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Journal of Chromatography A, 671 (1994) 63–71

JOURNAL OF CHROMATOGRAPHY A

Practical aspects on the use of organic solvents in ion chromatography

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Abstract

Organic solvents play an important role in ion chromatography. The advent of solvent-compatible ion-exchange stationary phases permit all proportions of organic eluents to be employed. Selectivity mediation for the ion-exchange process is the most important usage of solvents. Changing the retention characteristics of the column packing toward the analyte permits the analyst to alter retention order, peak efficiency and resolution to optimize the separation. Column clean-up of organic molecules is also dramatically enhanced by addition of solvent. Several examples are offered to describe the utility of organic solvents in modern ion chromatography.

1. Introduction

Ion chromatography (IC) has made great advances since the early days of the technique when it was used as a method of determining chloride and sulfate. The modern ion chromatographer has a variety of detectors and separation modes to perform analyses of inorganic and organic ions in a broad range of matrices. Ion-exchange separations followed by suppressed conductivity detection is still the mainstay of IC, and it remains a very powerful and versatile technique.

The advent of chemically suppressed conductance made IC practical. Beginning with the packed-bed suppressor developed by Small *et al.* [1] and proceeding through several generations to the current state-of-the-art device, an electrochemical suppressor that generates its own regenerant *in situ* from water in the detector cell effluent [2], suppression has advanced to the point where it is essentially invisible, can suppress very strong eluent concentrations including solvent-containing eluents, and creates very quiet baselines to further lower detection limits.

Ion-exchange column packings have also improved over the years since the inception of IC. Higher-capacity, higher-efficiency and faster columns have been developed [3], often in tandem with increased capacity and efficiency of the suppression devices. Stationary phases for IC have also become organic solvent compatible through development of highly cross-linked substrate beads which minimize swelling.

Solvents can play an important role in the modern day practice of IC. Column clean-up, sample solubility and selectivity optimization may all be enhanced through addition of solvents to the aqueous phase. This paper will discuss the use of solvents in IC, including description of the ion-exchange process as mediated by organic

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0021-9673/94/$07.00
SSDI 0021-9673(94)00002-Q

solvents, column clean up techniques and several examples of separation optimization.

2. Experimental

2.1. Chromatography systems

The chromatography systems used in this study were Dionex DX-300 and System 4500i ion chromatographs. The DX-300 uses a Dionex AGP quaternary gradient pump fitted with pistons to generate flow-rates compatible with either 2 or 4 mm I.D. columns. The System 4500i is equipped with Dionex GPM-2 quaternary gradient pump. Detectors for these systems were a Dionex CDM-II conductivity detector and a Dionex PED pulsed electrochemical detector operating in the conductivity mode. All data were collected and processed on Dionex AI-450 software.

2.2. Columns

Separation columns used in this study included the Dionex IonPac CS14 for cation determinations, and the Dionex IonPac AS4A-SC, AS5A, AS11, OmniPac PAX-500 and OmniPac PAX-100 for anion determinations. Mobile phase ion chromatography (MPIC) separations were performed with the Dionex IonPac NS1 column. Suppressors for this work included the Dionex CSRS, ASRS (both operating with external water feed for regeneration) and AMMS-II (with sulfuric acid regeneration).

2.3. Reagents

Methanesulfonic acid (MSA) eluents for cation determinations were prepared from the 99 + % puriss. acid (Fluka, Ronkonkoma, NY, USA). Sodium hydroxide eluents for anion separations were prepared by dilution from the certified grade 50% solution (Fisher, Pittsburgh, PA, USA). Tetrabutylammonium hydroxide (TBAOH; Dionex, Sunnyvale, CA, USA) was diluted from the MPIC-grade 0.10 *M* solution. Acetonitrile and methanol (both Optima grade, Fisher, Pittsburgh, PA, USA) were used as received. Deionized water (18 MΩ) was obtained from a Millipore (Bedford, MA, USA) Milli-Q water purifier.

Note: Non-spectral-grade acetonitrile (Burdick & Jackson, suitable for GC and pesticide analysis) was found to be unsuitable for IC. This solvent contained ionic impurities that caused poor separations and intolerably high backgrounds in the conductivity traces.

3. Results and discussion

3.1. Stationary phase development

In the early days of IC, stationary phases were not solvent compatible. The polymer beads that formed the substrate to which the ion-exchange latex were attached was lightly cross-linked ($<5\%$) polystyrene–divinylbenzene. The consequence of this was that the polymer beads would swell dramatically in the presence of even small amounts of organic solvent, creating high backpressure on the column and also creating headspace when the column had water pumped through it after use with the solvent. To produce a more solvent-tolerant resin, *i.e.* with reduced swelling characteristics, the degree of cross-linking had to increase. Today, advances in polymer chemistry yields greater than 50% cross-linked substrate beads that are 100% compatible with all common HPLC solvents [4]. Columns produced with these substrates exhibit very little swelling when exposed to solvents. These advances now allow the use of solvents for various purposes with IC.

3.2. Uses of solvents with IC

Selectivity mediation

Perhaps the most important usage of organic solvents for IC is mediation of the ion-exchange selectivity of a column. Changing the retention characteristics of a particular column may be important in separating otherwise co-eluting species, reducing the overall run time of the

chromatogram, or helping to reduce peak asymmetry and increase efficiency.

Ion exchange is a very complex process. Adding solvents to the eluent greatly increases this complexity as there are several competing effects that govern the overall retention. This section will discuss the major constituents that mediate ion exchange in the presence of organic solvents.

Solvents are commonly used to alter retention characteristics of ion-pair chromatography, where they have a long history [5]. While the use of organic modifiers to alter ion-exchange selectivity for IC is relatively new, it was not unknown before the advent of solvent-compatible stationary phases. Some ion chromatographers were willing to tolerate the high backpressures and swelling to get the advantages that solvent mediation had to offer [6]. With solvent-compatible columns, swelling is minimized, allowing the full use of solvents up to 100% concentration.

In solution, ions are surrounded by a solvation sphere, that is, a relatively ordered group of solvent molecules oriented around the ions in equilibrium with the bulk solvent phase [7]. In aqueous solution, ions are surrounded by water molecules (hydration). In a mixed aqueous–organic solvent system, however, the organic molecules can disrupt this hydration sphere, allowing intrusion of the organic solvent molecules in the solvation matrix. The degree of this intrusion is based upon several factors, most notably hydrophobicity, ability of the solvent to hydrogen bond to the ion, and polarizability of the ion [8]. This solvation sphere determines the ability of the ion to move in solution, as it must tow these molecules along with it. As the nature of the solvent sphere changes, so changes the movement of the ion in solution. For an ion to adsorb on an ion-exchange site, it must first rearrange and eventually, partially shed its solvation sphere to allow close approach to the ion-exchange site. The greater the degree of shedding, the closer the ion can approach the site; the closer it can approach, the more tightly bound it becomes. Likewise, the ion-exchange site must reorient its solvation sphere (it is essentially a permanently bound ion) to permit the ion to approach. Therefore, the ability of an ion to shed a part of its solvation sphere plays an important role in the ion-exchange process [9]. Combining these concepts leads to the notion of selectivity mediation. The degree of solvation and ability of an ion to remove the surrounding solvent molecules would then alter the affinity that ion has for the ion-exchange site, a significant part of the overall selectivity. Another related factor is the affinity that the eluting ion exhibits toward the stationary phase. These eluting ions are also solvated, and subject to the same effects that govern retention of the analyte. In essence, observed selectivity is a competition of relative affinities of the analyte and eluent ions for the ion-exchange sites, *i.e.* how easily can the eluent elute the analyte and *vice versa*.

Selectivity mediation is influenced by several factors, including choice of solvent mediator, concentration of solvent, and nature of the analyte. All of these factors can be exploited to enhance the IC separation.

The choice of solvent can be an important parameter when performing a separation. Solvating power and hydrophobicity of the solvent can influence the retention mechanism. Stillian and Pohl [4] have looked at different solvent types on the separation of various inorganic anions with a hydroxide eluent on a Dionex OmniPac PAX-100 column. They concluded that if a longer chromatogram with improved resolution is desired, a methanol-containing eluent should be used. The highly hydrated hydroxide ion would tend to lose waters of hydration less readily than the stationary phase or the analyte in the presence of methanol, thus decreasing the selectivity of the ion-exchange sites for hydroxide. For shorter retention times, acetonitrile can be used. They contend that swelling of the latex containing the ion-exchange sites is greater in acetonitrile, therefore creating a lower effective cross-link of the latex, which would spread out the ion-exchange sites, effectively reducing the number of these sites per unit area of ion-exchange polymer. To a much lesser degree, acetonitrile mediates the ion-exchange process due to a decrease in dielectric constant of the bulk solvent relative to water; this phenomenon is

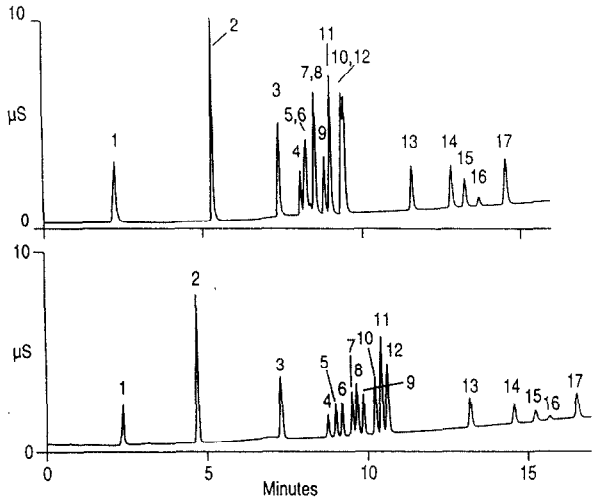

Fig. 1. Effect of methanol on retention on an IonPac AS11 column. Eluent: NaOH gradient: 0.5 to 5 m*M* in 5 min, to 38 m*M* in 15 min. Flow-rate: 2.0 ml/min. Detection: suppressed conductivity, ASRS suppressor. Peaks: 1 = acetate; 2 = chloride; 3 = nitrate; 4 = glutarate; 5 = succinate; 6 = malate; 7 = malonate; 8 = tartrate; 9 = maleate; 10 = fumarate; 11 = sulfate; 12 = oxalate; 13 = phosphate; 14 = citrate; 15 = isocitrate; 16 = *cis*-aconitate; 17 = *trans*-aconitate. Top: NaOH gradient with aqueous eluent; bottom: NaOH gradient with eluent containing 16% methanol.

more pronounced in longer-chain solvents such as isopropanol.

These effects are demonstrated in Figs. 1 and 2. Fig. 1 is a separation of various organic and inorganic anions on an IonPac AS11 column with a NaOH gradient. The top chromatogram does not contain methanol, while the bottom chromatogram contains 16% methanol. A longer retention time results with addition of methanol, yielding better resolution of closely eluting ions. In Fig. 2, various inorganic anions are eluted off of an OmniPac PAX-100 column. The first chromatogram contains 5% acetonitrile, the second 20%, and the third has 40% acetonitrile, all with 40 m*M* NaOH. As the acetonitrile concentration increases, the retention time decreases.

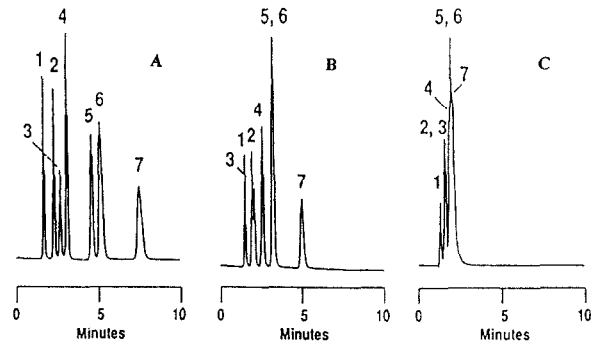

Fig. 2. Effect of acetonitrile on retention on an OmniPac PAX-100 column. All chromatograms contain 40 m*M* NaOH. (A) 5% MeCN; (B) 20% MeCN; (C) 40% MeCN. Flow-rate: 1.0 ml/min. Detection: suppressed conductivity, AMMS-II suppressor. Peaks: 1 = fluoride; 2 = chloride; 3 = nitrite; 4 = sulfate; 5 = bromide; 6 = nitrate; 7 = phosphate.

One thing to beware of when performing anion chromatography with a hydroxide eluent and acetonitrile is eluent decomposition. In the presence of base, acetonitrile slowly hydrolyzes to acetate and ammonia [10]. Acetate in particular is troublesome as it causes higher background conductivity and changes eluent composition; if running a gradient, acetate could build up on the column, then elute causing a large baseline disturbance. Therefore, to avoid eluent degradation, it is best to keep hydroxide and acetonitrile in separate bottles and proportion them together in the desired ratio(s) rather than mixing them together.

Other solvents can be used to enhance anion separations. Ethanol and isopropanol can be used as alternatives. The longer-chain, more hydrophobic alcohols such as isopropanol would tend to swell the matrix polymer to a greater degree than methanol. It has been reported [4] that addition of isopropanol to hydroxide eluents increases the k' for many ions at low alcohol concentrations, and then decreases k' as the isopropanol concentration increases. At low concentrations, isopropanol is more like methanol in its ion-exchange mediation properties, and at higher concentrations, it becomes a swelling solvent as is acetonitrile. Ethanol has properties midway between methanol and isopropanol. As depicted in Fig. 3, addition of ethanol to sodium hydroxide gives a shorter run time chromatogram than adding methanol. This can be explained in terms of solvation and dielectric constant effects. The dielectric constant for ethanol is significantly lower than methanol [11], therefore a solution of ethanol in water would be

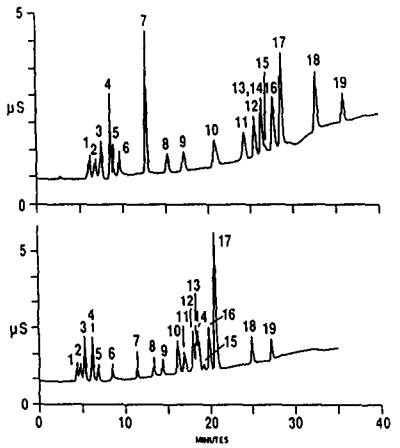

Fig. 3. Effect of solvent type on retention. Column: OmniPac PAX-500. Eluent: NaOH gradient–isocratic solvent; (top) 30% methanol, (bottom) 27% ethanol. Flow-rate: 1.0 ml/min. Detection: suppressed conductivity, AMMS-II suppressor. Peaks: 1 = fluoride; 2 = acetate; 3 = lactate; 4 = glycolate; 5 = formate; 6 = gluconate; 7 = chloride; 8 = galacturonate; 9 = glucuronate; 10 = nitrate; 11 = succinate; 12 = malate; 13 = sulfite; 14 = maleate; 15 = carbonate; 16 = tartrate; 17 = sulfate; 18 = phosphate; 19 = citrate.

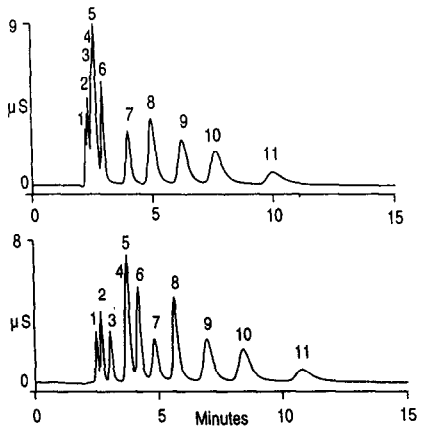

Fig. 4. Effect of solvent on separation of cations. Column: IonPac CS14. (Top) Eluent A: 40 m*M* MSA. (Bottom) Eluent B: 20 m*M* MSA + 1% acetonitrile. Flow-rate: 1.0 ml/min. Detection: suppressed conductivity, CSRS suppressor. Peaks: 1 = lithium; 2 = sodium; 3 = ammonium; 4 = potassium; 5 = magnesium; 6 = calcium; 7 = propylamine; 8 = *tert.*-butylamine; 9 = *sec.*-butylamine; 10 = isobutylamine; 11 = *n*-butylamine.

less polar than an equivalent methanolic solution, which in turn affects the degree of solvation and ability of ions to exchange.

The selectivity of cation chromatography can also be mediated by addition of organic solvents, for much the same reason as for anions. Fig. 4 shows two separations on a Dionex CS14 cation-exchange column. The top chromatogram contains no solvent, the bottom has half the acid concentration plus 1% acetonitrile. The retention times are nearly the same, yet peak resolution of the early eluting ions and tailing of the organic amines is greatly enhanced in the bottom chromatogram.

Cation-exchange columns with carboxylate functionality such as the Dionex IonPac CS12 and CS14 are difficult to use with alcohols. This is because the ion-exchange sites esterify in the presence of alcohol. This process is reversible, however it makes the use of alcohols impractical with this type of columns. Acetonitrile is the recommended solvent to use with carboxylate-functionalized resins.

There are some other effects of solvents on ion-exchange columns that play a small part in the mediation process. While solvent-compatible columns exhibit greatly reduced swelling relative to their lightly cross-linked predecessors, they still do swell to some degree, which would cause a slight increase in retention time as the ion-exchange sites are spaced further away.

Selection of column types in combination with solvents is dependent upon the type of analysis to be performed. As a general rule, hydrophilic ions are better separated on hydrophilic column packings, and hydrophobic analytes are better on hydrophobic packings. Ion-exchange capacity also can play an important role. For example, in a high-ionic-strength matrix, use of a high-ion-exchange-capacity column for overload prevention is desirable.

Column clean-up

Another important use of organic solvents with IC is for column clean-up. Many compounds, both ionic and non-ionic, can adsorb strongly on the ion-exchange sites and polymer backbone, respectively. These materials can cause loss of resolution and capacity, thus having a deleterious effect on the separation. While

pre-removal of these species through filtration or adsorption is always preferable, sometimes this is not possible and they find their way onto the column packing.

Before the days of solvent-compatible stationary phases, once the column became fouled, little could be done to recover it. High concentrations of acid or base could be used, sometimes with a small amount of organic modifier, but these often did not work, particularly if the fouling material was a bulky organic molecule. The use of solvents, however, allows another dimension in column clean-up as a combination of high ionic strength and organic modifier can often elute the fouling species from the stationary phase.

Humic acids have long presented a difficult analytical problem for IC because the acids adsorb strongly onto the ion-exchange stationary phases, gradually causing a loss of efficiency and capacity due to fouling of the ion-exchange sites. An example of this is shown in Fig. 5. Chromatogram A shows an injection of seven anions on an AS4A-SC column. After fouling with humic acid, chromatogram B demonstrates that the retention time and efficiency have dramatically decreased. After the first clean up step, some of the capacity has been recovered as shown on chromatogram C. Treatment with the final clean-up step gives nearly 100% recovery of both efficiency and retention time as depicted in chromatogram D.

Suppressed conductivity detection with solvents

Chemical suppression for IC serves three purposes: it increases the conductivity signal due to the analyte while reducing the background noise, thus it dramatically increases the signal-to-noise ratio to enhance sensitivity, as well as removing counterions, which serves to improve the resolution of early-eluting species. Until the advent of flat membrane suppressors such as the MicroMembrane Suppressor [12], solvent compatibility of suppressors was not possible due to solvent swelling effects encountered with the older devices. Today, modern suppressors are solvent compatible, allowing the combination of benefits that solvents have to offer regarding selectivity mediation with signal-to-noise enhancement of suppression.

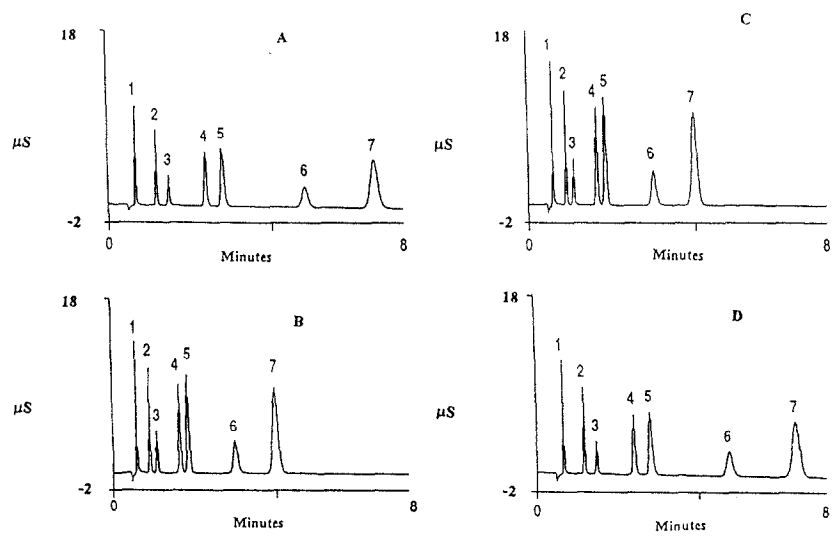

Fig. 5. Humic acid fouling and clean-up. Column: IonPac AS4A-SC. Eluent: 1.8 m*M* Na_2CO_3, 1.7 m*M* $NaHCO_3$. Flow-rate: 2.0 ml/min. Detection: suppressed conductivity, AMMS-II suppressor. Peaks: 1 = fluoride; 2 = chloride; 3 = nitrite; 4 = bromide; 5 = nitrate; 6 = phosphate; 7 = sulfate. Detection: suppressed conductivity. Clean-up steps: (1) 12 m*M* HCl, 50% acetonitrile for 1 h; (2) 200 m*M* HCl, 80% tetrahydrofuran for 1 h. Chromatograms: (A) before fouling; (B) after fouling; (C) after clean up step 1; (D) after clean up step 2.

The state-of-the-art suppression device, the Self Regenerating Suppressor (SRS), produces its own regenerant ions *in situ* from the electrolysis of water. The original design of the unit prevented use of organic solvents with electrochemical operation [13]. Design improvements now permit the use of all commonly employed IC solvents in all proportions while taking advantage of the ease of use of water electrolysis.

To operate the SRS when using solvents, a sufficient supply of water must be delivered to the suppressor to ensure good hydration of the membranes. To do this, an external supply of water must be supplied to the unit at flow-rates of 5–10 ml/min either by a pressure bottle, pump, or connection directly to a deionized water source. The other operational mode of the SRS, where the conductivity cell effluent is recycled back to the suppressor as the water source for electrolysis cannot be used with solvent-containing eluents. A sufficient supply of water must be available for the electrolytic reaction; addition of solvent to eluent reduces the bulk water concentration. Methanol is able to oxidize electrolytically, so the presence of this species in the electrolysis chambers would have a deleterious effect on suppression.

Following are several examples of separations using organic containing eluents and electrolytic suppression. Polarizable anions such as thiosulfate and perchlorate have long been a difficult separation problem due to their strong affinities for ion-exchange sites. Addition of methanol to a sodium hydroxide eluent on an IonPac AS11 allows easy separation of these species as shown in Fig. 6. Without addition of methanol, the more polarizable ions would have long run times and would exhibit greatly increased peak tailing. Kraft black liquor samples contain a substantial amount of thiosulfate. A separation shown in Fig. 7 demonstrates elution of thiosulfate in less than 10 min with good resolution of other oxyanions and chloride.

Ion-pair or mobile phase ion chromatography (MPIC) can also benefit from solvent gradients and electrolytic suppression. In the past, membrane suppression for anion MPIC separations required a specialized suppressor, the AMMS-

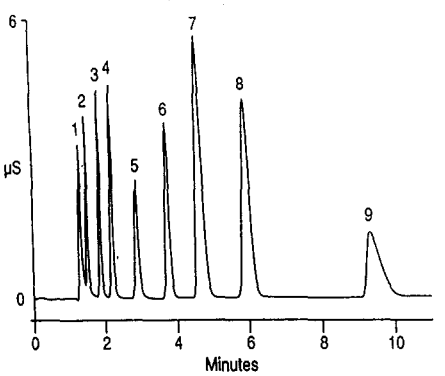

Fig. 6. Separation of polarizable anions. Column: IonPac AS11. Eluent: 45 m*M* NaOH, 40% methanol. Flow-rate: 1.0 ml/min. Detection: suppressed conductivity, ASRS suppressor. Peaks: 1 = fluoride; 2 = chloride; 3 = nitrate; 5 = phosphate; 6 = iodide; 7 = thiocyanate; 8 = thiosulfate; 9 = perchlorate.

MPIC. With the introduction of a solvent-compatible ASRS, MPIC can now be performed with the same device used for anion-exchange determinations. A demonstration of this is shown in Fig. 8. An acetonitrile gradient is used to elute various alkanesulfonic acids from C_3 to C_8 in less than 25 min. An ASRS is used in electrolytic mode with external water delivery to suppress the TBAOH eluent.

Organic solvents can aid cation determinations as well. Morpholine is a common additive to power plant waters to increase pH. The presence of morpholine often creates problems for determining other trace cations as it can coelute with ions of similar retention times, as well as exhibiting substantial band broadening and tail-

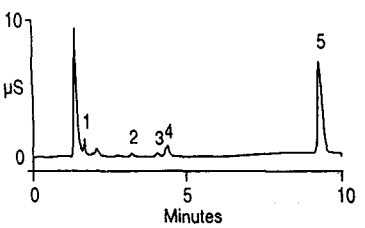

Fig. 7. Analysis of Kraft black liquor. Column: IonPac AS11. Eluent: 30 m*M* NaOH in 40% methanol for 3 min; gradient to 60 m*M* NaOH in 40% methanol at 5 min. Flow-rate: 1.0 ml/min. Detection: suppressed conductivity, ASRS suppressor. Peaks: 1 = chloride; 2 = sulfite; 3 = oxalate; 4 = sulfate; 5 = thiosulfate.

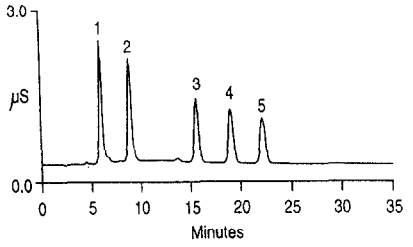

Fig. 8. Separation of sulfonic acids by MPIC with electrolytic suppression. Column: IonPac NS1. Eluent: 10 m*M* TBAOH, 20% acetonitrile to 40% acetonitrile in 20 min. Flow-rate: 1.0 ml/min. Detection: suppressed conductivity, ASRS suppressor. Peaks: 1 = 2-propanesulfonic acid; 2 = 1-butanesulfonic acid; 3 = hexanesulfonic acid; 4 = heptanesulfonic acid; 5 = octanesulfonic acid.

ing. The use of 5% acetonitrile in a methanesulfonic acid eluent can reduce the peak width and tailing to the point where determination of the other analytes is greatly simplified (see Fig. 9).

One thing to keep in mind when using conductivity detection with solvent-containing eluents is that peak response for ions will decrease somewhat as the solvent concentration increases. This is a function of the reduction of the dielectric constant of the overall solvent mixture. As the dielectric constant of the solvent system decreases, the resistance of the bulk solution increases [7], hence the specific conductivity of this solution decreases. Signal reduction is more pronounced in weak acids or bases, where the presence of solvent suppresses the dissociation of the ions relative to that of pure water. The decrease in peak response is offset somewhat by lower detector noise; unfortunately, however, sensitivity will suffer somewhat when using substantial amounts of organic solvent in IC eluents.

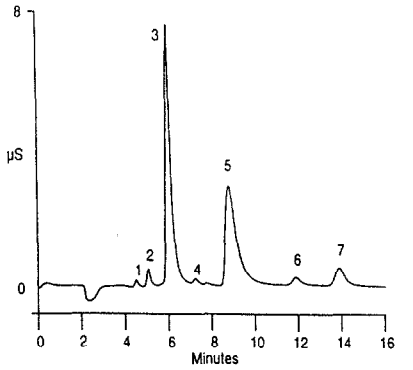

Fig. 9. Determination of trace cations in morpholine-treated power water. Columns: IonPac CG14, CS14 (2 mm). Eluent: 8 m*M* MSA, 5% acetonitrile. Flow-rate: 0.25 ml/min. Injection volume: 1.0 ml concentrated on a CG14 (2 mm) column. Suppressor: CSRS, external water supply. Peaks: 1 = lithium (0.5 µg/l); 2 = sodium (2.0 µg/l); 3 = ammonium (150 µg/l); 4 = potassium (2.0 µg/l); 5 = morpholine (2000 µg/l); 6 = magnesium (2.0 µg/l); 7 = calcium (10 µg/l).

4. Conclusions

Organic solvents are an important addition to one's IC "toolbox". Using solvent to mediate selectivity allows one to get maximal value from just a few columns. Through reduction of secondary effects, organic solvents also serve to improve chromatographic performance by decreasing tailing and peak broadening. Coupled with suppressed conductance, they provide a powerful problem-solving tool for the modern ion chromatographer.

5. Acknowledgement

The authors wish to thank Ruthann Kiser, John Statler, Charanjit Saini, Maria Rey, Rosanne Slingsby and Chris Pohl of Dionex for their helpful discussions and data collection.

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