INTRODUCTION

Mobile-phase ion chromatography (MPIC) is a technique that combines ion-pair chromatography with suppressed conductivity detection. Ion-pair chromatography is commonly used in combination with UV detection, in which case it is referred to as reversed-phase ion-pair chromatography (RPIPC). MPIC has the advantage of using conductivity detection, which serves as a universal detection mode for species likely to be separated by ion-pair chromatography.

MPIC is especially well suited to large molecules that carry localized charges (surfactants are a good example), as well as other ions that are not amenable to separation by ion-exchange chromatography (IC). For example, aliphatic quaternary ammonium ions can bind to ion-exchange resins during an IC separation, leading to inefficient peaks or low recovery. Due to the dynamic nature of the ion pair and since organic solvents are usually in the mobile phase, these ions do not adhere to the neutral resin used in MPIC. Also, when ion exchange does not have the required selectivity for a troublesome set of analytes, separation can often be achieved by switching to ion-pair.

Suppressed conductivity detection, which has been used with ion-exchange chromatography since 1975, offers the same advantages for MPIC as it does for IC. The suppressor simultaneously lowers background conductivity from the eluent and enhances the analyte conductivity. State-of-the-art suppressors are membrane-based (for high efficiencies), easy to use, and solvent-compatible. Special considerations for suppression of ion-pair mobile phases are discussed in this Technical Note.

Detergent and other chemical manufacturers routinely use MPIC for the analysis of compounds such as ionic surfactants or quaternary ammonium compounds. The technique is also of use to cosmetics manufacturers for the analysis of additives such as alkanolamines, and to the pharmaceutical industry for a variety of nonchromophoric, ionic species.

PRINCIPLES OF ION-PAIRING

Separation Mechanism

Ion-exchange selectivity is mediated by both the mobile and stationary phases. In contrast, the selectivity of an ion-pair separation is determined primarily by the mobile phase. The two major components of the aqueous mobile phase are the ion-pair reagent and the organic solvent; the type and concentration of each component can be varied to achieve the desired separation.

The ion-pair reagent is a large ionic molecule that carries a charge opposite of the analyte of interest. It usually has both a hydrophobic region to interact with the stationary phase and a charged region to interact with the analyte. Stationary phases used for ion-pair are neutral, hydrophobic resins such as polystyrene/divinylbenzene (PS/DVB) or bonded silica. A single stationary phase can be used for either anion or cation analysis.

Although the retention mechanism for ion-pair chromatography is not fully understood, three major theories have been proposed:

• Ion pair formation
• Dynamic ion exchange
• Ion interaction
In the first model, the analyte and the ion-pair reagent form a neutral “pair”, which then partitions between the mobile phase and the stationary phase. Retention can be controlled by varying the concentration of organic solvent in the mobile phase, as in classic reversed-phase chromatography.

The dynamic ion-exchange model maintains that the hydrophobic portion of the ion-pair reagent adsorbs to the hydrophobic stationary phase to form a dynamic ion-exchange surface. The analyte is retained on this surface, as it would be in classic IC. Using this scenario, solvents used in the mobile phase can be used to impede interaction of the ion-pair reagent with the stationary phase, thereby altering the “capacity” of the column.

Figure 1 summarizes the major interactions described in these two theories. In this example, a cationic analyte (i.e., C⁺) is being separated using an acetonitrile/water mobile phase containing octanesulfonate as the ion-pair reagent. A PS/DVB column acts as the stationary phase. The cations are retained by a combination of their interaction with the octanesulfonate that is adsorbed to the stationary phase (the hydrophobic environment), and by their interaction with the octanesulfonate ions in the mobile phase (the hydrophilic environment). When “paired” with the octanesulfonate in solution, the cations are able to partition between the mobile and stationary phases. Note that the acetonitrile in the mobile phase is also adsorbed to the stationary phase, thereby lowering the effective capacity of the column.

A third model describes an electrical double layer that is formed when the ion-pair reagent permeates the stationary phase, carrying with it an associated counterion. Retention of the analyte ion in this model is dependent upon a combination of factors, including those described in the first two models. References 1 and 2 are recommended for a more in-depth discussion of ion-pair mechanisms.

**Ion-Pair Reagents**

There are two simple rules when choosing the appropriate ion-pair reagent. The first rule is that hydrophilic ions are best separated using a hydrophobic ion-pair reagent, and that hydrophobic analytes are best separated with a hydrophilic ion-pair reagent. Common pairing reagents are listed in the order of increasing hydrophobicity:

- **For anion analysis**, ion-pair reagents include ammonium and tetramethyl-, tetraethyl-, tetrapropyl-, and tetrabutylammonium. Each of these must be in the hydroxide form to be suppressed for conductivity detection.

- **For cation analysis**, ion-pair reagents include hydrochloric, perchloric, and perfluorocarboxylic acids and pentane-, hexane-, heptane-, and octane sulfonic acids. Perfluorocarboxylic acids have the advantage of low conductance and are available in extremely high purity; they are useful for separating very hydrophobic cations. Cation-pairing reagents must be in the acid (hydronium ion) form to be suppressed for conductivity detection.

The second rule for choosing the appropriate ion-pair reagent is that smaller pairing reagents usually result in the best separation, since the structure and properties of the analyte will have a greater contribution to how the analyte-reagent complex partitions in the system.

**Reagent Concentration**

Because the effective capacity of the column is determined mainly by the ion-pair reagent, retention of the analyte will increase as the concentration of reagent is increased. However, electrostatic repulsion of reagent molecules on the stationary phase surface will ultimately limit the degree to which the capacity of the
column can be increased. The concentration of the ion-pair reagent is also limited by the capacity of the suppressor. Typical working concentration ranges are between 0.5 and 20 mM.\(^1\)

**Organic Modifiers**

Organic solvents are used to decrease retention times and to modify the selectivity of the separation. These modifiers work in the following two ways: (1) by competing with the pairing reagent for the stationary phase, thus decreasing the effective capacity of the column; and (2) by decreasing the polarity of the mobile phase, which affects partitioning of the analyte-reagent pair into the hydrophobic environment (see Figure 1).

The optimum organic solvent concentration for a given separation depends to a degree on the hydrophobicity of the ion-pair reagent. Figure 2 demonstrates that as the hydrophobicity of the ion-pair reagent increases, more organic solvent is needed to keep the run time to approximately 10 minutes. In this example, pairing the alkylsulfonate analytes with ammonium requires only 15% acetonitrile in the mobile phase, but 37% acetonitrile is required with tetrabutylammonium as the ion-pair reagent. Peak shapes for the more highly retained components are significantly improved, however, with the higher concentration of organic solvent.

Keep in mind that increasing the concentration of organic solvent will eventually compromise conductivity detection limits. If this becomes a problem, switching to a less hydrophobic ion-pair reagent is advisable.

Acetonitrile and methanol are by far the most common organic solvents used in MPIC. Higher concentrations of methanol are necessary to achieve results comparable to acetonitrile, but methanol has hydrogen-bonding properties which are sometimes useful. Other modifiers can be used as necessary, such as when sample solubility is an issue.

**pH Effects**

When it is necessary to adjust the pH of the mobile phase, choosing an acid or base that is compatible with suppressed conductivity detection is critical. For instance, when multivalent anions are too highly retained, lowering the pH will decrease their ionization and thus their interaction with the ion-pair reagent. Boric acid is a good choice because, although it is not suppressed, it does not raise the background conductivity of the mobile phase significantly.

The pH of the system can also be manipulated to enhance detection. This will be discussed in the “Example Separations” section of this note.

**Other Mobile Phase Additives**

Carbonate is commonly used to improve peak shape and reduce retention of multivalent anions.\(^1\) A dramatic decrease in \(k'\) for divalents relative to monovalents is observed with increased carbonate concentration. This is consistent with the ion-exchange retention model. Classical IC theory predicts that the slope of \(\log k'\) versus \(\log [\text{eluent ion concentration}]\) (the eluent ion being carbonate in this case) for divalents should be twice that for monovalents. Typical values for carbonate concentrations range from 0.1 mM to 1 mM \(\text{Na}_2\text{CO}_3\).
**DETECTION**

**Benefits of Suppression**

Figure 3 illustrates how chemical suppression is used to simultaneously reduce background conductance and increase the conductivity of the analyte ions. In this example, anions are being separated by an MPIC column with NR$_4^+$OH$^-$ representing the ion-pair reagent in the mobile phase. The quaternary ammonium ions are exchanged in the suppressor for hydronium ions, which results in the NR$_4^+$OH$^-$ being converted to water. At the same time, the NR$_4^+$ counterions to the analytes are being exchanged for hydronium ions. As seen in Table 1, the hydronium ion has a much higher equivalent conductance than typical ammonium pairing reagents, which means that the hydronium-analyte pair will produce a larger signal than it would without suppression (see Figure 3).

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**Table 1 Equivalent Conductances of Common Ion-Pair Reagents**

<table>
<thead>
<tr>
<th>Anion Analysis</th>
<th>$\lambda_a$ (µS cm$^{-1}$)</th>
<th>Cation Analysis</th>
<th>$\lambda_a$ (µS cm$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydronium</td>
<td>350</td>
<td>Hydroxide</td>
<td>199</td>
</tr>
<tr>
<td>Ammonium</td>
<td>73.5</td>
<td>Chloride</td>
<td>76.4</td>
</tr>
<tr>
<td>Tetramethylammonium</td>
<td>45.3</td>
<td>Perchlorate</td>
<td>67.9</td>
</tr>
<tr>
<td>Tetraethylammonium</td>
<td>33.0</td>
<td>Octanesulfonate</td>
<td>29.0</td>
</tr>
<tr>
<td>Tetrapropylammonium</td>
<td>23.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tetrabutylammonium</td>
<td>19.1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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**Suppression in MPIC**

Figure 4 details the suppression mechanism of a Cation Self-Regenerating Suppressor (CSRS$^\text{®}$). The suppressor for MPIC of cationic analytes is the same as that used for cation-exchange eluents and is used in the same manner. The CSRS consists of two anion-exchange membranes, across which the eluent anions are exchanged for hydronium. The hydronium ions are supplied by electrolytic hydrolysis of water, which is used as the regenerant. In this example, the analyte (C$^+$) is being separated using an alkylsulfonic acid mobile phase. The alkylsulfonate ion-pair reagent (RSO$_3^-$) moves across the membrane toward the anode and is replaced by hydronium ion being generated in the cathode chamber. The end result is a reduction in background conductivity with a corresponding increase in analyte signal.

Suppression for anion ion-pairing is a bit different. Figure 5 shows the separation of an analyte (A$^-$) using a quaternary ammonium compound (NR$_4^+$) as the ion-pair reagent. Again, the suppressor hardware is the same as that used in classical anion IC. However, there is one important difference when using the Anion Self-Regenerating Suppressor (ASRS$^\text{®}$) for MPIC. Hydrolysis of water at the anode supplies hydronium ions, which travel across the cation-exchange membrane to replace the NR$_4^+$ cations. Quaternary ammonium ions, however, are difficult to efficiently transfer across the suppressor membrane. Sulfuric acid is added to the regenerant to increase the driving force of these bulky cations through the membrane toward the cathode.
Figure 5. Suppression mechanism in anion ion-pairing.

**SPECIAL CONSIDERATIONS**

Complete equilibration of the system is critical to reproducible MPIC separations. For short-term shutdown, turning the flow rate down to 0.1 mL/min (rather than shutting off the pump completely) is recommended. To avoid long reequilibration times, it is also advisable to maintain separate SRSs for use with IC and MPIC separations.

Since MPIC is often used for separating large, hydrophobic ions, solubility can be an issue. Before beginning method development, check the analyte’s solubility in the mobile phase.

**REQUIRED EQUIPMENT**

Dionex DX-500 IC system consisting of:
- GP40 Gradient Pump
- CD20 Conductivity Detector
- Chromatography module (LC10, LC20, or LC30)
- Eluent organizer
- PeakNet® Chromatography Workstation
- IonPac® NS1 Analytical Column, 4 mm x 250 mm x 10 µm (P/N 035321)
- IonPac NG1 Guard, 4 mm x 35 mm x 10 µm (P/N 039567)
- ASRS or CSRS Self-Regenerating Suppressor

**EXAMPLE SEPARATIONS**

**Anions**

Figure 6 shows the separation of a series of straight-chain aliphatic sulfonic acids. In this example, tetrabutylammonium hydroxide is the ion-pairing reagent. An acetonitrile gradient is used to elute the more highly retained analytes. Method detection limits for these compounds are shown in Table 2.

<table>
<thead>
<tr>
<th>Column: IonPac NS1 (10 µm)</th>
<th>Detection: Suppressed conductivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eluent: 2 mM Tetrabutylammonium hydroxide, 24% to 48% Acetonitrile in 10 min</td>
<td></td>
</tr>
<tr>
<td>Flow Rate: 1.0 mL/min</td>
<td></td>
</tr>
<tr>
<td>Inj. Volume: 50 µL</td>
<td></td>
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<tr>
<td>Peaks:</td>
<td></td>
</tr>
<tr>
<td>1. Methanesulfonic acid 5.0 mg/L</td>
<td></td>
</tr>
<tr>
<td>2. 1-Propanesulfonic acid 8.6</td>
<td></td>
</tr>
<tr>
<td>3. 1-Butanesulfonic acid 8.7</td>
<td></td>
</tr>
<tr>
<td>4. 1-Hexanesulfonic acid 8.8</td>
<td></td>
</tr>
<tr>
<td>5. 1-Heptanesulfonic acid 9.1</td>
<td></td>
</tr>
<tr>
<td>6. 1-Octanesulfonic acid 9.2</td>
<td></td>
</tr>
<tr>
<td>7. 1-Decanesulfonic acid 9.1</td>
<td></td>
</tr>
</tbody>
</table>

**Table 2 Method Detection Limits for Aliphatic Sulfonic Acids**

<table>
<thead>
<tr>
<th>Analyte</th>
<th>MDL (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-Propanesulfonic acid</td>
<td>0.09</td>
</tr>
<tr>
<td>1-Butanesulfonic acid</td>
<td>0.10</td>
</tr>
<tr>
<td>1-Hexanesulfonic acid</td>
<td>0.11</td>
</tr>
<tr>
<td>1-Heptanesulfonic acid</td>
<td>0.11</td>
</tr>
<tr>
<td>1-Octanesulfonic acid</td>
<td>0.12</td>
</tr>
<tr>
<td>1-Decanesulfonic acid</td>
<td>0.18</td>
</tr>
</tbody>
</table>

*25-µL sample loop, determined as 3x noise.
Aromatic sulfonic acids are separated under the same set of conditions in Figure 7. Note that the benzenesulfonic acid (Figure 7) and hexanesulfonic acid (Figure 6), though both C6 sulfonic acids, have different retention times under these conditions. Steric and hydrophobic differences play significant roles in how anions interact with both the stationary phase and the ion-pair reagent.

Cations

Figure 8 shows the separation of a mixture of aliphatic, branched quaternary ammonium ions. Nona-fluoropentanoic acid was chosen as the ion-pair reagent and again an acetonitrile gradient was employed. The decrease in background as the run proceeds is due to the lower conductivity in acetonitrile versus water.
Using ion chromatography, alkali and alkaline earth metals often interfere with accurate quantification of alkanolamines. Ethanolamine, which coelutes with ammonium using IC, is well resolved from any interferences using the MPIC conditions shown in Figure 9. Although the inorganic cations themselves are not well resolved, ion-pairing helps retain the alkanolamines relative to the inorganic cations, making quantification of the alkanolamines easier. Method detection limits for these analytes are shown in Table 3.

The chromatogram in Figure 9 also demonstrates a unique way in which suppressors can be exploited in MPIC. In this example, 10 mM boric acid was used as the regenerant to enhance detection of the ethanolamines. After suppression, the ethanolamines are present as the free bases and are thus undetectable by conductivity whereas converting them to the borate salts raises their conductivity considerably. As a side benefit, the conductivity of interfering alkali and alkaline earth metals is reduced by converting them from the highly conductive hydroxide forms to the less conductive borate salts.

CONCLUSION

MPIC is a useful technique for the determination of surfactants, quaternary ammonium compounds, and other similar analytes. It is also a viable alternative when ion-exchange selectivities are inadequate. Method development is simple, and a wide variety of separations can be achieved by varying the ion-pairing reagent and organic solvent concentration. Suppressed conductivity offers sensitive, universal detection for most analytes likely to be separated by ion-pairing mechanisms.

REFERENCES
