13th Week

What are the ions in pristine snow? Antarctic snow provides a measure of global atmospheric chemistry because there are no local sources of pollution. One study found the following species by ion chromatography:

Ion	Concentrations observed (μg/L = ppb) Minimum	Maximum
F^-	0.10	6.20
Cl^-	25	40 100
Br^-	0.8	49.4
NO_3^-	8.6	354
SO_4^{2-}	10.6	4 020
$H_2PO_4^-$	1.8	49.0
HCO_2^-	1.1	45.7
$CH_3CO_2^-$	5.0	182
$CH_3SO_3^-$	1.1	281
NH_4^+	2.4	46.5
Na^+	15	17 050
K^+	3.1	740
Mg^{2+}	2.7	1 450
Ca^{2+}	12.6	1 010

SOURCE: R. Udisti, S. Bellandi, and G. Piccardi, "Analysis of Snow from Antarctica," *Fresenius J. Anal. Chem.* **1994**, 349, 289.

25-2 Ion Chromatography

Ion chromatography, a high-performance version of ion-exchange chromatography, is generally the method of choice for anion analysis.[5] In the semiconductor industry, it is used to monitor anions and cations at 0.1-ppb levels in deionized water.

Suppressed-Ion Anion and Cation Chromatography

In **suppressed-ion anion chromatography** (Figure 25-6a), a mixture of anions is separated by ion exchange and detected by electrical conductivity. The key feature of suppressed-ion chromatography is removal of unwanted electrolyte prior to conductivity measurement.

For the sake of illustration, consider a sample containing $NaNO_3$ and $CaSO_4$ injected into the *separator column*—an anion-exchange column in the hydroxide form—followed by elution with KOH. NO_3^- and SO_4^{2-} equilibrate with the resin and are slowly displaced by OH^- eluent. Na^+ and Ca^{2+} cations are not retained and simply wash through. Eventually, KNO_3 and K_2SO_4 are eluted from the separator column, as shown in the upper graph of Figure 25-6a. These species cannot be easily detected, however, because eluate contains a high concentration of KOH, whose high conductivity obscures that of analyte.

To remedy this problem, the solution next passes through a *suppressor,* in which cations are replaced by H^+. H^+ exchanges with K^+, in this example, through a cation-exchange membrane in the suppressor. H^+ diffuses from high concentration outside the membrane to low concentration inside the membrane. K^+ diffuses from high concentration inside to low concentration outside. K^+ is carried away outside the membrane, so its concentration is always low on the outside. The net result is that KOH eluent, which has high conductivity, is converted into H_2O, which has low conductivity. When analyte is present, HNO_3 or H_2SO_4 with high conductivity is produced and detected.

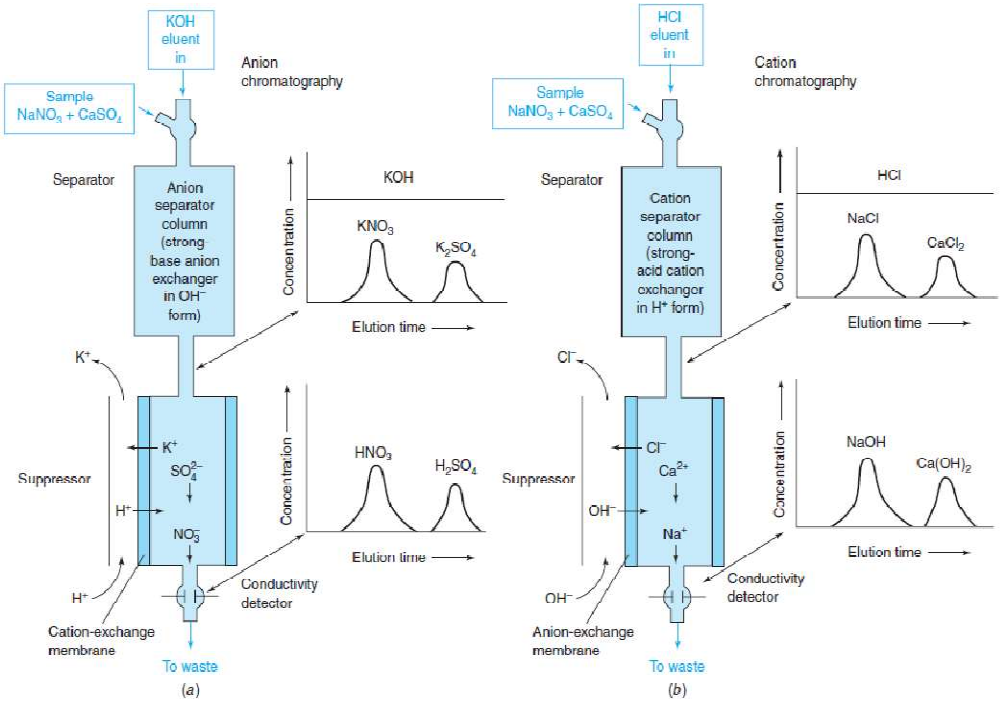


FIGURE 25-6 Schematic illustrations of (*a*) suppressed-ion anion chromatography and (*b*) suppressed-ion cation chromatography.

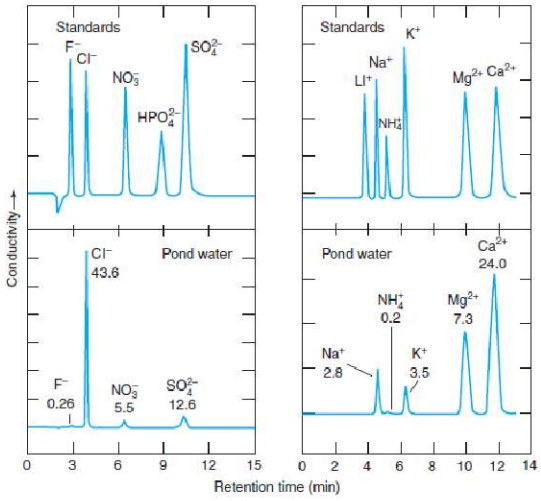


FIGURE 25-7 Ion chromatography of pond water. Upper chromatograms were obtained from mixtures of standards. Concentrations of ions in lower chromatograms from pond water are in units of μg/mL (ppm). Anion analysis was done with an IonPac AS14 column with 1.0 mM $NaHCO_3$/3.5 mM Na_2CO_3 eluent with ion suppression and conductivity detection. Cation analysis used an IonPac CS12A column with 11 mM H_2SO_4 eluent, ion suppression, and conductivity detection. [From K. Sinniah and K. Piers, "Ion Chromatography: Analysis of Ions in Pond Waters," *J. Chem. Ed.* 2001, *78*, 358.]

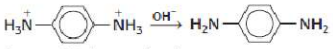
Suppressed-ion cation chromatography is conducted in a similar manner, but the suppressor replaces Cl^- from eluent with OH^- through an anion-exchange membrane. Figure 25-6b illustrates the separation of $NaNO_3$ and $CaSO_4$. With HCl eluent, NaCl and $CaCl_2$ emerge from the cation-exchange separator column, and NaOH and $Ca(OH)_2$ emerge from the suppressor column. HCl eluate is converted into H_2O in the suppressor.

Figure 25-7 illustrates a student experiment to measure ions in pond water. Eluent for the anion separation was $NaHCO_3/Na_2CO_3$ buffer. The product from eluent after passing through the suppressor is H_2CO_3, which has low conductivity.

In automated systems, H^+ and OH^- eluents and suppressors are generated by electrolysis of H_2O. Figure 25-8 shows a system that generates KOH for >1 000 hours of isocratic or gradient elution before it is necessary to refresh the reagents. Water in the reservoir of aqueous K_2HPO_4 reacts at the metal anode to produce H^+ and $O_2(g)$. H^+ combines with HPO_4^- to form $H_2PO_4^{2-}$. For each H^+ ion that is generated, one K^+ ion migrates through the cation-exchange barrier membrane, which transports K^+, but not anions, and allows negligible liquid to pass. The barrier membrane must withstand the high pressure of liquid in the KOH generation chamber destined for the chromatography column. For each H^+ generated at the

The separator column separates analytes, and the suppressor replaces ionic eluent with a nonionic species.

Benzene-1,4-diammonium cation is a stronger eluent that can be used instead of H^+ for suppressed-ion cation chromatography. After suppression, a neutral product is formed:



Benzene-1,4-diammonium ion

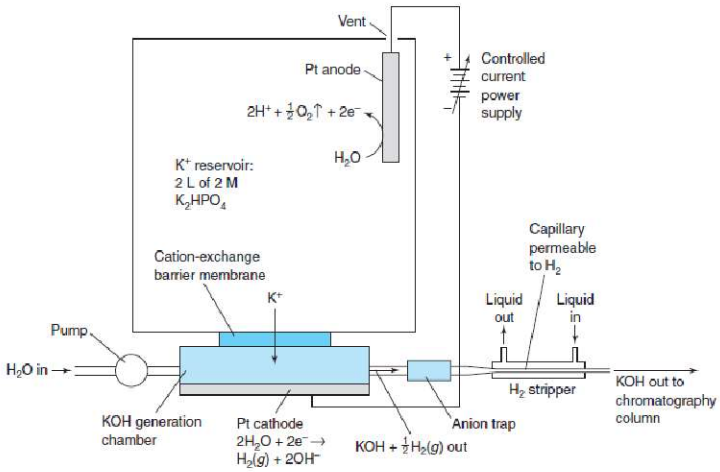


FIGURE 25-8 Electrolytic KOH eluent generator for ion chromatography. [Adapted from Y. Liu, K. Srinivasan, C. Pohl, and N. Avdalovic, "Recent Developments in Electrolytic Devices for Ion Chromatography," *J. Biochem. Biophys. Methods* 2004, *60*, 205.]

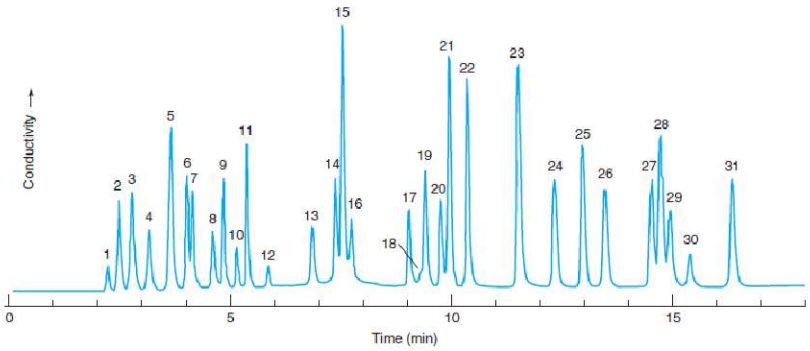


FIGURE 25-9 Anion separation by ion chromatography with a gradient of electrolytically generated KOH and conductivity detection after suppression. Column: Dionex IonPac AS11; 4 mm diameter; flow = 2.0 mL/min, Eluent: 0.5 mM KOH for 2.5 min, 0.5 to 5.0 mM KOH from 2.5 to 6 min; 5.0 to 38.2 mM KOH from 6 to 18 min. Peaks: (1) quinate, (2) F^-, (3) acetate, (4) propanoate, (5) formate, (6) methylsulfonate, (7) pyruvate, (8) valerate, (9) chloroacetate, (10) BrO_3^-, (11) Cl^-, (12) NO_2^-, (13) trifluoroacetate, (14) Br^-, (15) NO_3^-, (16) ClO_3^-, (17) selenite, (18) CO_3^{2-}, (19) malonate, (20) maleate, (21) SO_4^{2-}, (22) $C_2O_4^{2-}$, (23) tungstate, (24) phthalate, (25) phosphate, (26) chromate, (27) citrate, (28) tricarballylate, (29) isocitrate, (30) *cis*-aconitate, (31) *trans*-aconitate. [Courtesy Dionex Corp., Sunnyvale CA.]

anode, one K^+ flows through the cation-exchange barrier and one OH^- is generated at the cathode. Liquid exiting the KOH generation chamber contains KOH and H_2. The stream passes through an anion trap that removes traces of anions such as carbonate and degradation products from the ion-exchange resin. The trap is continuously replenished with electrolytically generated OH^-, which is not shown in the diagram. After the trap, liquid flows through a polymeric capillary that is permeable to H_2, which diffuses into an external liquid stream and is removed. The concentration of KOH produced by the apparatus in Figure 25-8 is governed by the liquid flow rate and the electric current. With computer control of the power supply, a precise gradient can be generated.

In the past, KOH eluent was usually contaminated with CO_3^{2-}. When CO_3^{2-} passes through the suppressor after the ion chromatography column, it is converted into H_2CO_3, which has some electrical conductivity that interferes with detection of analytes. In gradient elution with increasing KOH, the concentration of H_2CO_3 also increases, and so does the background conductivity. The feedstock for the electrolytic generator is pure H_2O, and the product is aqueous KOH containing very little CO_3^{2-}. Figure 25-9 shows a separation of 31 anions with a hydroxide gradient.

Suppressors in Figure 25-6 have been replaced by electrolytic units such as that in Figure 25-10, which generates H^+ or OH^- to neutralize eluate and requires only H_2O as feedstock. With electrolytic eluent generation and electrolytic suppression, ion chromatography has been

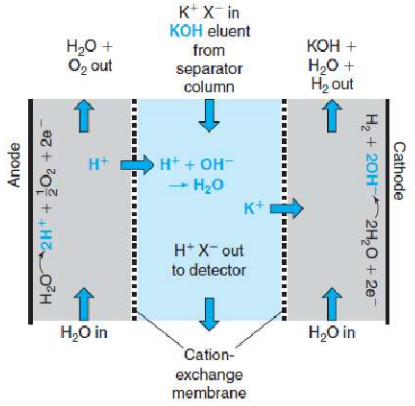


FIGURE 25-10 Electrolytic suppression for anion chromatography replaces KOH eluent with H_2O.

simplified and highly automated. Readily available software can be used to simulate and optimize ion chromatographic separations.[6]

Ion Chromatography Without Suppression

If the ion-exchange capacity of the separator column is sufficiently low and if dilute eluent is used, ion suppression is unnecessary. Also, anions of weak acids, such as borate, silicate, sulfide, and cyanide, cannot be determined with ion suppression, because these anions are converted into very weakly conductive products (such as H_2S).

For *nonsuppressed anion chromatography*, we use a resin with an exchange capacity near 5 μmol/g, with 10^{-4} M Na^+ or K^+ salts of benzoic, *p*-hydroxybenzoic, or phthalic acid as eluent. These eluents give a low background conductivity, and analyte anions are detected by a small *change* in conductivity as they emerge from the column. A judicious choice of pH produces an average eluent charge between 0 and −2 and provides control of eluent strength. Even dilute carboxylic acids, which are slightly ionized, are suitable eluents for some separations. *Nonsuppressed cation chromatography* is conducted with dilute HNO_3 eluent for monovalent ions and with ethylenediammonium salts ($^+H_2NCH_2CH_2NH_2^+$) for divalent ions.

Detectors

Conductivity detectors respond to all ions. In suppressed-ion chromatography, it is easy to measure analyte because eluent conductivity is reduced to near 0 by suppression. Suppression also allows us to use eluent concentration gradients.

In nonsuppressed anion chromatography, the conductivity of the analyte anion is higher than that of the eluent, so conductivity increases when analyte emerges from the column. Detection limits are normally in the mid-ppb to low-ppm range but can be lowered by a factor of 10 by using carboxylic acid eluents instead of carboxylate salts.

Benzoate or phthalate eluents enable sensitive (<1 ppm) **indirect detection** of anions. In Figure 25-11, eluate has strong, constant ultraviolet absorption. As each analyte emerges, nonabsorbing analyte anion replaces an equivalent amount of absorbing eluent anion. Absorbance therefore *decreases* when analyte appears. For cation chromatography, $CuSO_4$ is a suitable ultraviolet-absorbing eluent.

Ion-Pair Chromatography

Ion-pair chromatography uses a reversed-phase HPLC column instead of an ion-exchange column to separate polar or ionic compounds.[8] To separate cations (for example, protonated organic bases), an anionic *surfactant* (Box 25-1) such as *n*-$C_8H_{17}SO_3^-$ is added to the mobile

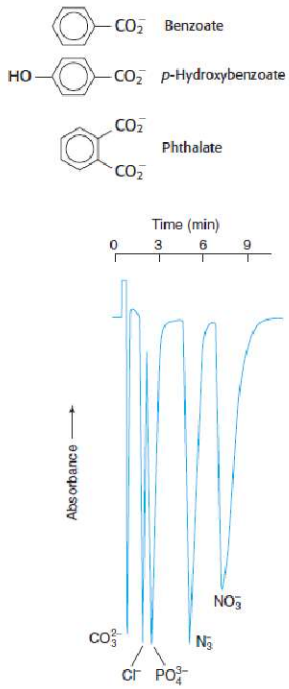


FIGURE 25-11 Indirect spectrophotometric detection of transparent ions. The column was eluted with 1 mM sodium phthalate plus 1 mM borate buffer, pH 10. The principle of indirect detection is illustrated in Figure 25-35.
[Reproduced from H. Small, "Indirect Photometric Chromatography," *Anal. Chem.* 1982, *54*, 462.]

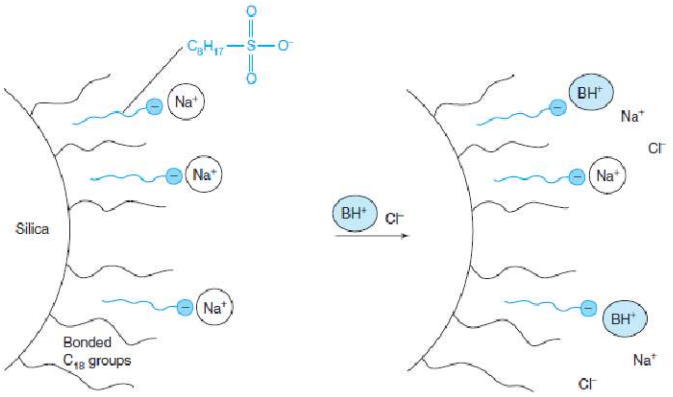


FIGURE 25-12 Ion-pair chromatography. The surfactant sodium octanesulfonate added to the mobile phase binds to the nonpolar stationary phase. Negative sulfonate groups protruding from the stationary phase then act as ion-exchange sites for analyte cations such as protonated organic bases, BH^+.

phase. The surfactant lodges in the stationary phase, effectively transforming the stationary phase into an ion exchanger (Figure 25-12). Analyte cations are attracted to the surfactant anions.[9] The retention mechanism is a mixture of reversed-phase and ion-exchange interactions. To separate anions, tetrabutylammonium salts can be added to the mobile phase as the ion-pair reagent (Figure 25-13).

Ion-pair chromatography is more complex than reversed-phase chromatography because equilibration of the surfactant with the stationary phase is slow, the separation is more sensitive to variations in temperature and pH, and the concentration of surfactant affects the separation. Methanol is the organic solvent of choice because ionic surfactants are more soluble in methanol/water mixtures than in acetonitrile/water mixtures. Strategies for method development analogous to the scheme in Figure 24-27 vary the pH and surfactant concentration with fixed methanol concentration and temperature.[10] Because of the slow equilibration of surfactant with the stationary phase, gradient elution is not recommended in ion-pair chromatography. Many ion-pair reagents have significant ultraviolet absorption, which makes ultraviolet detection of analytes problematic. Reversed-phase stationary phases with a *polar embedded group* (page 602) are a possible alternative to ion-pair chromatography for polar compounds.

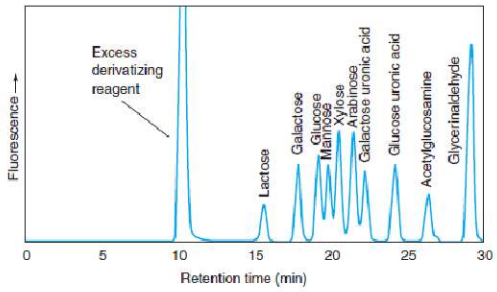


FIGURE 25-13 Separation of carbohydrates by ion-pair chromatography. Carbohydrates were *derivatized* by covalently attaching *p*-aminobenzoate ($H_2N—C_6H_4—CO_2^-$), which changes carbohydrates into fluorescent anions. The anions were separated on a 0.30 × 25 cm column of AQUA® C_{18}-silica, using tetrabutylammonium cation as the ion-pair reagent. Eluent was a linear 60-min gradient starting with 20 mM aqueous $(n\text{-}C_4H_9)_4N^+HSO_4^-$, pH 2.0 (solvent A) and ending with 50:50 A:methanol. The method was used to measure carbohydrates at 10- to 100-ng/mL levels in water leaching from landfills. [From A. Meyer, C. Raba, and K. Fischer, "Ion-Pair HPLC Determination of Sugars, Amino Sugars, and Uronic Acids," *Anal. Chem.* 2001, *73*, 2377.]