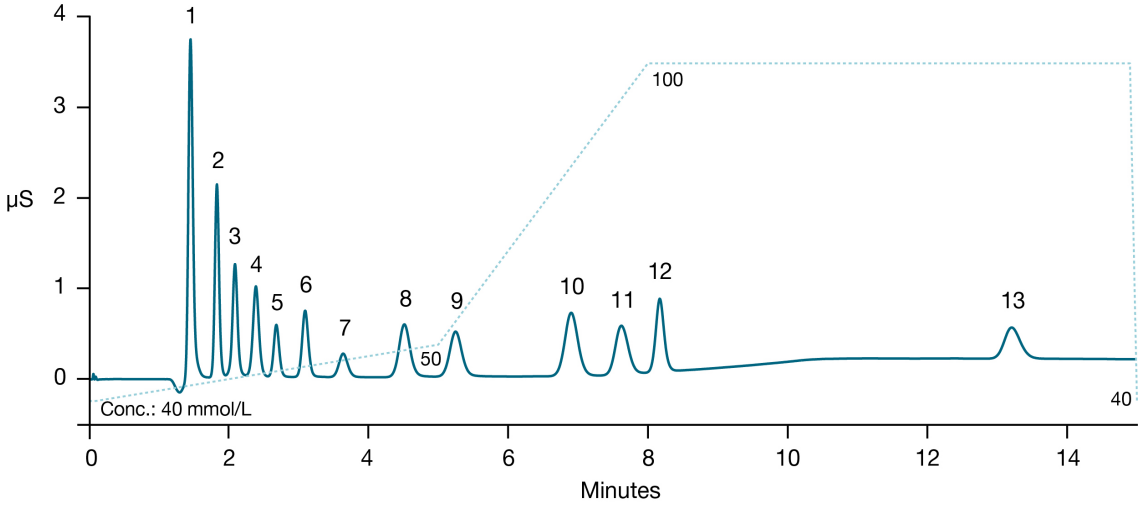


Figure 8.66 Separation of bisphosphonates, excipients, and standard inorganic anions by capillary IC. Separator column: Dionex IonPac AS18-Fast; column dimensions: 150 mm × 0.4 mm i.d.; column temperature: 40 °C; eluent: KOH (EG); gradient: linear, 40–50 mmol/L from 4 to 5 min, then to 100 mmol/L in 3 min; flow rate: 20 µL/min; detection: suppressed conductivity; injection volume: 0.4 µL; peaks: (1) fluoride, (2) chloride, (3) nitrite, (4) sulfate, (5) bromide, (6) nitrate, (7) ortho-phosphate, (8) benzoate, (9) *p*-hydroxybenzoate, (10) citrate, (11) etidronate, (12) clodronate, and (13) tiludronate.

Assays for active pharmaceutical ingredients (APIs) and counter ions Optimizing a pharmaceutical formulation is an important aspect of the drug development process. For an active pharmaceutical ingredient (API) with ionizable functional groups, the appropriate choice of salt forms is critical for a wide variety of physicochemical properties. Chromatographic methods are widely used in the salt selection process and in monitoring the quality of the formulation throughout the development process. Due to the large number of counter ions and their diversity in chemical properties (e.g., charge, polarizability, and absorption behavior), APIs and counter ions are usually analyzed by chromatographic methods that require different separator columns and/or different detection techniques. A more comprehensive analysis of APIs and their corresponding (nonvolatile) counter ions can be obtained using a trimodal separator column (Acclaim Trinity P1) in combination with charged aerosol detection (CAD). Trimodal stationary phase provide reversed-phase, anion-exchange, and cation-exchange retention mechanisms, allowing for the simultaneous separation of anions, cations, and neutral species. In case the APIs and counter ions lack or have only a weak chromophore, absorbance detectors cannot be used. Currently, such analytes are typically detected by aerosol-based detection techniques such as charged aerosol detection.

With the introduction of Acclaim Trinity P1 column, a broad range of hydrophobic and hydrophilic APIs that possess either acidic or basic functional groups can be separated along with their respective positively or negatively charged counter ions. As an example, the separation of the hydrophilic and basic API, 1,1-dimethylbiguanide (metformin), from its chloride counter ion can be achieved using a mobile phase with a relatively low amount of acetonitrile (20% *v/v*) under isocratic conditions (Figure 8.67). Metformin is an oral antidiabetic drug to treat diabetes mellitus type 2; it is believed to be the most widely prescribed antidiabetic drug in the world.

Increasing the organic content in the mobile phase to 75% or higher enables the separation of more hydrophobic and acidic APIs, such as naproxen and diclofenac, along with their sodium counter ions. The separation of naproxen and its sodium counter ion on an Acclaim Trinity P1 column is shown in Figure 8.68. Employing an eluent flow rate of 0.6 mL/min, both API and counter ion are eluted within 3 min under isocratic conditions. Because the Acclaim Trinity P1 column contains a 3 µm packing material and thus is UHPLC compatible, analysis time can be shortened to approximately 1 min by increasing the eluent flow rate to 2 mL/min. The elution order of the API and its counter ion very much depends on eluent pH. At pH 5, used for the separation in Figure 8.68, naproxen is partially dissociated and, therefore, is retained by reversed-phase and electrostatic interactions, resulting in a longer retention time in comparison with chloride. Lowering the eluent pH to 3.8, the elution order is reversed due to the lower degree of dissociation for naproxen, which is now predominantly retained by reversed-phase interactions. Chloride retention, on the other hand, remains the same as it is not affected by eluent pH.

Determination of impurities present at the 0.1% level or lower is often required by the pharmaceutical industry. The dynamic range and sensitivity of the CAD detector enables the simultaneous analysis of an API, its counter ion, and trace impurities. As an example, a sample solution of diclofenac sodium was spiked with ammonium chloride to achieve 0.1%, 0.2%, and 0.3% chloride levels. The chromatograms in Figure 8.69 clearly demonstrate that chloride is well resolved from both sodium and diclofenac and that impurity levels below 0.1% can readily be determined.

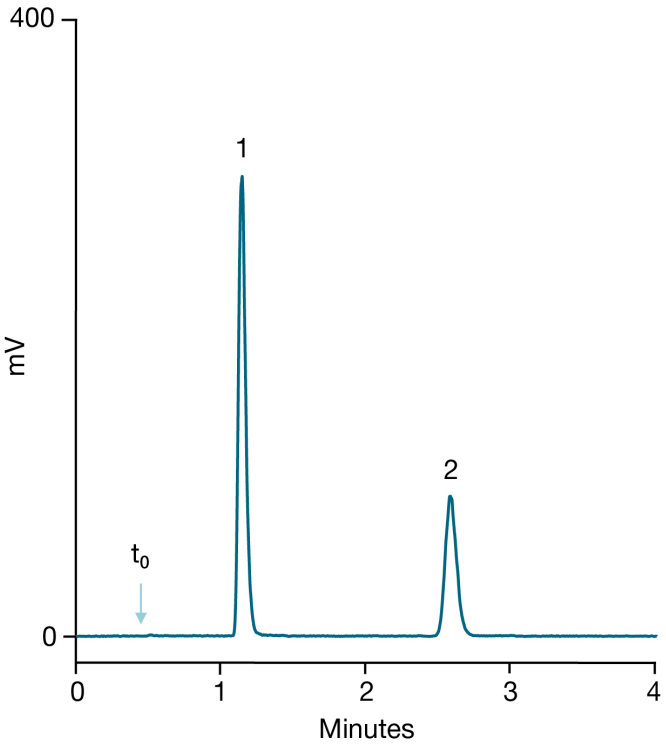


Figure 8.67 Simultaneous separation of 1,1-dimethylbiguanide (metformin) and its chloride counter ion by mixed-mode liquid chromatography. Separator column: Acclaim Trinity P1, 3 µm; column dimensions: 50 mm × 3 mm i.d.; column temperature: 30 °C; eluent: 20:80 (*v/v*) MeCN/40 mmol/L NH_4OAc, pH 5.2; flow rate: 0.6 mL/min; detection: CAD; injection volume: 2 µL; sample: 0.2 mg/mL 1,1-dimethylbiguanide•HCl; peaks: (1) 1,1-dimethylbiguanide and (2) chloride.

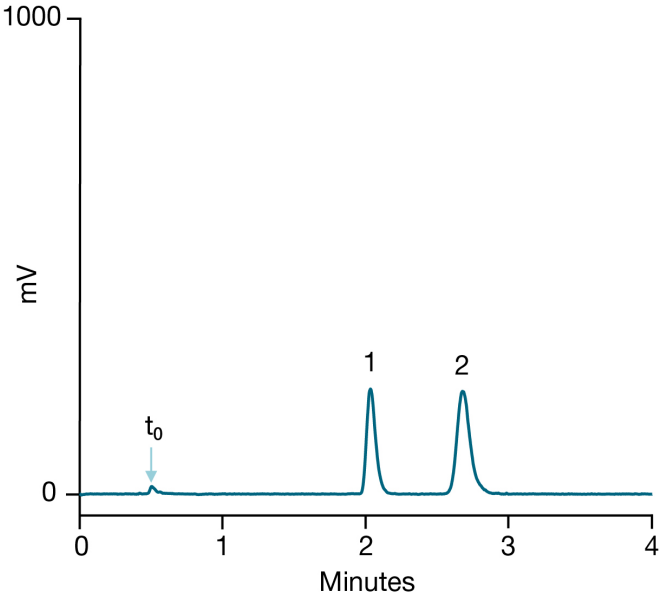


Figure 8.68 Simultaneous separation of naproxen and its sodium counter ion by mixed-mode liquid chromatography. Separator column: Acclaim Trinity P1, 3 µm; column dimensions: 50 mm × 3 mm i.d.; column temperature: 30 °C; eluent: 80:20 (*v/v*) MeCN/20 mmol/L NH_4OAc, pH 5; flow rate: 0.6 mL/min; detection: CAD (gain: 100 pA, filter: med., neb. temp.: 30 °C); injection volume: 2.5 µL; sample: 0.2 mg/mL naproxen-Na; peaks: (1) sodium and (2) naproxen.

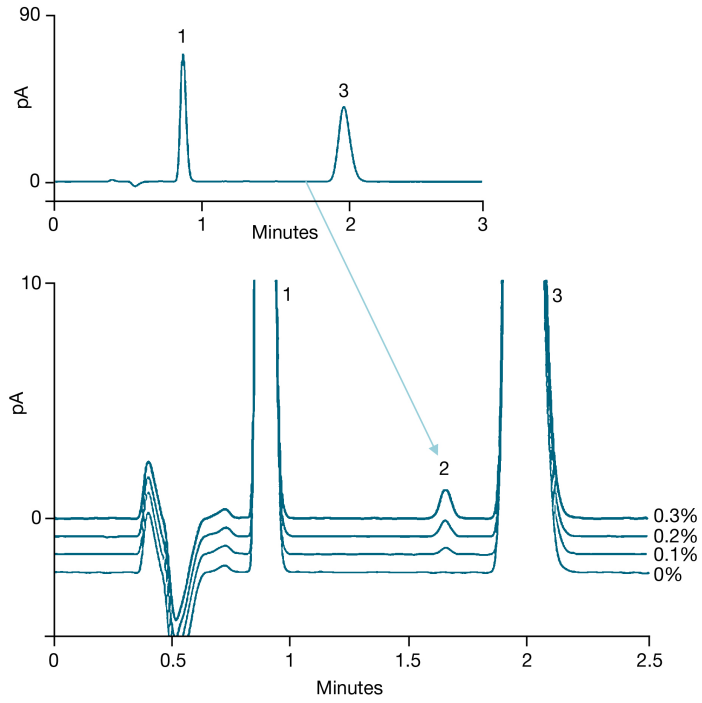


Figure 8.69 Simultaneous separation of diclofenac and its sodium counter ion by mixed-mode liquid chromatography. Separator column: Acclaim Trinity P1, 3 µm; column dimensions: 50 mm × 3 mm i.d.; column temperature: 30 °C; eluent: 75:25 (*v/v*) MeCN/200 mmol/L NH_4OAc, pH 4; flow rate: 0.8 mL/min; detection: CAD (gain: 100 pA, filter: med., neb. temp.: 60 °C); injection volume: 5 µL; sample: 1 mg/mL diclofenac-Na; peaks: (1) sodium, (2) chloride, and (3) diclofenac.

8.7
Chemical and petrochemical applications

8.7.1
Chemical applications

A real challenge for ion chromatography is the determination of ionic impurities such as bromide, iodide, sulfate, magnesium, calcium, and manganese in brine. Sodium chloride brines are used in chloralkali cells to produce chlorine and sodium hydroxide by means of electrolysis. As illustrated in Figure 8.70, saturated brine is passed into the first chamber of the cell where chloride ions are oxidized at the anode to produce chlorine gas:

$$2Cl^- \rightarrow Cl_2 + 2e^- \qquad (8.2)$$

At the cathode in the second chamber of the cell, water molecules are split to release hydroxide ions and hydrogen gas into the solution:

$$2H_2O + 2e^- \rightarrow H_2 + 2OH^- \qquad (8.3)$$

Both chambers in the cell are separated by a cation-exchange membrane that allows sodium ions to pass to the second chamber where they react with hydroxide ions to form sodium hydroxide.

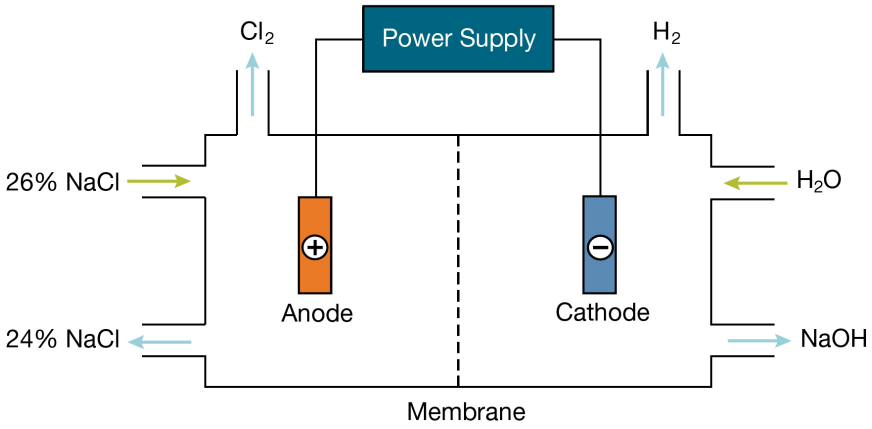


Figure 8.70 Schematic of a membrane cell used in the electrolysis of brine.

Thus, the overall reaction for the electrolysis of brine is:

$$2NaCl + 2H_2O \rightarrow Cl_2 + H_2 + 2NaOH \qquad (8.4)$$

Because of the corrosive nature of chlorine production, the anode is made from a nonreactive metal such as titanium, whereas the cathode can be made from a more easily oxidized metal such as nickel. The brine used in this process must be extremely pure because, for example, alkaline-earth metals reduce the lifetime of the membranes via interactions with iodide on the membrane surface.

The separation of nonpolarizable anions such as bromide and sulfate in brine is carried out on a conventional high-capacity anion exchanger. In the presence of extremely high chloride concentrations, the commonly employed conductivity detection is not suitable for determining bromide. In those cases, detection is performed via measuring the light absorption at 200 nm. For the sake of simplicity, both detection methods should be used in series. The determination of iodide in brine is also carried out by anion-exchange chromatography but coupled with pulsed amperometric detection [209]. Iodide is separated on a Dionex IonPac AS11 column with a dilute nitric acid eluent. Under those conditions, iodide can be eluted in less than 5 min and the detection limit for iodide using pulsed amperometric detection is in the low microgram/liter range.

Trace analysis of alkaline-earth metals such as magnesium and calcium in ultrapure brine is not possible without preconcentration because manufacturers of the membranes used for electrolytical processes specify the total concentration of alkaline-earth metals to be 50 µg/L at the most. According to the current ion chromatographic method [210], the alkaline-earth metals present in brine are selectively enriched on a Thermo Scientific™ Dionex™ MetPac™ column and subsequently separated on a weak acid cation exchanger. The required valve switching for this type of matrix elimination was discussed in Section 8.3 and exemplified by the analysis of anions in organic solvents. In contrast, the carrier liquid is not pure deionized water but dilute hydrochloric acid (c = 1 mmol/L). The hydrochloric acid concentration is dimensioned in such a way that after the successful preconcentration of the alkaline-earth metals, the high sodium excess is eluted off the column within 20 min without affecting the recovery of magnesium and calcium. To obtain optimal recovery rates for magnesium and calcium, sample pH must be adjusted to 11.8 because at this pH value the selectivity of the Dionex MetPac column for divalent cations is significantly higher than for monovalent cations. Separation of the preconcentrated alkaline-earth metals on the Dionex IonPac CS12A analytical column is carried out with 20 mmol/L methanesulfonic acid. The chromatogram of a 1:10 diluted ultrapure brine sample (30%) in Figure 8.71 shows that significant amounts of sodium are eluted from the

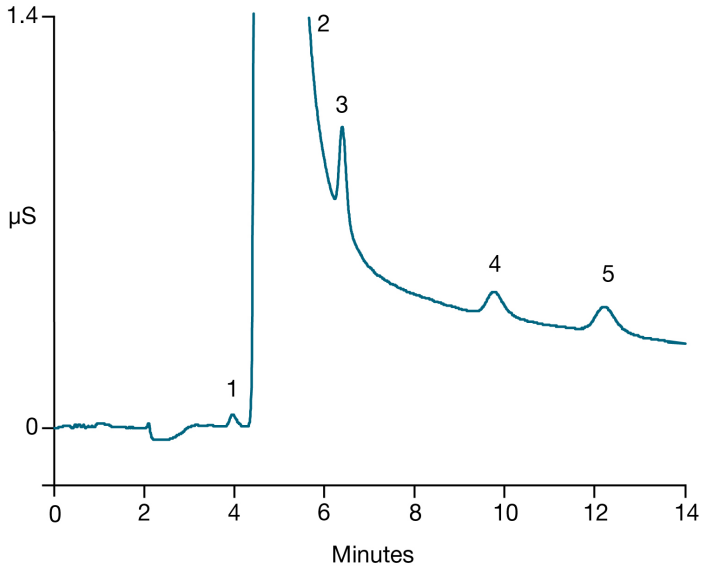
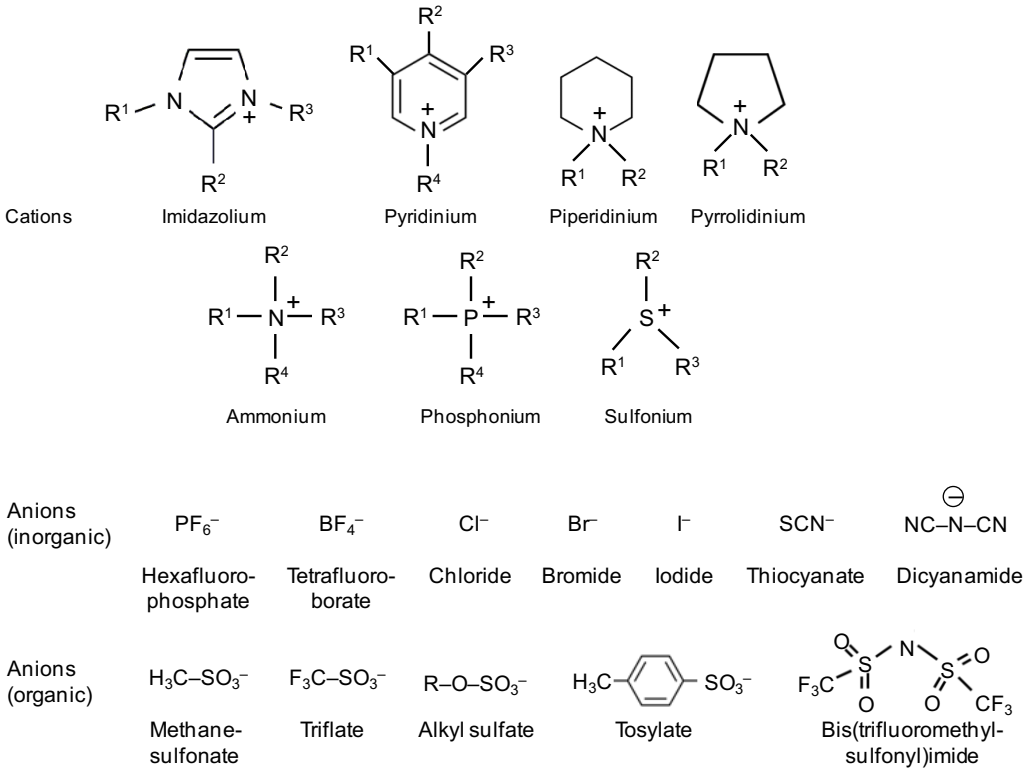


Figure 8.71 Separation of magnesium and calcium in ultrapure brine (30%). Separator column: Dionex IonPac CS12A; eluent: 20 mmol/L methanesulfonic acid; flow rate: 1 mL/min; concentrator column: Dionex MetPac CC-1; carrier and rinsing liquid: 1 mmol/L HCl; carrier flow rate: 2 mL/min; rinsing time: 20 min; detection: suppressed conductivity; injection volume: 100 µL; peaks: lithium (1), sodium (2), potassium (3), 25 µg/L magnesium (4), and 25 µg/L calcium (5).

analytical column despite the intensive rinsing process. However, this does not interfere with the evaluation of the two alkaline-earth metal peaks. The minimum detection limits for alkaline-earth metals in this matrix are on the order of 5 µg/L.

Reactive dyes, in which chloride and sulfate must be determined, also represent a difficult matrix for ion chromatography in the chemical industry. At first sight, this task does not seem to be that difficult. However, if aqueous solutions of such dyes are injected without any sample preparation, the peak areas or peak heights of chloride and sulfate decrease continuously with the number of injections. This phenomenon is not completely understood because reactive dyes are anionic compounds, too, and will eventually be eluted from the separator column. However, it might have something to do with their high affinity toward the stationary phase due to their aromatic character and the fact that they are blocking the ion-exchange functional groups. In any case, reproducible results are only obtained when the dyes are removed from the samples to be analyzed. Dionex OnGuard P solid-phase extraction cartridges are used for this purpose. They contain a polyvinylpyrrolidone resin on which interfering compounds are retained. Thus, the sample to be analyzed is injected through such a cartridge. It is important to flush these cartridges with deionized water before use to avoid chloride and sulfate contaminations. Furthermore, the sample should be pushed slowly through the cartridge to ensure complete extraction.

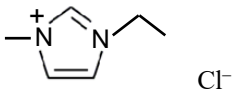
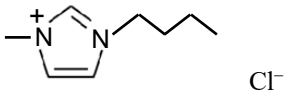
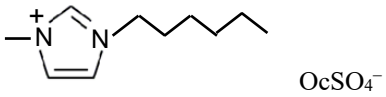
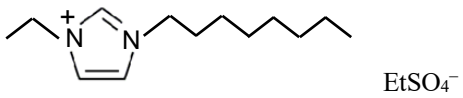
A relatively new and topical application area for ion chromatography in the chemical industry is the separation of complex electrolyte systems such as ionic liquids (ILs). The interest in their synthesis, characterization, and technical applications of ionic liquids is rapidly growing, as they are a new generation of solvents that might eventually replace conventional organic solvents. The term *ionic liquid* refers to organic salts with a melting point below 100 °C that have gained attention in nearly all fields of chemistry [211]. Ionic liquids have a very low vapor pressure, conduct electric current, and have selective dissolving properties. Some ionic liquids are exceptionally stable toward electrochemical redox processes and have low flammability. Ionic liquids generally consist of bulky, nonsymmetrical organic cations such as imidazolium, pyridinium, piperidinium, pyrrolidinium, ammonium, phosphonium, or sulfonium and numerous inorganic or organic anions.



1-Alkyl-3-methylimidazolium, for instance, is a representative of the best investigated group of ILs that is based on the imidazolium cation. Variation of the cationic parent substance, variation of the alkyl group of the side chains, and variation of the anions give rise to a large number of ionic liquids that differ in viscosity, density, hydrophobicity, and dissolving power. Consequently, the development of new analytical methods for characterization and purity determination has been concentrated on this type of IL. In addition to reversed-phase liquid chromatography with UV detection, ion-exchange chromatography with suppressed conductivity detection is a suitable analytical method for separating complex electrolyte systems. Since adsorption processes play an important role in the separation of these complex electrolytes, the separation of ILs on ion exchangers requires eluent profiles that have to be modified regarding ionic strength and organic solvent composition. Column temperature also plays a vital role. König et al. [212, 213] investigated the retention behavior of imidazolium-based cations that differ in the length of the carbon side chain. The structural features of the ILs investigated are summarized in Table 8.15.

The imidazolium-based ILs listed in Table 8.15 can be separated on the solvent-compatible Dionex IonPac CS17 weak acid cation exchanger and detected by suppressed conductivity. In case significant amounts of an organic solvent are used as a mobile phase additive, chemical regeneration of the cation suppressor is recommended. König et al. investigated the effect of adding acetonitrile as an organic modifier, keeping column temperature constant at the optimum value of $T = 70$ °C. As can be seen from the corresponding chromatograms in Figure 8.72, the larger imidazolium homologs, $HMIM^+$ and $OEIM^+$, cannot be eluted in an acceptable amount of time with a purely aqueous MSA gradient from 10 to 100 mmol/L (Figure 8.72a). Combining the ionic strength gradient with a linear acetonitrile gradient with a final amount of 20% (*v/v*), $HMIM^+$ and $OEIM^+$ can be eluted within 30 min (Figure 8.72b). Increasing the final amount of acetonitrile to 50% (*v/v*), all components can be eluted within 20 min (Figure 8.72c). In addition to the significant shortening of the retention times, peak tailing is greatly reduced. The negative baseline drift prior to the elution of $HMIM^+$ and $OEIM^+$ results from the addition of the organic solvent which, in turn, changes the dielectricity constant of the mobile phase. Thus, background conductance decreases with increasing acetonitrile concentration in the mobile phase.

Table 8.15 Structural feature of imidazolium-based ILs investigated by König et al. [212].

Systematic name	Abbreviation	Chemical formula
1-Ethyl-3-methylimidazolium chloride	EMIM-Cl	
1-Butyl-3-methylimidazolium chloride	BMIM-Cl	
1-Hexyl-3-methylimidazolium octylsulfate	HMIM-$OcSO_4$	
1-Octyl-3-ethylimidazolium ethylsulfate	OEIM-$EtSO_4$	

In a separate publication, König et al. [214] applied ion chromatography to the analysis of anions in typical ionic liquids, which can be of inorganic or organic nature. Like their cationic counterparts, these anions are also polarizable, so that adsorption effects are to be expected. Therefore, the most suitable anion exchanger for this type of application is the hydroxide-selective IonPac AS20 column, which was specifically developed for the rapid elution of polarizable anions in diverse matrices. Figure 8.73a shows the separation of a complex mixture of ionic liquids and conventional salts, using a sodium hydroxide gradient and suppressed

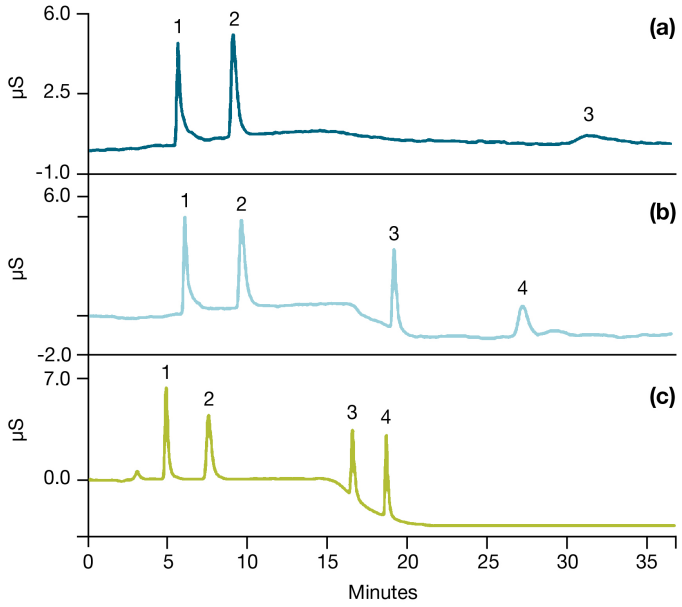


Figure 8.72 Separation of imidazolium-based ionic liquids by cation-exchange chromatography as a function of mobile-phase ionic strength and organic modifier content. Separator column: Dionex IonPac CS17; column temperature: 70 °C; eluent: MSA; gradient: 10–100 mmol/L; flow rate: 1 mL/min; detection: suppressed conductivity; peaks: (1) $EMIM^+$, (2) $BMIM^+$, (3) $HMIM^+$, and (4) $OEIM^+$ (reproduced with permission from Ref. [212]).

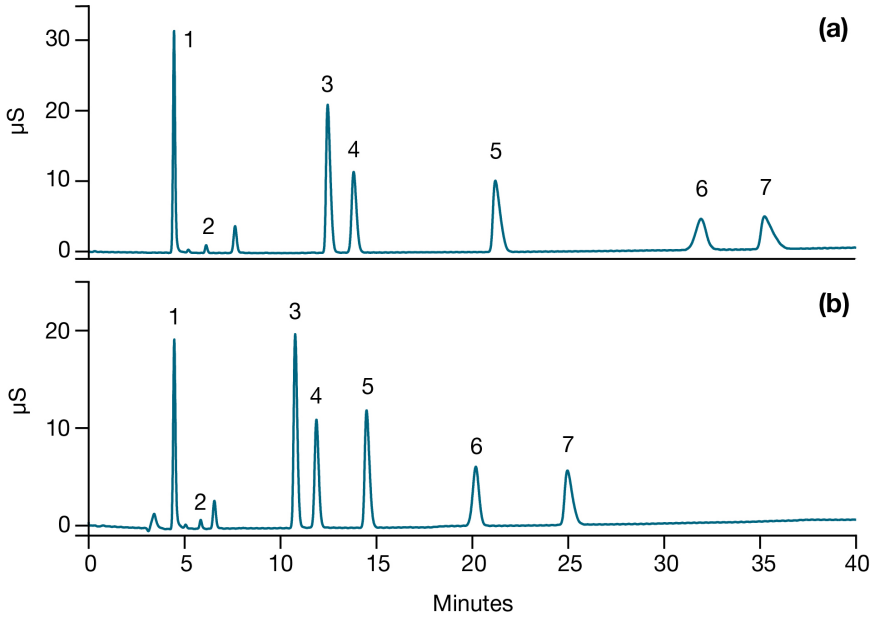


Figure 8.73 Separation of ionic liquids and conventional salts by anion-exchange chromatography as a function of mobile-phase ionic strength and organic modifier content. Separator column: Dionex IonPac AS20; column temperature: 40 °C; eluent: (a) 10–50 mmol/L NaOH, (b) 10–50 mmol/L NaOH/MeCN (80:20 *v/v*); flow rate: 1 mL/min; detection: suppressed conductivity; peaks: (1) fluoride, (2) chloride, (3) tetrafluoroborate, (4) triflate, (5) dicyanamide, (6) nonaflate, and (7) hexafluorophosphate (reproduced with permission from Ref. [214]).

conductivity detection. The concentration gradient from 10 to 50 mmol/L NaOH at a constant column temperature of $T = 40$ °C results in a baseline-resolved separation of all components under investigation within 40 min. The bottom chromatogram (Figure 8.73b) has been obtained after adding a constant amount of 20% (*v/v*) acetonitrile to the mobile phase, without changing the NaOH gradient. Comparing both chromatograms in Figure 8.73, it can be seen that the addition of an organic modifier affects the retention behavior of the analyte anions significantly. As expected, the shortening of the retention times is particularly drastic for the strongly retained nonaflate and hexafluorophosphate. Furthermore, the addition of acetonitrile as an organic modifier greatly improves peak geometry of the late-eluting species.

As an alternative to ion-exchange and reversed-phase chromatography, ionic liquids can also be separated by mixed-mode liquid chromatography. This analytical methodology is desired to meet different analytical goals. For example, the analysis of pharmaceutical formulations using ionic liquids as active ingredients and counter ions at high concentrations requires high throughput. This can be achieved with an LC–MS method that suits qualitative, confirmative, and semiquantitative applications. The LC–MS approach benefits from an Acclaim Trinity trimodal column, so that anions, cations, and neutral species can be separated in a single run. When using a trimodal column to retain and resolve ionic liquids, counter ions, and impurities, the chromatographic behavior is affected by the buffer concentration/ionic strength, the buffer pH, and the amount of organic solvent in the mobile phase as well as the column temperature. As shown in Figure 8.74, chromatography on an Acclaim Trinity P1 column was optimized to simultaneously separate imidazolium and lidocaine cations, inorganic anionic counter ions, and organic anions. The corresponding MS parameters are summarized in Table 8.16. When used in a confirmative analysis mode, this LC–MS method can detect submicrogram/liter levels of major ionic liquid analytes, submilligram/liter levels of halide impurities, and microgram/liter levels of cationic counter ions.

Ion chromatography has been successfully applied for the determination of inorganic anions and cations in liquid crystal materials [215]. Since the first application of liquid crystal materials

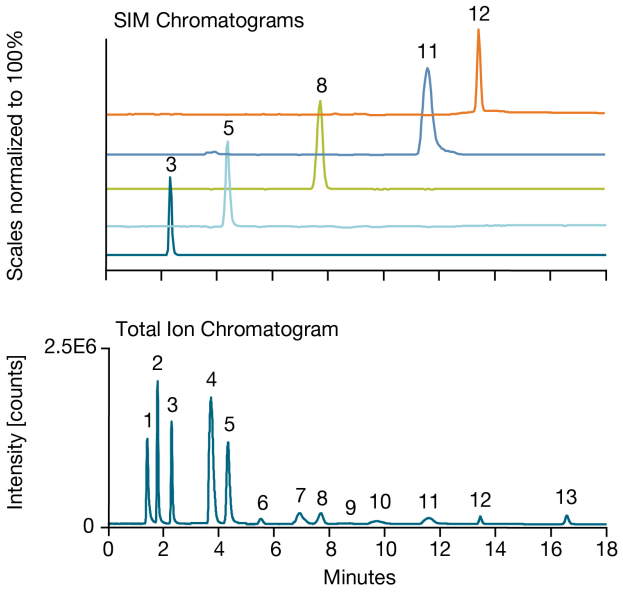


Figure 8.74 LC–MS for the simultaneous analysis of ionic liquids, counter ions, and impurities. Separator column: Acclaim Trinity P1, 3 µm; column dimensions: 100 mm × 2.1 mm i.d.; column temperature: 25 °C; eluent: (A) MeCN, (B) 100 mmol/L ammonium acetate, (C) water; gradient: 55% A, 5% B, 40% C for 2 min, then to 60% A, 5% B, 35% C in 8 min, then to 90% A, 5% B, 5% C in 1 min, isocratic for 7 min; flow rate: 0.4 mL/min; detection: MS, probe temperature: 500 °C, nebulizer gas: nitrogen at 85 psi, needle voltage: 1 kV; injection volume: 5 µL; peaks: (1) lidocaine, (2) $BMIM^+$, (3) $EMIM^+$, (4) sodium, (5) potassium, (6) methanesulfonate, (7) tetrafluoroborate, (8) hexafluorophosphate, (9) chloride, (10) bromide, (11) iodide, (12) tosylate, and (13) docusate.

Table 8.16 MS scan parameters for ionic liquids, counter ions, and impurities by LC–MS (see Figure 8.74).

Peak	Analyte	Retention time (min)]	SIM	Scan event	Polarity	Cone voltage (V)
1	Lidocaine	1.5	235	0-3	positive	55
2	$BMIM^+$	1.9	139	0-3	positive	55
3	$EMIM^+$	2.4	111	0-3	positive	55
4	Sodium[a)]	3.9	269	3.0-5.3	positive	50
5	Potassium	4.5	39	3.0-5.3	positive	100
6	Methanesulfonate	5.7	95	3.0-5.3	negative	95
7	Tetrafluoroborate	7.0	87	6.2-13.0	negative	95
8	Hexafluorophosphate	7.9	145	6.2-13.0	negative	80
9	Chloride	9.0	35	6.2-13.0	negative	90
10	Bromide	10.0	81	6.2-13.0	negative	90
11	Iodide	11.8	127	6.2-13.0	negative	90
12	Tosylate	13.6	171	6.2-13.0	negative	70
13	Docusate	16.9	421	15.0-18.0	negative	80

a) as $[Na + 6MeCN]^+$

in the early seventies, the commercial interest in this field of science has been steadily increasing. Nowadays, they play an important role in the electronics industry, such as in the production of liquid crystal displays. The electroconductibility of the liquid crystal material as determined by its structure and alignment is one of the most important properties. However, it is affected by inorganic anions and cations that are inevitably introduced in the material as impurities during synthesis. To guarantee the quality of the material, it is necessary to monitor the content of these ionic impurities. Ion chromatography is the most effective technique to determine both anions and cations due to its high sensitivity and ease-of-use. However, the presence of inorganic ions in an organic matrix requires sample pretreatment. Typical methods used to decompose organic matrices are dry ashing, combustion in an oxygen flask, or Kjedahl digestion, which are all time-consuming and prone to interferences. As an alternative method, oxidative UV photolysis has proven to be an attractive pretreatment method to decompose organic matter [216]. The grey chromatogram of inorganic anions in Figure 8.75 was obtained by Liu and Mou [215] on a Dionex IonPac AS16 column after irradiating a liquid crystal material dissolved in acetonitrile with a 300 W medium pressure mercury lamp for 2 h. As can be seen from this chromatogram, fluoride is interfered by a huge matrix peak when utilizing this kind of sample pretreatment. However, it can be concluded that the bonds between the halogens and the organic matrix, decomposed by the UV radiation as free halide ions, could not be detected in the sample before irradiation. Much larger signals for fluoride, chloride, and bromide can be achieved when adding hydrogen peroxide to the sample before irradiation. Hydrogen peroxide is a strong oxidizing agent that completely decomposes the organic matrix. Illustrated by the black chromatogram in Figure 8.75, the big matrix peak became much smaller and the signals for fluoride, chloride, and bromide were 6.6, 1.3, and 1.2-times higher than those in the sample treated without hydrogen peroxide, respectively. Even small amounts of iodide were found in the solution. This increase in response can be attributed to the formation of hydroxyl radicals that accelerate the decomposition of the organic matrix. Liu and Mou [215] used a similar procedure to determine inorganic cations in the liquid crystal material. Although free cations could be detected in the sample, a 2 h irradiation greatly increased the peaks for sodium, ammonium, potassium, magnesium, and calcium. In contrast to anion analysis, the addition of hydrogen peroxide does not have any significant effect on the size of the cation peaks.

Ion chromatography has successfully been applied for the analysis of battery electrolytes. Batteries are classified into primary and secondary forms. Primary batteries, or primary cells, irreversibly transform chemical energy into electrical energy. They are most commonly used in portable devices that have low current drain. Primary cells cannot be recharged because the chemical reactions are not easily reversible and active materials may not return to their original forms. Secondary batteries can be discharged and recharged multiple times. Examples are the lead–acid batteries used in vehicles and lithium ion batteries used for portable electronics. The most important and the oldest form of a rechargeable battery is the lead–acid battery, which is widely used because of its low initial cost and reliability. Stringent specifications are laid down for the quality of sulfuric acid used as battery electrolyte. However, the influence of impurities on battery aging is not well investigated. The transport of impurities seems to be a critical factor limiting cell performance.

Although ion chromatography seems to be the most effective analytical technique to determine anionic and cationic impurities in battery acid (usually 38% sulfuric acid), very few papers describe the application of IC for the analysis of ionic impurities in mineral acids. Buldini et al. developed a simple and accurate procedure for the determination of fluoride, chloride, bromide, nitrate, and orthophosphate in battery acid using anion-exchange chromatography on IonPac AS9-SC with suppressed conductivity detection [216]. However, the very acidic pH of the matrix has a very strong influence on the separation of anions. A

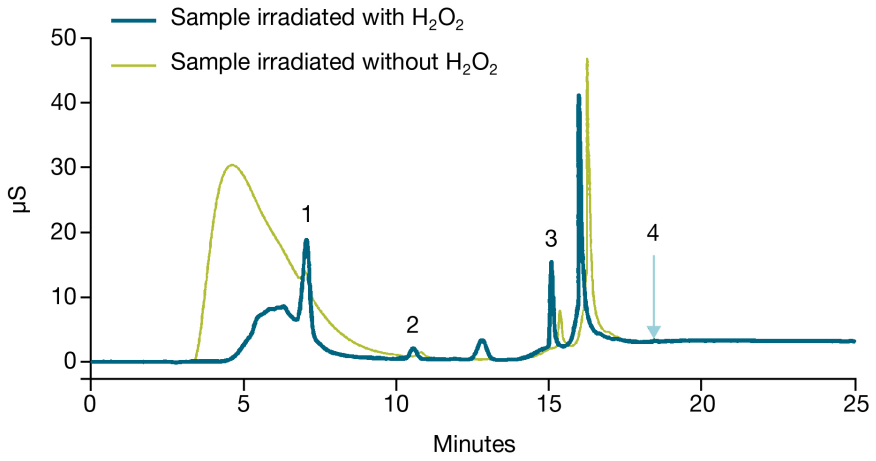


Figure 8.75 Determination of fluoride, chloride, and bromide in a liquid crystal material after oxidative UV photolysis. Separator column: Dionex IonPac AS16; column temperature: 25 °C; eluent: NaOH, linear gradient from 5 to 100 mmol/L; flow rate: 1 mL/min; detection: suppressed conductivity; injection volume: 150 µL; sample: 50 mg of liquid crystal material dissolved in 4 mL of MeCN, then diluted with water to 10 mL, injection through Dionex OnGuard-P and OnGuard-RP; peaks: (1) fluoride, (2) chloride, (3) bromide, and (4) iodide (reproduced with permission from Ref. [215]. Copyright 2003, Elsevier Science B.V.).

simple dilution of the sample with deionized water is not sufficient because fluoride and chloride determinations are strongly affected by the water dip. In addition, a spurious peak is observed behind the chloride peak, preventing bromide quantification. The best conditions for quantification of all anionic impurities are established when adjusting sample pH to between 3 and 6 by dilution with concentrated eluent. Buldini et al. also determined sodium, potassium, magnesium, and calcium in the battery electrolyte [216]. Initial experiments with a Dionex IonPac CS12A weak acid cation exchanger failed because the dissociation of the carboxyl functional groups strongly depends on sample pH. Even after sample dilution, the acid concentration in the sample is too high, so that this separator column is unsuitable for this application. Sulfonated cation exchangers such as the nanobead-agglomerated Dionex IonPac CS10 column, however, can tolerate an acid concentration of 125 mmol/L in the sample, which corresponds to an 80-fold dilution of the usual lead–acid battery electrolyte. Because significant amounts of sodium and potassium are common impurities in all the materials involved in cell assembly, the reduction in sensitivity caused by the dilution factor is acceptable. To elute mono- and divalent cations from this column a combination of HCl and 2.3-diaminopropionic acid (DAP) must be used as eluent, which requires chemical regeneration of the membrane suppressor.

Lithium ion batteries are the most popular rechargeable batteries and are predominantly used for portable electronics. They have the highest energy density of all secondary batteries, do not show a memory effect, and slowly lose charge when not in use. Generally, the negative electrode in a lithium ion battery is made of graphite; the positive electrode is typically a layered oxide such as lithium cobalt oxide. Both electrodes allow lithium ions to move in and out of their interiors. The electrolyte solution consists of a lithium salt in an organic solvent. Commonly used salts include lithium hexafluorophosphate, lithium perchlorate, lithium tetrafluoroborate, lithium hexafluoroarsenate, lithium hexafluorosilicate, and lithium tetraphenylborate. The most important organic solvents used in the electrolyte solution are ethylene carbonate, diethyl carbonate, dimethyl carbonate, methyl ethyl carbonate, and propylene carbonate. When a lithium ion battery is charged, the positive lithium ions move from the positive electrode to the negative one. The process to insert the lithium ions into the graphite electrode is called intercalation. When the cell is discharging, the reverse occurs.

The concentration of the electrolytes in the solvent ranges from 0.1 to 2 mol/L, with an optimal range of 0.8–1.2 mol/L. The anions of the added lithium salts can be determined by ion chromatography. Phesatcha et al. separated these anions in the same way as already outlined in this section for the analysis of ionic liquids [217]. Perchlorate, tetrafluoroborate, and hexafluorophosphate are polarizable anions that are strongly retained on conventional anion exchangers, exhibiting poor peak shapes. The Dionex IonPac AS20 stationary phase is the most suitable for a rapid elution of these anions with high chromatographic efficiencies. Its high anion-exchange capacity is another benefit when analyzing battery electrolytes in which these anions are present at high concentration. Phesatcha et al. prepared a simulated lithium ion battery sample to demonstrate that the anionic content can be accurately determined using reagent-free IC. Lithium tetrafluoroborate and lithium perchlorate were prepared as 1 mol/L solutions in a mixture of ethylene carbonate, diethyl carbonate, and propylene carbonate (1:1:1 *v/v/v*), and lithium hexafluorophosphate was prepared as a 1 mol/L solution in deionized water. Figure 8.76 shows the chromatograms of each of the three samples after dilution with deionized water. All three anions are well separated within 25 min. The first 12 min of the separation were included to allow common inorganic anions to elute so that they do not interfere with the determination of the analytes. Under these chromatographic conditions, tetrafluoroborate elutes between sulfate and orthophosphate, while perchlorate and hexafluorophosphate elute after orthophosphate.

8.7.2 Petrochemical applications

The exploration and production of petroleum products such as crude oil and natural gas involves numerous applications of ion chromatography in which inorganic and low-molecular weight

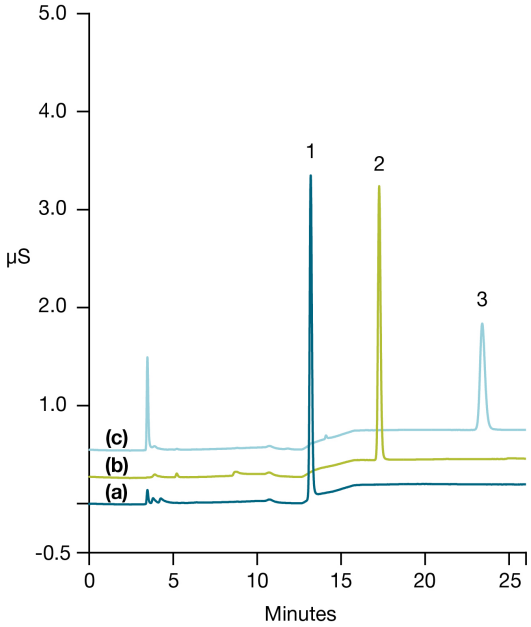


Figure 8.76 Determination of a lithium ion battery electrolyte. Separator column: Dionex IonPac AS20; column dimensions: 250 mm × 4 mm i.d.; column temperature: 35 °C; eluent: KOH (EG); gradient: 15 mmol/L from 0 to 10 min, 15–80 mmol/L from 10 to 13 min, isocratically for 13 min; flow rate: 1.2 mL/min; detection: suppressed conductivity; injection volume: 10 µL; sample: simulated lithium ion battery electrolyte diluted 1:10,000 with deionized water; peaks: (a) 9.5 mg/L tetrafluoroborate (1), (b) 9.7 mg/L perchlorate (2), and (c) 15.1 mg/L hexafluorophosphate (3).

organic ions are determined in fuels, lubricating oils, gas washing solutions, and the so-called formation water that is a by-product of crude oil drilling.

Natural gas often contains high concentrations of H_2S and/or CO_2, which are called sour gases in petrochemistry. Because they are very corrosive, sour gases must be removed from the natural gas before it is pumped into the pipelines. This is usually performed via a gas wash with an amine-containing solution. In most refineries, diethanolamine (DEA), monoethanol-amine (MEA), or methyldiethanolamine (MDEA) is used for the absorption of sour gases. Those ethanolamines are regenerated in a continuous process by the stripping of H_2S and CO_2 in the course of a heat treatment. In a refinery, ethanolamines are cost-intensive chemicals, so that losses due to leakages or degradation reactions increase operating expenses. In case of leakages, the amine used for the gas wash gets into the wastewater. An excellent separation of the various alkanolamines is obtained on the nanobead-agglomerated Dionex IonPac CS11 column with a methanesulfonic acid eluent under isocratic conditions utilizing suppressed conductivity detection [218]. Figure 8.77 shows the separation of inorganic cations and various ethanolamines in refinery wastewater. However, alkaline-earth metals are very strongly retained on this sulfonated cation exchanger. Because alkaline-earth metals are present in most wastewaters, their late elution can cause broad peaks in subsequent analytical runs. To avoid these problems, a column purge step with a much higher acid concentration has to be applied following the elution of *N*-methyldiethanolamine. The only disadvantage is the significantly longer analysis time of approximately 50 min per run. Much shorter analysis times can be achieved with modern weak acid cation exchangers such as the Dionex IonPac CS18 column, eluting mono- and divalent cations in the same chromatographic run.

Due to contamination and ethanolamine degradation, various anions are permanently enriched in the ethanolamine solution, thereby forming heat-stable salts (HSS) that cannot be regenerated. Anions observed in HSS originate from a variety of sources and are listed in Table 8.17. If the concentration of these salts exceeds 500 mg/L, a number of problems are observed with the gas wash, such as a decrease in absorption capacity of the ethanolamine for acid gases and an increase of corrosion. Thus, the content of iron sulfide increases which, in turn, leads to foam formation. To diagnose these problems, the amine solution is analyzed at different sites. Kadnar and Rieder [219] used different IC methods for analyzing carboxylic acids and other more strongly retained inorganic anions and oxalate, respectively. They achieved excellent resolution between fluoride and short-chain fatty acids on a Dionex IonPac AS10 column with a tetraborate eluent. However, the presence of other anions in the amine solution that cannot be eluted with this eluent requires a purge step after 20 min with 70 mmol/L $Na_2B_4O_7$ for 5 min.

The determination of nitrate, orthophosphate, sulfate, oxalate, and thiosulfate used to be performed on a Dionex IonPac AS9-SC column using a carbonate/bicarbonate eluent and suppressed conductivity detection. Weak points of this method are the rapid loss of capacity and the pH limitation of this column (application in the pH range 2–11 only). Using two different methods for short-chain fatty acids and other inorganic and organic anions is a disadvantage of the isocratic procedures. Nowadays, this problem can be solved by applying a gradient technique on a Dionex IonPac AS11 column (see Figure 8.78), with which all anions of interest can be separated in a single run in less than 20 min.

As an alternative to the Dionex IonPac AS11 column, polarizable anions can also be separated very effectively on a Dionex IonPac AS16 column, which was the first commercial anion exchanger designed for this application. Due to the electrostatic agglomeration of very hydrophilic quaternary ammonium groups on the substrate, polarizable anions can be eluted with high chromatographic efficiency and good peak shape, without the addition of organic solvents. Because the Dionex IonPac AS16 column is also hydroxide-selective, electrolytic eluent generation is recommended for producing high-purity carbonate-free eluents necessary for reproducible gradients. Figure 8.79 shows the separation of thiosulfate and other inorganic

anions in a refinery wastewater, using a multistep hydroxide gradient that starts at a low KOH concentration to enable the separation of fluoride from short-chain fatty acids [220]. For routine analysis of thiosulfate alone in such samples, gradient conditions can be adapted for shorter analysis times.

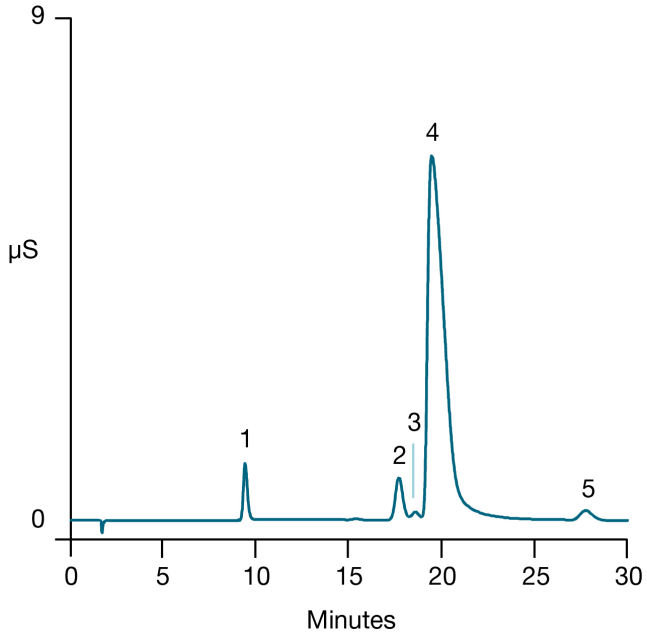


Figure 8.77 Separation of inorganic cations and alkanolamines in a refinery wastewater. Separator column: Dionex IonPac CS11; column dimensions: 250 mm × 2 mm i.d.; eluent: 35 mmol/L MSA; flow rate: 0.25 mL/min; detection: suppressed conductivity; injection volume: 2.5 µL; sample: refinery wastewater diluted 1:1000 with deionized water; peaks: 0.95 mg/L sodium (1), 2.3 mg/L mono-ethanolamine (2), 0.94 mg/L potassium (3), 290 mg/L diethanolamine (4), and 9.1 mg/L *N*-methyldiethanolamine (5).

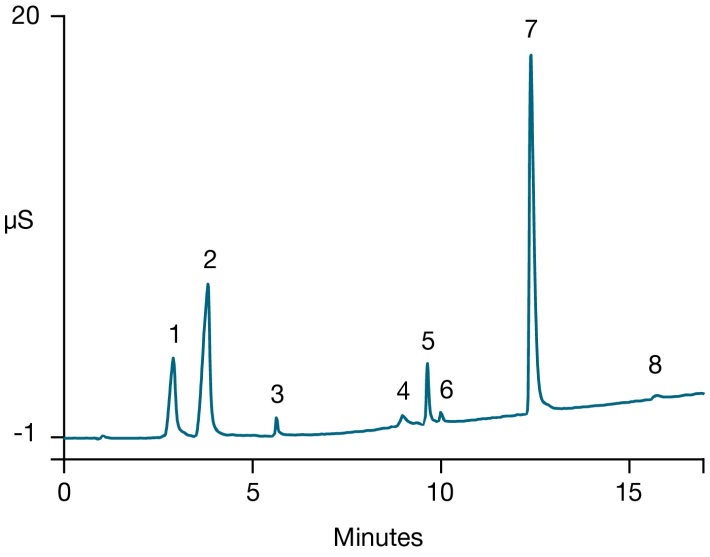


Figure 8.78 Separation of heat-stable salts in a "semi-lean" *N*-methyldiethanolamine solution applying a gradient elution technique. Separator column: Dionex IonPac AS11; eluent: NaOH gradient; flow rate: 2 mL/min; detection: suppressed conductivity; injection volume: 25 µL; peaks: 50 mg/L acetate (1), 55 mg/L formate (2), 6 mg/L chloride (3), carbonate (4), 12 mg/L sulfate (5), 4 mg/L oxalate (6), 110 mg/L thiosulfate (7), and 0.6 mg/L thiocyanate (8).

Table 8.17. Common sources of anions of heat-stable salts (HSS).

Anion	Source
Chloride	Makeup water Brine with inlet gas Well treatment chemicals
Nitrate, nitrite	Makeup water Corrosion inhibitors
Sulfate, sulfite, thiosulfate	Sulfur species oxidation products Component in gas
Formate, oxalate, acetate	Acid in feed gas Oxygen degradation Thermal degradation
Thiocyanate	Reaction product of H_2S and cyanide
Orthophosphate	Corrosion inhibitors Phosphoric acid activated carbon Cotton filters
Fluoride	Well treatment chemicals with inlet gas

Oil and gas reservoirs are often associated with so-called formation water that differs in composition depending on the geological formation. The major component is chloride at concentrations between 10 and 70 g/L. In comparison to seawater, formation waters exhibit higher contents of bromide, iodide, and nitrate as well as lower contents of sulfate and magnesium. Formation waters are characterized by the contents of bromide, sulfate, and iodide in addition to chloride; in some cases, nitrate, orthophosphate, fluoride, thiocyanate, and

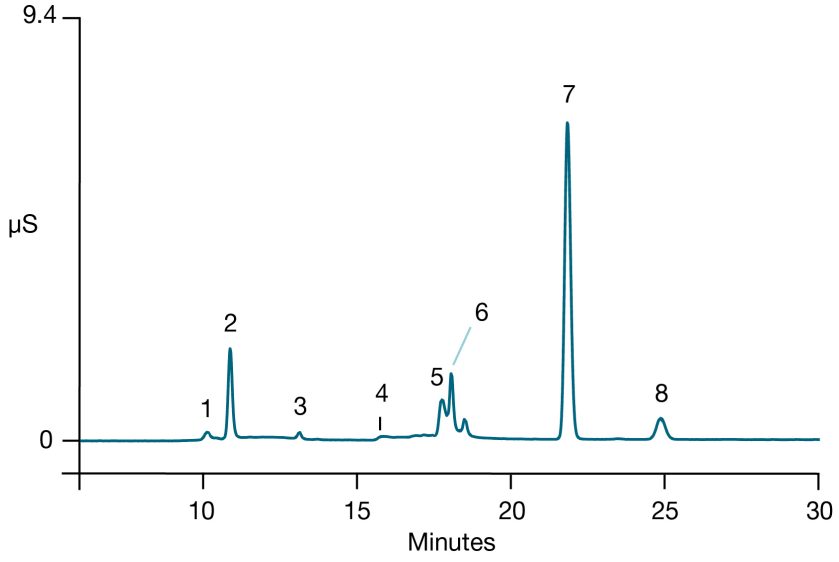


Figure 8.79 Separation of thiosulfate in a refinery wastewater. Separator column: Dionex IonPac AS16; column temperature: 30 °C; eluent: KOH (EG); gradient: 1.5 mmol/L from 0 to 8.2 min, then 1.5–10 mmol/L from 8.2 to 14.2 min, then 10–25 mmol/L from 14.2 to 40 min; flow rate: 1 mL/min; detection: suppressed conductivity; injection volume: 100 µL; sample: refinery wastewater 1:5 diluted with deionized water; peaks: 0.09 mg/L fluoride (1), 33 mg/L propionate (2), 0.5 mg/L bromate (3), 0.2 mg/L nitrate (4), carbonate (5), 1.1 mg/L sulfate (6), 34.8 mg/L thiosulfate (7), and 2.7 mg/L orthophosphate (8).

organic acids are also of interest. Application areas in the field of anion analysis include the analysis of water that was formed together with oil and gas, the composition of which allows conclusions about the original formation water. In addition, tracer studies are carried out, with which the influence of injection waters and drilling mud on the composition of the formation waters can be investigated. The high chloride excess in these samples is no longer an insuperable problem for modern ion chromatography. The most modern approach for analyzing inorganic anions in formation water uses a high-capacity hydroxide-selective anion exchanger such as the Dionex IonPac AS11-HC column in gradient mode (see Figure 8.80). Due to the focusing effect the late-eluting iodide can be detected with very high sensitivity.

In addition to the anions mentioned above, formation waters contain alkali and alkaline-earth metals, of which sodium is in high excess. With the introduction of weak acid cation exchangers for simultaneous analysis of mono- and divalent cations, ion chromatography became a welcome alternative to conventional methods such as flame AAS and complexometric titrations. However, the sodium content in formation waters can easily reach 50 g/L. If the samples are strongly diluted to avoid column overloading, the ammonium content is usually less than 1 mg/L and thus cannot be separated on low-capacity cation exchangers. Ammonium determination in the presence of a high sodium excess can be performed only with a high-capacity cation exchanger such as the Dionex IonPac CS16 column. A corresponding example of such a separation is shown in Figure 8.81. Although the total analysis time is much longer in this approach, all cations of interest can be analyzed in a single run.

Toxic anions such as sulfide and cyanide are of high environmental relevance for the petrochemical industry. Process liquors from catalytic cracking plants, for instance, sometimes contain very high concentrations of sulfide and cyanide. For ion chromatographic determination, the sample to be analyzed must be digested with boiling sulfuric acid to release cyanide from metal complexes. The resulting gases, H_2S and HCN, are trapped in an alkaline

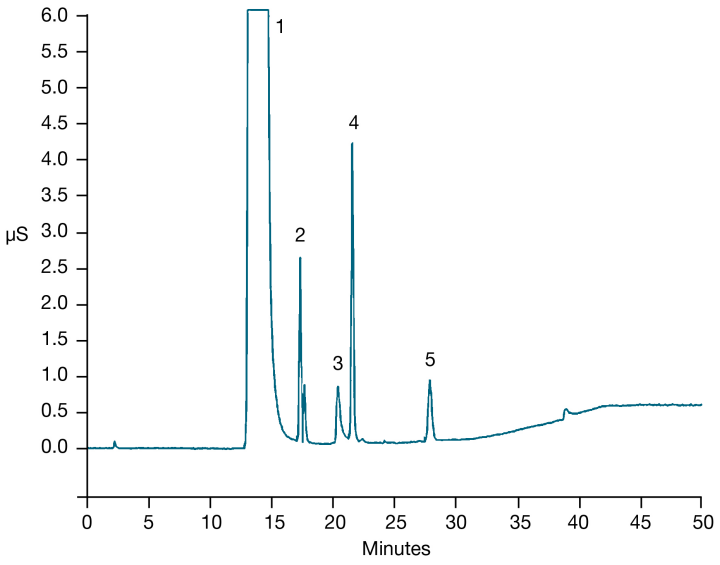


Figure 8.80 Anion analysis of a water sample from a gas well under gradient conditions. Separator column: Dionex IonPac AS11-HC; column dimensions: 250 mm × 2 mm i.d.; eluent: KOH (EG); flow rate: 0.25 mL/min; detection: suppressed conductivity; injection volume: 25 µL; sample: formation water diluted 1:25 with deionized water; peaks: (1) chloride, (2) bromide, (3) carbonate, (4) sulfate, and (5) iodide.

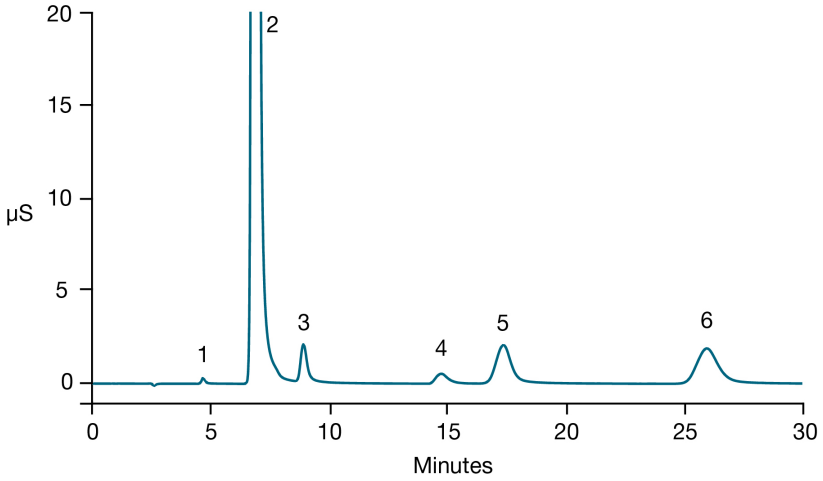


Figure 8.81 Analysis of alkali and alkaline-earth metals in a formation water on a high-capacity cation exchanger. Separator column: Dionex IonPac CS16; column dimensions: 250 mm × 5 mm i.d.; column temperature: 45 °C; eluent: 27 mmol/L methanesulfonic acid; flow rate: 1 mL/min; detection: suppressed conductivity; injection volume: 25 µL; sample: diluted formation water; peaks: (1) lithium, (2) sodium, (3) ammonium, (4) potassium, (5) magnesium, and (6) calcium.

absorber solution ($c = 0.5$ mol/L NaOH), which can be directly injected onto a Dionex CarboPac PA1 column (see Figure 7.10 in Section 7.1.2). Detection is carried out via DC amperometry on a silver working electrode.

Polyphosphonates are widely used as scale inhibitors in gas and oil wells that coproduce large amounts of brines. It has been shown that polyphosphonate concentrations as low as 0.2 mg/L can effectively prevent scale. In the application of these chemical inhibitors in field squeeze treatments, a decision to retreat a well is often effected when the inhibitor concentration drops below a predetermined threshold level. Therefore, a reliable and sensitive method for polyphosphonate determination in well water returns is required, which could lead to an extended squeeze lifetime with significant cost savings. The analysis of low concentrations of polyphosphonates in oilfield brines represents a difficult analytical problem and places limitations on current methods. In the classical ion chromatographic approach, polyphosphonates are separated on a high-capacity anion-exchange column using a nitric acid eluent. Due to partial protonation of the analytes in a nitric acid environment, the effective charge of the analytes is reduced, and their elution is possible. Because conductivity detection is not possible owing to the high acid concentration in the mobile phase, polyphosphonates are typically detected via postcolumn derivatization with ferric nitrate in acidic solution, followed by UV detection at wavelengths between 310 and 330 nm. However, this method suffers from chloride interference to the extent that when a brine sample is injected onto the column, the high chloride peak interferes with the polyphosphonate peaks. The combination of gradient anion-exchange chromatography using a Dionex IonPac AS11-HC column and phosphorus-specific detection effectively solves this problem. Equilibrating this column with 2 mmol/L nitric acid allows the polyphosphonates to concentrate on the column, even in the presence of high chloride concentrations. The nitric acid concentration is then increased to 200 mmol/L in 21 min, eluting the polyphosphonates from the column in order of increasing valency. Hydrolysis of the polyphosphonates to orthophosphate as the first postcolumn reaction is carried out with 4% ammonium peroxodisulfate at 100 °C. It takes about 2 min to ensure a complete reaction, so that a large reaction coil must be used to create such a large residence time.

The final molybdate reagent containing

- 10 mL of a 15% sodium dodecyl sulfate solution +
- 4.84 g sodium molybdate +
- 12.5 mL sulfuric acid +
- 5.4 g ascorbic acid in 1 L

can be added pneumatically. It forms the phosphomolybdenum blue complex with the released orthophosphate, which is measured at 800 nm. To ensure that inhibitors all concentrate well, the samples are diluted 1:10 with deionized water, and 5 mL of this diluted solution is then loaded onto a concentrator column, replacing the injection loop. As shown in Figure 8.82, diethylene-triamine-penta(methylenephosphonic acid) (DETMP) can be successfully pre-concentrated directly on-column, even in the presence of 20,000 mg/L NaCl. The five phosphonate groups of DETMP are the reason for its high affinity to the stationary phase of this separator column. The relative standard deviation for this analysis is below 3%. The detection limits for the inhibitor products tested at a level of 11,000 mg/L NaCl are 0.5 mg/L.

Combustion ion chromatography (CIC) Combustion IC [221] is predominantly used for the simultaneous determination of the various halogens and sulfur in all types of combustible samples. Especially petrochemicals are difficult to analyze with conventional ion chromatography. Thus, sample preparation is required to extract analytes or to eliminate interfering compounds, which is costly and time-consuming. Automated combustion IC reduces the time and labor for determining halogens and sulfur in complex matrices. Samples are pyrolyzed in an oxidative atmosphere, the resultant vapors are absorbed in an aqueous solution that is then introduced directly into the ion chromatograph for analysis:

Sample	Combustion tube	Absorption solution	Analysis
Organic sulfur compounds	SO_2, SO_3	SO_4^{2-}	IC
Organic halogen compounds	HX, X_2	X^-	IC

The schematic of a combustion IC system with the respective process flowchart is shown in Figure 8.83. Pyrolysis occurs in an Ar/O_2 atmosphere in the furnace at temperatures above 900 °C. Orthophosphate (2 mg/L P as PO_4^{3-}) may be added to the absorption solution as an internal standard to calibrate the system. The absorption solution (deionized water) also contains 10 mg/L hydrogen peroxide to oxidize SO_2, which results from the incomplete oxidation of S to SO_3. The entire system is controlled by the software of the combustion unit, which allows the pyrolysis of the next sample while the chromatogram of the current sample is being recorded.

Sulfur in petroleum products causes corrosion in pipelines, poisons catalysts, and affects stability during storage. Sulfur in fossil fuels is present in both inorganic and organic form. While inorganic sulfur is detrimental for the engine performance in terms of precipitating salts, organosulfur compounds are combusted to SO_2, which has a severe environmental impact. Whereas the water-soluble inorganic sulfate can be determined by conventional IC after extraction or in-line dialysis, the total sulfur content can be determined by IC. Chlorine also corrodes the equipment, clogs pipelines, and increases catalyst consumption during manufacturing. The anions in the absorption solution can be analyzed conventionally using either a carbonate/bicarbonate or a hydroxide eluent. Both approaches are carried out under isocratic conditions as the chromatograms of the combusted samples only contain a limited

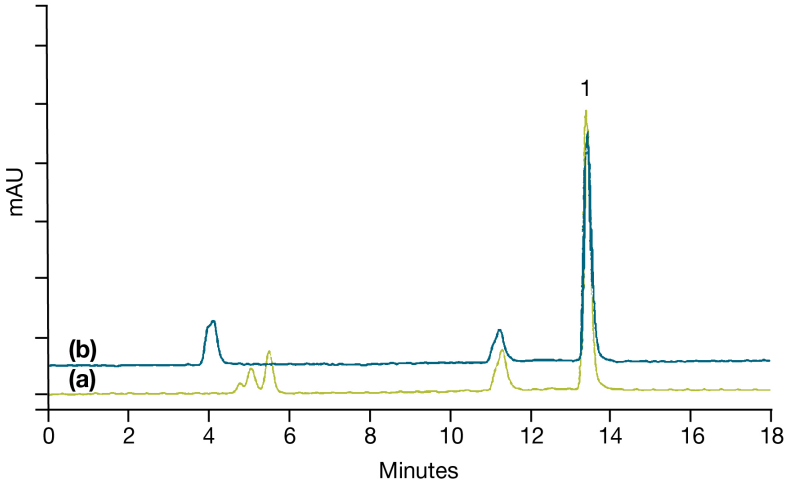


Figure 8.82 Analysis of diethylenetriamine-penta(methylenephosphonic acid) (DETMP) in formation water with on-column preconcentration on a high-capacity anion exchanger. Separator column: Dionex IonPac AS11-HC with NG1 guard column; eluent: nitric acid; gradient: linear, 2–200 mmol/L in 21 min; flow rate: 1 mL/min; detection: phosphorus-specific detection (see text); injection volume: 200 µL; sample: (a) 20 mg/L DETMP in deionized water, (b) 20 mg/L DETMP in formation water with 20 000 mg/L NaCl; peak: (1) DETMP.

number of peaks. Figure 8.84 illustrates combustion IC of a liquefied petroleum gas (LPG) sample on a Dionex IonPac AS11-HC column with a hydroxide eluent. If necessary, sample enrichment is achieved by either trapping analytes from multiple combustion cycles in the same absorption solution or by preconcentrating the volume of a 1 mL sample loop in the combustion unit onto a Dionex IonPac UTAC-ALP1 concentrator column installed in the ion chromatograph. In both cases, sulfur and totally speciated halogens can be determined by CIC down to 0.5 mg/kg.

Biofuel applications Biofuels are liquid fuels that are derived from organic matter, either directly from plants, or indirectly from agricultural, domestic, or industrial wastes. They are made by conversion of biomass to convenient energy containing substances in three different ways: thermal conversion, chemical conversion, and biochemical conversion.

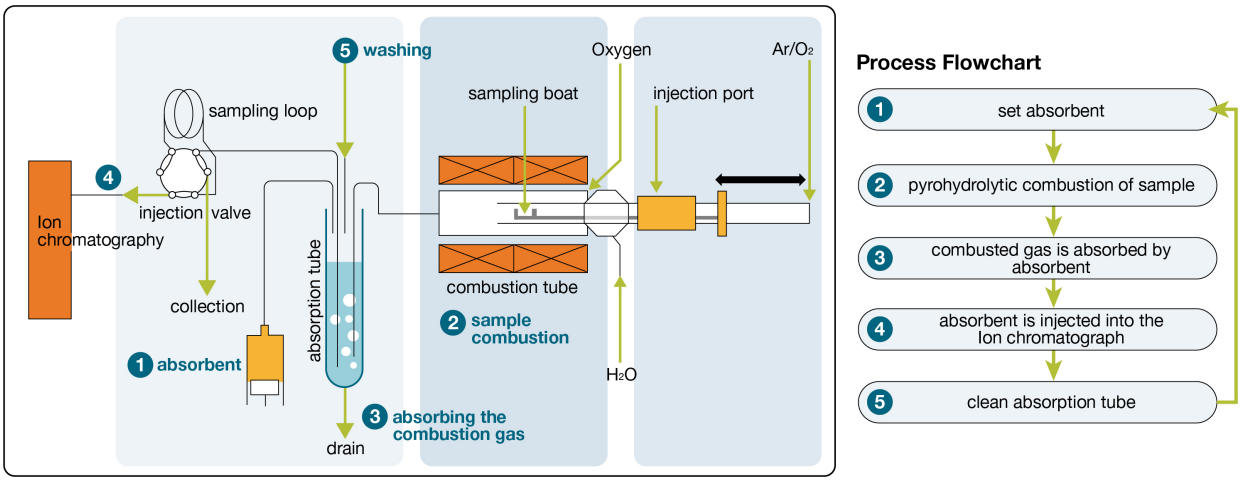


Figure 8.83 Schematic and process flow chart of a combustion IC unit.

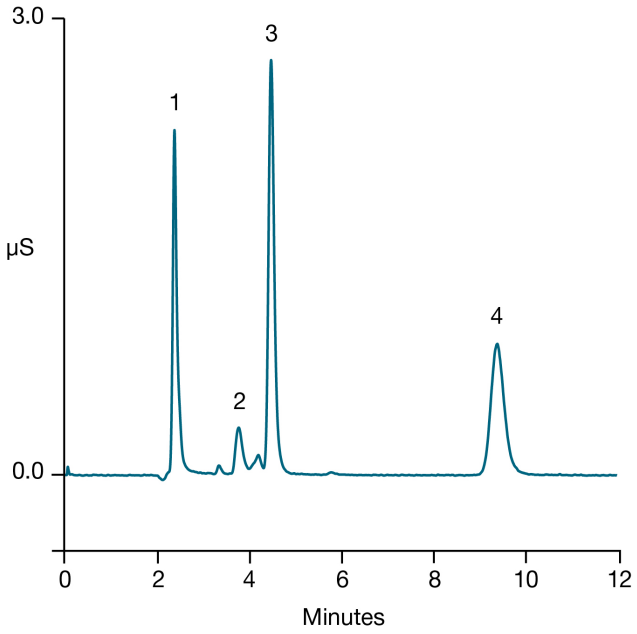


Figure 8.84 Combustion IC of a liquefied petroleum gas sample. Separator column: Dionex IonPac AS11-HC; column dimensions: 250 mm × 4 mm i.d.; eluent: 25 mmol/L KOH (EG); flow rate: 1.3 mL/min; detection: suppressed conductivity; injection volume: 100 µL; sample: LPG; peaks: 1.5 mg/L fluoride (1), 0.02 mg/L chloride (2), 3 mg/L S as sulfate (3), and 2 mg/L orthophosphate (as internal standard) (4).

Biologically produced alcohols, most commonly ethanol, and less commonly propanol and butanol, are made by fermentation of sugars, starches, or lignocellulosic biomass. Microbial fermentation of sugars derived from biomass via yeast is a well-established technology. Fermentation yields relatively dilute aqueous solutions of ethanol that must be distilled to provide 95% ethanol to be used as a fuel. Bioethanol is the most common biofuel worldwide; it can be used as a blending agent in gasoline. Ethanol has a smaller energy density than that of gasoline but a higher octane rating than ethanol-free gasoline, which allows an increase of an engine's compression ratio for better thermal efficiency. Bioethanol can be contaminated with chloride and sulfate that form plugging deposits and cause corrosion in automobile engines. If chloride and sulfate concentrations exceed the limits defined in ASTM Specification D 4806 (40 mg/L chloride and 4 mg/L sulfate), then the ethanol may be rejected as unacceptable for use in automotive spark-injection engine fuel. ASTM D 7319 [222] describes a direct injection ion chromatographic procedure for determining inorganic chloride and sulfate in denatured ethanol and butanol to be used in engine fuel applications. Both anions can be separated on either carbonate- or hydroxide-selective anion-exchange columns using suppressed conductivity detection [223]. If manually prepared carbonate/bicarbonate eluents are used, the Dionex IonPac AS22 column is the most suitable separator column. Electrolytically generated hydroxide eluents are typically used in combination with the Dionex IonPac AS18 column under isocratic conditions (Figure 8.85). For the analysis of trace level anions in bioethanol, a matrix elimination technique must be used, which has already been described in Section 8.3.

Biodiesel is another very common biofuel. Feedstocks for biodiesel include animal fats, vegetable oil, soy, rapeseed, jatropha, sunflower oil, palm oil, and others. Scientists are also studying the manufacture of biodiesel from algae oil. The advantage of algae is that it is relatively simple to cultivate in large quantities. In order to grow, algae need light and carbon dioxide, which they convert by photosynthesis into biomass. If a suitable process for extracting sufficient oil from algae is discovered, it will pose a solution to some of our current energy problems. Biodiesel is produced from oils or fats by transesterification of triglycerides. Because

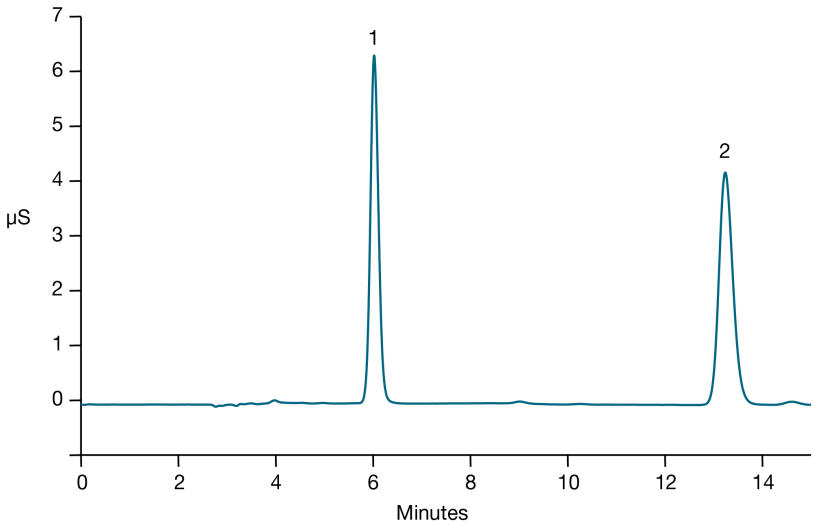


Figure 8.85 Reagent-free IC separation of chloride and sulfate in a denatured bioethanol sample. Separator column: Dionex IonPac AS18; column dimensions: 250 mm × 4 mm i.d.; column temperature: 30 °C; eluent: 30 mmol/L KOH (EG); flow rate: 1 mL/min; detection: suppressed conductivity; injection volume: 10 µL; sample: denatured bioethanol; peaks: 5 mg/L chloride (1) and 5 mg/L sulfate (2).

reaction rates under acid or enzyme catalysis are relatively slow, most producers use alkali- or alkoxide-catalyzed transesterification. Residues from these catalysts persist in the biodiesel and are responsible for elevated sodium and potassium levels in the fuel that must be washed out. In addition to the residual catalysts, glycerol is removed before the fuel can be used. Water is often added during these steps to separate the glycerol from biodiesel and to remove the residual catalysts. The water purity has an impact on the quality of the biodiesel. If the water used is hard, magnesium and calcium can transfer into the fuel during the removal of glycerol and the washing steps.

The major biodiesel standard (ASTM D 6751 [224]) applies to B100 biodiesel that is used for blending. Among the many parameters controlled by this standard are the concentrations of sodium, potassium, magnesium, and calcium in biodiesel. To prevent engine damage from the blended fuels, these cations are limited to a cumulative concentration of less than 5 mg/L for the alkali metals sodium and potassium and for the alkaline-earth metals magnesium and calcium. ASTM D 6751 specifies the use of ICP–OES for the determination of alkali and alkaline-earth metals in biodiesel. However, this method suffers several drawbacks, including spectral interferences from other elements in the sample, matrix effects, and complex sample preparation procedures to avoid interferences. On the other hand, ion chromatography is capable of simultaneously separating the target cations in biodiesel within a reasonable time frame after a simple liquid extraction [225]. The samples are extracted with deionized water (1:1 *v/v*) and filtered through a 0.2 µm Acrodisc syringe filter. To avoid any manual sample preparation, Trick et al. [226] developed an automated in-line sample preparation method in which an aliquot of biodiesel (1 mL) is mixed with 1 mL of 2-propanol and loaded onto a Dionex IonPac TCC-ULP1 concentrator column. (Dilution of the sample with 2-propanol is necessary for solubility support when dealing with water-insoluble and water-immiscible samples.) After the preconcentration step, the concentrator column is rinsed with pure 2-propanol to wash off the biodiesel matrix and reconditioned with ultrapure water. The preconcentrated cations can then be loaded onto the analytical column for separation. The fast separation of all target cations can be carried out on a Dionex IonPac CS12A-5µm column in less than 15 min. Figure 8.86a shows an example chromatogram of a B20 extraction. The four

target cations are well resolved from one another and easily quantified. The unidentified peak in this chromatogram is very likely a primary amine; however, it is well resolved from sodium and potassium and does not interfere with the determinations of the other cations. B20 contains only 20% biodiesel and would likely have low concentrations of cations after processing and blending with petroleum diesel. Figure 8.86b shows a representative chromatogram of B99 after extraction with deionized water. As expected, the concentration levels for the four target cations are significantly higher than those found in B20 but still well below the ASTM limits.

According to ASTM D 6584, the free and bound glycerol content affects the quality of biodiesel during storage in an engine's fuel system. If the free glycerol content is too high, it can partition from the biodiesel as a separate phase. If drawn into an engine, these viscous mixtures will cause immediate engine shutdown due to filter plugging. ASTM D 6751 specifies a maximum total glycerol content of 2400 mg/L (0.24%) and a maximum free glycerol content of 200 mg/L (0.02%). Free glycerol can be readily extracted from biodiesel or biodiesel blends with deionized water. After vigorous shaking for 5 min and phase separation, an aliquot of the aqueous phase can be analyzed directly after filtration with a 0.2 µm Acrodisc syringe filter. Total glycerol, defined as the sum of free and bound glycerol (unconverted triglycerides), is determined by saponification with sodium hydroxide and subsequent extraction of the generated glycerol with deionized water.

The determination of free and total glycerols in biodiesel is challenging. The current industry standard technique requires derivatization to produce trimethylsilylated glycerols for determination by gas chromatography with flame ionization or mass spectrometric detection. On the other hand, high-performance anion-exchange chromatography with pulsed amperometric detection (HPAE-PAD) can determine glycerol without derivatization [227]. The Dionex CarboPac MA1 column is the most suitable for the separation of glycerol. Employing a 100 mmol/L NaOH eluent, glycerol elutes from this column within 11 min and is fully

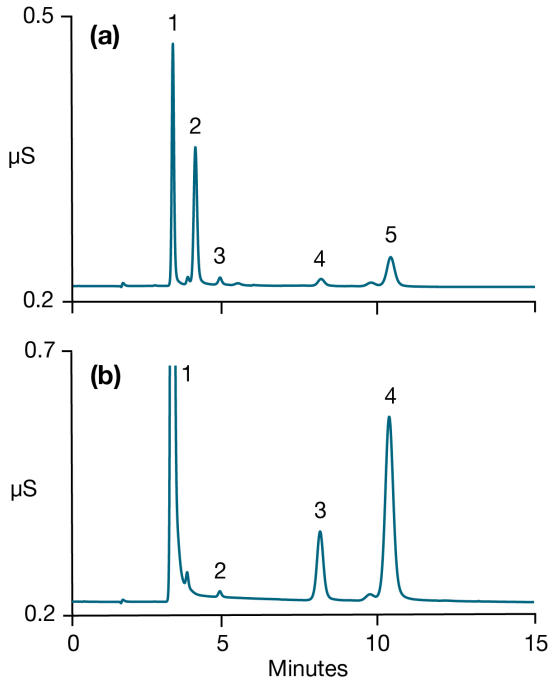


Figure 8.86 Separation of alkali and alkaline-earth metals in B20 and B99 biodiesel samples. Separator column: Dionex IonPac CS12A-5µm; column dimensions: 150 mm × 3 mm i.d.; column temperature: 30 °C; eluent: 20 mmol/L MSA (EG); flow rate: 0.5 mL/min; detection: suppressed conductivity; injection volume: 25 µL; samples: (a) B20 biodiesel, (b) B99 biodiesel, both after extraction with deionized water; peaks: (a) 0.05 mg/L sodium (1), unidentified (2), 0.005 mg/L potassium (3), 0.007 mg/L magnesium (4), and 0.03 mg/L calcium (5), (b) 0.96 mg/L sodium (1), 0.005 mg/L potassium (2), 0.036 mg/L magnesium (3), and 0.18 mg/L calcium (4).

separated from early-eluting peaks. The high sensitivity provided by the pulsed amperometric detection on a gold working electrode allows small amounts of sample to be injected to determine microgram/kilogram to milligram/kilogram concentrations of glycerol in biodiesel samples. Figure 8.87a shows chromatograms of free glycerol in an unspiked and a spiked B100 soy sample. The glycerol peak exhibits good symmetry and is well resolved from an earlier eluting unknown peak, which is likely ethylene glycol. As expected, the total glycerol concentration (Figure 8.87b) is 1–2 orders of magnitude higher than free glycerol so that the sample had to be diluted. Both free and total glycerol concentrations of this sample were within the ASTM specifications.

A common feedstock for biofuel alcohol production is corn stover. It consists of the leaves, husks, stalks, and cobs remaining in the field after harvest. Corn stover processing involves acid hydrolysis to release a water-soluble mixture of carbohydrates, typically consisting of arabinose, glucose, galactose, mannose, xylose, fructose, and cellobiose in 0.5–1.5% (*w/w*) sulfuric acid. The concentrations of these sugars can vary with the feedstock and the hydrolysis conditions. Knowledge of the monosaccharide content of acid-hydrolyzed corn stover allows an estimation of the product yield. Although determination of the individual monosaccharide concentrations is possible, the analysis time is typically long. Like glycerol, all the above-mentioned carbohydrates can be separated and detected by HPAE-PAD. A fast chromatographic analysis of the eight most common biofuel mono- and disaccharides without compromising resolution can be carried out on a Dionex CarboPac SA10 column [228]. Figure 8.88 shows the separation of carbohydrates in a 100-fold diluted acid-hydrolyzed corn stover sample using a 1 mmol/L KOH eluent. The major sugars present in this sample are arabinose, galactose, glucose, and xylose. This method can be used to measure xylose in biomass samples, which is typically used for monitoring the efficiency of biomass pretreatment and fermentation processes. Biomass samples typically have a sugar concentration of 100–200 g/L, and are analyzed after a 100- or 150-fold dilution. Peak area precision in the range 1.7–2.7% was determined for six replicate injections of a carbohydrate standard with 0.5 g/L for each of the biofuel sugars.

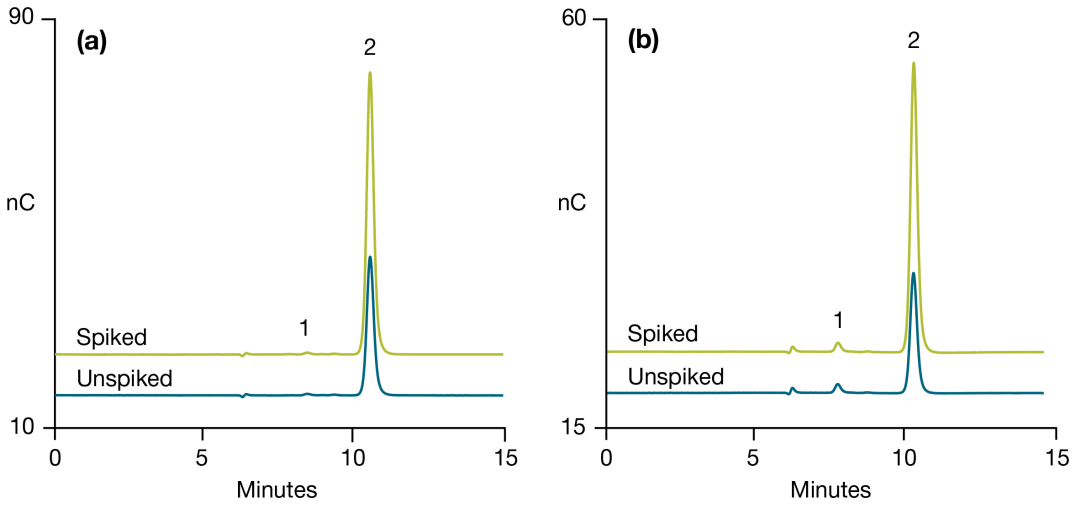


Figure 8.87 Separation of free and total glycerol in a B100 biodiesel sample derived from soy. Separator column: Dionex CarboPac MA1; column dimensions: 250 mm × 4 mm i.d.; column temperature: 30 °C; eluent: 100 mmol/L NaOH; flow rate: 0.4 mL/min; detection: pulsed amperometry on a gold working electrode; injection volume: 5 µL; sample: (a) B100 biodiesel (soy) after water extraction, (b) B100 biodiesel (soy) after water extraction, 1:100 diluted; peaks: (unspiked) unknown (1) and 1.1 mg/kg glycerol (2), (spiked with 1.2 mg/kg glycerol) unknown (1) and 2.2 mg/kg glycerol (2), (b) (unspiked) unknown (1) and 0.54 mg/kg glycerol (2), (spiked with 0.8 mg/kg glycerol) unknown (1) and 1.3 mg/kg glycerol (2).

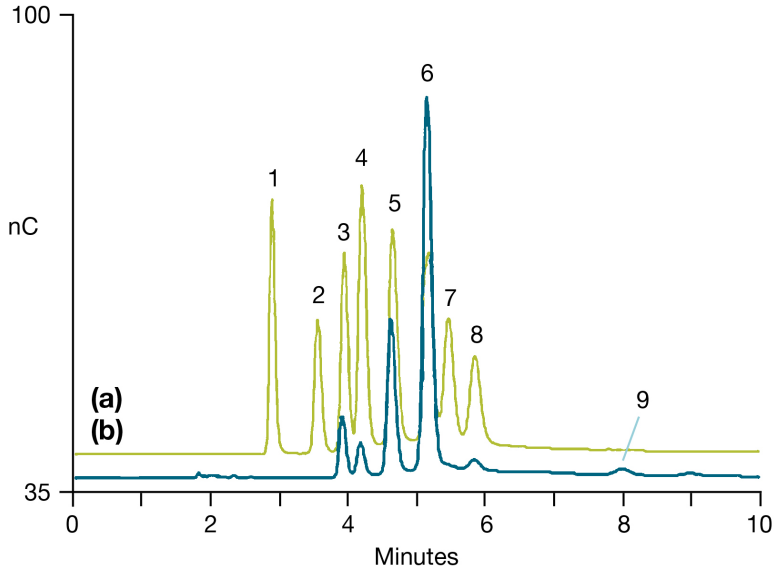


Figure 8.88 Separation of biofuel sugars in an acid-hydrolyzed corn stover sample. Separator column: Dionex CarboPac SA10; column dimensions: 250 mm × 4 mm i.d.; column temperature: 45 °C; eluent: 1 mmol/L KOH (EG); flow rate: 1.5 mL/min; detection: pulsed amperometry on a gold working electrode; injection volume: 0.4 µL; sample: (a) carbohydrate standard, (b) acid-hydrolyzed corn stover, 1:100 diluted; peaks: (a) 0.5 g/L each of fucose (1), sucrose (2), arabinose (3), galactose (4), glucose (5), xylose (6), mannose (7), and fructose (8), (b) <0.5 g/L arabinose (3) , <0.5 g/L galactose (4), <0.5 g/L glucose (5), 0.8 g/L xylose (6), and cellobiose (9).

8.8 Other applications

In addition to the main application areas summarized above, ion chromatography is of significant importance in other application areas, too. From the multitude of applications summarized in Table 8.18, only two examples with especially complex matrices will be dealt with here.

An interesting application in the field of *mineralogy* is the determination of ammonium in cement. Ammonium ions are a natural admixture in raw products of the cement industry. In the finished product they should not exceed a certain amount because cement is used, among other things, to line drinking water pipes. For the ion chromatographic analysis of ammonium ions, 0.1 to 0.5 g cement is extracted with 100 mL hydrochloric acid (c = 0.1 mol/L) for about 10 min by shaking and stirring. After a short sedimentation, the extract can be injected directly through a membrane filter (0.22 µm). Sample preparation is not a problem in this case, but the extremely high calcium content of such samples is. To obtain a satisfactory separation of ammonium ions from the alkali and alkaline-earth metals, a high performance nanobead-agglomerated cation exchanger is used as a stationary phase. Depending on the number of samples to be analyzed, it must be flushed every working day with concentrated hydrochloric acid to remove the more strongly retained alkaline-earth metals from the column, which increasingly occupy ion-exchange groups and thus lower the ion-exchange capacity. Chloride cancels out the passivation of steel surfaces in concrete and thus is only admitted up to a maximum content of 0.1%. For the ion chromatographic analysis of chloride in cement, samples are extracted with methane-sulfonic acid; the extracts can then be injected directly without any sample preparation

Table 8.18 Survey of other application areas and corresponding analytical examples.

Application area	Analytical examples
Pulp & paper industry: Dressing Bleaching processes Paper manufacturing Wastewater	Determination of sulfur- and chlorine species, alkali metals, and ammonium
Mining and metal processing: Potash mining Coal processing Phosphatation	Determination of inorganic anions, alkali- and alkaline-earth metals, transition metals, and metal complexes
Agriculture: Fertilizers Soil extracts	Determination of inorganic anions, alkali- and alkaline-earth metals, transition metals, and ammonium

on the high-capacity, hydroxide-selective Dionex IonPac AS15 anion exchanger. The chromatogram in Figure 8.89 reveals that a separation between the extraction agent and chloride is possible without any problem. Moreover, gradient elution with a simple linear hydroxide gradient offers the ability to detect mono-, di-, and tricarboxylic acids together with mineral acids within 30 min. In the present case, an eluent generator was used for contaminant-free hydroxide preparation, which helps to avoid a baseline drift during the gradient run.

A very topical subject in clinical analysis is the determination of 2-deoxy-2-[^{18}F]fluoro-D-glucose (FDG), which is the most widely used radiopharmaceutical for positron emission tomography (PET) studies. This approach is used in the diagnosis of a number of diseases and disorders including cancer, cardiovascular disease, and dementia. In addition, it is an important tool in neuroscience to enable the study of brain regional activation, and in drug research

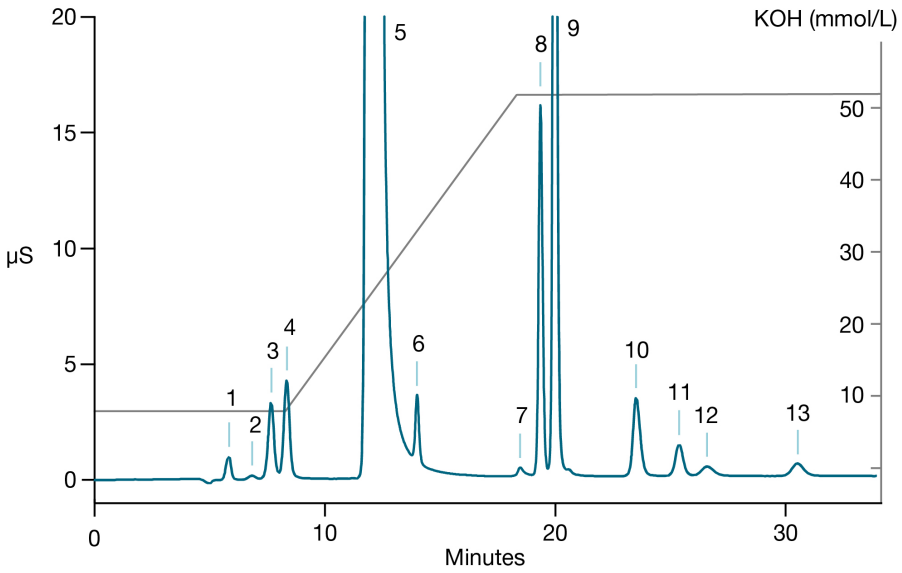


Figure 8.89 Gradient elution of inorganic and organic anions in cement. Separator column: Dionex IonPac AS15; eluent: KOH; gradient: see profile in the chromatogram; flow rate: 1.2 mL/min; detection: suppressed conductivity; injection volume: 25 µL; peaks: (1) unknown, (2) gluconate, (3) acetate, (4) formate, (5) methanesulfonate, (6) chloride, (7) carbonate, (8) tartrate, (9) sulfate, (10) nitrate, (11) orthophosphate, (12) unknown, and (13) citrate.

where it is used to evaluate drug biodistribution. FDG is a glucose analog in which the hydroxyl group at the 2' position of the glucose molecule is substituted by [^{18}F] fluorine. After administrating [^{18}F]FDG to a patient, it is incorporated into the cells by the same transport mechanism as in normal glucose. However, the 2' hydroxyl group in normal glucose is needed for further glycolysis, but this hydroxyl group is missing in [^{18}F]FDG. Thus, FDG cannot be further metabolized in cells. Because [^{18}F]FDG-6-phosphate is formed when [^{18}F]FDG enters the cell, it cannot move out of the cell before radioactive decay. As a result, the distribution of [^{18}F]FDG is a good reflection of the distribution of glucose uptake and phosphorylation by cells in the body (PET tomographic imaging). During the radioactive decay with a half-life of 110 min, [^{18}F]FDG is converted to glucose-6-phosphate that is labeled with a nonradioactive [^{18}O] in the hydroxyl group in the 2' position. The new presence of a 2' hydroxyl group allows it to be metabolized in the same way as normal glucose, producing nonradioactive end products.

Prostate cancer (PCa) is one of the major health problems in men in the Western world. In PET imaging, [^{18}F]FDG was one of the first tracers described; however, due to low glucose metabolism in PCa, its value is limited in PCa work-up. [^{18}F]fluorocholine (FCH) has shown clear superiority over [^{18}F]FDG as a tracer. The rationale for choline as a tracer labeled with [^{18}F] is that choline, an essential component of the phospholipids, is part of the cell membrane, whereby the increase in cell proliferation as well as the activity of the enzyme choline kinase in PCa cells is associated with an increase in choline as an indispensable component. The driving force for the production of [^{18}F]FCH over [^{11}C]choline is the substantially longer half-life of [^{18}F]FCH. [^{18}F]FCH is synthesized by ^{18}F-fluoralkylation of *N*,*N*-dimethylaminoethanol (DMAE) using gaseous ^{18}F-fluorobromomethane. This labeling reaction results in high levels of residual DMAE.

Quality control of both [^{18}F]FDG and [^{18}F]FCH can be done by ion chromatography in a fast and sensitive way. As a representative example, Figure 8.90 shows the HPAE-PAD separation of the glucose precursor, the carrier-free [^{18}F]FDG, and 2-deoxy-2-chloro-D-glucose, a by-product resulting from the production of [^{18}F]FDG. The separation was carried on a Dionex CarboPac PA1 column with 0.22 mol/L NaOH as the eluent. [^{18}F]FCH can be separated on any weak acid cation exchanger utilizing suppressed conductivity detection. Besides nanomole quantities of the [^{18}F]FCH radiotracer, considerable amounts of the DMAE reactant and trace levels of calcium impurities are typically detected in these reaction mixtures.

A relatively new application area for ion chromatography is the separation and identification of metabolites in biological samples. The idea that biological fluids reflect the health of an individual has existed for a long time. Reversed-phase liquid chromatography covers a wide range of hydrophobic compounds, while hydrophilic compounds can be separated using hydrophilic interaction liquid chromatography. However, ionic and ionizable compounds such as organic acids, carbohydrates, nucleotides, and amino acids are difficult to separate or even to retain on traditional reversed-phase columns. Hence, ion-exchange chromatography is by far better suited for the separation of these compounds. The problem of this separation mode to be applied in metabolomic studies is the high concentration of base in the mobile phase being incompatible with mass spectrometers. Because mass spectrometry is the preferred detection mode due to its exceptional sensitivity and its ability to provide additional information for the identification of compounds, eluent suppression is of utmost importance to MS detection as it converts the hydroxide eluent to MS-compatible pure water. As the eluent from the analytical anion-exchange column is converted to pure water, direct coupling to a mass spectrometer is possible. An organic solvent may be added as a makeup flow prior to entering the electrospray interface to aid the desolvation process.

Publications surrounding IC–MS are emerging in literature. Bruggink et al. reported on the use of anion-exchange chromatography with MS detection for carbohydrate analysis [229] using eluent suppression. Carbohydrate analysis by anion-exchange chromatography is usually coupled to pulsed amperometric detection, which does not require eluent suppression.

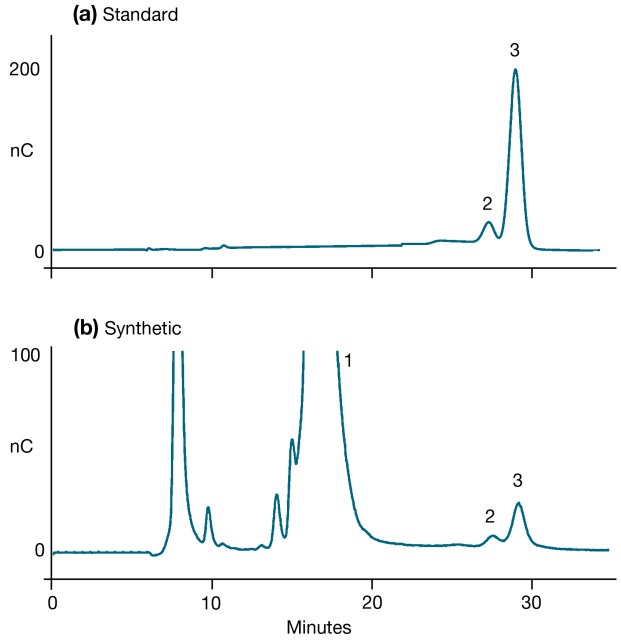


Figure 8.90 Separation of [^{18}F]FDG from its precursor glucose and its by-product 2-deoxy-2-chloro-D-glucose. Separator column: Dionex CarboPac PA1; eluent: 220 mmol/L NaOH; flow rate: 0.2 mL/min; detection: pulsed amperometry on a gold working electrode; injection volume: 25 µL; sample: (a) standard with 1 mg/L [^{18}F]FDG (2) and 40 mg/L 2-deoxy-2-chloro-D-glucose (3), (b) synthetic sample with glucose (1), [^{18}F]FDG (2), and 2-deoxy-2-chloro-D-glucose (3).

However, the transformation of the hydroxide/acetate eluent to volatile acetic acid in the suppressor system is used to allow the coupling of the very efficient separation system to ESI–MS. Despite this key application of IC–MS, very few publications have been seen that describe the use of this technique in the field of biological metabolites where the sample matrix is very complex and the number of potential ionic metabolites is very high. Cook et al. [230] separated ionic and polar compounds such as organic acids, carbohydrates, and nucleotides on a hydroxide-selective anion-exchange column with a simple potassium hydroxide gradient. The corresponding chromatogram in Figure 8.91 clearly shows the applicability of ion-exchange chromatography to this range of metabolite classes. The upper trace (Figure 8.91a) was obtained using suppressed conductivity detection, while the lower trace (Figure 8.91b) represents UV detection at 260 nm. Utilizing eluent suppression that allows for MS detection, ion-exchange chromatography provides a new analytical platform to study metabolisms. Amine-containing compounds can be targeted using cation-exchange chromatography coupled with MS detection. Saccani et al. have demonstrated this for the analysis of underivatized biogenic amines such as cadaverine, putrescine, histamine, agmatine, phenylethylamine, and spermidine in processed meat using a Dionex IonPac CS17 cation exchanger with suppressed conductivity and mass spectrometric detection [231].

Cook et al. [230] employed reversed-phase, HILIC, and anion-exchange chromatography with high-resolution MS to investigate the metabolic profile of biological samples. Their investigations of urine samples showed that there is an expected overlap between the different chromatographic techniques in terms of compounds identified. However, there would be a significant drop in the coverage of identified metabolites if any of the three chromatographic methods would not be employed. Anion-exchange chromatography particularly contributes to the increase in visibility of several classes of compounds such as amino acids, aromatic acids, keto acids, carboxylic acids, nucleosides, purines, carbohydrates, and glucuronides. These

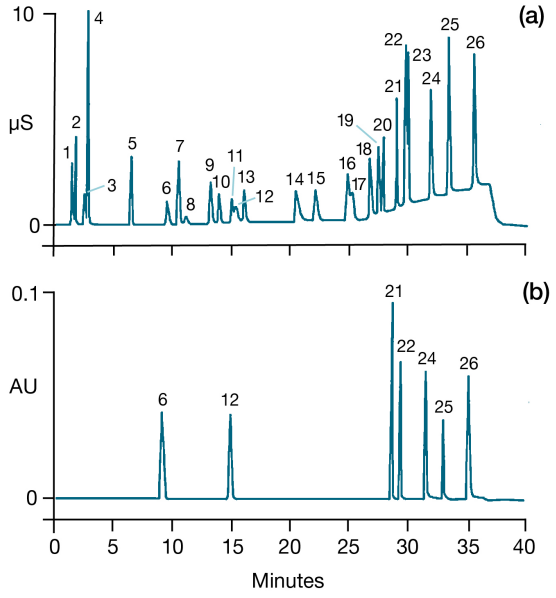


Figure 8.91 Separation of various metabolic intermediates by anion exchange chromatography. Separator column: Dionex IonPac AS11; eluent: NaOH; gradient: 2–12 mmol/L in 15 min, then to 20 mmol/L in 10 min, then to 70 mmol/L in 10 min; flow rate: 2 mL/min; detection: (a) suppressed conductivity, (b) UV at 260 nm; peaks: 2.5 nmol each of acetate (1), pyruvate (2), glyoxylate (3), chloride (4), nitrate (5), cAMP (6), succinate (7), unknown (8), glucose-6-phosphate (9), fructose-6-phosphate (10), ribose-5-phosphate (11), AMP (12),sedoheptulose-7-phosphate (13), orthophosphate (14), 6-phospho-gluconate (15), 3-phosphoglycerate (16), 2-phosphoglycerate (17), citrate (18), isocitrate (19), phosphoenolopyruvate (20), ADP (21), dCTP (22), FDP (23), ATP (24), dTTP (25), and dGTP (26).

classes of compounds play an important role in several biological pathways. IC–MS has been extremely useful in identification and quantification of the glycolytic pathways, which is not surprising as intermediates containing carboxyl and phosphate groups are well separated by anion-exchange chromatography. With the combined approach, 379 out of 652 intermediates of several pathways were reliably identified with minimum effort. Full coverage will probably never be achieved in some biological pathways due to the extremely short half-life and turnover of some intermediates. The availability of high-resolution reversed-phase, HILIC, and anion-exchange columns provides enormous flexibility in the chromatography of metabolites today. Together with modern high-resolution mass spectrometry and recent developments in capillary IC using ion-exchange columns with 0.4 mm internal diameter that allow the analysis of much smaller sample volumes, the physical tools for the separation, detection, and identification of polar and ionic metabolites have improved significantly.

8.9
Sample preparation in ion chromatography

Sample preparation embraces all operations that help to bring the samples into a form appropriate for analysis. These processes include a possible crushing of the sample, its homogenization, digestion, dissolution, stabilization, and filtration. All these steps are important and are usually interconnected. At this point, various techniques will be summarized, with emphasis on procedures that are typical for ion chromatographic analyses.

After the analyte sample has been dissolved, a number of working steps are often required before the sample can be injected into the ion chromatograph. Weakly contaminated aqueous samples with moderate total ionic strength such as drinking water can usually be injected directly after filtration. However, there are numerous application problems that require removal of interfering matrix components from the sample. Also, a modification of the chemical nature of the analyte species is sometimes necessary for obtaining a better separation or detection. Considering the large number of scientific publications dealing with ion chromatography, it is surprising that the subject of sample preparation usually does not gain a lot of attention even though it is a very critical step of the entire analytical procedure. Each manipulation of a sample can falsify the analytical result; therefore, the care taken in sample preparation directly affects the quality of the analytical result. In addition, sample preparation is often difficult to automate and thus the limiting factor in sample throughput. In most cases, sample preparation steps take up most of the required analysis time and thus contribute substantially to the analysis cost.

Sample preparation is usually performed prior to the chromatographic analysis, but it is often coupled directly to it in the form of an intermediate first step (automated in-line sample preparation). Sample preparation aims to avoid overloading effects by appropriately diluting the sample, removing interfering matrix constituents, and/or making ions that are present in very low concentrations accessible to analysis via preconcentration. Thus, sample preparation methods are multifaceted and, depending on the required sample preparation steps, associated with a widely different instrumental setup and time expenditure.

Sample filtration and preservation As with all liquid chromatographic techniques, the solutions to be injected for ion chromatography must be free of particulate matter to avoid plugging of the tubings and, above all, the frits in the guard or separator column head. Disposable filters are generally used for the filtration of sample solutions. Owing to their Luer connector, they are also suited for manual injection, for which they are attached to the injection syringe. To preclude sample contamination, membrane filters should always be rinsed with deionized water prior to their use. While membrane filters with a pore diameter of 0.45 µm are sufficient in most cases, sterile filters with a pore diameter of 0.22 µm should be used for samples with biological activity in order to avoid, as much as possible, a change in sample composition by bacteriological oxidation or reduction. While ions such as chloride, bromide, sulfate, and alkali/alkaline-earth metals are chemically stable, nitrite, nitrate, orthophosphate, and ammonium *cannot* be preserved. In contrast to what many people assume, chlorinated hydrocarbons and mercury salts have *no* stabilizing effect on the above-mentioned non-preservable ions. Sterile filtration, excluding oxygen during sampling, and storing samples in a refrigerator at 4 °C under light exclusion are the only measures to stabilize samples. In some cases, sample stabilization can be carried out by adding chemicals. Known examples are the addition of formaldehyde solution or mannitol for stabilizing sulfite and the acidification of samples if alkaline-earth metals are to be measured.

Sample filtration can also be carried out in-line, either by using an autosampler with dedicated vials and individual filter caps or by using in-line high-pressure filters [232]. Both methods are completely automated, making the filtration process simple, reproducible, and inexpensive. The Thermo Scientific™ Dionex™ AS-DV Autosampler accommodates vials with filter caps. After a sample is dispensed into an inert sample vial, a plunger cap with an integrated 20 µm filter is installed in the top of the vial. The autosampler arm applies a sampling tube that forms a hermetic seal with the filter cap. The cap is then pushed into the vial, displacing the sample upwards through the filter. The filtered sample is transferred hydraulically to the injection loop or a concentrator column. Because filtration occurs from the top down, the filtration efficiency is assisted by gravity, which forces larger particles to be

deposited at the bottom of the vial. Each sample has its own individual filter, eliminating sample carryover related to the filter.

On the other hand, a low-volume filter can be placed in the high-pressure flow path of the ion chromatograph between the injection valve and the guard column to remove particulates from the sample and thus prevents the guard column from clogging due to particulate accumulation in the inlet frit. The low dispersion volume permits filtration of just the injected sample volume without significant band broadening. However, this approach is not recommended for highly contaminated samples, for which the autosampler filter caps described above are better suited. In-line filtration installed on the high-pressure side of the injection valve provides two major benefits:

- The filter is constantly exposed to eluent flow, so that carryover is essentially eliminated.
- The system pressure can be used to monitor the filter condition.

Maintenance of the filter is very simple: one of the tubing connectors is removed, allowing that half of the filter housing to be unscrewed. After the filter frit is replaced, the housing is screwed back together and the tubing reinstalled. Alternatively, a filter backflush can be implemented if concerns about sample carryover remain. As shown in Figure 8.92, the plumbing scheme for this approach requires an auxiliary 10-port high-pressure valve, which can be integrated in modern ion chromatographs. Two filters are installed on this auxiliary valve along with an auxiliary pump. When plumbed as illustrated in Figure 8.92, backflush flow traverses the filter frit in the direction opposite to the sample introduction so that particulates are flushed off the frit to waste, which dramatically extends filter lifetimes. The effectiveness of the filter backflush process can be seen by observing the system pressure over a series of injections.

In conclusion, it has to be emphasized that no matter whether or not any of the sample in-line filtration techniques described above are applied, the sample has to be filtrated with a 0.2 μm filter at the point of sampling if ions are to be analyzed that are affected by bacteria!

In addition to conventional sample filtration using disposable membrane filters or in-line filtration techniques, ultrafiltration is often used when samples with complex matrices are to be analyzed. In ultrafiltration, samples are pressed through a membrane under pressure. The required pressure gradient is provided by either a vacuum pump or an ultracentrifuge. In this approach, particulates as well as colloidal sample components and, depending on the pore size of the filter membrane, dissolved molecules with high molecular weight are retained. However, ultrafiltration is a filtration technique in which filter membranes with much smaller pore sizes than in conventional membrane filtration are used. Ultrafiltration membranes are typically not characterized by their pore size but by their molecular weight cutoff (MWCO). This value refers to the approximate molecular weight of a dilute globular solute (i.e., a typical protein) that is 90% retained by the membrane. In ion chromatography, ultrafiltration is recommended to remove proteins from, for instance, blood sera, milk-based, and meat-based samples. Such samples must be de-proteinated because proteins exhibit such a high affinity towards the stationary phase of an ion exchanger that cleaning the separator column is very time consuming. For proteins, it is recommended that a MWCO be selected that is three to six times smaller than the molecular weight of the solute being retained.

Sample dilution In environmental water analysis, it is often necessary to dilute samples that exceed the working range of ion chromatography which, in turn, depends on the ion-exchange capacity of the separator column. High analyte concentrations necessitate loading less sample volume or diluting the sample prior to injection to avoid exceeding column capacity and to ensure that the concentrations determined fall within the calibration range. The latter is particularly important when regulated methods are used, which typically prescribe a concentration range for each analyte. Exceeding column capacity usually results in poor chromatography, a

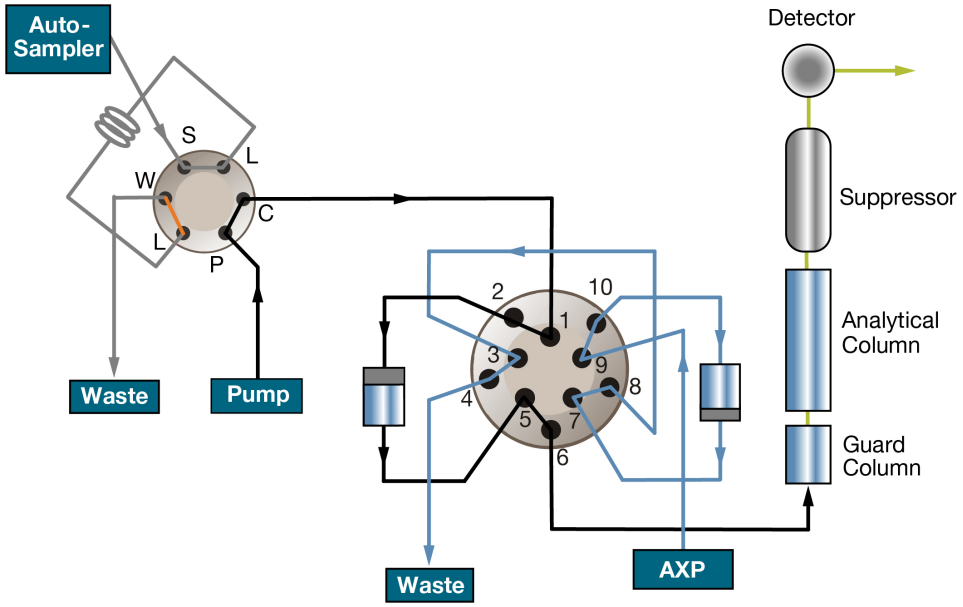


Figure 8.92 In-line high-pressure filter schematic with two filters and back flush.

decreased column lifetime, and inaccurate reporting of analyte concentrations. There are several ways to determine sample concentrations and thus the need for dilution or for lowering the amount of sample injected before reanalysis:

- Samples are injected undiluted and then, based on the peak area of the analytes in the resulting chromatogram, diluted post-chromatographically before reinjection.
- Manual conductivity measurement pre-chromatographically followed by dilution.

Both methodologies are labor intensive and prone to errors. Alternatively, AutoDilution, an important feature of the Chromeleon CDS software, provides a less time-consuming approach to analyze out-of-calibration range samples by utilizing

- a smaller sample loop as compared to the one used for standards [233, 234]
- a smaller volume by partial loop injection [235]
- dilution by autosampler [236]
- in-line conductivity measurement using the Sample pH and Conductivity Accessory for the Thermo Scientific™ Dionex™ AS-AP Autosampler [237]. In this approach, sample conductivity is determined prior to injection and, if the measured conductivity exceeds a specified value, the sample is either automatically diluted or less sample is injected (as mentioned above).

In AutoDilution, samples are first analyzed using a full-loop injection from a large injection loop while the Chromeleon CDS software monitors the sample concentration. If the sample concentration is outside the specified range, AutoDilution will automatically reinject the sample using a smaller injection loop. All AutoDilution runs can be stored in either the original sequence or a separate sequence for easy identification of reanalysis results. To perform AutoDilution with two injection loops, an auxiliary 10-port valve is needed, which can be integrated either in the autosampler or in the ion chromatograph. The two injection loops are mounted on the 10-port valve, for instance a 25 µL loop and a 2.5 µL loop. The program for AutoDilution is created using a Chromeleon built-in wizard that guides users through the main steps required to create an AutoDilution program. Up to a maximum of 10 target compounds can be entered that are searched in the AutoDilution process. It also can be programmed how the target compounds are identified. Any connected detector can be selected from the drop-

down list. Peaks may be selected by name, retention time, greatest height, or greatest area. Mass spectrometry settings are not used for this application. When using a Dionex AS-DV Autosampler, a rinse step is programmed to remove the residual sample from both injection loops. Without the rinse, sample carryover will occur in the sample run.

As an example for AutoDilution with two injection valves, Figure 8.93a shows the analysis of a wastewater sample with sulfate clearly being outside the calibrated range. Chromeleon automatically detects this and adds another line at the end of the sequence. After all other samples have been analyzed, this sample is reanalyzed by switching the 10-port valve with the smaller injection loop in-line with the analytical separator column. The chromatogram of the reinjected sample is illustrated in Figure 8.93b with the analytical result for sulfate being well within the calibrated range.

As an alternative to AutoDilution with two injection loops, automated dilutions can also be carried out using the more sophisticated Dionex AS-AP Autosampler in combination with the Dionex Sample Conductivity and pH Accessory [237]. With this device, an in-line sample conductivity measurement is determined prior to injection and, if the measured conductivity exceeds a specified cutoff value, the sample is automatically diluted. The Chromeleon CDS

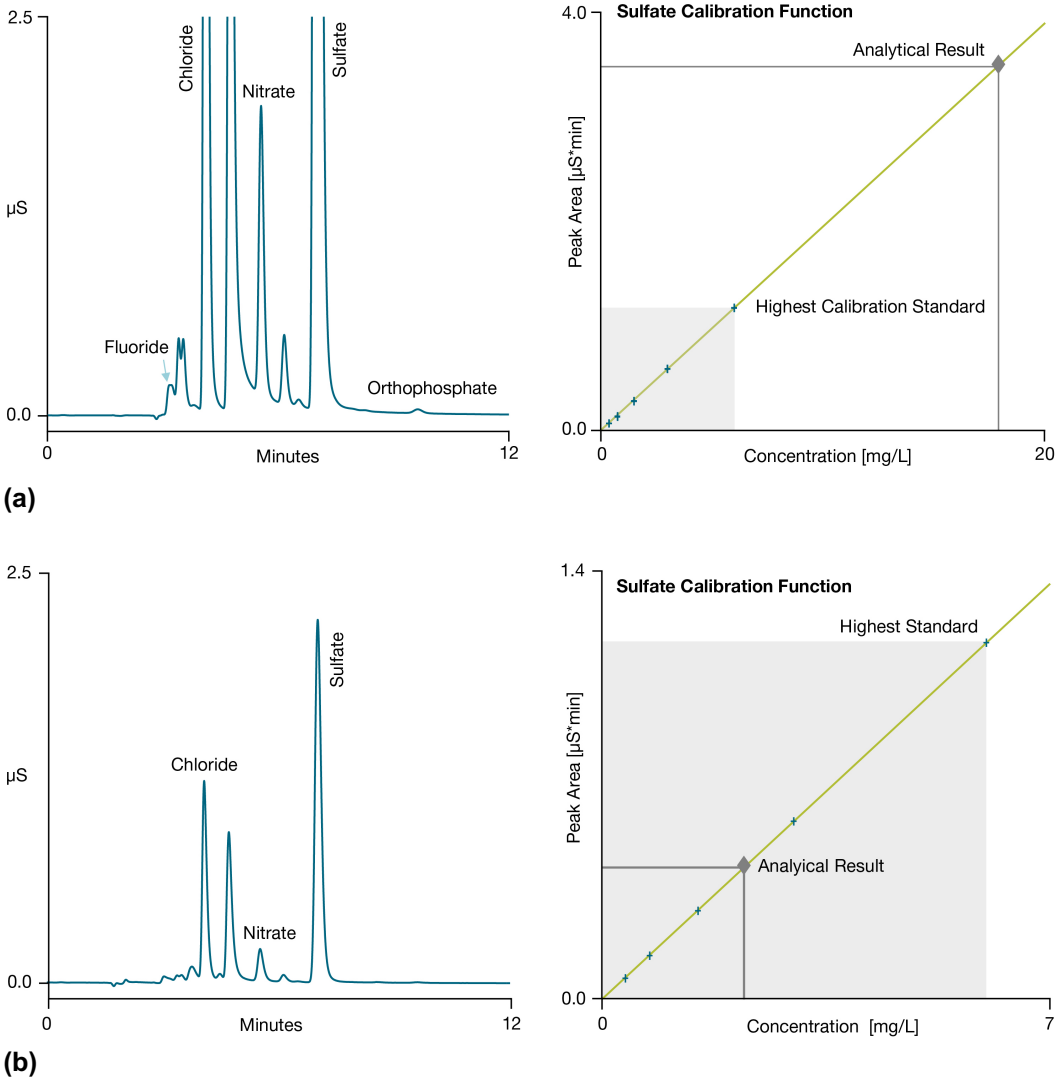


Figure 8.93 AutoDilution of a wastewater sample with conditional large loop/small loop injection. (a) Large loop injection, (b) small loop injection. Separator column: Dionex IonPac AS18; column dimensions: 250 mm × 4 mm i.d.; eluent: 33 mmol/L KOH (EG); detection: suppressed conductivity.

software uses a simple Program Wizard to set up the conductivity measurement parameters and its application to preinjection decisions for sample handling. High and low limits for the sample conductivity can be specified and, if the determined value lies outside of this range, the sample can be skipped or processed with another program that specifies an automated dilution. With the Chromeleon CDS software, programs can be created to perform dilutions into a single vial or sequentially into multiple vials using the Dionex AS-AP Autosampler.

Sample pretreatment cartridges Today, the most convenient form of a fast and selective sample preparation is the use of small disposable cartridges containing a solid sorbent, which allow the selective removal of interfering matrix components or the enrichment of individual sample components from a complex matrix. In the former case, it is important that only matrix components are retained, while in the latter case only solute ions should exhibit a high affinity toward the stationary phase. The technique called *s*olid *p*hase *e*xtraction (SPE) is currently the most widely used sample preparation technique in liquid chromatography [238]. In comparison with liquid/liquid extraction, SPE offers a number of advantages, including high recovery, efficient sample cleanup, ease-of-use, and compatibility with all analytical methods for liquid samples. SPE is compatible with EPA-approved clean water and safe drinking water methods.

When SPE is used to remove interfering matrix components, the process is divided into four separate steps:

- *Activation*. Methanol or acetonitrile are typically used to activate the functional groups of the sorbent material, allowing the use of nonpolar sorbents for polar media such as water. Without this step, matrix components could not bind to the sorbent.
- *Conditioning*. The conditioning step with deionized water removes the organic solvent. It can also be used to convert the sorbent into the desired form.
- *Sample loading*. Sample loading is carried out with a controlled flow rate to avoid a break-through of the component to be separated due to slow mass transfer. If the analytes of interest pass through the SPE cartridge, the sample is “loaded to collect”.
- *Elution*. The eluate is collected for further sample handling.

By using a standard Luer-tip syringe, samples can easily be treated individually. They may be injected through the SPE cartridge directly into the injection valve of the ion chromatograph or into autosampler vials. The most common manual SPE device is a vacuum driven accessory for simultaneous pretreatment of up to 12 samples. Such a sample preparation station has individual stopcock valves for each 5 cm^3 sample reservoir to allow control of individual flow rates. Samples can also be transferred directly to various autosampler vials.

In general, when using sample preparation cartridges, care must be taken to ensure that they are rinsed thoroughly with deionized water prior to their use to avoid sample contamination by ionic constituents that possibly remained in the cartridges from the production process. Furthermore, it should be noted that the sample to be analyzed is passed through the cartridge as slowly as possible. Due to their low packing densities, the cartridges are designed for flow rates up to 50 mL/min, but it is widely recognized that the required pretreatment is much more efficient at significantly lower flow rates (<10 mL/min). For practical execution of the sample pretreatment it is advisable to fill the injection syringe with the solution to be analyzed. This solution is then pushed continuously through the cartridge that has been activated and rinsed in advance. However, the first two to three cartridge volumes should be discarded, so that the sample does not become diluted with liquid remaining in the cartridge. The subsequent effluent can be collected or injected directly into the chromatograph.

Sample pretreatment cartridges such as the Dionex OnGuard II family of cartridges are available in a wide range of packings and in two sizes (1 cm^3 and 2.5 cm^3) for various sample

pretreatment needs. The respective column hardware is designed with Luer inlets for easy and secure connections. The cartridge design eliminates leaks and channeling and contains a sample distribution frit that maximizes complete resin bed usage. Cartridges can be used singly or in series, depending on the matrix interferences to be removed. Table 8.19 summarizes the various Dionex OnGuard II packing materials and their typical applications.

The Dionex OnGuard II A cartridge contains a styrene-based strong anion-exchange resin in the bicarbonate form. This cartridge is ideal for the removal of anionic contaminants from sample matrices. It is also effective for the neutralization of highly acidic samples. The styrene-based Dionex OnGuard II Ag resin has a sulfonic acid functionality in the silver form to easily remove chloride, bromide, and iodide from highly saline samples such as sea water and brines. Samples treated with this cartridge should be passed through a Dionex OnGuard II H cartridge to remove silver counter ions that otherwise would foul the separator column. Therefore, it is recommended that a cation exchanger in the silver form always be used in combination with a cation exchanger in the hydrogen form. The two-layer Dionex OnGuard II Ag/H combination cartridge can be used in place of two single cartridges in series and has the added advantage of a higher capacity for silver ions. Strong acid cation exchangers are also offered in the

Table 8.19 Dionex OnGuard II cartridges for solid-phase extraction and their typical applications.

Cartridge	**Functionality**	**Capacity (mequiv/cartridge) 1 cm³ / 2.5 cm³**	**Mode of use**
OnGuard A	Anion exchange bicarbonate form	0.70/1.75	Removal of anions; pH adjustment of acidic samples
OnGuard Ag	Cation exchange silver form	2.0–2.2/5.0–5.5	Removal of chloride, bromide, iodide by precipitation
OnGuard Ba	Cation exchange barium form	2.0–2.2/5.0–5.5	Removal of sulfate by precipitation
OnGuard H	Cation exchange hydronium form	2.0–2.2/5.0–5.5	Removal of alkaline-earth and transition metals; pH adjustment of basic samples
OnGuard Na	Cation exchange sodium form	2.0–2.2/5.0–5.5	Removal of alkaline-earth and transition metals
OnGuard M	Iminodiacetate ammonium form	0.4/1.0	Concentration of transition metals by chelation (2.5 cm³ format); removal of transition metals (1 cm³ format)
OnGuard P	Polyvinylpyrrolidone	6.0/not available	Removal of phenols, azo dyes, humic acids by complexation
OnGuard RP	Polydivinylbenzene	0.3 g/0.75 g	Removal of surfactants, high-molecular weight organic acids, aromatic dyes by adsorption
OnGuard Ag/H	Cation exchange silver form / cation exchange hydronium form	Not available/4.6 (Ag) 0.8 (H)	Removal of chloride, bromide, iodide by precipitation; removal of alkaline-earth and transition metals; pH adjustment of basic samples
OnGuard Ba/Ag/H	Cation exchange barium form / cation exchange silver form / cation exchange hydronium form	Not available/2.2–2.6 (Ba) 2.2–2.6 (Ag) 0.8 (H)	Removal of chloride, bromide, iodide by precipitation; removal of alkaline-earth and transition metals; pH adjustment of basic samples; removal of sulfate by precipitation

hydronium form. This resin is designed to have a very high selectivity for divalent cations such as calcium and transition metals. It is also used for the neutralization of highly alkaline samples such as sodium hydroxide and sodium carbonate. The Dionex OnGuard II Na cartridge contains a 16% cross-linked styrene-based resin in the sodium form to also remove alkaline-earth and transition metals from sample matrices without acidifying the sample. This attribute ensures good recovery of acid-labile analytes such as nitrite. The styrene-based Dionex OnGuard II Ba cartridge is used to remove high concentrations of sulfate from sample matrices. For reproducible, quantitative determinations in low-ionic strength samples, this cartridge should be activated with a calcium chloride solution. Samples treated in such way should be passed through a Dionex OnGuard II Ag cartridge to remove chloride from the activation solution and then passed through a cation exchanger in the hydronium form to remove the silver counter ions. Thus, the layered Dionex OnGuard II Ba/Ag/H combination cartridge can be used in place of three single cartridges used in series. The Dionex OnGuard II M cartridge contains an iminodiacetate resin in the ammonium form, which is ideal for removing high levels of transition metals. For matrix elimination of transition metals with this cartridge, samples should be buffered to pH greater than 4. This technique allows the recovery of alkali metals while retaining transition metals. The Dionex OnGuard II P cartridge contains a polyvinylpyrrolidone (PVP) resin that has a very high selectivity for phenolics such as humic acids and lignins, tannins, azo dyes, aromatic carboxylic acids, and aromatic aldehydes. In addition, the Dionex OnGuard II P cartridge and all other Dionex OnGuard II cartridges are compatible with all common HPLC solvents and are stable in the pH range between 1 and 10. Hydrophobic packing materials such as divinylbenzene or chemically modified silica (C_8, C_{18}) are recommended for removing hydrophobic matrix constituents, including unsaturated or aromatic organic substances, long-chain fatty acids, and hydrocarbons from the sample. It is also ideal for the removal of lipids and small amounts of proteins, which can foul ion-exchange columns, from food or physiological samples. In contrast to ODS materials, divinylbenzene-based resins have the advantage of being stable over a broad pH range (pH 0–14). Also, divinylbenzene shows a higher selectivity for aromatic and unsaturated compounds.

While Dionex OnGuard cartridges are used for manual sample pretreatment, Thermo Scientific™ Dionex™ InGuard™ cartridges are installed in-line between the autosampler and the IC injection valve, facilitating immediate, automated sample pretreatment. After loading a sample onto an injection loop, the sample is pushed through one or more Dionex InGuard cartridges onto a concentrator column and is ready for analysis. These cartridges are designed with standard 10-32 fittings for easy and secure installation in an IC system. Like with Dionex OnGuard cartridges, Dionex InGuard cartridges are designed to eliminate leaks and channeling and contain a sample distribution frit for maximum resin bed usage. The Dionex InGuard line of sample pretreatment cartridges is available in a wide range of packing materials for various sample pretreatment needs. Table 8.20 summarizes the various packing materials and their typical applications.

The Dionex InGuard HRP cartridge contains a hydrophilic reversed-phase resin based on divinyl-benzene. The material is water-wettable, so that 100% aqueous samples can be pre-treated without disrupting the column bed. The most important application for this cartridge is the removal of organic material over a wide range of hydrophobicity, including fats from whole milk. It contains a blend of a sulfonated resin in the sodium form and HRP resin to provide dual functionality of removing both cations (including metals) and organic contaminants from a sample for a general-purpose cleanup of samples. Optimum performance is achieved when a Dionex InGuard cartridge is used to treat the contents of an injection loop; the treated sample is then loaded onto a concentrator column. The configuration shown in Figure 8.94 can be accomplished on any IC system with two 6-port valves.

Table 8.20 Dionex InGuard cartridges for solid-phase extraction and their typical applications.

Cartridge	Functionality	Capacity (mequiv/cartridge)	Mode of use
InGuard Ag	Cation exchange silver form	5.0–5.5	Removal of chloride, bromide, iodide by precipitation
InGuard H	Cation exchange hydronium form	5.0–5.5	Removal of alkaline-earth and transition metals; pH adjustment of basic samples
InGuard Na	Cation exchange sodium form	5.0–5.5	Removal of alkaline-earth and transition metals
InGuard HRP	Hydrophilic divinylbenzene	2 g	Removal of hydrophobic species, surfactants, high-molecular weight organic acids, aromatic dyes by adsorption and π-π bonding
InGuard Na/HRP	Cation exchange sodium form / hydrophilic divinylbenzene	50% Na/50% HRP	Removal of calcium (Na) and lipids (HRP) from dairy products

Sample neutralization One of the most frequent sample modifications for ion chromatography is the neutralization of strongly acidic or strongly alkaline samples. This applies particularly to digestion solutions. They should not be injected directly, as high concentrations of acid or base in the sample can result in severe baseline instabilities, which are attributed to the huge pH difference between sample and eluent. The neutralization of samples may be accomplished with AutoNeutralization [239], an automated neutralization of acidic or basic samples with a special membrane suppressor. The AutoNeutralization system performs an in-line neutralization step on a concentrated acid or base in two-dimensional ion chromatography. This replaces cumbersome manual dilution and neutralization procedures that would otherwise be required. While dilution can work to reduce the concentration of the interfering matrix ions to a level that does not affect separation, it also reduces the concentration of target ions and consequently compromises sensitivity. AutoNeutralization solves the analytical problem of detecting trace amounts of ions in concentrated acids or bases by neutralizing the sample using an electrolytically operated membrane-based neutralizer device (SRN). As illustrated in the plumbing diagram for AutoNeutralization of concentrated bases for anion analysis in Figure 8.95, the integrated metal-free pump transports the content of the 25 µL sample loop with deionized water through the collection loop into the SRN neutralization unit, which is connected to a valve, so that the sample to be analyzed can be passed a second time through the neutralization unit via time control of the valve to complete the neutralization. As in AutoSuppression, neutralization is based on the electrolysis of water yielding hydronium and hydroxide ions, which act as a regenerant for the ion-exchange membranes.

An important application for this sample preparation technique is anion analysis in a concentrated sodium hydroxide solution as formed during chloralkali electrolysis. The two chromatograms in Figure 8.96 show the results of anion analysis in such a sample with and without neutralization. As can be seen from the chromatogram of the untreated sample in Figure 8.96a, a significant negative dip in the baseline is observed due to the injection of the concentrated base. This matrix interference results in poor chromatography, so that the detection of target anions is virtually impossible. Conversely, the chromatogram in Figure 8.96b shows the result after applying AutoNeutralization. The large negative dip is completely eliminated, and distinct anion peaks can be readily measured. In the same way, purity control of amines in the semiconductor industry can be carried out. In the area of cation analysis, the

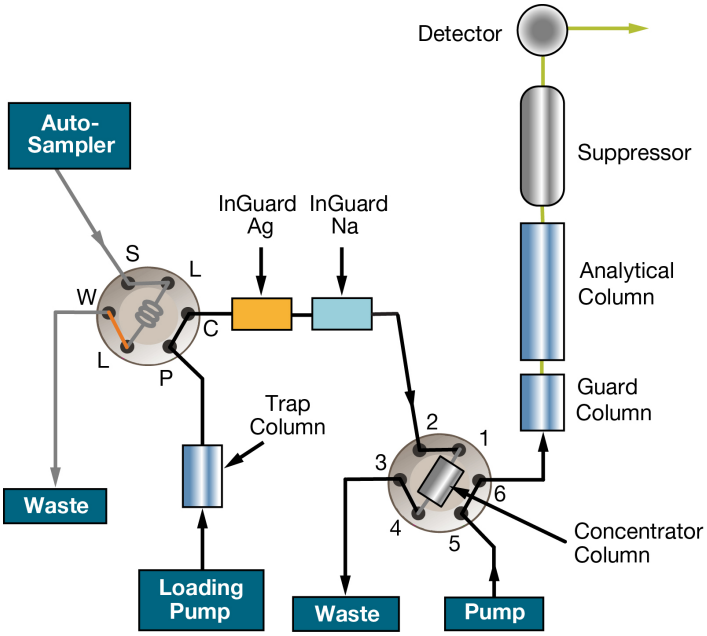


Figure 8.94 Instrumental setup with two 6-port valves and two pumps for the use of Dionex InGuard cartridges.

determination of alkali and alkaline-earth metals in high-purity acids such as hydrofluoric acid, hydrochloric acid, sulfuric acid, and orthophosphoric acid is the most important application, predominantly in the semiconductor industry [240].

Column maintenance One of the most effective methods for protecting separator columns is the use of guard columns, which normally contain the same stationary phase as the analytical columns. Compounds with high affinities toward the stationary phase being used are retained on the guard column and, therefore, poisoning of the analytical separator column is avoided. Fouling of the analytical column is typically indicated by a loss of separation efficiency. Because the capacity of guard columns is limited, they must be rinsed periodically and eventually replaced.

The type of rinsing agents mainly depends on the type of stationary phase. Details are found in the column manuals. In general, guard and separator columns should be stored sealed when they are removed from the chromatograph. Drying out of the column packing can lead to alterations in the column bed and result in a reduction of separation efficiency. Furthermore, guard and separator columns should not be exposed to direct sunlight and should be stored free from vibration. Fats, oils, surfactants, humic acids, and lignins as well as cellulose, proteins, and other high-molecular compounds are considered—particularly in ion exchangers—to be column poisons. Those compounds must be removed by one of the sample preparation techniques described above. Organic polymer-based ion exchangers should only be rinsed with organic solvents if the materials are 100% solvent compatible. Otherwise, swelling and shrinking phenomena may result, depending on the kind of separation material. In general, only water-soluble solvents such as methanol, acetonitrile, and acetone should be used. In any case, the guidelines of the manufacturers are to be followed.

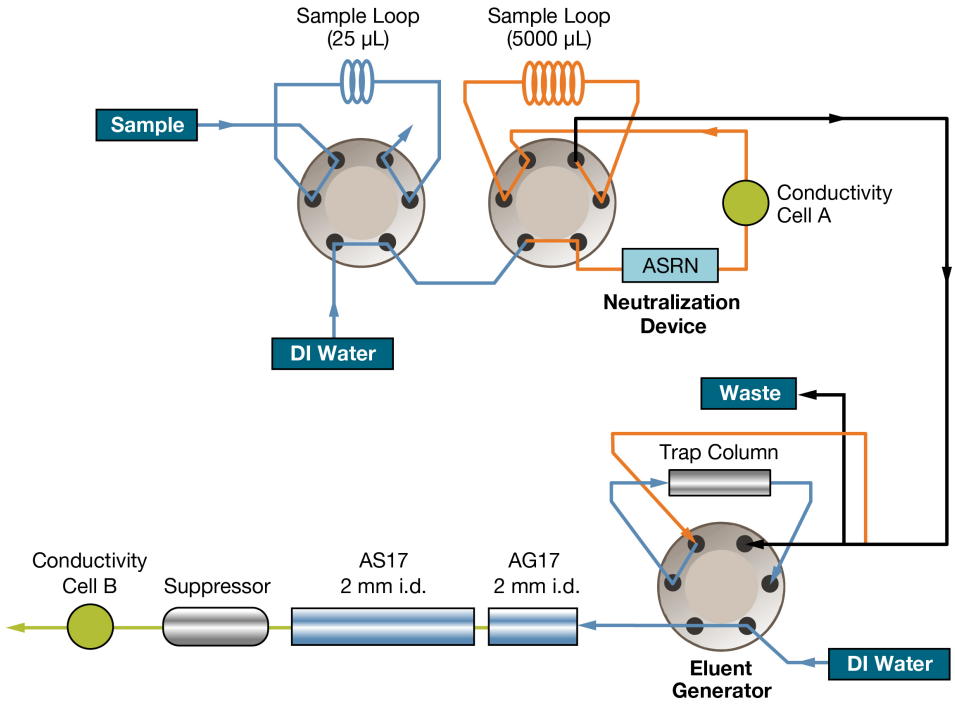


Figure 8.95 Instrumental setup for AutoNeutralization of concentrated bases for anion analysis.

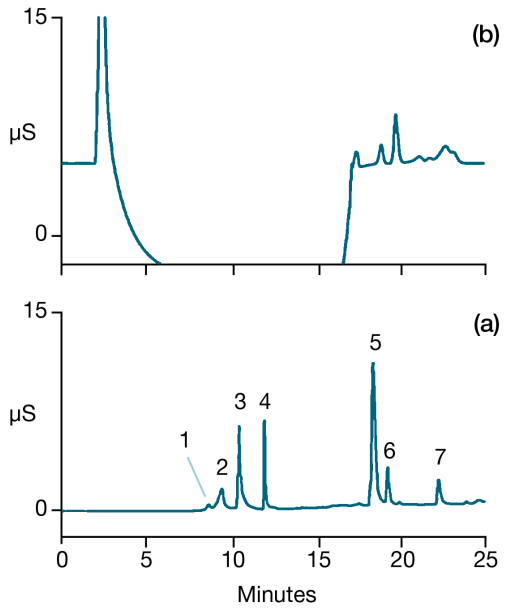


Figure 8.96 Anion analysis in 50% sodium hydroxide (a) with and (b) without AutoNeutralization. Separator column: Dionex IonPac AS17 with guard; column dimensions: 250 mm × 2 mm i.d.; eluent: KOH gradient (EG); flow rate: 0.38 mL/min; detection: suppressed conductivity; injection volume: 25 µL; neutralization device: ASRN; peaks: 0.06 mg/L fluoride (1), (2)–(3) unknown, 0.68 mg/L chloride (4), 2.8 mg/L sulfate (5), (6) unknown, and 1.4 mg/L orthophosphate (7).

Concluding remarks A multitude of separation and detection methods developed in the past years for analyzing ionic compounds have been summarized within the scope of this eBook. However, it should be noted that none of the described methods are universally applicable; none can be considered the method of choice in every case. Hence, for any given analytical problem in this field, the following factors should be taken into account when selecting an appropriate analysis method:F

- Type and concentration of the species to be analyzed
- Required resolution and precision
- Speed and cost of analysis
- Ease-of-use
- Automation

It was the objective of this eBook to provide a summary of the method of ion chromatography, which has rapidly evolved over the past 45 years, and to assist as much as possible in choosing individual techniques.

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After his graduation in chemistry in 1979 from the Technical University of Berlin (Germany) he worked in the field of Liquid and Gas Chromatography at the Hahn-Meitner-Institute in Berlin and received his Ph.D. in Analytical Chemistry in 1982 from the Technical University of Berlin. In 2000, Prof. Guenther Bonn appointed him Visiting Professor at the Leopold-Franzens University in Innsbruck (Austria). Weiss habilitated in Analytical Chemistry at the Leopold-Franzens University in 2002. In 2011, 2014, and 2016, Prof. Jacek Namiesnik appointed him Visiting Professor at the Technical University of Gdansk (Poland).

In 1982, Dr. Weiss started his professional career as an applications chemist at Dionex Corporation in Germany. From 1996 to 1997, he worked in the field of Scanning Probe Microscopy at TopoMetrix GmbH in Germany as a European Distributors Manager and rejoined Dionex Corporation (now part of Thermo Fisher Scientific) in 1998. He currently holds the position of International Technical Director for Dionex Products within the Chromatography and Mass Spectrometry Division (CMD) of Thermo Fisher Scientific, located in Dreieich (Germany).

Dr. Weiss is recognized as an international expert in Analytical Chemistry (especially in the field of Liquid/Ion Chromatography). The 4th edition of his *Handbook of Ion Chromatography* was published in 2016. In 2015, he was awarded the Maria Sklodowska Curie Medal of the Polish Chemical Society for his achievements in separation science.