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ADVANCES IN ION CHROMATOGRAPHY

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Advances in
Ion Chromatography

April 2013

Articles

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Speciation Analysis by Coupling IC with ICP/MS

Separate and Quantify Different Elemental Oxidation States

The need to distinguish between chemical forms of an element such as Cr III/VI, As III/V, and organic v. inorganic mercury has become critical for food, environmental, and pharmaceutical analysis. In the past, measuring the total amount of an element was sufficient. Unfortunately, the effects of an element extend far beyond its absolute amount, since different forms of an element can exhibit very distinct levels of toxicities. The process of separation and quantification of different oxidation states of an element, more specifically termed speciation analysis, can be utilized to determine an element's various chemical forms. Speciation analysis can be split into two components: separation of individual ionic species by ion chromatography followed by trace elemental detection and quantification using ICP/MS. The combined method is termed Ion Chromatography – Inductively Coupled Plasma Mass Spectroscopy (IC – ICP/MS). Using ICP/MS as a sensitive elemental detector in combination with a selective separation technique such as Ion Chromatography provides complete information on the chemical form (species) of an element.

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Arsenic	As^{+3}, As^{+5}
Chromium	Cr^{+3} , Cr^{+6}
Mercury	Hg^{+2}, $EtHg^{+}$, and $MeHg^{+}$
Selenium	Se^{+4}, Se^{+6}

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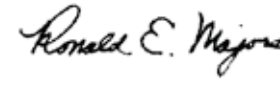
Ion Chromatography: Yesterday, Today, and Tomorrow

As a liquid chromatographer, one tends to lump ion-exchange chromatography (IEC) and ion chromatography (IC) in the same bucket, but there are actually significant differences in the practice of these methodologies. Both techniques use columns with ionogenic functionality, mobile phases with various buffer compositions, and separate ionic compounds in simple to complex matrices. Early on, IEC had its biggest success in the separation of amino acids, helping Moore and Stein win the Nobel Prize in chemistry in 1972 and in the separation of transuranium elements during the development of the atomic bomb back during World War II. However, the biggest difference between the two techniques is based on the needs of the early detection principle of conductivity — the removal of the buffer from the mobile phase before detection. In the early 1970s, Hamish Small and coworkers, while working at Dow Chemical in Midland, Michigan, envisioned a method with a fast separation of non-chromophore-containing ionic compounds but in a nonconducting mobile phase, water. The concept of buffer removal (stripping), later termed *eluent suppression*, was a key element in the development and differentiation of IC from other ion-exchange separations. It spurred the development of specialized low specific exchange capacity packings, sophisticated suppressors with regeneration capabilities, and new detection principles such as indirect photometric and pulsed amperometric detection. Throughout the decades, alongside high performance liquid chromatography (HPLC), IC has seen parallel developments in separating ionic compounds in a variety of matrices.

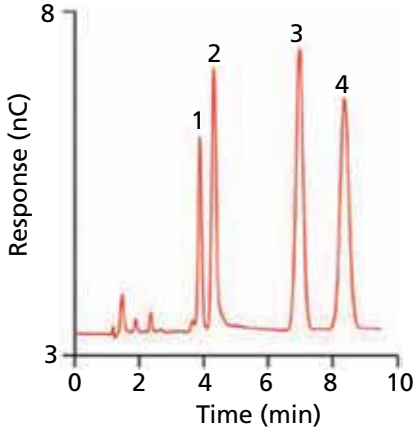
IC has solved many practical problems where problematic cationic and anionic compounds at the parts-per-billion level were more easily measured such as trace chloride in water causing corrosion in power-generation turbines, trace electrolytes in semiconductor processing water, and in environmental water analysis. IC has progressed beyond simple ions in solution and can now measure ionized carbohydrates and alcohols at high pH using electrochemical detection in diverse and difficult samples such as encountered in the food and brewing industries.

In this special issue, a supplement to both *LCGC North America* and *LCGC Europe*, we have managed to assemble experts from the top IC research and development laboratories from across the world to provide state-of-the art reviews surrounding elements of this well established separation technique. Hamish Small, now a consultant, gives a fascinating historical prospective, from the development of IC at Dow through its commercialization, and explains why and how certain improvements were made along the way. In encountering difficult matrices, sample preparation is equally important in IC as it is in HPLC. Rosanne Slingsby and coworkers from Thermo Scientific (formerly Dionex) provide some practical advice on sample preparation for IC. Chris Pohl, also of Thermo Scientific, who has contributed to many of the advances in the technology, especially from the commercial side, brings us up to date on the most widely used columns in modern IC. Chuck Lucy and Farooq Wahab from the University of Alberta in Edmonton, Alberta, Canada, have been instrumental in advancing high-speed and high-resolution IC and provide examples on the use of shorter, smaller particle columns. Sandy Dasgupta and students from the University of Texas in Arlington discuss the most widely used detectors — the conductivity detector and the charge detector — the latter developed in their own laboratory. Finally, Paul Haddad and his group from the University of Tasmania in Hobart, Australia, discuss their development of simulation software tools for the method development and optimization of separations in IC.

I sincerely hope you find this special issue of interest and think of IC the next time you encounter a challenging problem in the separation and measurement of ionic compounds.



Landmarks in the Evolution of Ion Chromatography



A personal perspective on the milestones in the development of ion chromatography. From the invention of eluent suppression to today's "just add water" concept, pivotal developments over the last 40 years are chronologically highlighted from a chemical and instrumental viewpoint. Suggested key references on applications and detailed developments are provided.

In the late 1950s, a few of us at the Dow Chemical Company began thinking about a new form of chromatography that would challenge the old methods of inorganic ion analysis. Those ideas and subsequent research were the precursors of what became known as *ion chromatography* (IC). I have been privileged to be closely linked to those early endeavors and to be at least an interested observer of the many other important events that followed. From this long perspective I will attempt to identify ideas, inventions, and innovations that have altered the pace of development and the trajectory of IC in the years since our early imaginings. I call these events *landmarks*.

In other publications (1,2) I have described in some detail how quite unrelated experiences in my early years at Dow influenced the first IC inventions, so for brevity's sake I will jump directly to the first landmark, in 1971.

1971: The Invention of Eluent Suppression

By 1971, we had decided that what inorganic ion analysis needed was a chromatographic technique that would supplant many of the tedious, time-consuming classical wet-chemical methods. At this time, ion-exchange chromatography had made significant contributions to inorganic analytical chemistry (3) but it was usually as an adjunct to wet-chemical methods; chromatography segregated the analytes of interest from interfering species, fraction collectors made "cuts" of the effluent from the ion-exchange column, and the cuts were analyzed by the classical methods. Because the analytes often had widely diverse chemistries and required special analytical techniques, the task of analysis was handled by specialists. It was a slow business and days could go by between the separation and the results. We envisioned a method that would couple fast separation with prompt detection and measurement of analytes by a monitor placed at the column outlet. Desirably, this detector should be "universal" — that is, capable of quantifying ions of widely diverse chemistries. But what might we use as a detector? Many inorganic ions of interest such as alkali and alkaline earth metal ions, ammonium, halides, nitrate, sulfate, and phosphate were notably "bland" in not having useful chromophores or a general postseparation means of generating chromophores, as Moore and Stein had done for amino acid analysis (4), so spectrophotometric methods of detection seemed a no-go at that time. We recognized, however, that a universal property shared by aqueous electrolyte solutions was electrical conductance and a notable landmark was our decision to exploit that property. But therein we anticipated a problem.

With the considerable knowledge available to us (3), we were convinced that ion-exchange chromatography could separate any mixture that challenged us. We

Hamish Small

Direct correspondence to:
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Capillary Ion Chromatography—Bringing a new level of speed and resolution

Always On, Always Ready, Better Results

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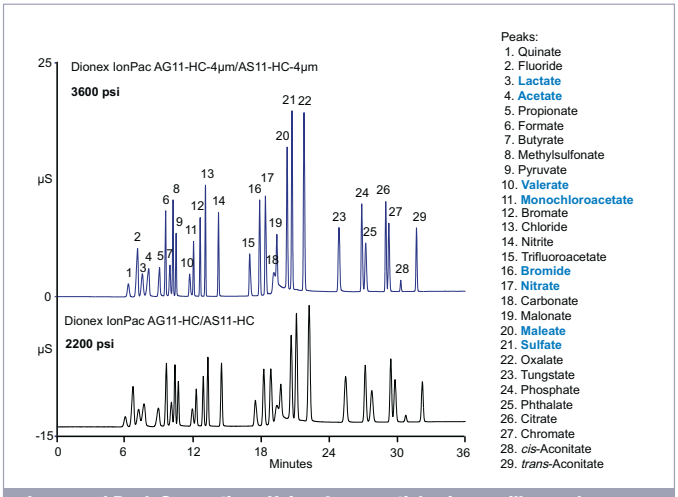

Improved Peak Separations Using 4 µm particle size capillary columns

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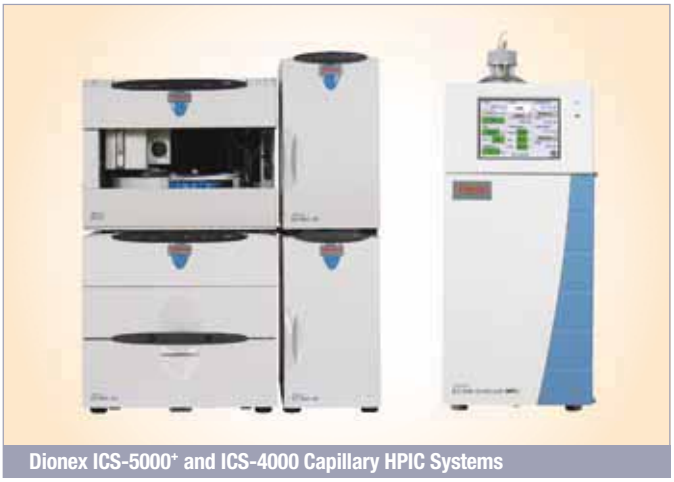

Dionex ICS-5000⁺ and ICS-4000 Capillary HPIC Systems

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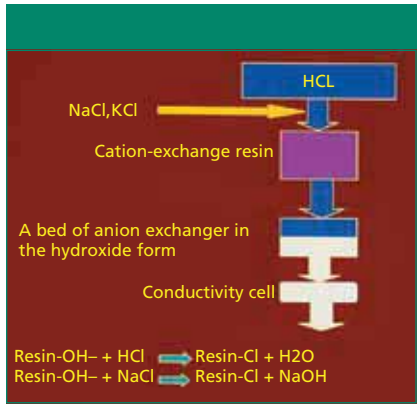

Figure 1: Workflow devised in 1971 using a "stripper" column between the separator column and the conductivity cell, as applied to the separation of the alkali metals on a cation-exchange resin using hydrochloric acid as the eluent.

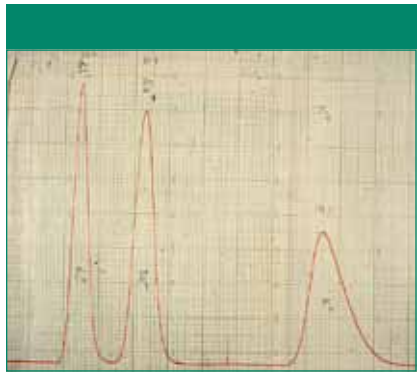

Figure 2: The first chromatogram using eluent suppression and conductometric detection. The sample injected was 0.1 mL of a mixture of LiCl, NaCl, and KCl, 0.01 M of each. The eluent was 0.02 M HCl. From author's laboratory notebook, dated November 9, 1971.

were also aware that using conventional high-capacity ion-exchange resins would in many cases require quite concentrated (several molar) solutions to displace the more intractable species and any conductance changes imparted to the effluent by the appearance of the analyte ions could easily be overwhelmed by the conductance "noise" of the eluent. So we decided — before 1971 — to abandon ion-exchange chromatography and instead develop stationary phases that would separate electrolytes using water as eluent, because water was the perfect background for sensitive conductance measurement. Our early efforts to devise such media were spasmodic and mostly barren of success.

In the fall of 1971, we made a number of significant decisions that led to a breakthrough: We would revert to ion-exchange chromatography as the separation mode and to conductometry as the detection and measurement mode, but instead of placing the conductivity cell right after the separating column, we would insert a second column between the separator and the cell. Initially we called this column the *stripper* because its purpose was to strip the effluent of the highly conducting eluent and leave just the analytes in water, the ideal background for detection. For example, using the workflow depicted in Figure 1, we would separate the alkali metals on a cation-exchange resin using hydrochloric acid as the eluent, then pass the effluent from the separating column through a bed of anion-exchange resin in the hydroxide form, which would strip out the acid and present the alkali metals to the conductivity cell as the metal hydroxides in a water background. Analogously, anions could be separated on an anion exchanger using sodium hydroxide as eluent, while a following bed of cation-exchange resin in the hydronium form would remove the sodium hydroxide, thus presenting the anions (such as the halides) to the conductivity cell as their acids in a water background.

Thus, we would solve the problem of eluent conductance noise simply by removing the eluent and retaining the analytes in a water background.

As a first implementation of this new idea, we proposed using a strong acid cation-exchange resin (Dowex 50, Dow Water and Process Solutions) as separator and a weak-base resin (Dowex 30) to absorb the HCl eluent. But that idea was never tried — for the following reason.

In using the stripper, we were proposing something radically new for chromatography, a component that would become partially depleted with each sample injected and eventually would have to be regenerated. We felt that for this technique to succeed, this regeneration would have to be as unobtrusive as possible; users should be able to run many samples before stripper regeneration became necessary. It became clear that if we used our first proposal we could easily encounter cases where the elution of even a single sample would exhaust a stripper that at the same time had to be massively larger in volume than the separator to supply enough stripping capacity. The large void volume of the stripper bed would be the source of two serious defects: It would greatly prolong elution times and seriously degrade chromatographic efficiency. Clearly, this was not going to fly.

But the problem suggested a solution (5,6): Use separator columns with very low specific capacity, thus enabling elution with low concentrations of eluent; this in turn would prolong the life of the stripper and thus allow the analyses of many samples before regeneration became necessary. And we envisioned that an effective suppressor could be about the same size as the separator, thus minimizing the problems caused by the suppressor void volume. These concepts were central to our invention of IC with eluent suppression and conductometric detection (7).

Separation Media and Eluents for Early IC

The first successful separation by IC was the separation of a mixture of lithium, sodium, and potassium using a lightly sulfonated styrene–divinylbenzene (DVB) polymer as the separator, dilute hydrochloric acid as eluent, and Dowex 1 (hydroxide form) as the stripper. I had used much more separator than necessary so elution times were unnecessarily long, but otherwise the separation looked excellent, and for that era, the detectability levels were impressive (Figure 2).

Because of the very basic eluents anticipated for anion analysis, styrene-based anion exchangers with their great chemical stability were preferred over the silica-based media that were being widely used in high performance liquid chromatography (HPLC). However, although surface sulfonation of styrene-based polymers gave a useful cation separator and surface quaternization of a styrene–DVB polymer seemed the obvious route to a low-capacity anion-exchange resin, I saw it as a route fraught with problems. If I used the same synthetic steps as were used to produce high-capacity resins, I anticipated serious obstacles to creating, on a styrene-based substrate, a thin anion-exchanging shell that would not have a diffuse boundary, and diffuse boundaries were anathema to efficient chromatography. Instead, a prior experience had impressed on me the Velcro-like attachment that anion-exchange resins formed with their cation-exchanging counterparts (1,2) so I created the first useful anion separator by treating a surface-sulfonated styrene–DVB resin with a suspension of a colloidal anion exchange resin (Figure 3). Because of the manifold

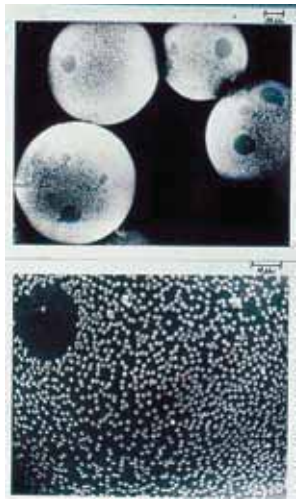

Figure 3: (Bottom) Colloidal anion-exchange particles (about 300 nm in diameter) on surface-sulfonated styrene–DVB particles (about 50 µm in diameter) (top).

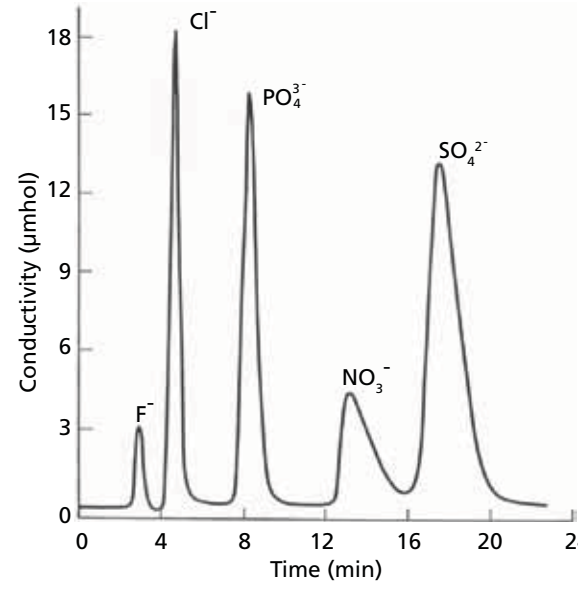

Figure 4: Status of anion IC using carbonate eluent, circa 1974.

advantages of preparing IC media in this way (1,2), particularly to the manufacturer, stationary phases prepared by this means (8) would become, and for many years remain, the workhorse separating media for anion analysis by IC.

Improvements in cation IC came rapidly in our first year but progress in anion analysis was slower. Although sodium hydroxide was an excellent choice in that the stripper action produced the ideal background, the hydroxide ion was an anion of low ion-exchange affinity for anion-exchange resins of that time and high concentrations were required to displace many analyte ions. This high concentration of sodium hydroxide placed a heavy burden on the stripper. This burden was greatly alleviated by using the phenate ion as the eluting anion (5); the phenate ion in the hydronium-form stripper was converted to phenol, a very weak acid, which contributed little to the conductivity of the background. The most significant development in anion eluents, however, was the discovery of carbonate eluents that converted to the weak and therefore feebly conducting carbonic acid in the second column (9). Thus, carbonate eluents became widely used for anion IC for many years to come (Figure 4).

With these developments we introduced a new vocabulary to IC; we realized that the second bed was really converting the eluent to a less conducting form rather than removing it entirely ("stripping") so *suppressor* and *eluent suppression* seemed more appropriate terms and we adopted them from then onwards.

By 1975, we had established the foundations of IC. Particularly, we had developed the procedures for producing anion exchangers in colloidal form, a keystone of stationary-phase production.

1975: IC Goes Commercial

In 1975, the Dow Chemical Company, which by this time had applied for several patents on the new technology, established a licensing agreement with Durrum Chemical, a small company whose main product was amino-acid analyzers. A separate business unit was formed within Durrum to pursue the commercialization of IC. This unit was later spun out of Durrum to become Dionex Corp., surely a major landmark in the evolution of IC. In September of 1975, Dionex signaled IC's public availability by demonstrating the first commercial instrument at the fall meeting of the American Chemical Society. It was also at this time that the term *ion chromatography* was used for the first time. (In 1975, the term *ion chromatography* referred exclusively to the combination of ion-exchange separation, eluent suppression, and conductometric detection. In later years it came to embrace a wide variety of techniques of separation and detection.)

That same fall we published the first article on the new technique (5).

Although the center of gravity of development now moved to Dionex, our small group at Dow stayed involved and had much still to contribute.

1979: IC without Suppression

Although packed-bed suppressors had enabled sensitive conductometric detection, they had some drawbacks. Notably, there was the drifting of elution times for certain analyte peaks (5,6) and a few analytes such as nitrite were degraded by interactions with the resin in the suppressor. Also, there were the interruptions for suppressor regeneration, even though we had made the interruptions to regenerate less obtrusive by arranging for the suppressor to last about an 8-h day and regenerate overnight. The picture on suppressors changed in 1979 when it was shown that ion chromatography could be accomplished using ion exchange and conductometric detection without a suppressor (10–12). This new development turned the spotlight on the suppressor and the drawback of the interruptions for its regeneration. Additionally, this new version of IC was often promoted as avoiding the "complexity" that the suppressor added. The interruptions argument was a legitimate one; the complexity argument was much less so. It is true that for samples sufficiently burdened with analyte, suppressorless IC is an adequate performer, but it has been demonstrated in practice and in theory (13) that the so-called

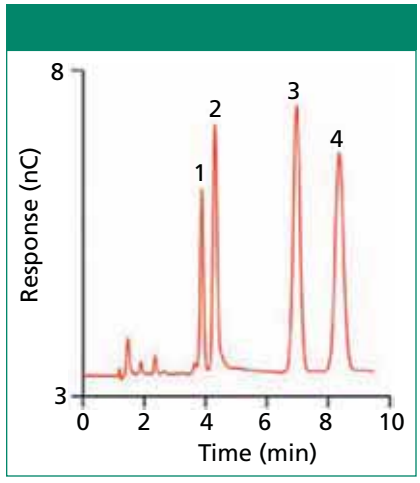

Figure 5: Determination of carbohydrates by IC: 1 = glucose, 2 = fructose, 3 = lactose (internal standard), and 4 = sucrose. Adapted from reference 43 with permission.

complexity of adding a suppressor is the price of extracting the maximum sensitivity from conductometric detection. (Please note: In the context of this article, the term *sensitivity* describes a technique's ability to detect low levels of analyte.) This new development did, however, ignite efforts to devise better suppressors.

Continuous Suppression

When I performed the first anion separation in 1971, I used as suppressor a coil of sulfonated polyethylene tubing immersed in a stirred suspension of a cation-exchange resin (Dowex 50) in the hydronium form. While the sodium hydroxide effluent from the separator was passed through the lumen, sodium ions diffused across the wall of the tubing and exchanged with hydronium ions from the resin as its particles made bumping contacts with the exterior wall of the tubular membrane. The hydronium ions in turn diffused in the opposite direction and united with hydroxide ions to form water. These membrane devices worked quite well as a continuous suppressor but were fragile and prone to bursting, and because the bed suppressors were quite robust and we had more pressing priorities, we shelved the continuous tubular suppressor. When suppressorless IC emerged, we revived the membrane concept and succeeded in fabricating a number of more rugged devices (14,15) and they became the first in a series of continuous, chemically regenerated eluent suppressors. At about the same time, Ban and others obtained a patent on a similar continuous suppressor (16).

Dionex's 1991 introduction of a flat membrane continuous suppressor, the MicroMembrane Suppressor (MMS), was a landmark event. In this device, using anion analysis as the example, the effluent from the separator passed through the narrow channel between closely spaced, flat cation-exchange membranes in the hydronium form. The outside of the membranes was bathed by a continuous stream of sulfuric acid that supplied hydronium ions in exchange for the sodium extracted from the intermembrane channel. These devices were very rugged, could suppress higher concentrations of eluent than their predecessors, and, with their low-volume intermembrane channels, they did not degrade the efficiency of the chromatography to an appreciable extent.

With these developments, the suppressor had evolved from being a conspicuous part of IC, and something of a bother, to being practically invisible to the user.

But further important developments in suppressors lay ahead.

Electrochemical Regeneration of Suppressors

Although the flat membrane continuous suppressor was a major advance, it still had some limitations. In the first place, it required a continuous supply of a chemical regenerant. Secondly, although the membranes were preferentially permeable to the suppressing ion, they did allow some leakage of its co-ion; in an anion suppressor, for example, some regenerant sulfuric acid leaked into the mainstream, raising the background conductivity and compromising the measurement of analytes.

As early as 1984, Jansen and others had shown that electrochemistry could be used in membrane devices to effect eluent suppression (17). By placing electrodes in the regenerant compartments of a device of MMS-like construction, ion transport across the intermembrane space was assisted by the electric potential applied to the electrodes. However, because electrolyte was used in the electrode chambers, these devices could be expected to show undesirable electrolyte leakage into the mainstream.

Another landmark in the development of suppressors was the electrochemically regenerated suppressor or the Self-Regenerating Suppressor (SRS) of Dionex (18).

While these devices used an arrangement of membranes and electrodes similar to the Jansen device and to the MMS, the electrode compartments of the SRS were flushed with deionized water. Using anion analysis with sodium hydroxide eluent as the example, when the electrodes were DC-polarized, hydronium ions produced at the anode were driven by the applied field across the cation-exchange membranes, forcing sodium ions into the cathode compartment where they united with the cathodically generated hydroxide ions and the sodium hydroxide was flushed to waste. By eliminating the chemical regenerant, the SRS eliminated the problem of regenerant leakage. And in an improved embodiment of the SRS, the water effluent from the IC operation was directed to the electrode compartments, thus eliminating the need for an extra water pump (19).

Electrochemistry also revived the packed-bed suppressor (20,21). In the Dionex Atlas suppressor, a small packed bed of ion-exchange resin, embraced by ion-exchange membranes, is continuously regenerated by polarizing the bed.

Revival of the Packed-Bed Suppressor with Chemical Regeneration

By the 1990s, analytical chemistry was augmented by a powerful ally, the computer. With the computer came the ability to automate many operations. Initially, we had introduced the "dogma" that a packed bed needed to suppress many samples before regeneration, but we now realized that with automated valve switching, a small suppressor with just single-sample capacity was viable and would require little intervention from the user (22). This basic idea was later implemented by manipulating three small suppressor beds in a clever three-compartment-revolver device (23) and marketed by Metrohm. Two major advantages of this small-bed approach over the earlier large suppressor beds are the virtual elimination of peak drifting and minimal degradation of chromatographic efficiency by peak spreading in the void space of the small suppressor bed.

Electrochemical Generation of Eluents for IC

While electrochemistry was a boon to suppression, it had another important role to play in IC.

Pioneering work by Dasgupta and others (24,25) had demonstrated that while membrane systems could remove eluent in IC they could also be used to introduce eluent in a controlled way, simply by pumping water to a suitable electrically polarized membrane device. They also recognized that the production of "pure" sodium hydroxide by such a system could provide major advantages for anion analysis by IC. In the early years of IC, carbonate eluents were successful and widely used but they had a few significant shortcomings. One was that carbonate suppressed to carbonic acid, which has low conductivity but orders of magnitude higher conductivity than pure water. Another was that the carbonate background conductivity was lowered by the presence of analyte; this was not a big issue when the analyte was abundant, but caused nonlinear responses at lower analyte levels. A third drawback was that gradient elution, as it became more of a requirement, was complicated by the ramping baseline conductivity of the carbonic acid background. Sodium hydroxide (or potassium hydroxide) as eluent had always been a sort of holy grail for IC because it could be suppressed to the ideal background, water, but two issues delayed its adoption: Hydroxide ion was a relatively ineffective displacing ion, thus demanding high suppression capacities, and it was notoriously difficult to prevent its contamination by omnipresent carbon dioxide that altered its eluting power in unpredictable ways and led to unstable backgrounds. Although the new MMS suppressors had much greater suppression capacity, thus diminishing the first issue, the carbonate-in-the-eluent problem remained. The membrane-based electrochemical generator alleviated the contamination problem to a great extent because the generator could be provided with ultrapure water and the generator was intrinsically a generator of pure carbonate-free base (26).

The new eluent generators had these positive features::

- The operator was freed from the task of frequently preparing eluents with its attendant problems of contamination and occasional operator error.
- Eluent concentration was controllable simply by controlling the current in the generator or the flow rate of the water stream, or both.
- They greatly simplified the creation of gradients.

Concurrent with these developments in hydroxide generators, new separation media with much greater affinity for hydroxide (see a later article in this issue) enhanced the impact of the electrochemical generators in anion analysis.

Ion Reflux and Eluent Recycling

While the new electrochemically based eluent generators have made a major change to the trajectory of IC, they do exhaust and have to be replaced at a cost to the user. In the late 1990s, we invented a hybrid of suppression and regeneration where the effluent from the suppressor is not discarded to waste but instead is recaptured and used again as eluent. In principle, these systems could work perpetually simply by pumping water to the IC system. In one embodiment, called *ion reflux,* the three components of an IC operation — eluent generation, separation, and suppression — were performed continuously within a single column, with just water as the pumped phase. In another embodiment of ion reflux the separator phase was uncoupled from the other two functions, allowing any stationary phase to be used (27,28).

Another invention, called *eluent recycling* (29), also enabled reuse of eluent.

Reference 30 provides a comprehensive and detailed review of these developments in electrochemistry as applied to suppression and eluent generation in IC.

Other Detection Methods in IC

Although conductometric detection was the method that launched IC, it became obvious that other detectors could be used with the new separation media. UV detectors were effective when the analytes were UV-absorbing. And of course, UV detectors did not require a suppressor, although it should be added that conductometric detection was often the preferred method when the analyte mixture contained target ions, some of which were UV-absorbing and others not.

It had been a sort of dogma in detection that UV detectors were usable only if the analytes were UV-absorbing. We showed, however, that using ion-exchange separation coupled to UV detectors could indeed be used to detect and sensitively measure UV-transparent ions (31). We called this combination *indirect photometric chromatography* (IPC) and the detection principle *indirect photometric detection* (IPD). Although IPD had eliminated the need for a suppressor and performed well in many applications (32), it lacked the sensitivity reach of conductometric detection and received little promotion in IC. However, the principle became widely used in capillary electrophoresis and somewhat in HPLC. Our work was rewarded by recognition as a milestone in analytical chemistry (33).

Carbohydrates and alcohols are not usually thought of as ionics, but Rocklin and Pohl discovered how their intrinsic ionicity could be expressed and used to chromatographically separate them (34). Because carbohydrates are extremely weak acids they express their ionicity only at very high pH, but Pohl and coworkers saw the opportunity of exploiting this and separated carbohydrates by ion exchange using strongly basic eluents (Figure 5). The anion-exchange resins of IC, with their great stability in high-pH environments, were important facilitators of this new technique. Of course, suppressed conductometric detection was a nonstarter because the carbohydrate anions would revert to their non-conducting form in the typical suppressor, so amperometric detection methods, particularly pulsed amperometric detection (PAD) (35,36), became important adjuncts to IC and enabled IC separation of a wide variety of analytes (37).

This application of IC to nonionics was a distinctly new and different trajectory for the method and certainly a landmark event.

IC with Water as Eluent

Although in the early years we made little progress using simply water as the eluent, others have been notably more successful. A significant landmark in the evolution of IC is the work of Lamb and others using macrocylic species that form selective and reversible complexes with electrolytes in pure water (38).

Applications of IC

It is impossible, in an article of this length, to do justice to the pioneer users of IC and the many landmark applications that expanded the method's usefulness. Therefore, I will restrict my choice to two: the first symposium on IC and the series of

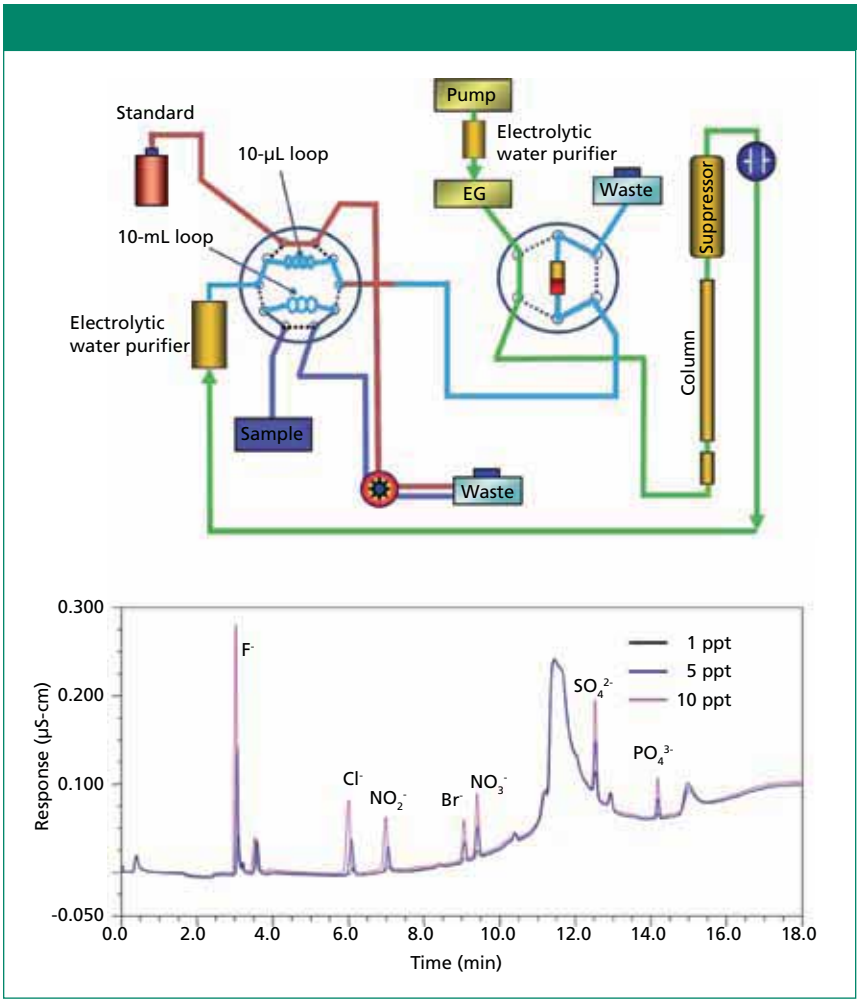

Figure 6: A dual-loop injection valve and concentrator column are used to automate calibration and sample loading. Electrolytic deionization of the conductivity cell waste produces ionically pure water for in-line standard dilution, sample loading, and loop rinsing. This system results in extremely low detection limits (parts per trillion), low blanks, and high precision. The chromatogram was generated using a Dionex ICS3000 system with a KOH generator and ASRS300 suppressor (2 mm). Columns: IonPac AG17 and AS17 (2 mm); flow rate: 0.5 mL/min; loop volumes: 10 µL and 10 mL. Concentrator column: UTAC-ULP2 low pressure anion concentrator. (Adapted with permission from Trovion Singapore Pte Ltd.)

developments that have produced astounding advances in the detectability limits of IC. (References 32 and 37 are recommended for accounts of the myriad applications of IC.)

After the introduction of IC in 1975, it took a certain boldness to embrace this brand-new technology; there were many who said it would not prosper, or as one guru expressed it — so I've been told — "IC was fatally flawed" by the suppressor. But many did embrace it, and some of their early creative applications are recorded in the proceedings of that first symposium at Gatlinburg organized by the U.S. Environmental Protection Agency (39).

There are at least two industries that depend on water of the highest available purity: the power-generating industry and the electronics industry. The first must protect its critically important boilers from the corrosive effects of ions, notably chloride, while in the second, sensitive electronic components can be irreparably damaged by traces of electrolytes in processing water. Careful monitoring of water streams is vital to both these industries so there is great demand to extend the sensitivity of IC as much as possible. By adhering to scrupulously clean procedures, such as the *in situ* production of ultrapure water by electrochemical deionizers, adventitious contamination of samples and eluents can be avoided and techniques have been developed that enable the detection and measurement of ions at the parts-per-trillion level (Figure 6).

In our earliest embodiments of IC we were proud of our ability to measure at the parts-per-million level; all the efforts that have extended detectability of ions by a million-fold are truly landmarks in the evolution of IC.

Speculations on the Future of IC

It is to be expected that IC will continue its penetration into ion analysis and it is likely that special cases will emerge, like sulfate in the early days or perchlorate more recently, where IC will display its unique ability to solve urgent analytical problems.

As to the future of IC technology, the indicators already point in the direction of smaller-scale instruments, with their many benefits (40). From our earliest days in IC we were aware that the conductivity cell was unique among chromatographic detectors in its amenability to miniaturization and recent developments in detectors, where electrodes make capacitive contact with the contents of capillaries (41), are a significant step in this direction.

IC and other forms of liquid chromatography use elution times of analytes as the defining measure of their chromatographic behavior. The elution time of an analyte is not a fundamental property in chromatography, but time has the great advantages of ease of measurement and of almost indefinite subdivision. However, for elution time to be a reliable definer of the chromatogram, the chromatographic pump must deliver very stable and precisely controlled flow. The pump is thus a flowmeter as well as a mover of eluent. As a result, the pump is often the most highly engineered and costly component in the system. Further, reductions in pump size do not seem to be keeping pace with the miniaturization of stationary phases and detectors.

A more fundamental property than an analyte's elution time is its elution volume, and development of volume flow meters as a separate chromatographic device would relieve the pump of this task. This should, in turn, lead to a significant reduction in pump size and complexity. We have recently taken a step toward reducing pump size by inventing an electrochemically driven pump (42).

In IC there is a property of analyte elution that is even more fundamental than

elution volume. Assuming that the eluent and the stationary phase have been established, then, with one important caveat, the number of equivalents of displacing ion required to elute an analyte is fixed; or the number of coulombs to elute is fixed if an eluent generator is being used. (This is true only if the analyte and the displacing ion are of the same valence; it is more complicated if they are not, but there are solutions for this complication.) So IC chromatograms might be defined not by time or volume but by the number of coulombs applied. Does this mean not only that the pump need not be supremely stable but that we might also in some cases even dispense with a flow meter? In IC, the field is open for innovation in how eluent is delivered and its flow measured.

IC eluent generators include a reservoir of concentrated acid or base as the source of the eluent and the membrane or membranes that separate this reservoir from the mainstream must be of substantial thickness to prevent leakage of the base (or acid) into the mainstream. A reservoir of ion-exchange resin, the perfectly nondiffusible electrolyte, would offer a remedy for this problem and is worth examining. And the neglected area of eluent recycling (27–29) is likely to be re-examined in the years ahead.

In the next several years, the landscape of IC will change as dominant patents expire and other players enter the field. Where this might lead is beyond speculation, but we can be certain that new inventions and innovations will emerge to challenge the status quo, just as they have since the beginning of IC.

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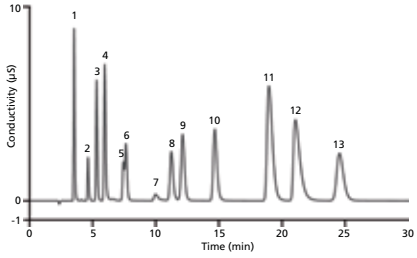
Recent Developments in Ion-Exchange Columns for Ion Chromatography



Ion-exchange chromatography is a relatively mature area of chromatographic separation yet advances in this technique continue unabated. This article provides a summary of the latest in new ion-exchange phases for ion chromatography. It starts by focusing on general aspects of phase design and then reviews anion-exchange and cation-exchange columns introduced in the past few years.

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Ion chromatography (IC) continues to be the chromatographic technique most widely used for the separation of ionic and ionizable compounds, with a special focus on the analysis of inorganic anions, inorganic cations, small hydrophilic organic acids, and aliphatic amines. Although a number of separation modes are included under the umbrella term of *ion chromatography,* ion exchange is by far the most widely used technique in IC. Although use of ion-pair techniques in conjunction with reversed-phase columns remains a viable alternative to ion exchange for IC applications, ion exchange continues to be the focus of development when it comes to new stationary phases designed for specific applications involving the separation of ionic compounds. There are a number of reasons why ion exchange has proven to be the preferred separation technique in IC. These include a broad range of available selectivities, the ability to tailor selectivity for specific applications, the exceptional chemical stability of polymeric ion-exchange materials, the ability to separate ions of similar size, and rapid equilibration when operated in the gradient mode.

Stationary-Phase Architecture

Stationary-phase construction for IC columns comprises nine basic architectures: silane-based modification of porous silica substrates, electrostatic-agglomerated films on nonporous substrates, electrostatic-agglomerated films on ultrawide-pore substrates, polymer-grafted films on porous substrates, chemically derivatized polymeric substrates, polymer-encapsulated substrates, ionic molecules adsorbed onto chromatographic substrates, step-growth polymers on polymeric substrates, and hybrid materials based on a combination of a silane-modified silica substrate with a polymeric exterior surface coating. Five of these — electrostatic-agglomerated films on ultrawide-pore substrates, polymer-grafted films on porous substrates, chemically derivatized polymeric substrates, polymer-encapsulated substrates, and step-growth polymers on polymeric substrates — represent the architectures most widely used in recently introduced phases. Hence it's worth taking a deeper look at these five architectures to better understand their relative strengths and weaknesses.

Electrostatic agglomerated films on ultrawide-pore substrates: For

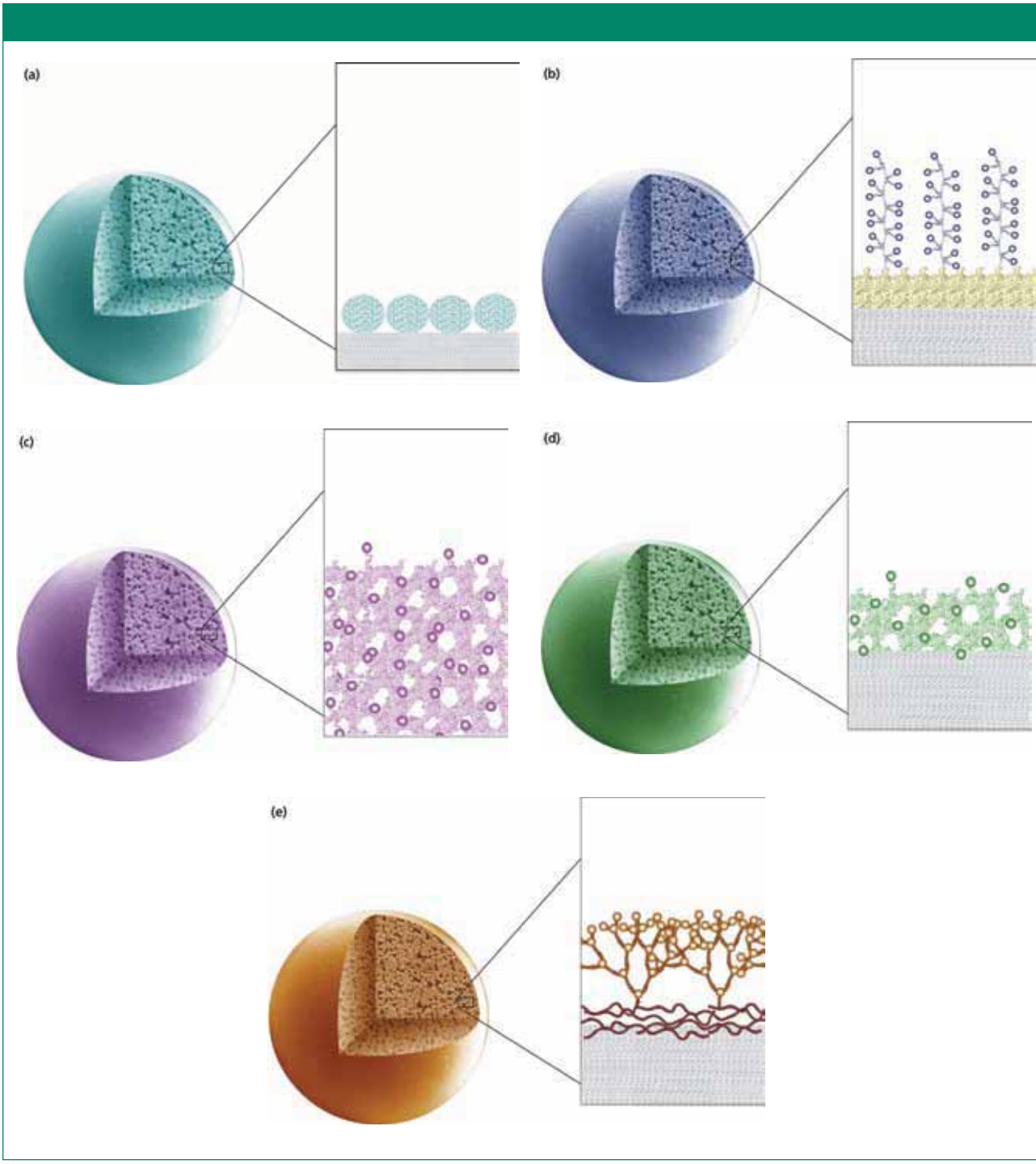

Figure 1: Ion chromatography stationary-phase architectures most widely used in recently introduced phases: (a) electrostatic agglomerated ultrawide-pore substrates, (b) polymer-grafted film on porous substrates, (c) chemically derivatized polymeric substrates, (d) polymer-encapsulated substrates, and (e) step-growth polymers on polymeric substrates.

the most part, electrostatic agglomerated films on nonporous substrates have been largely supplanted by higher capacity versions utilizing ultrawide-pore substrates (Figure 1a). By using an architecture similar to that based on nonporous substrates, but making use of substrates with pore sizes in the 100–300 nm range, it is possible to construct materials with substantially higher capacity (1). The pore size of the ultrawide-pore substrate and the particle size of the colloidal ion-exchange material are chosen such that the pore size is large enough to accommodate a coating of ion-exchange colloid on both the interior and the exterior surfaces of the porous substrate. With the optimal ratio of substrate pore size to colloidal particle size, the resulting material can exhibit 6–8 times the capacity achievable on an identical particle size nonporous substrate (that is, 30–150 µEq/mL for materials using an ultrawide pore format compared to 5–30 µEq/mL for materials using a nonporous format).

Given the increasing importance of high capacity chromatographic materials in IC and the availability of high capacity suppressor devices, this stationary phase architecture has seen wide application in recent years.

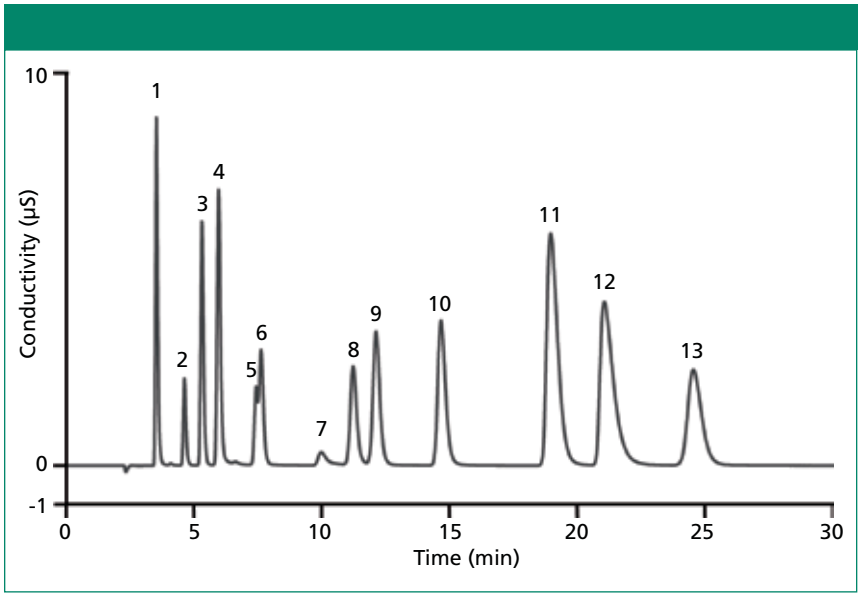

Figure 2: Isocratic separation of sulfur species and inorganic anions. Column: IonPac AS25, 4 mm; eluent: 37 mM potassium hydroxide; eluent source: EGC III KOH; flow rate: 1 mL/min; injection volume: 25 µL; temperature: 30 °C; detection: suppressed conductivity, ASRS 300 4 mm, AutoSuppression recycle mode, 92 mA. Peaks: 1 = fluoride, 2 = bromate, 3 = chloride, 4 = nitrite, 5 = bromide, 6 = nitrate, 7 = carbonate, 8 = sulfite, 9 = sulfate, 10 = iodide, 11 = thiocyanate, 12 = perchlorate, 13 = thiosulfate.

Polymer-grafted films on porous substrates: This type of material (Figure 1b) is widely used to prepare high capacity packings where crosslinking is not required for selectivity control. Chromatographic materials of this sort are prepared through attachment of polymer strands to the surface of a substrate (2,3). To prepare such materials, the substrate is either prepared with polymerizable groups on the surface, the surface is modified to introduce polymerizable groups, or the surface is modified to introduce an initiator species. Resin, monomer (or monomers), and initiator are then allowed to react to produce a composite polymer graft with polymer strands projecting from the substrate surface. Because including a crosslinking monomer into the reaction mixture will cause the reaction mixture to form a gel with substrate particles suspended in the gel, this synthesis approach precludes the use of crosslinking monomers. The fact that no crosslinker can be used in grafted polymer films limits the ability to control

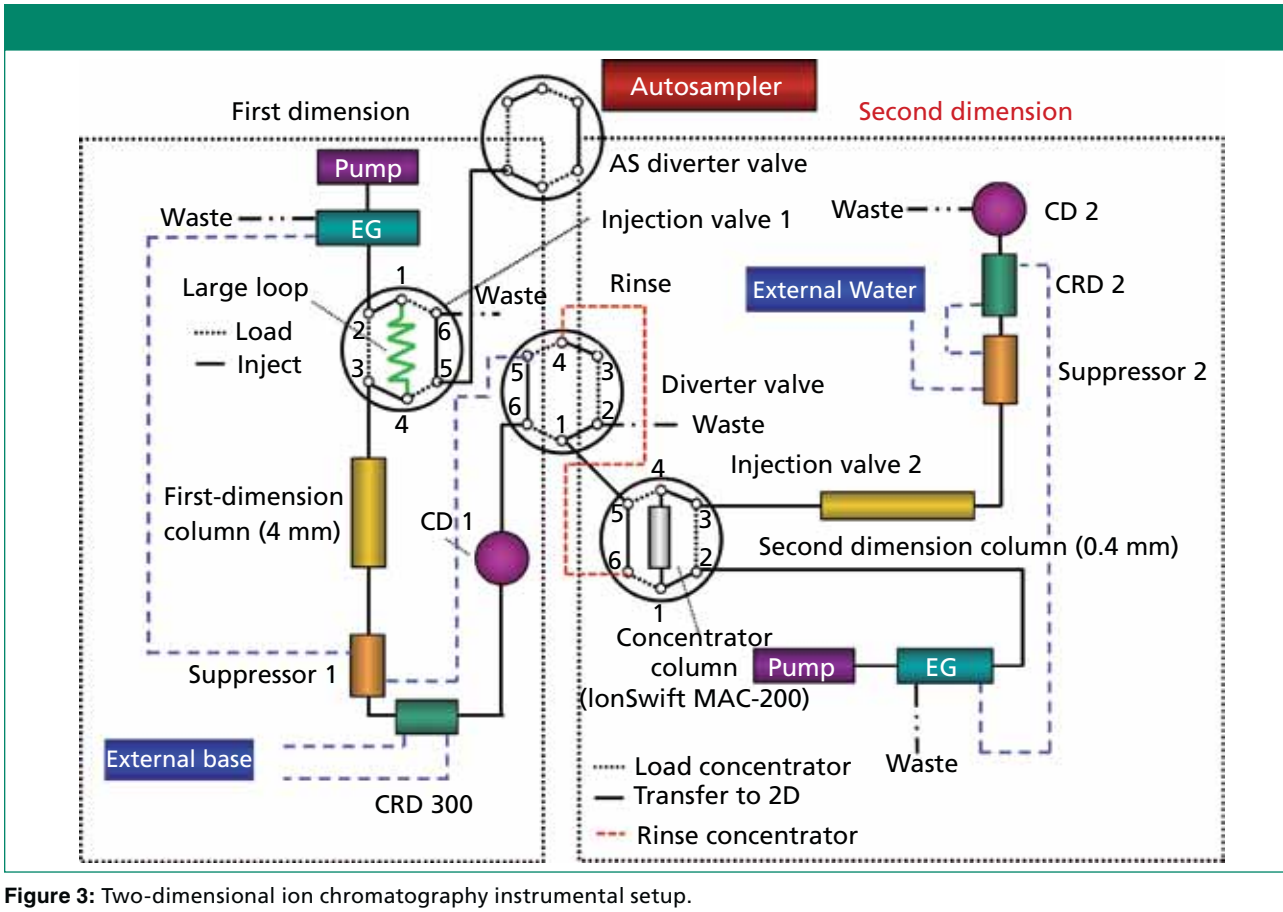

Figure 3: Two-dimensional ion chromatography instrumental setup.

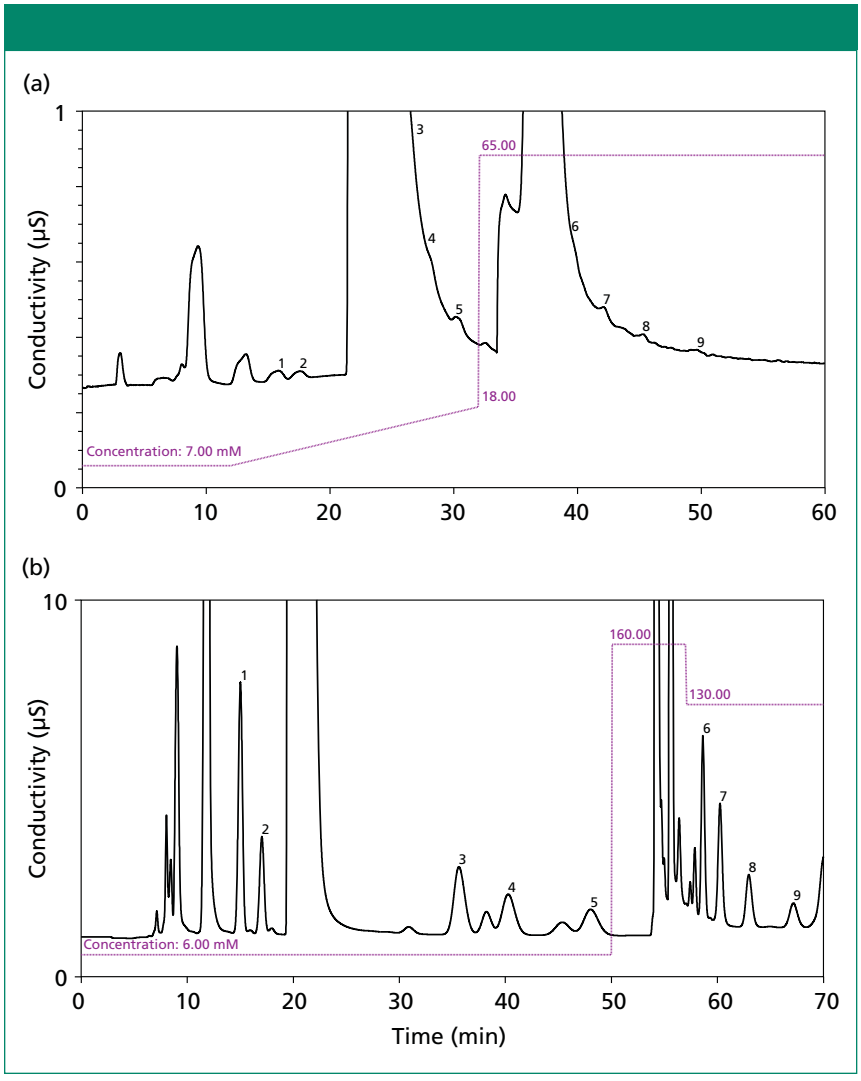

Figure 4: Two-dimensional ion chromatography analysis of haloacetic acids: (a) D1 columns: Dionex IonPac AG24A, AS24A, 4 mm; flow rate: 1.0 mL/min; eluent: potassium hydroxide, 7 mM (0–12 min), 7–18 mM (12–32 min), step to 65 mM at 32.1 min; suppressor: Dionex ASRS 300, 4 mm; current: 161 mA; loop: 500 µL; oven: 15 °C. (b) D2 columns: Dionex IonPac AG26, AS26, 0.4 mm; flow rate: 0.012 mL/min; eluent: potassium hydroxide, 6 mM (0–50 min), step to 160 mM at 50 min, step to 130 mM at 57 min; suppressor: Thermo Scientific Dionex ACES anion capillary electrolytic suppressor; current: 25 mA; concentrator: MAC-200; oven: 14 °C. Matrix: A. HIW (250 ppm Cl, 250 ppm SO_4, 150 ppm HCO_3, 10 ppm NH_4Cl). Peaks: 1 = MCAA, 2 = MBAA, 3 = DCAA, 4 = BCAA, 5 = DBAA, 6 = TCAA, 7 = BDCAA, 8 = CDBAA, 9 = TBAA.

selectivity in such grafted films. This architecture is mainly used in applications that require a stationary phase with relatively high capacity and high water content. Such materials can be prepared from either polymer-based or silica-based substrates, but in practice nearly all such materials are produced using polymeric substrates.

Chemically derivatized polymeric substrates: This type of material (Figure 1c) tends to involve proprietary synthesis techniques, so the actual chemistry used for the derivatization reaction is generally unknown in commercial products. In general, chromatographic materials of this sort have substantial capacity because functional groups are not necessarily limited to the surface of the substrate. Such materials have become popular in recent years as column capacities have shifted higher. The critical difficulty with this stationary-phase synthesis methodology is the requirement that the derivatization be constrained to the surface to achieve good chromatographic performance. Reactions that take place beneath the surface in the dense polymer matrix of the substrate will exhibit sluggish mass transport and relatively poor chromatographic performance. Early examples of this stationary-phase architecture exhibited relatively poor performance but newer materials such as the IC SI-52 4E column (Showa Denko) illustrate that high performance materials can indeed be constructed in this manner.

Polymer-encapsulated substrates: Professor Gerard Schomburg of the Max Planck Institute in Mulheim-Ruhr, Germany, pioneered this type of material (Figure 1d) as a means of preparing materials for reversed-phase chromatography using alumina as the base material. Synthesis of polymer-encapsulated materials is accomplished by combining the substrate, a preformed polymer with residual double bonds, and a suitable free radical initiator dissolved in an appropriate solvent, stripping off the solvent to leave a polymer film on the surface of the substrate, and then curing the film at elevated temperature to yield a crosslinked film permanently encapsulating the substrate. The advantage of this architecture is that chemical attachment to the surface of the substrate is not required, allowing it to be used with inorganic substrates not amenable to covalent modification. Although initially developed as a means of producing a reversed-phase material based on alumina, the technique was later adapted by Schomburg's group as a means of preparing a weak-cation-exchange phase using a preformed butadiene-maleic acid copolymer as the encapsulating polymer (4). The first commercial introduction of stationary phases based on this approach brought about a major shift in stationary-phase design as applied for the separation of inorganic cations. Before the introduction of this new synthesis method, nearly all separation products were based on strong-acid cation-exchange stationary phases. Since that time, nearly all stationary phases utilized for the separation of inorganic cations have used weak-cation-exchange carboxylic acid–based stationary phases. A disadvantage of this synthetic approach is

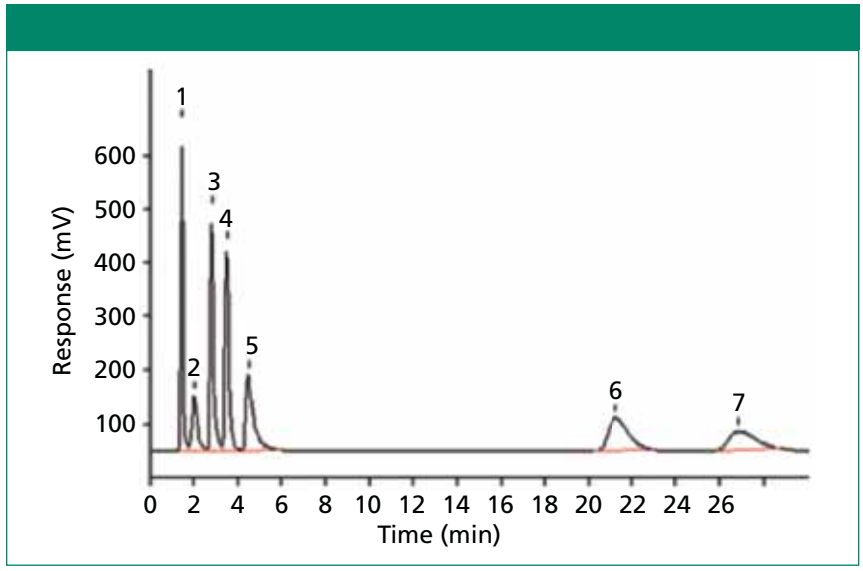

Figure 5: Ion chromatography separation of transition metal cations. Column: 150 mm X 4.6 mm Metrosep C5; eluent: 6 mM oxalic acid, 3 mM citric acid adjusted to pH 4.2 (KOH); flow rate: 1 mL/min; PCR reagent: 0.15 mM 4-pyridylazoresorcinol, 0.4 M ammonium hydroxide, 80 mM nitric acid; PCR flow rate: 0.5 mL/min; detection: absorbance (vis) at 530 nm. Peaks: 1 = copper (5.00 mg/L), 2 = nickel (3.00 mg/L), 3 = zinc (4.00 mg/L), 4 = cobalt (5.00 mg/L), 5 = lead (30.0 mg/L), 6 = manganese (4.00 mg/L), 7 = cadmium (7.00 mg/L).

the possibility of swelling and shrinking of the phase during gradients or temperature programming depending on the cure conditions of the film. In addition, even if the coating is free of surface defects, alkaline reagents can still attack the underlying silica by penetrating to the surface coating, resulting in bed collapse.

Step-growth polymers on polymeric substrates: This simple yet versatile synthesis method has seen wide use in recent years (Figure 1e). Over the past decade, more than 10 anion-exchange columns have been introduced using this stationary-phase architecture. This synthesis approach is a hybrid of the first and second architectures described above. Stationary-phase preparation begins with functionalization of a wide-pore substrate to introduce anionic surface charges (5,6). Then, an epoxy-amine copolymer is formed in the presence of this material, producing an amine rich "basement" polymer that is electrostatically bound to the resin surface. Finally, in a repetitive series of reactions, this polymer-coated substrate is allowed to react with first an epoxy monomer containing at least two epoxy functional groups and then an amine or ammonia. By using a primary amine or ammonia or a trifunctional epoxy monomer, it is possible to introduce branch sites. The resulting surface composite can be exceptionally hydrophilic because the epoxy monomer and the amines used in its construction contain only aliphatic substituents. And yet, such materials are completely compatible with high-pH mobile phases that tend to damage most hydrophilic stationary phases.

New Anion-Exchange Chromatography Columns

A number of new anion-exchange columns were introduced in the last few years. Thermo Fisher Scientific extended its Dionex IonPac stationary phases with the introduction of IonPac AS25 polymeric anion-exchange columns with alkanol quaternary ammonium functionality. This column uses the type 5 architecture described above but unlike prior versions of this chemistry it uses alternating reactions with a di-epoxide and a di-tertiary amine to produce linear projecting strands. Earlier versions of this architecture had relatively poor selectivity for sulfate and sulfite. By controlling the gap between the tertiary amine sites in the polymer chain, the selectivity for these two anions can be controlled. Figure 2 shows an example application of this column for the analysis of sulfur species, an application not possible on previous columns using this architecture.

In addition, two new specialty high capacity columns, the IonPac AS24A (4 mm i.d.) and IonPac AS26 (0.4 mm i.d.) columns (Thermo Fisher Scientific), were introduced. These columns are specifically designed for analysis of haloacetic acids via two-dimensional (2D) IC. The technique of 2D chromatography uses two columns in a switching arrangement and is useful to achieve improved separations of complex mixtures by selecting (heart-cutting) unresolved components from the primary column and further separating them on the secondary column that has a different selectivity than the primary column. The technique is also useful for samples where there is a mismatch in concentration levels such that quantitation of the smaller concentration solutes is jeopardized. Figure 3 shows a schematic representation of the instrumental setup that allows these two columns to be used in combination for the analysis of trace levels of haloacetic acids in the presence of high levels of common inorganic anions such as chloride and sulfate. On the left side of the schematic is shown the configuration for the 4-mm columns. Analytes of interest are separated at least partially on the 4-mm column after which they pass through suppressor 1, a carbonate-removal device (CRD), a conductivity cell, the diverter valve, and finally onto a concentrator column located in injection valve 2 where the analyte bands are refocused before reinjection onto a capillary column. Because the second-dimension column has a 100-fold smaller cross-sectional area, a 100-fold increase in detection sensitivity is achieved compared to using columns of identical internal diameter for both portions of the separation. Figure 4 shows an example separation using these two columns in this 2D IC application. The two columns were developed in collaboration with a team of scientists from the United States Environmental Protection Agency (USEPA). Because drinking water samples vary widely in terms of

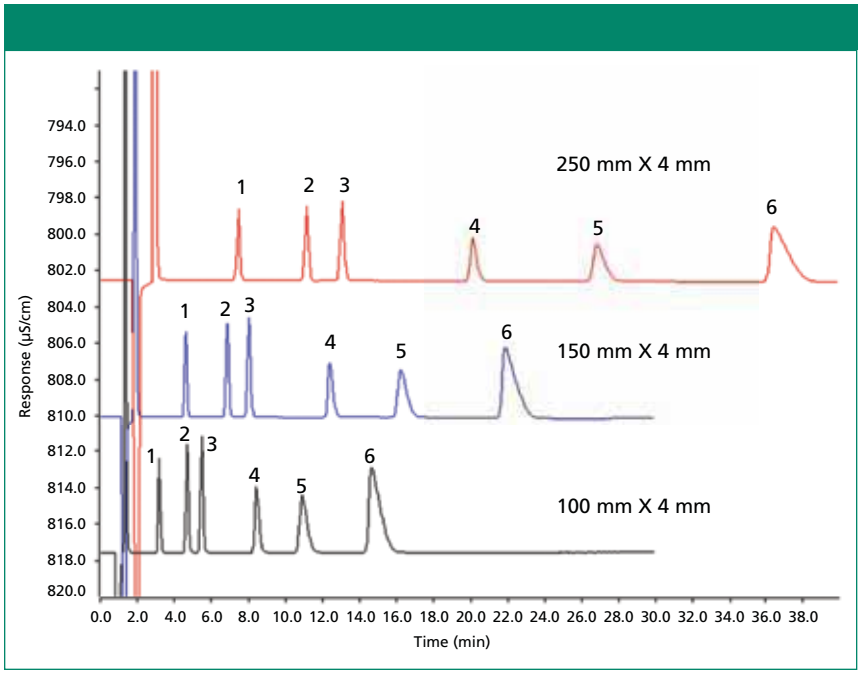

Figure 6: Ion chromatography separation of alkali metals and alkaline earths. Column: Metrosep C6, 4 mm; eluent: 1.7 mM nitric acid, 1.7 mM dipicolinic acid; flow rate: 0.9 mL/min; temperature: ambient; detection: conductivity. Peaks: 1 = lithium, 2 = sodium, 3 = ammonium, 4 = potassium, 5 = magnesium, 6 = calcium.

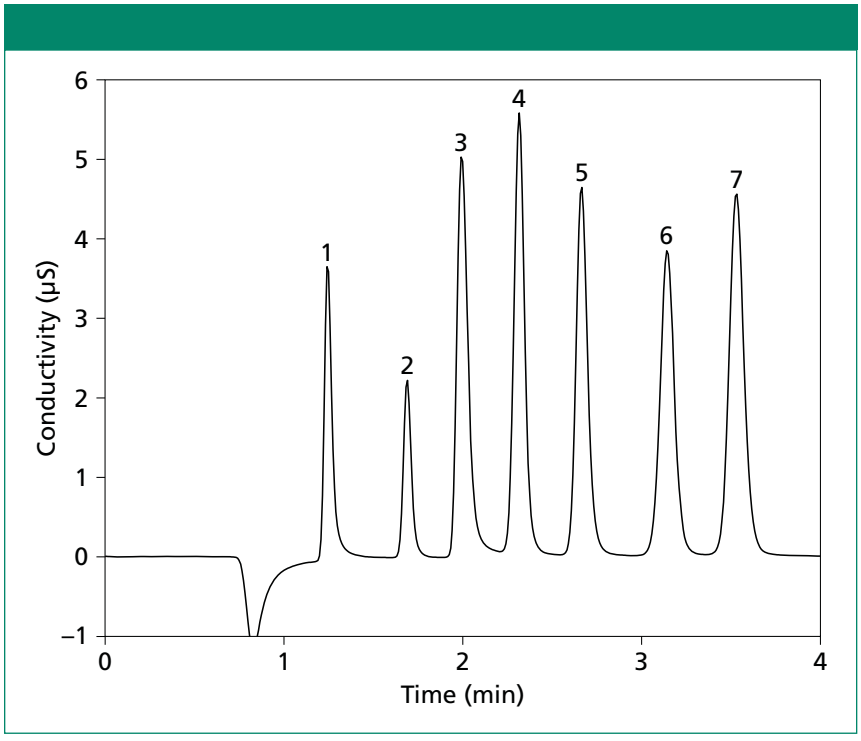

Figure 7: Fast ion chromatography analysis of common inorganic anions. Column: 100 mm X 4.6 mm TSKgel SuperIC-Anion HS; eluent: 3.8 mM sodium bicarbonate and 3 mM sodium carbonate; flow rate: 1.5 mL/min; injection volume: 25 µL; temperature: 30 °C; detection: suppressed conductivity, ASRS 300 4 mm, AutoSuppression, recycle mode, 77 mA. Peaks: 1 = fluoride (1 ppm), 2 = chloride (3 ppm), 3 = nitrite (5 ppm), 4 = bromide (10 ppm), 5 = nitrate (10 ppm), 6 = phosphate (15 ppm), 7 = sulfate (15 ppm).

ionic composition and ionic strength, the first of these two columns used in the 2D configuration was developed in a high-capacity format. The second column is of somewhat lower capacity as most of the matrix components are diverted to waste rather than being passed through the second column. Both columns use type 5 architecture to achieve good selectivity and excellent peak shape for haloacetic acids, which are highly polarizable anions and tend to exhibit poor peak shape on most polymeric anion-exchange columns.

New Cation-Exchange Chromatography Columns

In the area of cation-exchange columns, Metrohm recently introduced two new cation-exchange columns: the Metrosep C5 column and more recently, the Metrosep C6 column. The Metrosep C5 is a strong-acid cation column with a polystyrene–divinylbenzene substrate and is based on type 3 stationary-phase architecture. The column allows the separation of transition metal cations when using chelating mobile-phase components, as shown in Figure 5. This column is designed to be used in conjunction with postcolumn addition of colorimetric metal chelating agents such as 4-pyridylazoresorcinol (PAR), allowing low parts-per-billion detection limits. In addition, Metrohm also recently introduced the Metrosep C6 column, which is based on type 4 stationary-phase architecture. The column is well-suited to samples with analytes exhibiting extreme concentration differences. The utility of the column can be demonstrated, for example, by environmental water samples, where low levels of ammonium can be quantified in the presence of 12,500-fold higher concentrations of sodium. With the Metrosep C6 column, all of the more common alkali metals and alkaline earths can be determined in a single run (see Figure 6).

Another new cation-exchange column targeting suppressed IC applications is the IonPac CS19 column (Thermo Fisher Scientific). This weak-cation-exchange column makes use of an ultrawide-pore substrate with type 2 stationary-phase architecture. It is the first column in the IonPac cation-exchange column family to use ultrawide-pore substrate morphology. The substrate enables exceptionally good chromatographic performance for inorganic cations while providing excellent

peak shape for common aliphatic amines, which is more difficult to achieve with higher surface area media with small pore size.

Columns with a Smaller Internal Diameter

In addition to the new columns mentioned above, there is an increasing trend toward the use of smaller internal diameter columns in IC. Microbore (1–2 mm i.d.) and capillary columns (<1 mm i.d.) have two main advantages. The first is higher sensitivity in analyses with a limited amount of sample. If the same mass of sample is injected onto a column with a smaller internal diameter, the peak height will increase, perhaps allowing one to measure smaller concentrations of substance. Second, to maintain the same separation time, the linear velocity must be the same. For a column with a smaller internal diameter, this means that the flow rate must be decreased proportionally to the inverse radius-ratio squared. The result is lower usage of solvent.

Over the past few years there has been a significant expansion in the range of column chemistries available in 2-mm i.d. columns. Metrohm added four new chemistries to its 2-mm column portfolio: the Metrosep A Supp 10, Metrosep A Supp 15, Metrosep A Supp 16, and Metrosep C4 column chemistries. Thermo Fisher Scientific added the IonPac AS24A, IonPac AS25, and IonPac 26 column chemistries to its already extensive range of 2-mm column chemistries. In addition, over the past few years, Thermo Fisher Scientific has expanded its column portfolio to include more than 20 different column chemistries in capillary (400 µm i.d.) column formats.

Columns Based on Reduced-Particle-Size Media

With the advance of ultrahigh-pressure liquid chromatography (UHPLC), the high performance liquid chromatography (HPLC) community has seen a significant increase in the number of small-particle-size columns available for HPLC applications. In recent years, this trend has begun to influence new column introductions in IC as well. Smaller particles tend to provide improved separation efficiencies compared to larger particles and allow the use of shorter columns to achieve similar separations. For example, Tosoh Bioscience recently introduced the TSKgel SuperIC-Anion HS column, which it describes as a "hypervelocity anion analysis column." Based on 3.5-µm particle size media, the new column is available in a 100 mm × 4.6 mm format, optimized to take advantage of the highest plate count possible with small particle media for fast analysis of common inorganic anions (see Figure 7). In addition, Shodex has introduced IC SI-35 4D column, which is based on 3.5-µm particle size polyvinylalcohol media in a 150 mm × 4 mm column format. Taking advantage of the small particle size and associated high chromatographic efficiency, this phase enables fast analysis of the common anions and oxyhalide disinfection by-products in less than 14 min. The same column is useful for the analysis of a number of common aliphatic carboxylic acids. Following the same trend, over the past 12 months, Thermo Fisher Scientific has made three popular column chemistries available with 4-µm particle size substrates: the IonPac AS18-4µm, IonPac AS11-HC-4µm (both using type 1 architecture), and IonPac CS19-4µm chemistries. Each of these three columns was separately optimized targeting specific application areas. The IonPac AS18-4µm column was optimized for fast analysis of common inorganic anions; the IonPac AS11-HC-4µm column was optimized for high-resolution separations of inorganic anions and carboxylic acids; and the IonPac CS19-4µm column was optimized for high-resolution separations of inorganic cations and aliphatic amines.

Conclusions

New ion-exchange columns for IC continue to be introduced each year, as improvements in column selectivity progress. The growth in ion exchange is spurred by new environmental regulations and growing concerns over possible food contamination. Most new columns have been packed with rugged polymeric-based materials, and this trend will undoubtedly continue. Most ion-exchange columns tend to have relatively high ion-exchange capacities. Increased ion-exchange capacity is important for challenging applications where analytes span a wide concentration range. A trend toward the use of columns with smaller internal diameters is clearly apparent with the 2-mm i.d. columns now widely available and capillary column formats available in most common column chemistries. In addition, particle sizes for IC columns have decreased in recent times, following the trend in HPLC and UHPLC.

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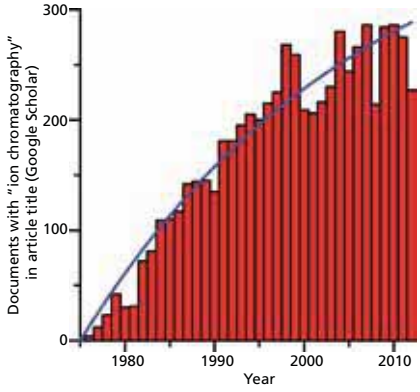
Ion Chromatography Yesterday and Today: Detection



Here, we look at the current status of detection in ion chromatography (IC), focusing on the most popular detectors in IC: the conductivity detector and the charge detector. Conductivity detection, including the capacitively coupled contactless conductivity detection, is discussed. Charge detectors developed in the author's laboratory are shown to complement conductivity detectors.

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The great power of ion-exchange chromatography to bring about complex separations was aptly demonstrated by Moore and Stein (1); this work eventually led to their receiving the 1972 Nobel laurel. They were able to separate all 50 amino acids in 175 h (slightly more than a week) using a 100 cm × 0.9 cm column packed with 25–37 µm cation-exchange resin, at a flow rate of 67 µL/min. They recognized that not only pH affects the retention of amino acids by controlling their ionization but that temperature can also profoundly affect such equilibria. They used a pH gradient from 4.25 to 11.0, and temperatures of 25–75 °C at various points during the profile. Interestingly, in this paper Moore and Stein thanked William Bauman of the Dow Chemical Company for supplying them with an ultrafine cation-exchange resin (not then commonly available).

Detection in the Moore and Stein work was off-line with fractions collected and reacted with ninhydrin before colorimetric measurement. Two decades later, amino acid analyzers with postcolumn ninhydrin reaction and flow-through colorimeters and high performance liquid chromatography (HPLC) with flow-through UV absorbance detectors were in existence. For the analysis of simple inorganic ions, however, many major anions of interest (such as sulfate) have no useful optical absorption or, in the case of chloride and other anions, absorb very poorly except at very low wavelengths where eluents are also likely to absorb. Conductivity detectors, on the other hand, can sense all ions, but there was no simple way to use them. For analyte ions to be eluted from available (high-capacity) ion-exchange columns in a reasonable period, other (eluent) ions are needed in significant concentrations. With a high conductivity background, minor conductivity changes accompanying the elution of analyte ions would have been impossible to detect. It was the genius of Small, Stevens, and Bauman (2) that solved this problem through a unique solid-phase postcolumn reactor that literally made the separation visible to the conductivity detector, and a unique electrostatically agglomerated stationary phase that was both efficient and of sufficiently low capacity to be used in that configuration. The stationary phase was akin to pellicular ion-exchanger stationary phases proposed earlier by Horvath and colleagues (3) but far more robust and functionally no different in its attributes than present-day superficially porous particles.

The original discovery and the principles of suppressed conductometric ion chromatography (IC) are discussed in detail by Small in the present issue (4). The solid-phase postcolumn reactor survives in the form of a unique three-position alternating revolving device (5) but has largely been supplanted by electrodialytically regenerated membrane devices (6). Here, we focus on the evolution and the current status of the two detectors unique to IC: the conductivity detector and the charge detector.

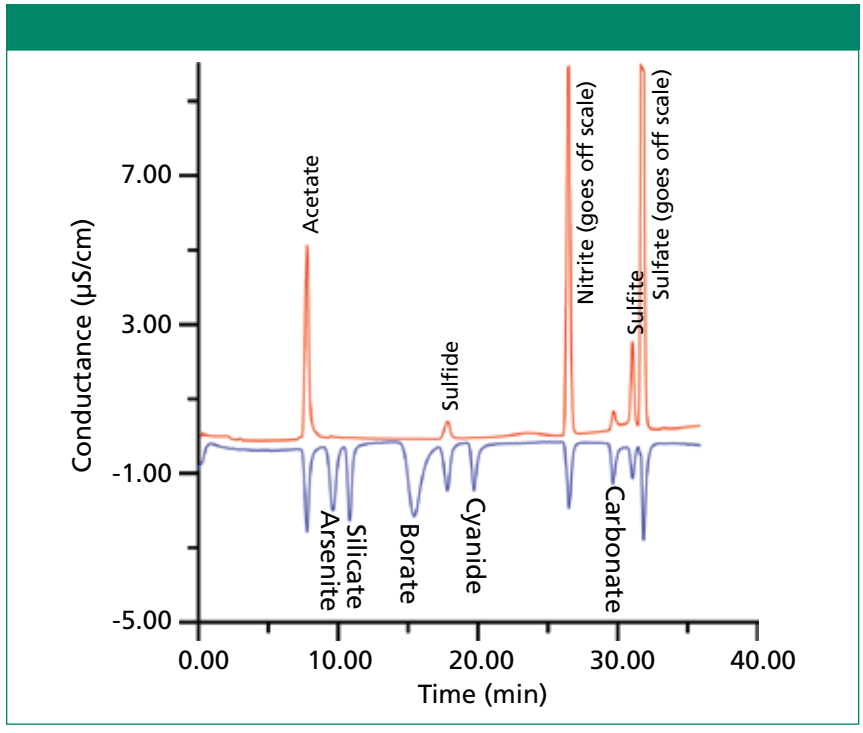

Figure 1: Two-dimensional gradient hydroxide elution ion chromatogram. Red trace: suppressed conductivity detector; blue trace: second-dimension detector (after baseline offset, background ~25 µS/cm). Column: AS11HC; mobile-phase gradient: 0–3.0 mM KOH in 10 min, hold at 3.0 mM until 15 min, ramp to 10 mM 15–20 min, ramp to 20 mM 20–30 min, ramp to 30 mM 30–40 min. Analyte amounts: 6.25 nmol for nitrate, borate, acetate, and sulfate; 12.5 nmol for the others, except carbonate (deliberately not added). Adapted from reference 17 with permission.

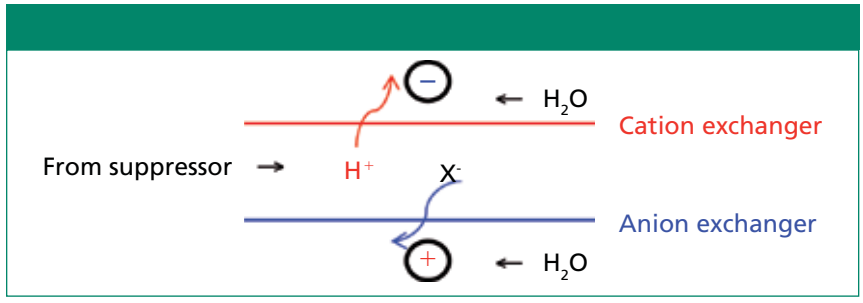

Figure 2: Basic schematic for the charge detector.

Conductivity Detection

Conductivity detection has been and continues to be the mainstay of IC. In the analyte concentration range of interest, conductivity is linearly related to ionic concentration: The slope is dependent on the specific ion or ions. At high concentrations, the response is less than linear, but this area typically is not of interest in IC.

Conductivity detectors are fundamentally of two types, differentiated by whether or not the electrodes are in galvanic contact with the solution. It is possible to make measurements from outside a glass or polymeric tube without electrodes contacting the liquid. Currently available IC equipment does not yet use very small capillaries (<100 µm bore), whether packed or wall-coated. With such dimensions, it is not practical to make measurements in a separate cell, external to the separation system, because of excessive dispersion from connecting tubing and other flow-path components. The measurement must be made directly on-capillary. Although fine wire electrodes in galvanic contact with the solution inserted through holes drilled through the capillary walls (7) or at the end of the capillary (8) have been demonstrated for capillary electrophoresis (CE) systems, a more elegant and generally applicable solution is capacitively coupled contactless conductivity detection (C4D).

In C4D, a pair of ring-shaped electrodes is put on the separation or measurement capillary ~1 mm apart. An excitation voltage is applied to one electrode; this voltage usually has a frequency of several hundred kilohertz, but some operating at a frequency as low as 200 Hz have been reported (9). This excitation signal is capacitively coupled through the capillary walls to the solution inside and travels to the other electrode. There are many different approaches to measure the conductance. In the simplest approach, a current-to-voltage converter is connected between the pickup electrode and ground. The resulting signal is amplified and rectified and is directly proportional to the solution conductance. Kuban and Hauser have repeatedly reviewed design and application of C4D techniques; the latest appeared in 2013 (10). A readily available inexpensive capacitance-to-voltage high resolution digital converter behaves very much like a C4D (11,12) but saturates at specific conductance values of ~100 µS/cm. As yet, C4D has not been much used in conventional IC. But it has many virtues and it is merely a matter of time before it is used more extensively in IC.

In galvanic conductivity measurements, there are two basic arrangements: a four-electrode and a two electrode arrangement. The four-electrode arrangement uses an outer pair of electrodes to apply a constant current through the system and then the voltage drop across the inner pair is monitored; this voltage drop is reciprocally related to the conductance. Although the four-electrode technique is the gold standard for resistance measurements in the solid state and used in some CE instruments, it has seen little use in IC.

A common geometry for a flow-through electrical conductivity detection cell consists of two disk-shaped stainless steel electrodes (~1–1.5 mm in diameter) that are spaced ~1 mm apart, or two ring-shaped electrodes spaced 4–5 mm apart. A temperature sensor, usually a low thermal mass thermistor, is also placed in the thermal block containing the cell to measure and correct for the temperature dependence of the measured conductance. A typical correction coefficient assumes that conductivity increases 1.7% per Celsius degree, but most detectors allow user-selectable temperature compensation values to be input. (Two major vendors of IC equipment both specify temperature constancy better than 0.001 °C. We remain somewhat skeptical about how well these dry cell block temperature specifications relate to the temperature constancy of the

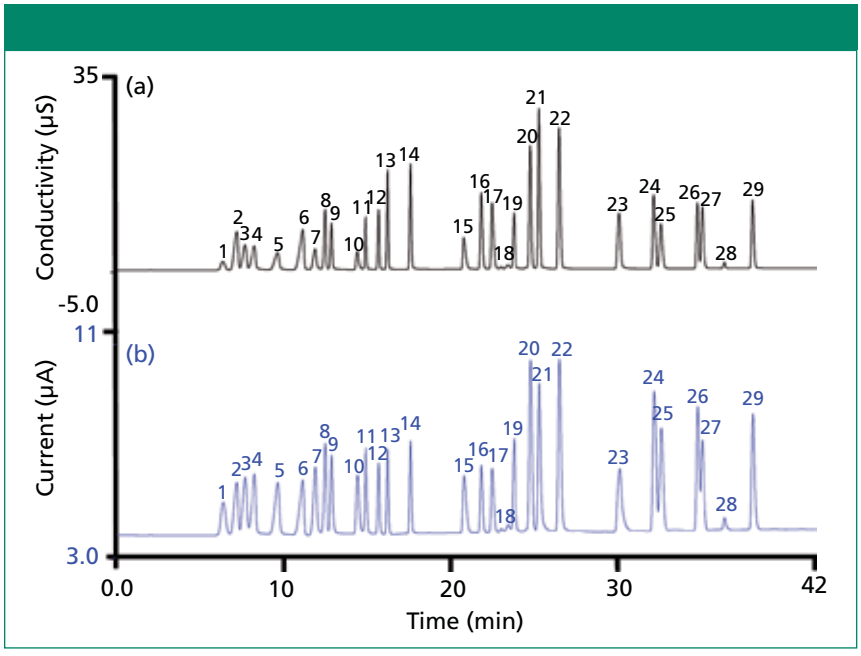

Figure 3: Gradient hydroxide elution on a 250 mm X 0.4 mm, 4-µm AS11HC column with a flow rate of 15 µL/min and temperature set at 30 °C. (a) conductivity detector, (b) charge detector. Injection volume: 0.4 µL. Peaks: 1 = quinate (10 mg/L), 2 = fluoride (3 mg/L), 3 = lactate (10 mg/L), 4 = acetate (10 mg/L), 5 = propionate (10 mg/L), 6 = formate (10 mg/L), 7 = butyrate (10 mg/L), 8 = methanesulfonate (10 mg/L), 9 = pyruvate (10 mg/L), 10 = valerate (10 mg/L), 11 = monochloroacetate (10 mg/L), 12 = bromate (10 mg/L), 13 = chloride (5 mg/L), 14 = nitrite (10 mg/L), 15 = trifluoroacetate (10 mg/L), 16 = bromide (10 mg/L), 17 = nitrate (10 mg/L), 18 = carbonate (20 mg/L), 19 = malonate (15 mg/L), 20 = maleate (15 mg/L), 21 = sulfate (15 mg/L), 22 = oxalate (15 mg/L), 23 = tungstate (20 mg/L), 24 = phosphate (20 mg/L), 25 = phthalate (20 mg/L), 26 = citrate (20 mg/L), 27 = chromate (20 mg/L), 28 = *cis*-aconitate (20 mg/L), 29 = *trans*-aconitate (20 mg/L). (Courtesy of Thermo Scientific.)

flowing liquid. If thermal noise were the only noise, in a measured background conductance of 1 µS/cm, the noise level will be 20 pS/cm, an order of magnitude less than the 100–300 pS/cm observed in practice. As a reference point, the best refractive index detectors in HPLC claim a temperature constancy of 0.005 °C.) In the simplest arrangement, an alternating voltage, 1–20 kHz in frequency, is applied across the electrodes and the resulting current is measured, rectified, and converted to a voltage signal. The ratio of the current to applied voltage is the conductance.

Both the above measurements are, however, affected by the capacitance at the electrode–solution interface. A bipolar pulse conductance measurement technique is not affected by the presence of capacitance, either serially or in parallel to the resistive element. Two successive voltage pulses of equal magnitude but opposite polarity are applied to the measurement cell. The current passing through the cell at the end of the second pulse in measured. This is the technique typically used in high-end conductivity detectors. DC voltages are generally not used, to avoid electrochemical processes at the electrodes. Conductometry is not an electrochemical technique — no chemistry occurs at the electrodes. However, when the background conductance is low, as with hydroxide eluents or a carbonate eluent with a carbon dioxide removal device, DC measurements have been shown to provide a very simple inexpensive alternative (13). In the stopped flow mode, a DC detector serves as a chronoamperometric device. The temporal signal profile reflects the ionic mobility of the anion in the cell; this helps identify a peak beyond retention time (14).

A major shortcoming of suppressed conductometric IC is its reduced response to weak acids. For acids with a $pK_a \leq 7$, practically no response is observed. On the other hand, hydroxide eluent nonsuppressed IC can provide a response (15); however, chromatographically such eluents are essentially unusable with real samples. Ideally, the best of both worlds can be attained if, after the first (conventional) suppressed conductivity detector, a small amount of hydroxide (such as sodium hydroxide) is introduced electrodialytically (16) or by Donnan breakdown (17) and then detected with a second detector. The detection principle of this second detector will be the same as that in a nonsuppressed hydroxide eluent IC. The results of such a two-dimensional detection are shown in Figure 1. This is a powerful adjunct to conventional suppressed IC. While not commercially available, commercial systems are now available that have two independent IC systems, each equipped with an eluent generator. Such systems can be configured to carry out such dual detection. Note that the ratio of the peaks in the two detectors is indicative of the pK_a of the acid and thus serves as an independent identifier.

Charge Detection

The charge detector (18) is the most recently introduced detector for IC. It is generically responsive to ions but the detection principles differ from those of a conductivity detector and hence a charge detector is a good adjunct to the latter. The basic configuration of the charge detector is the same as that of an electrodialytic suppressor except that it uses a cation-exchange membrane (CEM), which is held negative, and an anion-exchange membrane (AEM), which is held positive, while the suppressor effluent flows between the two membranes. The current between the electrodes (applied voltage is 2–12 V DC, typically 6 V DC) is the analytical signal. There is a small background current from the residual ionic impurities in the water and also from the autodissociation of water itself.

When an electrolyte (for suppressed anion IC this is necessarily an acid, but in principle it can be any electrolyte) moves into the detector, H^+ and X^-, proceed through the CEM and AEM, respectively, to the oppositely charged electrodes; it is the charge carried by these ions that is measured (Figure 2). This allows universal calibration for fully ionized electrolytes given that 1 µM SO_4^{2-} produces the same signal as 2 µM Cl^-, and so on. The charge detector is a destructive detector: It is essentially a deionizer; the charge transport occurring during deionization is what is measured. Interestingly, compared to the conductivity detector, the charge detector has a relatively higher response for weak electrolytes. Whereas a conductivity detector responds only to the ionized portion of a weak electrolyte, as deionization occurs in the charge detector, more of the undissociated material ionizes to maintain equilibrium. To what

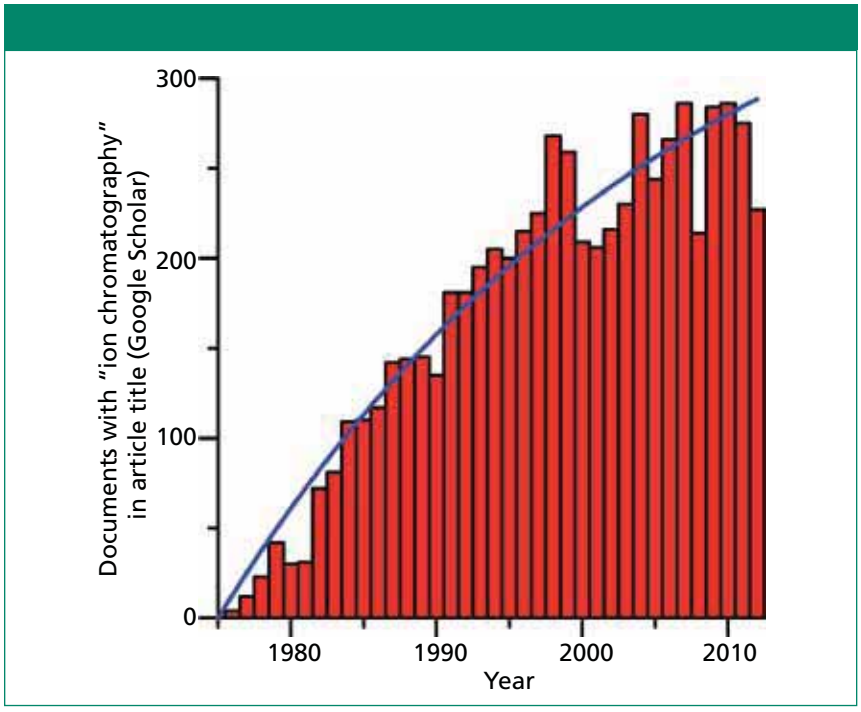

Figure 4: Number of documents retrieved by Google Scholar with the phrase "Ion Chromatography" in the title. The solid line indicates the best fit to an asymptotic approach to a plateau. Data from 2012 are incomplete and were not used in the fit.

degree it is ultimately deionized depends on the applied electric field and the residence time in the detector. As such, the effect of the eluent flow rate (reciprocally related to the detector residence time) is far greater for a weak acid anion than a strong acid anion. Even at normal chromatographic flow rates, the charge detector has enhanced response for weak electrolytes like organic acids, as shown in Figure 3.

Conclusions

Undoubtedly, as has been proven in the past, IC and its modes of detection will continue. In Figure 4, we plot the number of documents Google Scholar retrieves as having the exact phrase "Ion Chromatography" in the title. The best fit to an asymptotical approach to a plateau model is indicated by the solid line. The predicted plateau ordinate value is 420, which would suggest that we are only two-thirds of the way to the plateau; when we reach that, the time for the next paradigm-shifting technology will have arrived.

Acknowledgments

We thank Hamish Small first of all for planting the seed from which this tree has grown. We also thank him for sharing a draft version of his article that also appears in this issue. We are indebted to Kannan Srinivasan, Yan Liu, Christopher Pohl, and Andrea Willie for their generous and prompt help with sought-after information. We apologize that space constraints precluded much else that should otherwise have been included.

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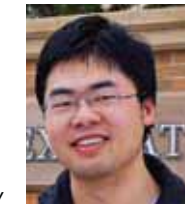

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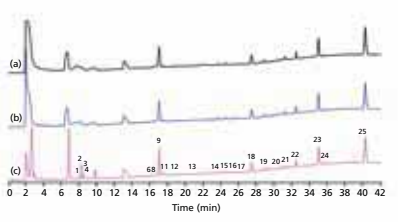
Practical and Popular Sample Preparation Techniques for Ion Chromatography



Techniques that could be included in a discussion of sample preparation for ion chromatography are as diverse as the panorama of sample types containing charged or ionizable targets. In this article, we present the most common and fundamental techniques that address common matrix issues, and discuss the critical chemistry considerations. We show the expected improved data quality by using sample preparation.

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Ion chromatography (IC) is first and foremost a chromatographic analytical technique used for the analysis of ionizable as well as permanently ionized target analytes. Components of the sample can interfere directly with the targets or with the eluent components, thus altering the elution behavior. They also can foul the analytical column or compromise analyte detection. The interferents can be charged or neutral, so knowledge of the chemistries involved is important to minimize time spent optimizing a method. The interferents can be identified (known) or be unknowns characterized by their behaviors. Many of the most common application problems in IC and their sample preparation solutions have been described (1,2).

Have the Right Toolbox of Columns

The goal of sample preparation, in any form, is to achieve good quantification, reproducibility, accuracy, and long system life. Sample preparation is a cost component in an analysis and is used because data quality or system ruggedness can be unacceptable without it. The good news is that there are quite a number of separation columns available for IC, each developed to meet a key analytical need. These columns may have higher capacity or optimized selectivity, or both. For some samples, choosing the right column that can separate analytes of interest from matrix interferences can eliminate the need for sample preparation. Likewise, some detection principles such as inductively coupled plasma–mass spectrometry (ICP-MS) can eliminate or minimize sample preparation through the technique's inherent selectivity.

The capabilities available in a well stocked "toolbox" of columns can be invaluable in time and money savings. For example there are a number of "high-low" applications where ratios as high as 10,000:1 sodium:ammonium can be managed isocratically simply by choosing the separation column designed for the analysis (3). In such a case, when the best column is selected for the job, there is no additional cost for sample preparation. As most any tradesperson

Table I: Cartridge-type sample preparation products

Ions Removed	Chemistry	Example of Commercial Products
Cations and neutralization	Sulfonic acid, H-form	OnGuard II H and InGuard (Thermo Scientific), IC-H (Alltech), IC-H (Metrohm)
Cations without pH change	Sulfonic acid, Na-form	OnGuard II Na and InGuard Na (Thermo Scientific), IC-Na (Alltech & Metrohm)
Transition metals without other cations	Iminodiacetate	OnGuard II M (Thermo Scientific), IC-Chelate (Alltech)
Chloride and other halides by precipitation	Sulfonic acid, Ag-form	OnGuard II Ag and InGuard Ag (Thermo Scientific), IC-Ag (Alltech & Metrohm)
Sulfate	Sulfonic acid, Ba-form	OnGuard II Ba (Thermo Scientific), IC-Ba (Alltech)
Halides and cations	Sulfonic acid, Ag- and H-forms, two-layer	OnGuard II Ag/H (Thermo Scientific)
Anions and neutralization	Quaternary ammonium, OH-form	IC-OH (Alltech)
Anions and neutralization	Quaternary ammonium, bicarbonate form	OnGuard II A (Thermo Scientific)
Halides, sulfate, and cations	Sulfonic acid, Ag-, Ba-, and H-forms	OnGuard II Ba/Ag/H (Thermo Scientific)
Hydrophobic species	Styrene–divinylbenzene	OnGuard II RP (Thermo Scientific), IC-RP (Alltech and Metrohm)
Hydrophobic species	Styrene–divinylbenzene, hydrophilic	InGuard HRP (Thermo Scientific)
Hydrophobic species	Octadecylsilane	IC-C18 (Metrohm)
Phenolics, azo dyes, humic- and tannic-acids	Polyvinylpyrrolidone	OnGuard II P (Thermo Scientific)

will say, having the right tool for the job makes all the difference.

Added concepts that expand the column selection option are the use of heart-cutting and two-dimensional heart-cutting. Heart-cutting takes advantage of column selectivity to direct only the targets to a detector such as a mass spectrometer. Two-dimensional heart-cutting combines (usually) two column formats of two orthogonal chemistries to eliminate the matrix while also concentrating the target analytes in one method (4).

The present discussion picks up at that point: What can one do when the need is beyond column selection?

The most commonly used sample preparation technique for IC other than filtration is matrix elimination, with and without off-line or in-line concentration. In this article we review two of the most commonly used techniques for sample preparation in IC and discuss the various formats of their use. Exemplary applications are used to illustrate the key concepts of data quality and ruggedness.

Sample Preparation Phase Chemistries

In IC, the most common sample preparation concept (after filtration) is matrix elimination, which is the concept of selectively removing matrix species while flowing the target analytes into a vial or to a concentrator column. The sample preparation chemistries take advantage of differences in chemical properties between target species and matrix species to accomplish matrix removal. The stationary phase of the sample preparation device is designed for maximally selective retention for the matrix species while exhibiting no retention for the species passing through the resin bed. Table I includes the selective retention principles for sample preparation phases used in ion chromatography. The most common format is an off-line cartridge with Luer connectors for use with a syringe or a vacuum manifold. The technique using this format is often referred to as *solid-phase extraction*, in this case, of the matrix. Various stationary phases are chosen to extract ionic and organic compounds while letting target components pass through the cartridge. After the samples are "prepared" they can be analyzed by a variety of techniques including IC, high performance liquid chromatography (HPLC), ICP, gas chromatography (GC), nuclear magnetic resonance (NMR) spectroscopy, and hyphenated techniques such as IC–MS and others.

Matrix elimination products are available in several formats that allow off-line or in-line matrix removal of species including chloride (halides), sulfate, azo dyes, fats, transition metals (by several different selectivity phases), common anions, humic acids (polyphenolics), general neutral hydrophobic species, and others. These devices can be used alone or in combination and can have other uses, including pH adjustment. Most of the available chemistries were developed to solve specific application problems, such as the determination of nitrate in a 1.6% NaCl brine solution or the removal of fat from milk to eliminate

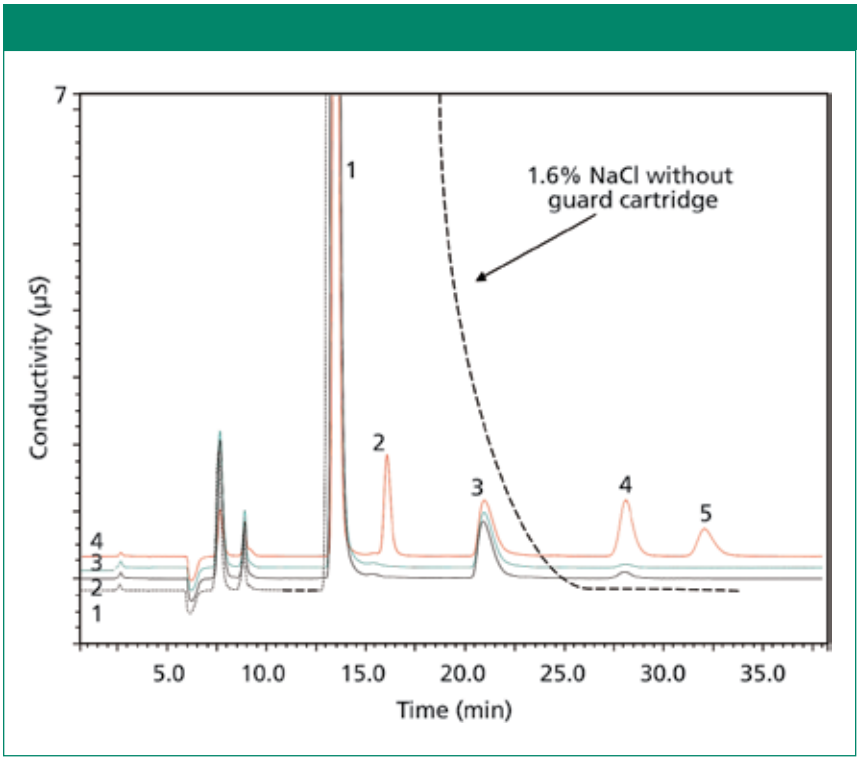

Figure 1: Determination of nitrite in brine with the aid of matrix elimination sample preparation. Column: IonPac AG/AS15, 4 mm; eluent: 23 mM KOH; flow rate: 1 mL/min; sample preparation: InGuard Ag and Na; injection volume: 100 µL; detection: suppressed conductivity, ASRS 300. Chromatogram 1: 1.6% NaCl brine after sample preparation; chromatogram 2: water blank; chromatogram 3: standard of 2 ppm nitrite, sulfate, nitrate in 1.6% brine after sample preparation. Peaks: 1 = chloride, 2 = nitrite, 3 = carbonate, 4 = nitrate, 5 = sulfate.

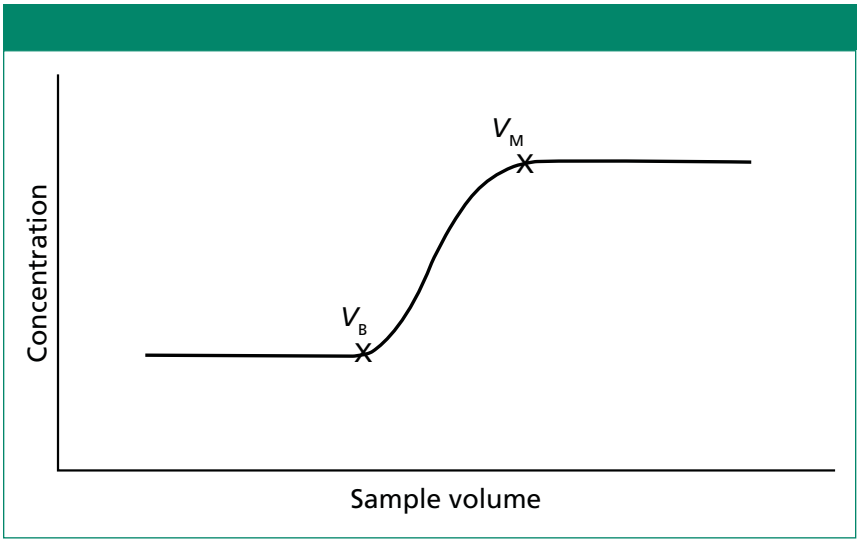

Figure 2: A typical breakthrough curve for a sample preparation cartridge.

detector electrode fouling. Table I is a summary of the commercially available chemistries and the off-line products that use them.

Practical Calculations

Use of matrix elimination requires a few calculations to ensure that the device can treat the required sample, in terms of concentration and volume. The overall goal is to determine if the sample preparation cartridge has enough capacity to treat the required volume of sample so that the matrix is actually removed from the sample. Following is an example of a possible sample:

Given: The sample contains 1% NaCl as matrix. The target is nitrite, at about 1 mg/L.

Issue: Nitrite is eluted close to chloride, so at high concentration, the chloride peak swamps the nitrite peak, reducing its recovery and rendering quantification impossible.

Solution: Nitrite does not precipitate with silver. A silver-form resin cartridge, such as the OnGuard II Ag cartridge (Thermo Scientific), can remove chloride from a sample matrix by precipitation of silver chloride in the resin bed.

Calculations: 1% NaCl is 1 g per 100 g or 10 g/L NaCl. The chloride portion of this is 35.45/58.45, 6.06 g/L, or about 0.17 mEq/mL. One OnGuard II Ag cartridge (1-cc) contains about 2.5 mEq of capacity. Dividing the cartridge capacity by the number of milliequivalents per milliliter of the sample, one can theoretically treat about 14 mL of 1% NaCl. The cleanest sample is generally obtained by using about 20% less than the maximum capacity. A white precipitate is visible in the resin bed and can guide the analyst in the determination of breakthrough volume. This cartridge is used in series with a sodium-form resin cartridge (such as the OnGuard II Na cartridge, Thermo Scientific) so that any silver ion from dissolved AgCl can be trapped on the sodium-form resin without any pH change. As a comparison, if the silver-form resin is followed by a hydrogen-form resin, then any trapped silver ion displaces hydronium ion, thus lowering pH and causing oxidation of nitrite to nitrate. Figure 1 shows an overlay of chromatograms showing 2 mg/L nitrite, carbonate, nitrate, and sulfate in 1.6% NaCl brine, the standard, and the blank.

An alternative experimental approach to measure capacity is to determine the "breakthrough volume" of a stationary phase–sample combination. A solution of sample of known concentration is passed through the cartridge and the output of the cartridge is collected in fractions for analysis or monitored with a detector that can measure matrix

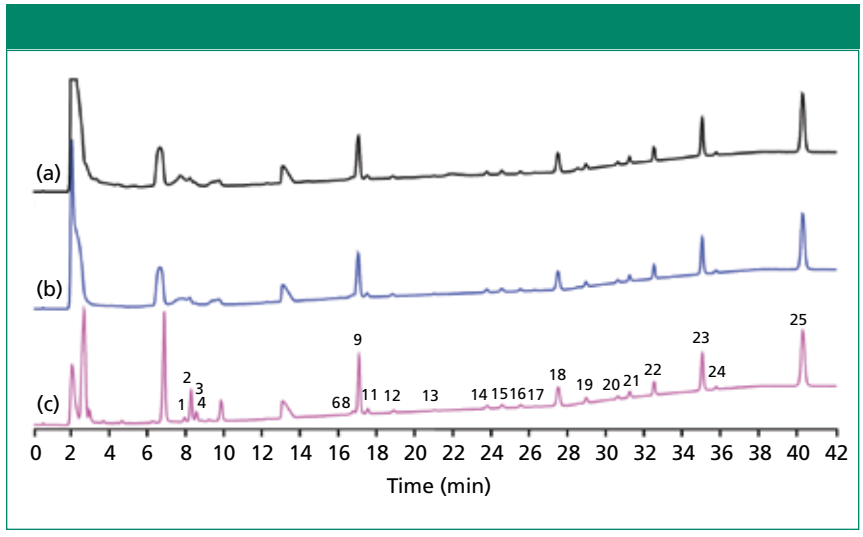

Figure 3: Chromatograms of a dried distillers grains with solubles (DDGS) sample showing improvements in peak shapes and recovery of inositol phosphates. Chromatograms: (a) without treatment, (b) with OnGuard II RP only, and (c) with OnGuard II RP and Ag/H. Columns: Dionex CarboPac PA100 Guard and Analytical (250 mm × 4 mm); eluent: 25–500 mM HCl; flow rate: 1 mL/min; temperature: 30 °C; postcolumn derivatization; ferric nitrate in perchloric acid; detection: UV absorbance at 290 nm. Peaks: 1–4 = InsP2, 5–13 = InsP3, 14–20 = InsP4, 21–24 = InsP5, 25 = InsP6.

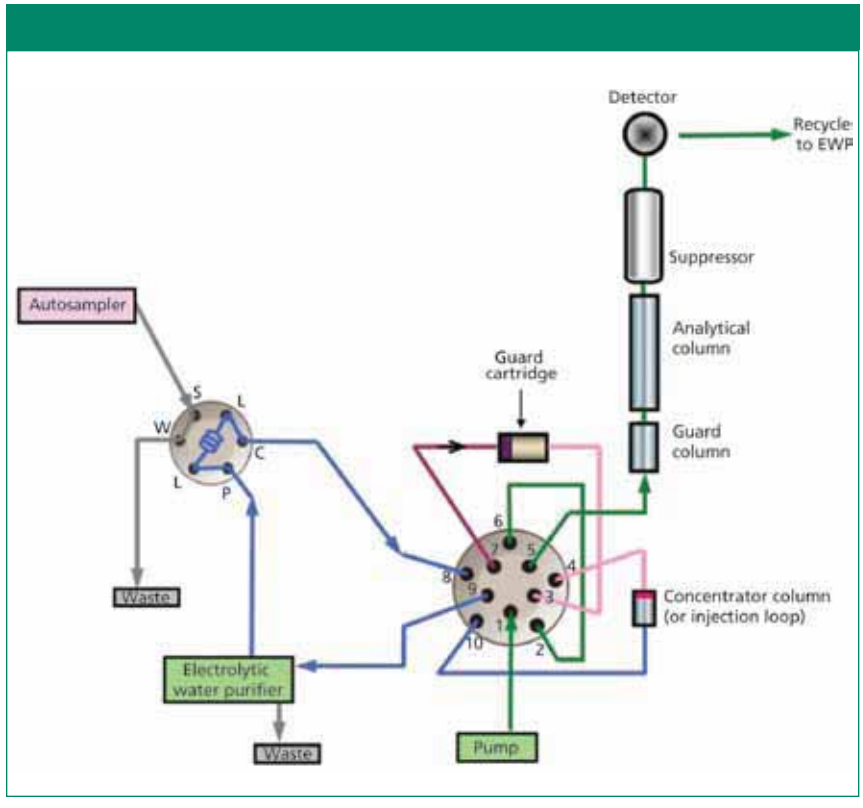

Figure 4: Example analytical setup for use of in-line sample preparation followed by preconcentration of the target analytes. The water for the loading pump should be cleaned using an electrolytic water purifier for minimal background.

components. Figure 2 shows a typical breakthrough curve. Initially, as the sample is passed through the cartridge, matrix components are retained by the resin until all sites are occupied. Then, the sample matrix components begin to be eluted (V_B), resulting in an increase in the baseline until the original concentration of sample is being eluted continuously (V_M). By knowing the volume of sample passed through the cartridge or the flow rate and the initial sample–matrix concentration, one can determine the loading capacity of the cartridge resin and thus make sure that one doesn't inject a sample concentration that will exceed this value (maximum sampling volume or calculated mass). Similarly to the calculation approximation, one should back off on the matrix mass by about 20% to take into account matrix variability and to obtain the cleanest sample.

Off-Line and In-Line Matrix Elimination

The most common need for matrix removal arises from the presence of sample matrix components that interfere with the elution of the targets. Such interference can mean that matrix components are co-eluted with the target or otherwise change the retention, elution, or recovery of the target. Off-line matrix removal sample preparation has been applied to a wide variety of sample types (5–21).

Figure 3 demonstrates a recent study on the determination of inositol phosphates in dried distillers grains with solubles (DDGS). Because the eluent is HCl, chloride needed to be removed from the sample so that the early elutions could occur only from the eluent, and not be affected by the chloride in the sample matrix. The off-line, two-layer silver–hydronium cartridge (OnGuard II Ag/H, Thermo Fisher Scientific) contains silver-form resin to remove chloride and also has a layer of hydronium-form cation-exchange resin at the outlet to trap any breakthrough Ag^+ ion that might redissolve. The sample also contains hydrophobic species that are co-eluted with the targets. A reversed-phase resin (OnGuard II RP, Thermo Scientific) removed those species (22). The cartridges were used in series with the reversed-phase cartridge followed by the two-layer silver–hydronium cartridge. Recovery of phytate was monitored at every

Table II: Retention time of polyphosphates in shrimp at the first and 300th injection, with and without using a sample preparation cartridge

Analyte	Retention Time Without Cartridge (min)		Retention Time With Cartridge (min)	
	First Sample Injection	300th Sample Injection	First Sample Injection	300th Sample Injection
Orthophosphate	2.74	2.50	3.34	3.32
Citrate	3.55	3.12	4.18	4.14
Pyrophosphate	6.10	5.78	6.66	6.63
Trimetaphosphate	7.10	6.66	7.61	7.55
Triphosphate	9.77	9.13	10.32	10.28

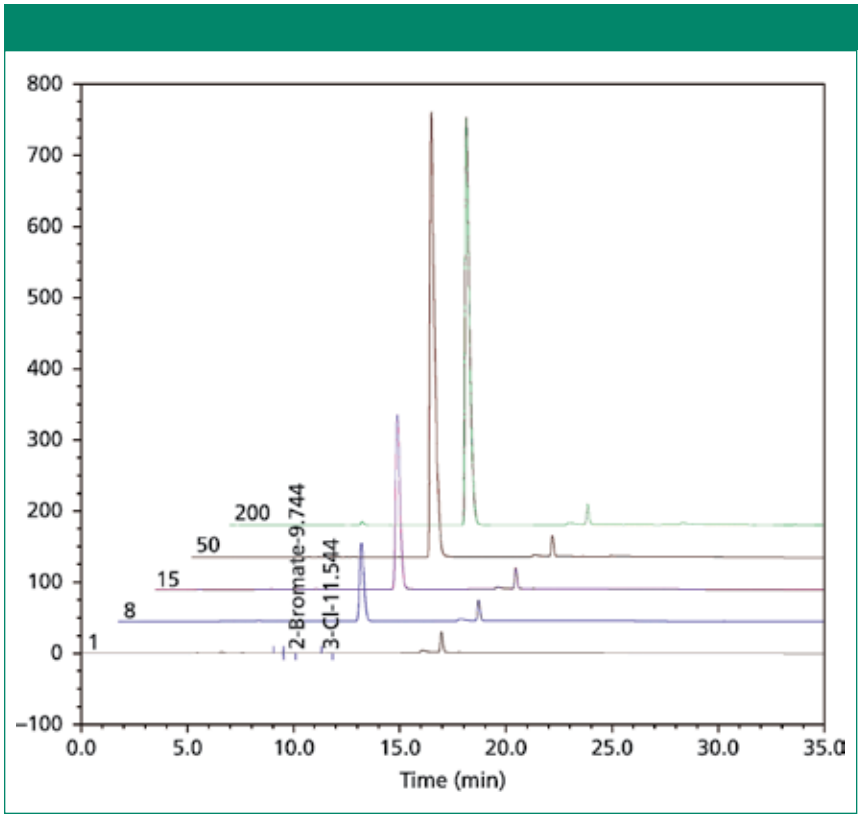

Figure 5: Comparison of lifetime chromatograms for 10 ppb bromate in 300 ppm chloride using sample preparation. Columns: IonPac AG24/AS24 (250 mm × 2 mm); eluent: 15–50 mM KOH gradient; flow rate: 0.3 mL/min; suppressor: ASRS 300 (2 mm); concentrator: TAC-ULP1; injection volume: 500 µL; sample preparation: InGuard Ag/InGuard H; sample loading: deionized water, 0.5 mL/min, 6 min.

step in the method development, and the overall loss on recovery was 4.5% using this order of cartridges. Interestingly, the loss on recovery using the reverse order of cartridges was 22%.

An in-line version of sample preparation chemistries was introduced in the last couple of years, and its acceptance is growing (23). Use of in-line sample preparation chemistries has the advantage of requiring much less sample, as only the injection volume is treated, and can therefore be used repeatedly until the capacity is depleted. A concentrator column is used to "recollect" the targets for injection onto the analytical column. This arrangement minimizes operator time and saves money.

Figure 4 shows a flow diagram for one configuration (of many) for use of in-line sample preparation. It is important to note that for low level determinations of common ions, the loading pump is usually supplied with electrolytically purified water (Electrolytic Water Purifier, Thermo Scientific or CIRA, Trovion Pte Ltd. Singapore) to minimize ions in the blanks from trace contamination in the loading water. Figure 5 shows the results of 200 injections of 10 ppb bromate in a matrix of 300 ppm chloride using in-line sample preparation. The relative standard deviation for retention time on 200 injections was 0.062%, the bromate peak area was 0.309%, and the amount (parts per billion), was 0.309%.

A general application of in-line matrix removal in the food industry is the use of a hydrophilic styrene–divinylbenzene cartridge (InGuard HRP, Thermo Scientific) for the in-line removal of hydrophobic matrix components. Hydrophobic matrix components are usually unidentified but can be retained on reversed-phase stationary phases. One example is the determination of polyphosphates in shrimp (24). The goal was to prolong column life as evidenced by stable retention times over hundreds of injections. Table II shows the retention time data for the target polyphosphate species found in shrimp, comparing the first and 300th injection, with and without the use of in-line matrix removal.

Concentrators

Use of concentrators can lower detection limits by several orders of magnitude depending on the sample volume.

Critical parameters include sample volume, capacity of the concentrator stationary phase, and composition of the sample matrix. Concentrators are used when the concentration of the target in the sample is too low for good quantification or when the sample band entering the analytical column is diffused, as in the case of some applications of in-line sample preparation. Some of the most common (and successful) uses of concentrators are in the determination of ultratrace targets in ultrapure water. In this case, the matrix to be eliminated is the water. Sometimes guard columns can be used as concentrators because the selectivity matches the analytical column. However, specifically designed concentrator columns are available with important features including ultralow back pressure for compatibility with an autosampler. Some concentrators have ultralow background of some analytes, such as sulfate (25). So once again, choices save time and money.

Summary

In this short review we have tried to cover the most commonly used sample preparation techniques for ion chromatography. The reference list provides many details that were outside the scope of this article. We began with the concept of choosing the best analytical column for the job; then, by understanding the chemistry issues associated with the sample, chose the best off-line or in-line matrix elimination solution.

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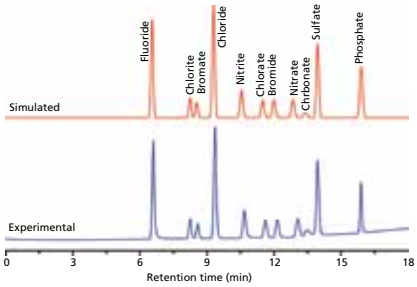
Tools for Simulation and Optimization of Separations in Ion Chromatography



Users of ion chromatography (IC) are faced with lengthy method development times because of the relatively slow equilibration of IC columns to each new eluent. Method development becomes even more demanding when multistep elution profiles (containing sequential isocratic and gradient steps) are used. Given that modern IC instruments are designed to generate these multistep elution profiles, computer-based methods to simulate separations for a wide range of analytes, columns, and eluents are highly desirable. The tools available for computer-assisted method development are discussed here.

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Ion chromatography (IC) is used widely for the detection and determination of ionic species in various samples. With the advent of eluent generators and electrolytic suppressors, IC is now operated routinely using both isocratic elution and multistep eluent profiles comprising sequential isocratic and gradient steps. Frequently, an eluent profile consisting of multiple steps (commonly up to five) is applied, typically when the separation is difficult or the number of analytes to be separated is large. The practical implementation of these multistep eluent profiles is simplified greatly by the use of an eluent generator in which the eluent is generated electrolytically from an input stream of water. The desired eluent concentration profile can be created simply by applying a suitable profile of electrical current to the eluent generator.

The development of a new IC separation method involves many decisions. Which column should be used? Which eluent type should be used and what is the optimal eluent composition? As with most chromatographic techniques, the development of a new IC method can be a time-consuming process. Trial-and-error optimization of the separation is therefore both tedious and challenging. For these reasons, there has been a strong interest in the use of computational tools to facilitate the method development process.

Considerable work has been undertaken to understand the retention processes that apply in IC. Over the years, there have been numerous attempts to produce mathematical retention models for isocratic elution (1–5), gradient elution (6), and multistep elution profiles (comprising sequential isocratic and gradient steps) (7–10) in IC. These models aim to provide a mathematical relationship between the analyte retention factor and measurable properties of the analyte (such as its charge), the eluent driving ion (such as its concentration and charge), and the stationary phase (such as its ion-exchange capacity and phase ratio). Many of the published models are remarkably accurate in their prediction and in theory, they could be used for the *a priori* calculation of retention factor for any analyte without the need for experimentation, provided that values for all of the above properties are known. This situation rarely occurs in practice because the process of determining values for all the necessary analyte, eluent, and stationary-phase properties would normally take longer than a straightforward trial-and-error manual optimization. For this reason, computer-assisted method development in IC

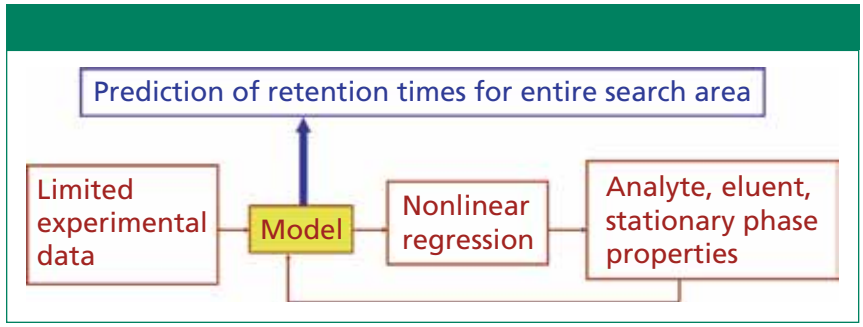

Figure 1: Schematic overview of computer-assisted optimization in IC.

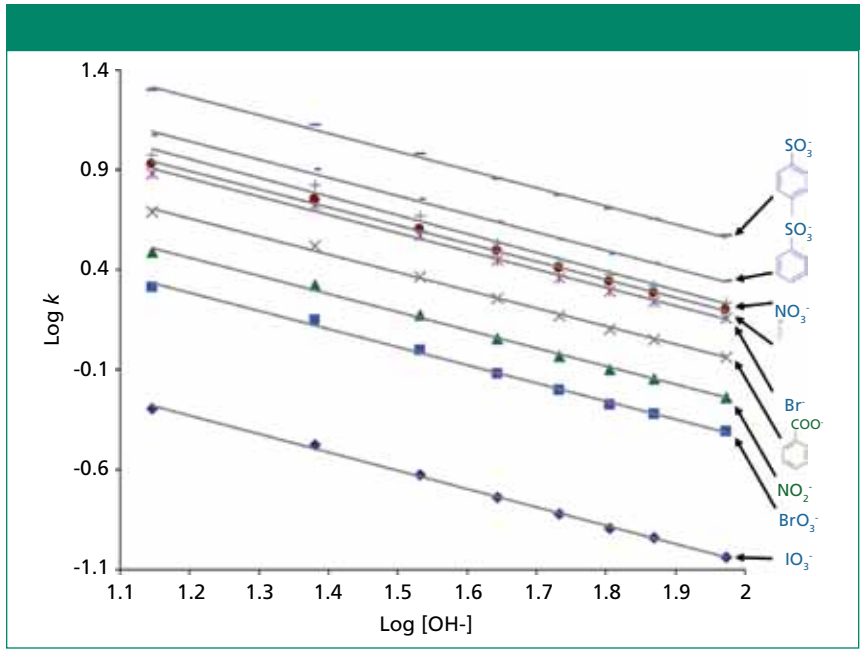

Figure 2: Plot of log k versus log [OH^-] for inorganic and organic anions.

almost invariably involves a combination of experimentation and computer prediction. The experimentation step is done to acquire some limited retention data using, for example, three eluent conditions, and then applying the chosen mathematical retention model to calculate analyte retention for all possible eluent conditions. This process is represented schematically in Figure 1. This process shows that, provided the retention model is accurate and sufficient experimental data are available to permit reliable implementation of the model, it should be possible to quickly produce a retention map for all analytes over all eluent combinations (the "search area"), leading to straightforward simulation and optimization of separations.

Retention Models in IC

It is not the intention of this article to discuss in any detail the theoretical basis underlying IC retention models. However, it is pertinent to show which models are most useful for the process outlined in Figure 1. It is also often the case that the most useful models are surprisingly simple.

Isocratic elution in IC is described accurately by the following relationship:

$$\log k_A = \frac{1}{y}\log(K_{A,E}) + \frac{x}{y}\log\left(\frac{Q}{y}\right) + \log\left(\frac{w}{V_m}\right) + \frac{x}{y}\log[E^{y-}] \quad [1]$$

where k_A is the retention factor, $k_{A,E}$ is ion-exchange selectivity coefficient between the analyte and the eluent competing ion, x is the charge of the analyte, y is the charge on the eluent, Q is the effective ion-exchange capacity of the stationary phase, w is the mass of the stationary phase, V_m is the volume of the eluent species, and $[E^{y-}]$ is the concentration of the eluent.

If this model is employed for isocratic separations consisting of a single competing ion, $k_{A,E}$, Q, w, and V_m can be treated as constants and thus the model can be simplified to

$$\log k = a - b\log[E^{y-}] \quad [2]$$

where a and b are both constants.

A plot of log k versus log[E^{y-}] will give rise to a linear relationship with the effective charge of the analyte relative to the competing ion as the slope, b, and the intercept, a, indicating the degree of interaction between analyte and stationary phase. This linear relationship is illustrated in Figure 2 for a univalent eluent ion (OH^-) and a series of inorganic and organic univalent analyte ions.

Retention modeling for gradient elution IC or for separations involving multistep elution profiles is understandably more difficult than is the case for isocratic separations. Generally, two approaches can be used. In the first, a mathematical gradient retention model is derived, but this generally involves quite complex derivations, requires accurate gradient retention data to solve the model, and can also be challenging computationally when calculations of retention factors under numerous eluent conditions are undertaken. The second, and preferred, approach is to break up the gradient or multistep elution profile into a series of small, successive isocratic segments, to then apply an isocratic retention model to each segment, and finally to integrate the individual segments into the overall elution profile. This process is illustrated schematically in Figure 3. The chief advantages of this approach are that only isocratic retention data are required (because an isocratic retention model is used), the same model can be used for both isocratic and gradient steps of an elution profile, and computational demands are less for isocratic models.

Simulation and Optimization of Isocratic Separations in IC

It is clear from Figure 2 that the isocratic retention model shown in equation 2 gives a very accurate description of retention behavior. It is also evident from Figure 2 that the retention data for any of the analytes shown could be predicted if a minimum of two experimental data points were used to calculate the intercept (a in equation 2) and slope (b in equation 2) of the plots shown in Figure 2. Thus, when

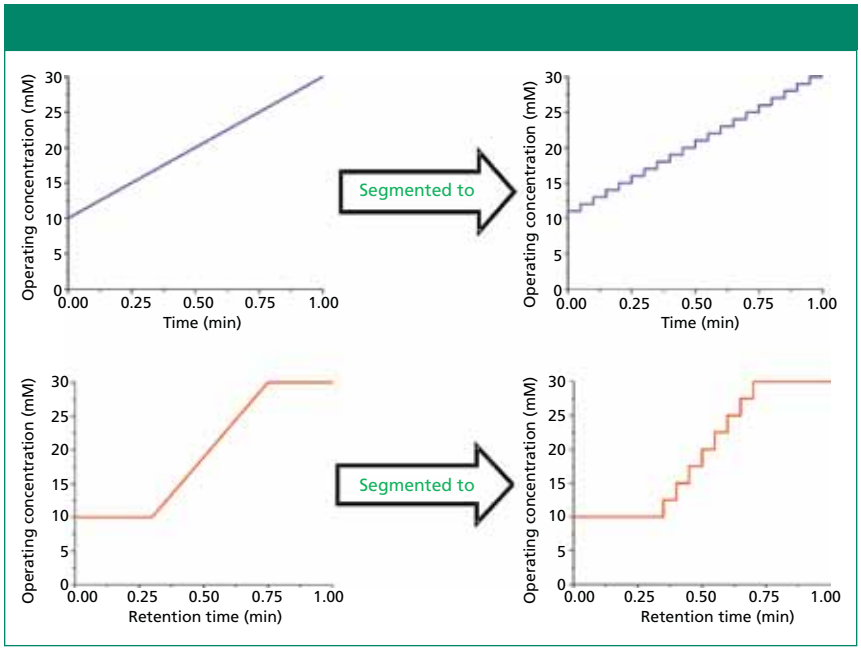

Figure 3: Schematic overview of segmenting gradients and multistep profiles to isocratic segments.

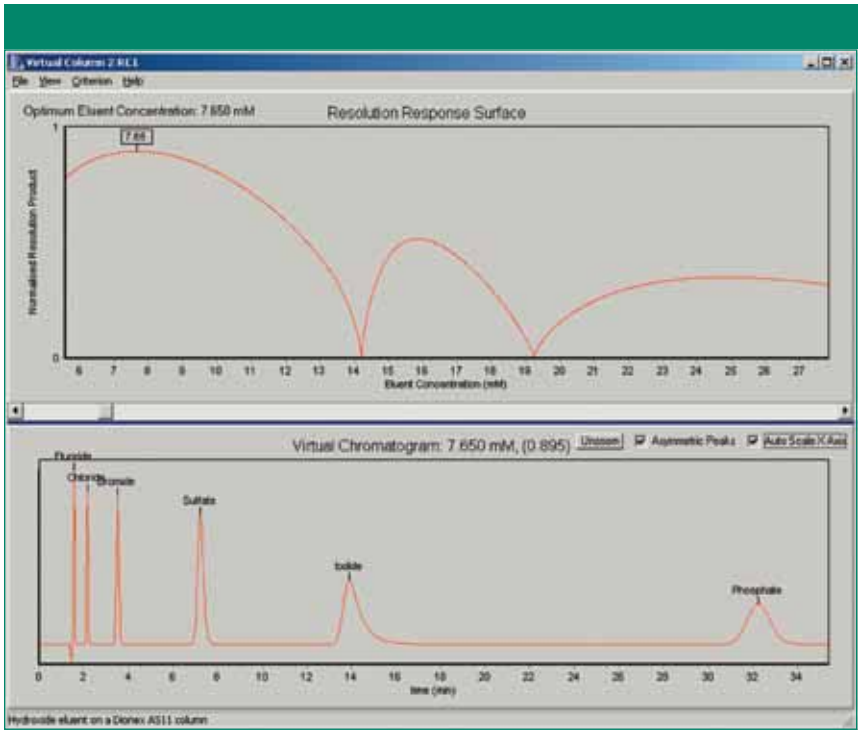

Figure 4: Screen display from Virtual Column software for isocratic separations.

applied to the simulation of isocratic IC separations, the process depicted in Figure 1 would have the following steps:

- Retention factors for all analytes in the test mixture would be determined using at least two eluent concentrations.
- These retention data would be used to solve equation 2 for each analyte to obtain *a* and *b* values for the retention plot for each analyte.
- The *a* and *b* values can then be used to calculate the retention factors for each analyte at any isocratic eluent concentration. In this way, the chromatograms obtainable for any isocratic elution composition can be simulated.

After the ability to predict retention has been achieved, it is then a simple further step to include optimization. This involves using a mathematical algorithm that can assign a numerical value to an entire chromatogram, according to its alignment with a desired goal. For example, this goal might be to maximize the separation of all peaks, to maximize the separation of a problematic pair of peaks, or to achieve the separation in the fastest possible time. Such an algorithm is referred to as an *optimization criterion,* and many such algorithms have been proposed.

It is clear from the above comments that reliable isocratic retention data are essential for the successful implementation of simulation and optimization of isocratic separations in IC. Of course, these retention data can be acquired as needed for particular combinations of analytes, eluents, and stationary phases. An alternative approach is to compile an extensive set of reliable and accurate isocratic retention data for a wide range of analytes, eluents, and columns and to store these data in a suitably accessible database. In this way, users could simulate and optimize separations by accessing the database, without the need to undertake any experimentation. The authors have worked with Thermo Scientific Dionex (hereinafter referred to as Dionex) over the past 10 years to compile such a database and this has formed the basis of the Virtual Column software for IC simulation and optimization, marketed by Dionex. The database underlying this software contains retention data for more than 75 analytes (anions and cations) on more than 20 Dionex IC columns using a range of eluents, column diameters, and temperatures. The user simply needs to select the analytes that are to be separated and the software can then be used to simulate all possible chromatograms on all columns and to identify the optimal combination of eluent and column for the desired separation.

One example of the output (in its simplest form) generated by the Virtual Column software is shown in Figure 4. In this case, fluoride, chloride, bromide, sulfate, iodide, and phosphate are being separated on a Dionex AS11 column using hydroxide eluents in the range 4.5–28 mM. The top panel shows a plot of the optimization criterion (in this case, the normalized resolution product, which reaches a maximum value of 1.0 when all peaks are spaced evenly over the chromatogram, and has a value of zero when any two peaks are coeluted). It can be seen that

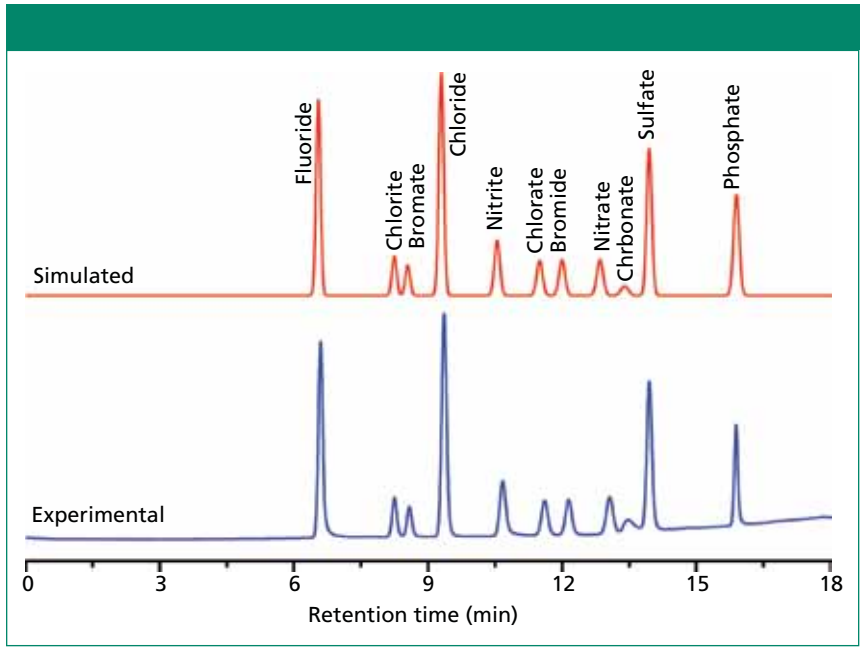

Figure 5: Simulated and experimental separations on a Dionex AS19 column under a three-step gradient profile consisting of: 3.75 mM KOH for 0.8 min, 3.75 to 33.75 mM KOH for 10 min, followed by 33.75 to 99.75 mM KOH for 4 min at 1 mL/min at 30 °C.

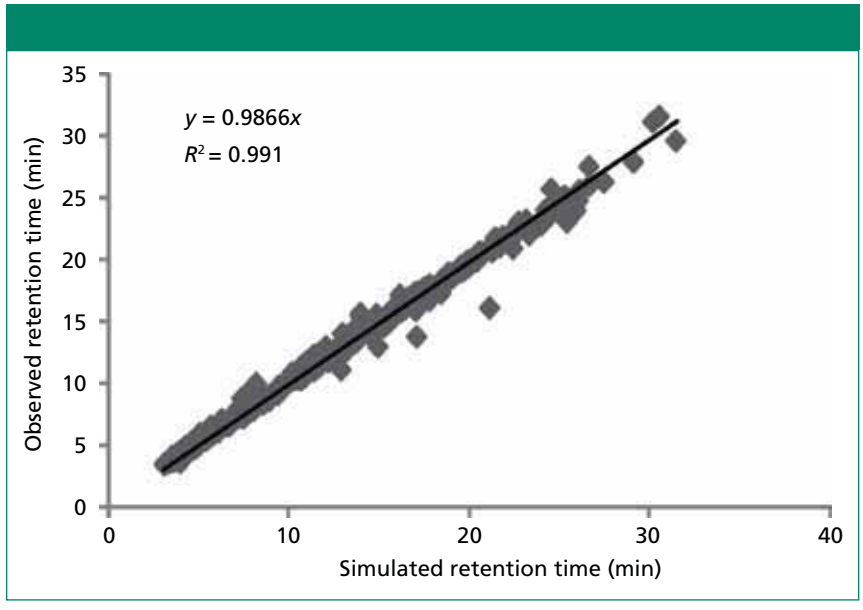

Figure 6: Correlation of experimental and simulated retention times for 24 anions and 13 cations on Dionex AS11 HC, AS16, AS19, CS12A, and CS16 columns for four 5-step complex eluent profiles.

the maximum value of this criterion is reached when the eluent concentration is 7.65 mM. The bottom panel displays the chromatogram obtained with this eluent concentration. There is a slider bar at the bottom of the top panel and the user can slide this to any desired eluent concentration and the chromatogram obtainable at this concentration will appear in the bottom panel. The software also allows other columns, diameters, and temperatures to be investigated, as well as the use of other optimization criteria. In addition, two-component eluents (such as mixtures of carbonate and bicarbonate) can be simulated, in which case the top panel reverts to a response surface plotted against the concentrations of the two eluent components on the horizontal and vertical axes.

Simulation and Optimization of Multistep Elution Profiles in IC

Because of the ease with which multistep eluent profiles containing sequential isocratic and gradient steps can be generated, and the versatility that they offer in developing new separations, such profiles are now the preferred mode of operation in modern IC. However, the selection of an optimal eluent profile is a very challenging and time-consuming task if trial-and-error procedures are used. For this reason, there has been very strong interest in extending the isocratic approaches described above for use with multistep elution profiles.

The earlier discussion of retention models highlighted the potential use of isocratic retention data for gradient elution by dividing the gradient into successive small isocratic segments. If this approach is taken, only isocratic retention data are needed for calculation of retention factors, so the same isocratic database used for the Virtual Column isocratic software can be applied. Simulation performed in this way is highly successful, as evidenced by Figure 5, which shows the simulated and experimental chromatograms for the separation of 11 anions on a Dionex AS19 column using a three-step elution profile. Figure 6 shows the overall correlation between experimental and simulated retention times for 37 analytes (anions and cations) on several columns using four different five-step elution profiles.

Figures 5 and 6 show that accurate simulation of retention under multistep elution profiles is possible. However, optimizing the separation by finding the optimal elution profile is an exceedingly difficult task because of the large number of parameters that need to be optimized. For example, in a simple three-step elution profile comprising an isocratic step, a gradient step, and then a final isocratic step, it is necessary to optimize the initial eluent concentration, the duration of the first step, the slope and duration of the gradient step, and the duration of the final isocratic step. The complexity increases when more steps are included in the eluent profile. Currently, there are no published methods for this type of optimization. However, it is possible to simulate the separation using the approach depicted in Figure 7. Here, a four-step elution profile is depicted, with an initial isocratic step, a shallow gradient, a steeper gradient, and a final isocratic step. The square boxes represent points where the shape of the profile can be adjusted on a computer screen. For example, the length of the first isocratic

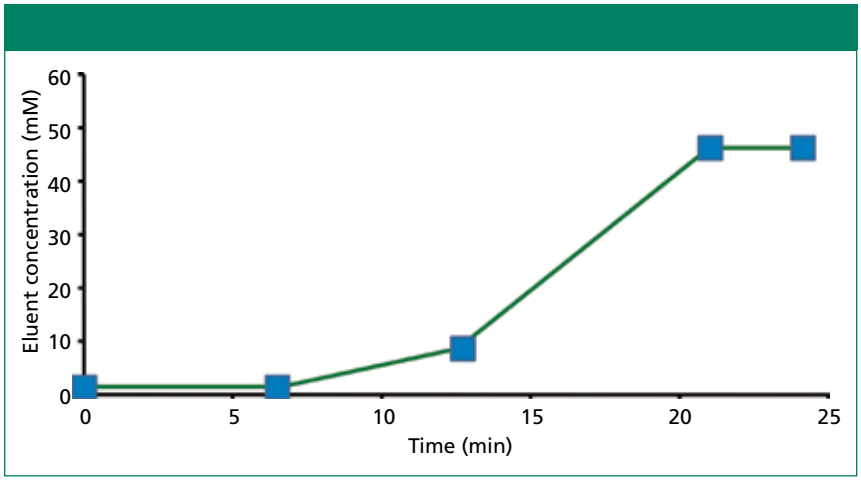

Figure 7: Screen display for manipulation of a multistep elution profile.

step can be adjusted by moving the first box left or right. Any change made to the profile will lead to the instant simulation of a new chromatogram by the software. Users can thereby simulate any desired eluent profile and can optimize the separation manually without experimentation.

Updating the Retention Database

An obvious potential limitation of the above approaches to simulation and optimization is that they rely on retention data stored in the Virtual Column database, which may become dated. For example, a new column might not behave in the same manner as the column used to acquire the data some years previously. We have addressed this issue by devising a system where the database can be updated (or "ported") to a new column by making adjustments to the stored data, based on some actual measurements obtained on the new column on which the IC method is to be used (11). In this way, the database can be continually adapted for use with newer versions of the columns on which the original data were obtained.

Conclusions

The ability of modern IC instruments to apply multistep elution profiles has placed severe demands on method development procedures designed to exploit the full advantages of these elution profiles. Retention models derived for simple isocratic IC separations can be applied to multistep eluent profiles by segmenting these profiles into small isocratic steps. This approach simplifies the retention modeling process and enables rapid calculation of retention factors based only on isocratic data. The computational simplicity of these calculations means that real-time simulations can be performed. Historical isocratic retention databases can be easily updated with a small amount of experimentation. These new tools are not yet available in the commercial Virtual Column software, but work is currently under way to update this software.

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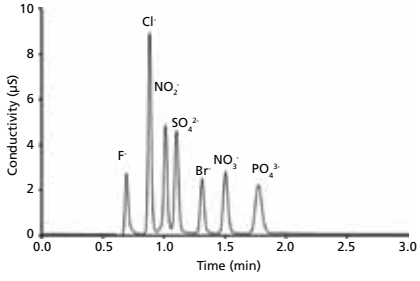
Advances in High-Speed and High-Resolution Ion Chromatography



Compared to modern high performance liquid chromatography (HPLC) and ultrahigh-pressure liquid chromatography (UHPLC), separation speeds in ion chromatography (IC) have lagged behind. In recent years, dramatic strides have been made by the development of PEEK flow components, eluent generators and membrane suppressors with increased pressure and lower volumes, smaller particle size packings that permit shorter columns and higher flow rates, and capillary columns with higher efficiencies. Here, we review these developments and show examples of faster IC separations. For simple systems, separation speeds rival those of modern LC. For complex samples, separation times are still slow, but the use of 2D techniques allows more rapid analysis of complex samples in high ionic-strength matrices.

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Just four decades ago, the analysis of an individual ion required that the analyst perform a time-consuming titration or gravimetric measurement. Each additional ion to be analyzed magnified the effort required. In 1975, Small and coworkers (1) introduced ion chromatography (IC). This technique enabled the simultaneous analysis of multiple ions with low detection limits. Compared to the hours of labor needed for classical methods, IC yielded rapid measurements. For instance, Li^+, Na^+, and K^+ could be baseline-resolved in 134 min (2). Similarly common anions could be analyzed in 20 min (1,2).

Today, IC is widely used for the analysis of inorganic ions and many ionizable organic compounds. More than 70 commercial IC columns are available (3). Until recently, however, developments by IC column manufacturers focused on increasing the ion-exchange capacity, reliability, and selectivity of stationary phases (4) rather than the speed of analysis. Thus, up to just a few years ago the typical separation time of anions differed little from that demonstrated by Small and coworkers in 1975 (1,2)

In contrast, in the last decade, rapid developments have taken place in reversed-phase liquid chromatography. Smaller particles have enabled faster analysis by allowing shorter column lengths while maintaining high efficiency and resolution (5). Introduction of sub-2-µm particles required ultrahigh-pressure (up to 15,000 psi) metallic pumps because of the dramatically greater back pressure generated by these smaller particle diameters (5,6).

Why has IC lagged behind this trend? First, IC was limited by the hardware (7). The stainless steel pumps, tubing, and fittings used in high performance and ultrahigh-pressure liquid chromatography (HPLC and UHPLC) are not compatible with the highly corrosive acidic or alkaline eluents required in IC. Early IC systems were constructed with glass columns and polyoxymethylene (Delrin) components (8). The introduction of polyether ether ketone (PEEK) pumps and tubing increased the pressure capabilities of IC to near that of conventional HPLC systems. Second, severe baseline drift and ghost peaks were experienced with gradient elution in IC because of the inherent background conductivity of carbonate eluents and impurities in the carbonate or hydroxide eluents. The generation of ultrahigh-purity eluents such as carbonate-free sodium hydroxide was made possible through membrane-based

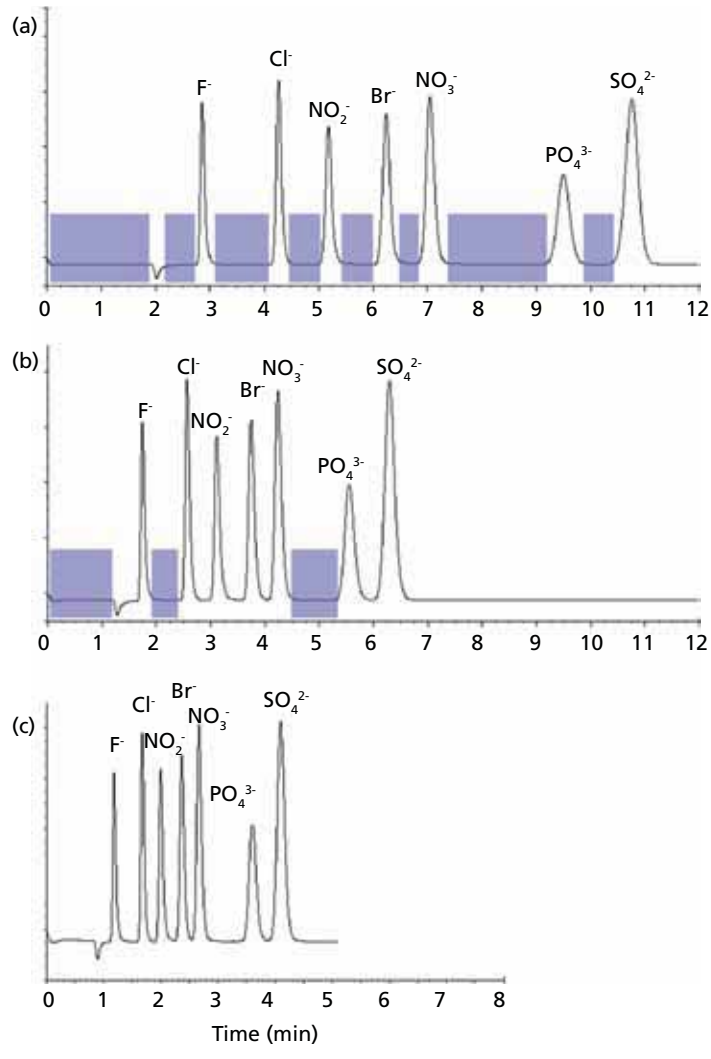

Figure 1: Comparison of separations obtained using 25- and 15-cm columns with the same particle size (6.5 µm) and column diameter: (a) 25 cm × 0.4 cm AS22 (flow rate: 1.2 mL/min), (b) 15 cm × 0.4 cm AS22-Fast (flow rate 1.2 mL/min), (c) 15 cm × 0.4 cm AS22-Fast (2.0 mL/min). The typical working pressure of AS22 and AS22-Fast is ~1900 psi. The excessive resolution is shown in blue boxes. Eluent: 4.5 mM sodium carbonate, 1.4 mM sodium bicarbonate; injection volume: 10 µL; suppressed conductivity detection. (Courtesy of Thermo Fisher Scientific.)

electrodialytic eluent generators (9,10). The fragility of the eluent generators restricted the upper pressure limit to 3000 psi (11). The maximum flow rate in IC was further limited by the suppressor to 1–3 mL/min (for 2 mm to 4 mm columns, respectively) to protect the membranes from leaking. Thus the standard IC hardware was pressure- and flow rate–limited as late as 2011.

Because of these pressure and flow rate limits, IC columns continued to be characterized by large particle sizes (7–13 µm) and long (20–25 cm) columns. For instance, Figure 1a shows a typical ion chromatogram using a 25-cm IonPac AS22 column packed with 6.5-µm particles. Seven common anions are separated in ~12 min with 9600 plates at a column pressure of <2000 psi.

However, there is actually excessive resolution in Figure 1a, leading to "wasted" time, which is highlighted in blue in the figure. Clearly, some of this excess resolution could be traded for analysis time by shortening the column while keeping the particle size and other parameters the same. The first such column was the 15-cm IonPac AS22-Fast column, which was introduced at Pittcon 2010. Figure 1b shows a 7-min separation on the AS22-Fast column — a 40% reduction in run time. The efficiency is reduced from 9600 to 5000 because of the shorter column length, but it is still sufficient for baseline resolution. The lower back pressure of the shorter column also allows use of higher flow rates while staying within the pressure limits of the IC instrumentation. Figure 1c shows a 4.5-min separation of seven common inorganic anions using a 15-cm AS22-Fast at 2 mL/min. Using even shorter columns while keeping the particle size the same can further reduce the analysis time. For instance, Haddad's group used 3–5 cm columns packed with 7.5-µm resins to demonstrate 15-min separations of highly retained perchlorate and thiocyanate ions (12). Such ions are typically retained for more than 1 h on standard 25-cm IC columns.

The next evolution in the speed of IC analysis was associated with the redesign of the commercial hardware for capillary columns (~0.4 mm i.d.). Such columns allow the same linear velocity (*u,* cm/min) as a normal 4-mm i.d. column at a 100-fold lower volumetric flow rate (13), enabling extended operation with a single bottle of eluent. An immediate consequence of the lower volumetric flow rates needed for capillary IC was a decrease in the surface area of the electrodialytic membrane in the eluent generator (14,15), which yielded a higher burst pressure for the membrane. Thus, a capillary IC system such as the Thermo Scientific Dionex ICS-5000 has a pressure limit of 5000 psi (compared to a limit of 3000 psi for a conventional IC system). Capillary separation systems also produce greater column efficiencies (reduced plate heights <2) than large-bore counterparts (13). Figure 2 shows the separation of seven common anions in less than 2 min using a 15-cm capillary column packed with 4-µm particles (16). At 25 µL/min, the back pressure was only 3480 psi. For the sake of comparison, this capillary separation in Figure 2 takes 17 s/ion compared to 98 s/ion for the AS22-Fast column in Figure 1c. Thus, capillary systems have the potential of high-speed IC separations by employing smaller particles in 15-cm capillaries. A number of such "fast" IC capillaries are commercially available for cations and anions. In January 2013, a redesign of the eluent generators for conventional flow IC yielded the Thermo Scientific Dionex ICS-5000$^+$ system, which also has a 5000-psi pressure limit (17).

Given the ubiquitous presence of UHPLC systems in the industry,

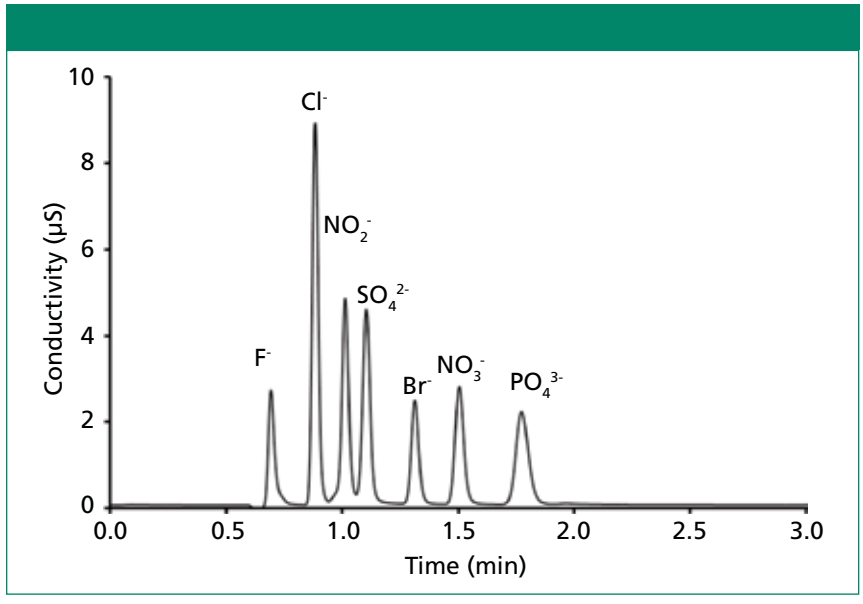

Figure 2: Fast separation of seven common anions on an IonPac AS18 capillary column (150 mm × 0.4 mm) packed with 4-µm particles. Eluent: 35 mM OH^- with a flow rate of 25 µL/min; operating pressure = 3480 psi. Suppressed conductivity detection. Adapted from reference 16.

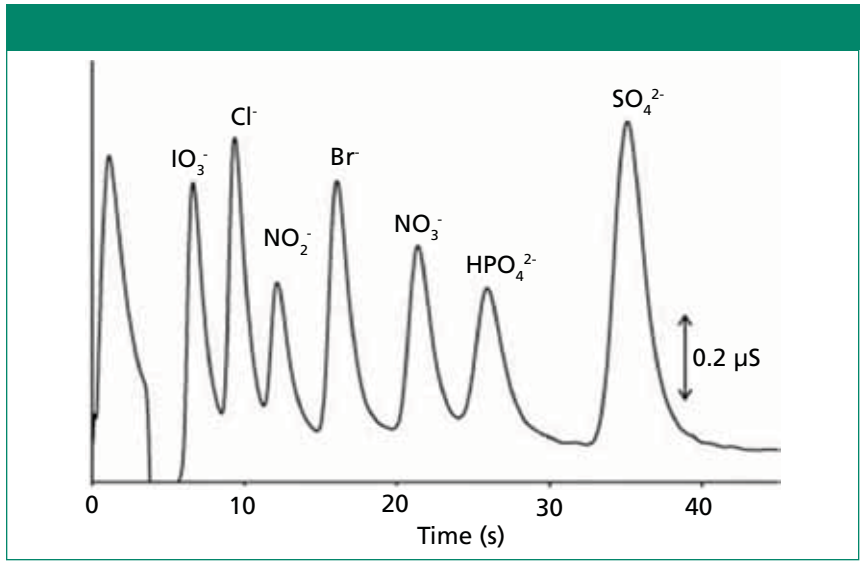

Figure 3: Ultrafast separation of seven common anions on very short column (1.3 cm × 0.46 cm) with suppressed conductivity detection. Column: Cationic surfactant (didodecyldimethylammonium bromide) coated Extend-C18 column; flow rate: 2.0 mL/min; particle size: 1.8 µm; eluent: 2.5 mM 4-hydroxybenzoic acid (pH 10.1). Adapted from reference 20.

chromatographers have begun to ask "Can IC be as fast as UHPLC?" Although the terms "fast" and "ultrafast" separations are relative, a survey of the literature and commercial catalogs shows that these terms imply subminute separations. Subminute IC separations were pioneered by the groups of Paull (18) and Lucy (19). For instance, Figure 3 shows the ultrafast separation of seven common anions in just 40 s using an ultrashort 1.3-cm column packed with 1.8-µm C18 silica particles (20). Although significant developments have taken place in achieving fast separations in commercial IC columns, we have yet to see subminute IC separations in commercial columns. Technology exists to synthesize 2-µm highly crosslinked polymer particles that can withstand pressures of >5000 psi (21). However, there are still significant challenges in using smaller particles. First, as columns become more efficient, extracolumn band broadening effects come into play (20), and those effects are particularly challenging in IC where postcolumn devices such as suppressors are essential. Second, optimum packing of sub-2-µm particles is not a trivial task. Even 4.4-µm charged polymeric particles display unexpected behavior in the packing process (22). Third, the mechanical strength of PEEK limits the pressure of current IC systems to <5000 psi. IC certainly has room to catch up with the efficiencies and speed of UHPLC.

In the above discussion, we provided examples of fast separations of a few ions in relatively simple samples by employing smaller particles or very short columns, or both. Speed becomes a secondary factor, however, when analyzing difficult and ill-characterized samples such as food, biological samples, or environmental waste. In these cases, the target is to fully resolve all the ions of interest. The factors that affect the resolution *(R)* of critical pair of analytes in a given separation are described by

$$R = \left(\frac{\sqrt{N}}{4}\right)\left(\frac{k}{1+k}\right)\left(\frac{\alpha-1}{\alpha}\right) \quad [1]$$

The plate number, *N,* is easily increased by using longer columns or smaller particles. The selectivity factor, α, and retention factor, *k,* become important for high resolution. Both of these variables are dependent on the stationary phase chemistry and the eluent (4,23). Software packages for the simulation and optimization of separations are available (24). For instance, the Virtual Column Separation Simulator (Thermo Fisher Scientific) uses a database of experimental retention data to predict retention using the linear solvent strength model–empirical approach. Resolution maps are also generated by the software for a critical pair of ions. Retention times have been predicted within 3% of the observed behavior for 33 anionic and cationic analytes across a variety of column formats and complex gradients (25).

Thus, by using specially designed high capacity columns and small particles, one can achieve very high resolution. Figure 4 illustrates a high-resolution separation of 44 inorganic and organic ions in 40 min using a long 25-cm capillary and 4-µm particles. This corresponds to a separation speed of 53 s/ion.

However, in most real samples not all ions are in similar concentration. For example, bromate is a human carcinogen whose concentration is regulated by various government environmental agencies around the world (26). In IC, bromate is eluted early, close to the chloride peak. In clean, low

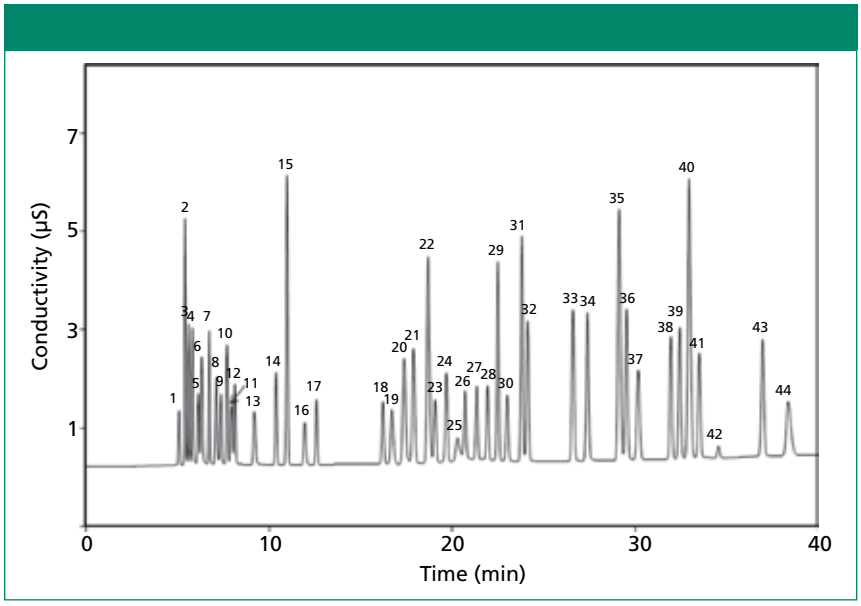

Figure 4: High-resolution gradient separation of 44 anions on a specially tailored high-capacity capillary column (prototype IonPac AS11-HC, 250 mm × 0.4 mm, 4-µm particle size). Eluent: KOH: 1–14 mM KOH in 16 min, 14–55 mM KOH in 24 min, 15 µL/min. Injection volume: 0.4 µL. Peaks: 1 = quinate, 2 = fluoride, 3 = lactate, 4 = acetate, 5 = 2-hydroxybutyrate, 6 = propionate, 7 = formate, 8 = butyrate, 9 = 2-hydroxyvalerate, 10 = pyruvate, 11 = isovalerate, 12 = chlorite, 13 = valerate, 14 = bromate, 15 = chloride, 16 = 2-oxovalerate, 17 = nitrite, 18 = ethylphosphonate, 19 = trifluoroacetate, 20 = azide, 21 = bromide, 22 = nitrate, 23 = citramalate, 24 = malate, 25 = carbonate, 26 = malonate, 27 = citraconitate, 28 = maleate, 29 = sulfate, 30 = alpha-ketoglutarate, 31 = oxalate, 32 = fumarate, 33 = oxaloacetate 34 = tungstate, 35 = molybdate, 36 = phosphate, 37 = phthalate, 38 = arsenate, 39 = citrate, 40 = chromate, 41 = iso-citrate, 42 = *cis*-aconitate, 43 = *trans*-aconitate, 44 = iodide. (Courtesy of Thermo Fisher Scientific.)

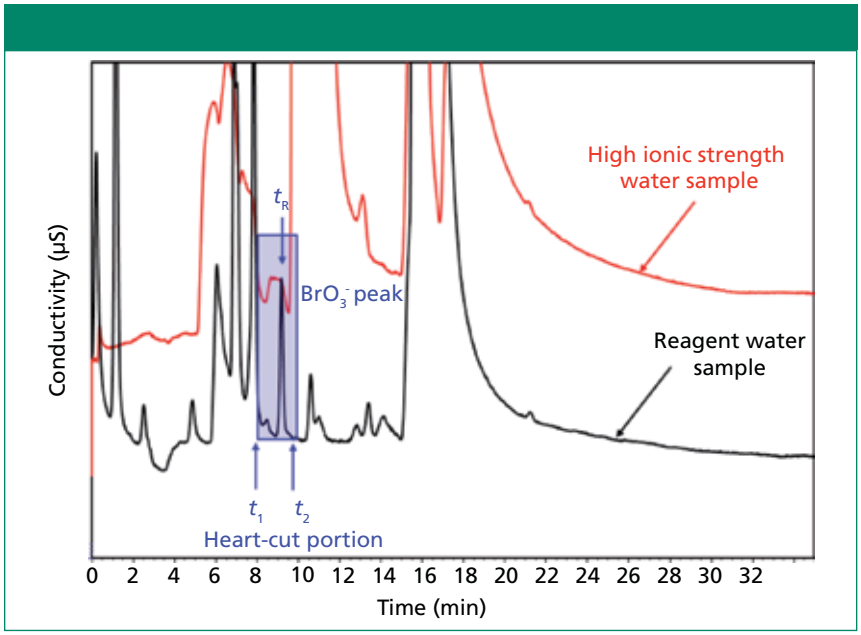

Figure 5: First-dimension analysis of a bromate peak in reagent water and high ionic strength water. The bromate concentration is 15 µg/L. The bromate retention time (t_R) is 9.2 min. The start of the cut window (t_1) is 8.0 min and the end of the cut window (t_2) is 9.8 min. Column: 25 cm × 0.4 cm IonPac AS19; eluent: 10 mM KOH, step changed to 65 mM KOH following the elution of bromate at 1.0 mL/min; injection volume: 1.0 mL. (Courtesy of Thermo Fisher Scientific.)

ionic strength samples, baseline resolution is satisfactory, as shown in the lower trace in Figure 5. In high-ionic-strength water samples, such as seawater or wastewater, the resolution of bromate with the adjacent peak is severely compromised, as shown in the upper trace of Figure 5 (27). The high concentration of the matrix ions overloads the analytical column. The resulting wide fronted or tailed peak broadens and shifts the retention time of the trace (~10 ppb) bromate peak (28). Eventually, the broadening compromises accurate quantification and degrades the resolution of bromate from the overloaded peak.

One ingenious way to obtain high-resolution separation in complex matrices is matrix elimination IC (27,29,30) based on high-resolution two-dimensional IC (2D IC). In this heart-cutting approach for bromate analysis (EPA Method 302.1), the bromate ion is separated in the first dimension using a high-capacity column capable of handling the high-ionic-strength matrix. A ~2 mL portion of the suppressed eluent (the heart-cut portion) containing the bromate (t_1 to t_2 in Figure 5) is passed through a concentrator column positioned in place of the sample loop of the injection valve for the second-dimension separation. The trapped analyte is eluted off the concentrator column into a second-dimension IC column of different selectivity than the first-dimension column. For high-resolution applications, a second-dimension column with a lower cross-sectional area is used to enhance sensitivity (31). Figure 6 shows the second-dimension high-resolution separation of trace levels (15 ppb) of bromate in both reagent and a high-ionic-strength water sample. This heart-cutting matrix elimination approach is generic and can easily be applied on IC samples that give poor resolution of trace components as a result of matrix overload. For instance, perchlorate has similarly been resolved in highly complex water samples (31). Research is ongoing on comprehensive 2D IC for complete analysis of complex ionic samples (32).

Conclusions

Until recently, the speed of IC was limited by the pressure and flow rate limits of the IC hardware. These conditions required the use of large particles and long columns. Numerous recent advances have enabled faster IC separations by reducing the column length while using large particles, or by reducing the particle size to 4 µm. However, commercial IC has not yet achieved the ultra-fast subminute separations that have been demonstrated by sub-2-µm particles in very short columns. Thus, further advances in IC speed for simple samples are anticipated.

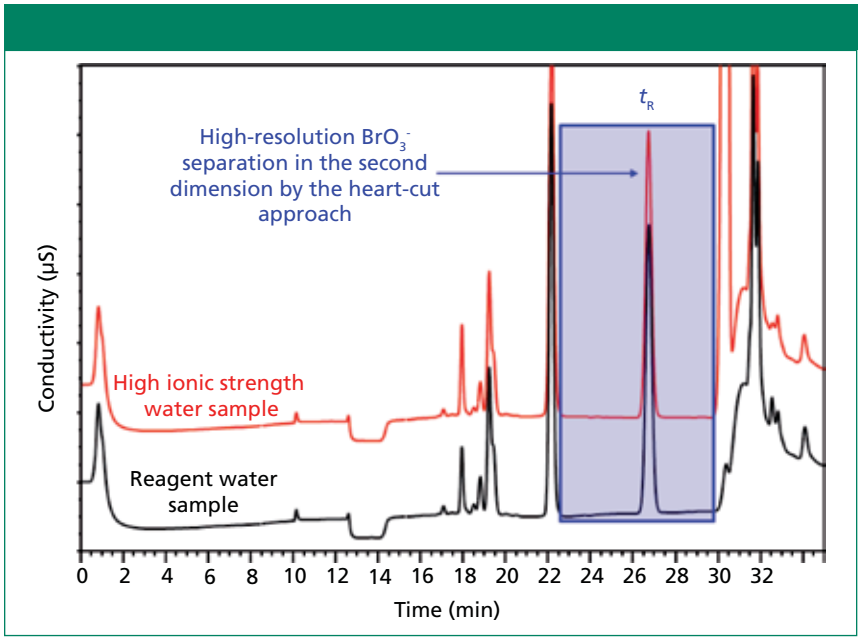

Figure 6: High-resolution separation of bromate ion in second-dimensional analysis of 15 µg/L bromate in reagent water and high ionic water matrix. Column: IonPac AS24 analytical column (25 cm × 0.2 cm) and AG24 guard column (5 cm × 0.2 cm) were used for the second dimension analysis; eluent: isocratic 10 mM KOH changed to 65 mM KOH following the elution of bromate for approximately 10 min and re-equilibrate at 10 mM hydroxide before injection; eluent flow: 0.25 mL/min. (Courtesy of Thermo Fisher Scientific.)

For complex matrices, the enhanced pressure capabilities of modern IC are enabling greater one-dimensional peak capacities. Commercial equipment has made targeted two-dimensional IC separations routine.

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