Simultaneous Separation of Neutral Organics and Inorganic Anions by Coupling Reversed-Phase and Ion-Interaction Chromatography

Sheila J. Maness[a]; John R. Jezorek[a]

[a] Department of Chemistry and Biochemistry, The University of North Carolina at Greensboro, Greensboro, NC, USA

PLEASE SCROLL DOWN FOR ARTICLE

Simultaneous Separation of Neutral Organics and Inorganic Anions by Coupling Reversed-Phase and Ion-Interaction Chromatography

Sheila J. Maness and John R. Jezorek

Department of Chemistry and Biochemistry, The University of North
Carolina at Greensboro, Greensboro, NC, USA

Abstract: Ion-interaction chromatography is coupled with reversed-phase HPLC to effect simultaneous separation of inorganic anions and neutral organic species. The column is a standard C_{18}, and the mobile phase is an aqueous combination of typical ion-interaction and reversed-phase constituents, an ion-interaction reagent and an organic cosolvent (methanol). Anions and small, polar, organic mixtures were separated in relatively low methanol content mobile phases (40–60%, v/v) with tetra-butylammonium and tetraheptylammonium ion-interaction reagents. Mixtures of anions and nonpolar organics (PAHs) required higher methanol content with a relatively hydrophobic ion-interaction reagent, cetyltrimethylammonium chloride. In all cases, mobile phase conditions could be adjusted to allow anions and neutral organics to be separated both between and within classes.

Keywords: Ion-interaction, Ion-pairing, Anion-exchange, Mixed-class separation, Mobile-phase composition

INTRODUCTION

Most chromatographic procedures are designed for only one analyte class, for example, inorganic ions or neutral organic compounds. But the potential to separate and determine analytes both within and between classes in one chromatographic run holds the promise of reduced cost and analysis time. Our lab-

Address correspondence to John R. Jezorek, Department of Chemistry and Biochemistry, The University of North Carolina at Greensboro, P.O. Box 26170, Greensboro, NC 27402-6170, USA. E-mail: jjezorek@uncg.edu

oratory has been interested for some time in adapting existing single-class procedures and stationary phases for the simultaneous separation and detection of two or more analyte classes. We have employed both weak[1] and strong[2,3] ion-exchange HPLC columns to simultaneously separate mixtures of inorganic ions and neutral organic molecules. The latter two references describe work by other groups as well, in which serial, mixed-bed, and mixed-mode columns were used.

An approach, which has gotten relatively little attention for simultaneous ion-neutral separations is that of ion-interaction or paired-ion HPLC. In this technique, a standard reversed-phase HPLC column is used and either permanently or dynamically coated with a charged surfactant, which affords ion-exchange or ion-interaction properties to the system. In the dynamic approach, the ion-interaction reagent (IIR) is contained in the mobile phase and is in equilibrium with sorbed IIR on the nonpolar stationary phase surface.

Our laboratory has published a report[4] that describes work using the ion-interaction approach for the simultaneous separation of divalent metal cations and neutral organic compounds, in aqueous mobile phases that contained both ion-exchange and reversed-phase constituents. That is, an ion-eluting agent and methanol cosolvent were both present in addition to the cation-interacting IIR. The present report describes a similar approach for mixtures of inorganic anions and neutral organic analytes, again using methanol as the reversed-phase cosolvent, and a variety of anion-interacting IIRs.

EXPERIMENTAL

Apparatus

The chromatographic system consisted of a Kratos Spectroflow 400 dual-piston pump; a Rheodyne Model 7010 injection valve fitted with a 10 μL sample loop; an ISCO V^4 variable wavelength ultraviolet-visible detector; and in some cases, an Alltech Model 320 conductivity detector in series with the UV-visible. UV detection was typically done at 264 nm when potassium hydrogen phthalate (KHP) was included in the mobile phase for indirect UV detection of anions and at 230 nm when it was absent. Chromatograms were recorded on a Fisher Recordall Series 5000 dual-pen recorder. Mobile phase pH data was obtained using a Fisher Model 915 pH meter. The analytical column was a 250×4.6 mm Alltech Econsil C_{18} filled with 5 μm irregular particles.[4]

Reagents and Solutions

Mobile phases and analyte solutions used water purified with a Barnstead Nanopure II or a Sybron/Barnstead PCS cartridge system using deionized feed water. Reagents were either HPLC or ACS-grade. Mobile phase and

sample solutions were filtered through 0.45 μm membrane filters. Anion analyte stock solutions were made up in 100% water; organics in 100% methanol or a methanol-water mixture. Stock solutions were typically 100 mM. Analyte concentrations in samples were about 1 mM. Dilution of stock solutions was made with mobile phase.

Six different surfactants were employed as IIRs: tetrabutylammonium bromide (TBABr); tetrabutylammonium fluoride (TBAF); tetrabutylammonium acetate (TBAAc); tetrabutylammonium salicylate (TBASal); tetraheptylammonium bromide (THABr); and cetyltrimethylammonium chloride (CTACl). TBABr and THABr were obtained from Eastman; TBAF from ACROS; CTACl from Aldrich. The TBAAc and TBASal were prepared from tetrabutylammonium hydroxide (Aldrich) by adding acetic acid or salicylic acid, respectively, in a 1 : 1 mole ratio.

Chromatographic Procedures

All chromatography was performed at ambient temperatures. The flow rate was 1.00 mL min^{-1}. Injections of samples were made through 0.45 μm syringe filters. Mobile phases were degassed by helium sparging. Column equilibration generally required about one hour. The column was cleaned at least once a week using water and then methanol to strip off dissolved salts and IIR.

RESULTS AND DISCUSSION

Chromatographic analysis of samples containing ionic analytes is usually done with an ion-exchange column, while neutral organic species are typically separated in the reversed-phase mode on a C_8 or C_{18} column. As mentioned above, these two separation modes can be combined and ion-neutral separations done simultaneously, using a nonpolar stationary phase column and an ion-interaction reagent in the mobile phase.

Haddad[5] describes three proposed mechanisms of interaction of analyte ions with the IIR as: (1) ion-pairing in the mobile phase and subsequent interaction with the nonpolar stationary phase; (2) interaction with IIR that is already sorbed on the stationary phase; and (3) a broader perspective intermediate between these two, proposed by Bidlingmeyer.[6] This ion-interaction model describes the situation in terms of an electrical double layer created by the sorbed IIR and its co-ion, whereby the analyte ion enters the double layer but is not associated with any particular IIR moiety. This model seems to account for the observed behavior of analyte ions and mobile-phase constituents better than the other two. But whatever the mechanism of ion-exchange via the ion-interaction approach, the reversed-phase interactions with the nonpolar stationary phase (and/or the nonpolar portion of the IIR) appear to remain and are available for the separation of neutral species.

Ion-interaction reagents generally contain common ion-exchange groups, sulfonate for cations and quaternary ammonium for anions. These reagents may contain long-chain hydrocarbon groups, e.g., in a dodecyl sulfate cation exchanger; or more bulky nonpolar moieties, e.g., in tetrabutylammonium bromide. Of course, the greater the extent of the hydrocarbon portion the more hydrophobic the IIR will be, and the greater the extent of its sorption onto the nonpolar stationary phase. This, in turn, is expected to increase retention of ions,[5], as is shown below.

As both neutral organics and ions are to be separated, the aqueous mobile phase needs to contain, in addition to the IIR, an organic cosolvent in typical reversed-phase fashion. The present study employed methanol as the cosolvent. When analyte anions are UV absorbing (e.g., I^-, NO_3^-) the counter ion of the IIR can serve as the ion-eluting species, e.g., the Br^- of tetrabutylammonium bromide. To visualize non-UV absorbing anions, indirect UV detection may be used by incorporating a UV-absorbing, competitive anion in the mobile phase, for example hydrogen phthalate.[2,7] Both approaches were used in this study. Of course, both UV and conductance detectors can be used in series, to monitor UV-absorbing organics and ions, respectively. That approach was also used here.

Ion-Interaction Reagents Used

Six IIRs were examined in this study. The commonly used[5] tetrabutylammonium IIR was employed, accompanied by four different counter ions: bromide, fluoride, acetate, and salicylate. Fluoride ion proved to be a strong eluent[5] of analyte ions, with the result that very little retention was observed at the TBA concentration normally required for ion-interaction. Salicylate was used as a UV-absorbing counter ion to allow indirect UV detection of nonabsorbing anions, but appeared to have no advantage over KHP. Therefore, most of our studies with TBA used the bromide or acetate counter ions, with added KHP for anion visualization, if necessary.

We also employed two IIRs that are more hydrophobic, tetraheptylammonium (bromide) and cetyltrimethylammonium (chloride), a C_{16} containing surfactant. The tetraheptylammonium ion did not reach mobile-stationary phase equilibrium but continued to build up on the C_{18} phase with continued mobile-phase flow at low methanol concentrations, as described below. However, at high methanol concentrations it proved to be a useful IIR. The cetyltrimethylammonium IIR was also useful at high methanol concentrations.

## Effect of Mobile Phase Constituents on Analyte Retention

It was of interest to examine the effect of IIR and KHP concentrations on analyte retention in the mixed-class mobile phases used in this study.

Earlier reports indicate that ion retention increases with increasing concentration of IIR in the mobile phase.[4,5,8] Retention of anions was found to reach a maximum, around 4 mM with TBA in an all-aqueous mobile phase, and then decrease as IIR concentration is further increased. Essentially, the stationary phase is thought to become saturated with IIR, and further addition to the mobile phase introduces more competing counter ions, which reduce anion retention.[8] We observed similar behavior in the methanol-containing mobile phases in the present study. Retention of iodide and nitrate ions increased as the concentration of TBABr increased, up to about 15 mM in a 30 : 70 (v/v) methanol-water mobile phase. No decrease in retention was seen up to that point. A similar study with TBAAc in 40 : 60 methanol-water yielded an apparent retention maximum between 15 and 25 mM, but not enough data was obtained to specify the position of the maximum with certainty. Of course, overall anion retention is lower at higher methanol and a given IIR concentration. For example, k for iodide is about 4 in 30% methanol, 15 mM TBABr, but is reduced to a k of about 1.2 in 40% methanol, 15 mM TBABr. The additional methanol reduces the extent of sorption of IIR on the stationary phase, thus reducing the exchange capacity.

Addition of KHP to the mobile phase (to effect indirect UV detection of anions) also reduces anion retention, of course, as the hydrogen phthalate ion competes with analyte anions for exchange capacity. The k for iodide was reduced from 2.2 to 1.5 in a 30% methanol, 5 mM TBABr IIR solution when 2 mM KHP was introduced.

We were also interested in separating larger neutral organic analytes such as polynuclear aromatic hydrocarbons (PAHs) simultaneously with inorganic anions. This necessitates using relatively high methanol cosolvent concentrations to elute these hydrophobic molecules, in turn decreasing the sorption of IIR on the stationary phase and lowering k values for anions. Therefore, we investigated IIRs that are more hydrophobic than the TBA species discussed above. One IIR chosen was THABr, and was used for the separation of three anions and phenol. As seen in Figure 1, retention of the ions increased with continued flow of the 40% methanol, 0.5 mM THABr, 2.5 mM KHP mobile phase. After about 750 mL of mobile phase had been passed, the column was flushed with about 250 mL total of, first water, then 100% methanol to remove sorbed IIR. The k values for anions went to near zero. Then mobile phase containing the THABr was again started, and k values increased steadily through another 1500 mL of eluent. When this experiment was repeated in 60% methanol, mobile phase k values remained constant with eluent volume passed (not shown). Apparently the lower methanol concentration (40%) allowed buildup of THA IIR on the C_{18} stationary phase. This was not observed at 40% methanol for the less-hydrophobic TBA IIR.

Note that k for phenol in Figure 1 also increased as IIR built up on the stationary phase, possibly indicating interaction with sorbed IIR in addition
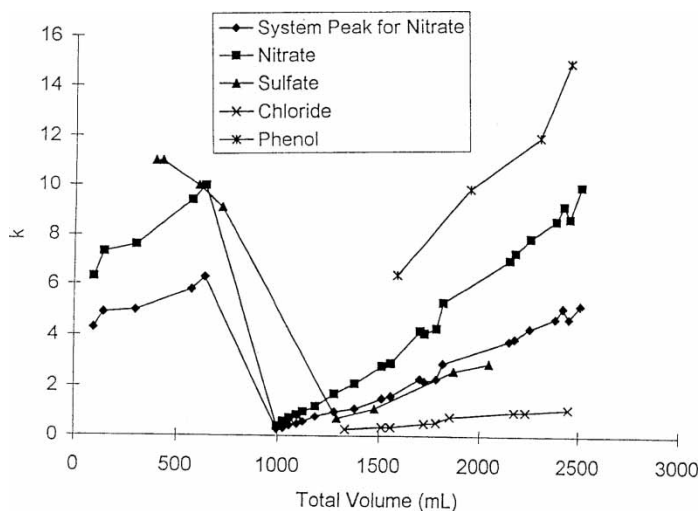
Figure 1. Retention increase due to IIR (THABr) buildup during mobile-phase flow. Column was washed to desorb IIR at about 750 mL, and eluent containing IIR was begun again at 1000 mL. Mobile phase: methanol-water (40 : 60, v/v), 0.5 mM THABr, 2.5 mM KHP.

to C_{18} groups. A different situation resulted from the retention of phenanthrene in 80% methanol mobile phase with CTACl (another rather hydrophobic IIR) at concentrations of 1, 2.5, 5, and 10 mM. The k for phenanthrene remained constant at about 3.5 ± 0.1 as long as the methanol concentration did not change and IIR did not build up on the C_{18} phase.

In general, then, ion retention increases with increasing IIR concentration in the mobile phase, but decreases at higher organic cosolvent concentration and with added ion-eluting agents. Although not fully addressed in this study, changes in IIR concentrations have little effect on neutral organic analyte retention as long as build up of IIR on the stationary phase does not occur. These effects and others are fully described in earlier works.[4,5]

Simultaneous Anion-Organic Separations

Good separation of several anions and neutral polar organics was obtained in relatively low-methanol mobile phases using either TBABr or TBAAc IIR. However, relatively large concentrations of the IIR were needed to get reasonable k values for anions. For example, 15 mM TBABr or TBAAC was needed in 30 : 70 and 40 : 60 methanol-water mobile phases. Typical k values with 15 mM TBABr in 30% methanol were: 2.2 for NO_3^-; 4.2 for I^-; 5.4 for phenol; and 14.7 for p-nitrophenol.

To achieve similar results with lower IIR concentrations in the mobile phase a more hydrophobic IIR was needed. The 28-carbon THABr was chosen. A mobile phase of 0.5 mM THABr and 2.5 mM KHP in 40 : 60 methanol water resulted in changing analyte k values, as noted above in Figure 1. However, in 60 : 40 (v/v) methanol-water the IIR did not build up on the C_{18}, and a useful separation of anions and neutral organics resulted (Figure 2). At 60% methanol the analyte classes are separated, with the polar organics eluting as a group before any anions. Note, that the negative peaks for the anions are a consequence of the indirect UV detection mode employed, whereby the UV-absorbing hydrogen phthalate eluting ion replaces an analyte ion in the secondary layer, giving a decreased UV absorbance proportional to the concentration of the nonabsorbing analyte ion. The
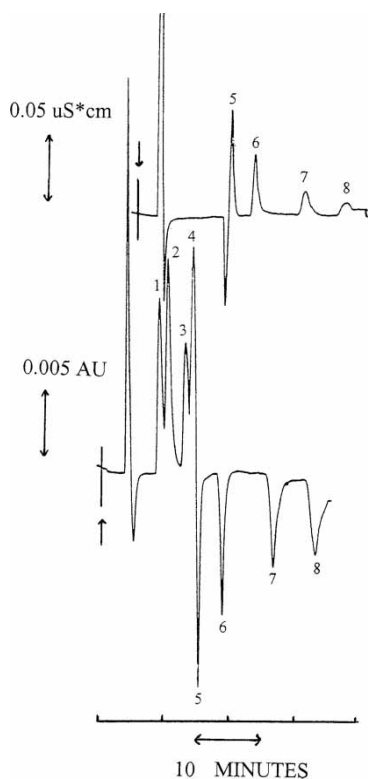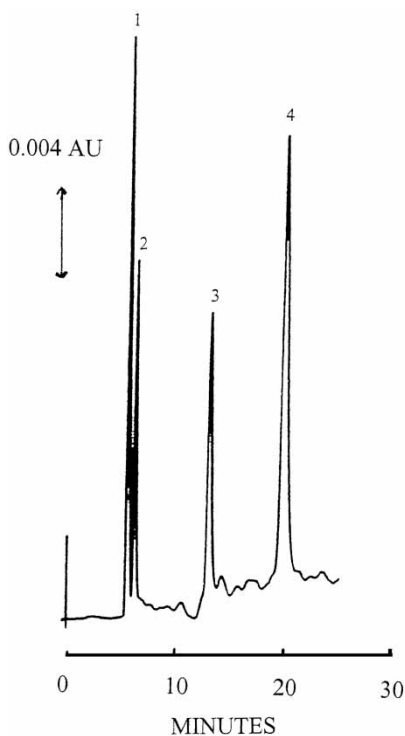

Figure 2. Simultaneous separation of a mixture of polar, neutral organics and inorganic anions by combined reversed-phase and ion-interaction chromatography. Mobile phase; methanol-water (60 : 40, v/v), 0.5 mM THABr, 2.5 mM KHP. Top, conductivity; bottom, UV detection. Analytes: (1) phenol, (2) p-nitroaniline, (3) p-nitrophenol, (4) system peak, (5) nitrate, (6) iodide, (7) perchlorate, (8) sulfate.

top chromatogram in Figure 2 was from the conductivity detector (in series with the UV) which senses only the ionic species.

It was of interest to assess the feasibility of quantitating the analytes in this mixed-mode system. Six standard solutions of mixtures of four of the analytes in Figure 2 were prepared, with concentrations ranging from 10^{-2} to 5×10^{-5}M. The analytes chosen were phenol and p-nitroaniline (peaks 1 and 2); and nitrate and iodide (peaks 5 and 6). The mobile phase was the same as that in Figure 2. Linear regression of log peak height versus log concentration yielded correlation coefficients of at least 0.999 for all but nitrate ($R^2 = 0.996$) and slopes close to the unity value expected for a log-log plot, from 0.98 to 1.01, except for nitrate (0.92). Standard deviations of the capacity ratio values in this calibration study were in the $1-6\%$ (relative) range, and standard deviations of peak height for the four analytes were in the $2-15\%$ (relative) range, the poorer values for the lower concentrations, as expected. It would appear that reasonable quantitation can be expected for both neutral organic and ionic analytes in this mixed-mode system.

Figure 3.   Simultaneous separation of anions and polynuclear aromatic hydrocarbons by combined ion-interaction and reversed-phase chromatography. Mobile phase: water-methanol (80:20, v/v), 2.5 mM CTACl. Analytes: (1) nitrite, (2) nitrate, (3) phenanthrene, (4) pyrene.

In order to elute larger, nonpolar organic analytes such as the PAHs, a higher methanol concentration in the mobile phase is required, as well as a rather hydrophobic IIR. Figure 3 shows the separation of NO_3^- and NO_2^- from each other and from phenanthrene and pyrene in an 80% methanol, 2.5 mM CTACl mobile phase. As the two anions absorb UV light no KHP was needed here.

CONCLUSIONS

Under mobile-phase conditions suitable for both ion-interaction (exchange) and reversed-phase separations, we have shown that analyte anions behave as they normally do in all-aqueous eluents, retention increasing with IIR concentration. As the organic cosolvent content increases, anion retention decreases as less IIR sorbs on the stationary phase. IIR mobile phase concentration has little effect on neutral organic analyte retention.

Polar organic analyte-anion mixtures were separated in low-methanol mobile phases, 30–60% methanol, while higher methanol content was needed for larger, nonpolar organic analytes, as well as relatively hydrophobic IIRs such as those containing tetraheptyl or cetyl groups. It appears that ion-interaction chromatography coupled with reversed-phase provides a useful approach to anion-organic mixed class separations.

REFERENCES

1. Muenter, M.M.; Stokes, K.C.; Obie, R.T.; Jezorek, J.R. Simultaneous separation of inorganic ions and neutral organics on ion-exchange stationary phases. J. Chromatogr. A **1999**, *844*, 39–51.
2. Dean, T.H.; Jezorek, J.R. Demonstration of simultaneous anion-exchange and reversed-phase behavior on a strong anion-exchange column. J. Chromatogr. A **2004**, *1028*, 239–245.
3. Gu, R.-F.; Jezorek, J.R. Demonstration of simultaneous cation-exchange and reversed-phase mechanisms on a strong cation-exchange column. J. Chromatogr. A **2001**, *919*, 21–28.
4. Jezorek, J.R.; Wagner, K.A. Simultaneous separation of metal ions and neutral organics by reversed-phase ion-pair liquid chromatography. J. Chromatrogr. A **1997**, *776*, 221–231.
5. Haddad, P.R.; Jackson, P.E. *Ion Chromatography: Principles and Applications* J. Chromatography Library; Elsevier: Amsterdam, 1990; Vol. 46, Chapter 6.
6. Bidlingmeyer, B.A. Separation of ionic compounds by reversed-phase liquid chromatography: an update of ion-pairing techniques. J. Chromatogr. Sci. **1980**, *18*, 525–539.
7. Small, H.; Miller, T.E. Indirect photometric chromatography. Anal. Chem. **1982**, *54*, 462–469.

8. Xianren, Q.; Bayens, W. Retention and separation of inorganic anions by reversed-phase ion-interaction chromatograpy on octadecyl silica. J. Chromatogr. **1988**, *456*, 267–285.