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Simultaneous separation of metal ions and neutral organics by reversed-phase ion-pair liquid chromatography

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Abstract

Mixtures of divalent metal ions and neutral organics (phenols, anilines, polynuclear aromatics) were separated and detected by isocratic high-performance liquid chromatography in one run using standard cation-exchange ion-pairing or ion-interaction reagents (IIRs) and water–methanol–tartrate mobile phases on a C_{18} reversed-phase column. The column could be coated with the more hydrophobic (C_{18}, C_{20}) IIRs but severe bleeding occurred in mobile phases containing more than 30% methanol. Most work was done with the IIR incorporated in the mobile phase. In eluents containing about 30% methanol the C_8 (octanesulfonate) IIR was most useful; the C_{12} (dodecyl sulfate) IIR proved best around 50% methanol; and the C_{18} (octadecyl sulfate) IIR was most effective above 70% methanol. IIR concentrations in the 1–10 m*M* range were used. Detection of organics was carried out at 254 nm. Metal ions were post-column derivatized with 4-(2-pyridylazo)resorcinol downstream from the UV detector and monitored at 510 nm at a second detector. Metal ion retention increased with decreasing pH and methanol concentration and increasing IIR concentration. The presence of the IIR had only a slight effect on organic analyte retention as compared to the same water–methanol mobile phase with no IIR. Both peak area and height were linear with analyte concentration from about 10^{-2} to 10^{-5} *M* for both metal ions and organics. © 1997 Elsevier Science B.V.

Keywords: Retention mechanisms; Metal ions; Phenols; Anilines; Polynuclear aromatic hydrocarbons

1. Introduction

Virtually all liquid chromatographic methods are developed for only one class of analyte, because different types of analytes are separated by different mechanisms, requiring different stationary phases and/or mobile phases. Even the so-called mixed-mode stationary phases that have begun to appear on the market have been utilized for only one class of analyte at a time [1]. However, separation and quantitation of mixed classes of analytes in one run would be useful in situations involving complicated samples, for example an industrial effluent or leachate from a toxic waste site. In order to accomplish this goal both stationary and mobile phases must be chosen or designed with each class of analyte in mind. Possible approaches are serial columns, mixed-bed, mixed ligand [2] and the mixed-mode or multisite stationary phases where each type of stationary phase site provides a mechanism for a particular class of analyte. The intent of the present research is the simultaneous separation and detection of mixtures of metal ions and neutral organic analytes. The metal ions would normally be separated

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on a cation-exchange phase, and the organics in the reversed-phase mode on a nonpolar stationary phase. This research addresses methods of combining the two types of mechanisms in one column with one mobile phase employed in the isocratic mode. A typical C_{18} column was employed for the reversed-phase mechanism along with an aqueous tartrate–methanol mobile phase containing an ion-interaction reagent (IIR) to produce the ion-exchange mechanism. The organics were monitored with an UV detector while the metal ions were post-column complexed and detected in the visible region [3]. This specific detection approach allowed a separate chromatogram to be obtained for each of the two classes of analytes even if the metal ions were not completely resolved from the organics.

2. Experimental

2.1. Apparatus

The chromatographic system was composed of a Shimadzu LC-10AD solvent delivery module; a Rheodyne 7010 injection valve with a 10-μl sample loop; a Schoeffel Model 770 detector for organic compound monitoring (usually 254 nm) and an ISCO Model V^4 detector set at 510 nm for metal-ion monitoring after post-column derivatization. A Waters Model 740 data module was employed to obtain peak area data. The post-column derivatizing reagent was blended with the column effluent after it had passed through the organic-monitoring detector. The reagent was delivered from a helium-pressurized vessel and blended with the effluent stream in a low-volume mixing tee. The combined streams were then directed to the visible-region detector. Details of the delivery system have been published [4]. The column used was an Alltech Econosil C_{18}, 250×4.6 mm, packed with 5-μm particles. Water used for mobile phases and samples was purified with a Barnstead Nanopure II system which used deionized feed water.

2.2. Reagents and solutions

The derivatizing reagent, 4-(2-pyridylazo)resorcinol (PAR), was obtained from G.F. Smith. Preparation of the solution has been previously described [5]. The pH was adjusted with "InstraAnalyzed" aqueous ammonia from J.T. Baker. ACS-grade methanol was used for mobile phases and as the solvent for organic analytes. Sodium octane sulfonate (98%), sodium dodecyl sulfate (98%) and sodium octadecyl sulfate (93%) were obtained from Aldrich. Sodium eicosyl sulfate (purity unknown) was from Regis. Organic analytes were typically 10^{-3}–10^{-4} *M* in methanol solvent; metal-ion analytes were made up in a methanol–water (30:70, v/v) solvent and were generally 10^{-4} *M* for qualitative studies. Mobile phases and the PAR solutions were filtered through 0.45-μm nylon membrane filters. Mobile phases were degassed either by membrane filtration, helium sparging, or by an Alltech on-line vacuum degassing module. Mobile phase pH was adjusted with dilute sodium hydroxide solution. The pH meter was calibrated with standard aqueous buffers so that pH values in methanol-containing mobile phases are apparent only.

2.3. Chromatographic procedures

Column equilibration was effected by passing 15–30 column volumes of mobile phase through the system or until a steady baseline was achieved. Low concentrations of the more hydrophobic IIRs (e.g., the octadecyl sulfate) in low-methanol mobile phases required long equilibration times, up to 50 or 60 column volumes. The eicosyl sulfate-coated column was made by passing about 225 ml of a $5 \cdot 10^{-4}$ *M* solution in a water–methanol (45:55, v/v) solution (close to saturation) through the column at 1 ml/min. The amount coated was determined by measuring the IIR solution conductivity before and after passage through the column (Alltech Model 320 conductivity detector). Knowing the total amount of IIR originally present and the fractional decrease in conductivity, the amount of eicosyl sulfate IIR sorbed on the column was determined to be 67 μmoles (27 mg). This compares favorably to the 60 μmoles of sodium eicosyl sulfate sorbed by a 150×4.6 mm C_{18} column from an acetonitrile–water (25:75) solvent as reported by Ito et al. [6]. It also is of interest to compare these amounts to that of sodium dodecyl sulfate (SDS) reported by Grohs et al. [7] to be taken up by a 150×3.9 mm C_{18} column. They reported

about 70 μmoles (20 mg) sorbed from a water–methanol (80:20) SDS mobile phase. However, practically no SDS was sorbed from a 55% methanol mobile phase such as was used in the present study. Clearly the larger, more hydrophobic C_{20} IIR is much more strongly sorbed than the C_{12} species. In all cases, however, low exchange-capacity columns were obtained. The column was then equilibrated with mobile phase (containing no eicosyl sulfate) in the usual way. Bleeding of the IIR from coated columns was monitored by comparing metal-ion retention over the course of the study.

All separations were done at a flow-rate of 1 ml/min at ambient temperature under isocratic conditions. Gradient operation (increasing the methanol concentration) resulted in irreproducible post-column derivatization results and so was not employed in this study.

Column efficiency was calculated based on peak width at half-height. Peak asymmetry was determined as the ratio of the distances from trailing and leading edges of the peak to the vertical dropped from the peak apex, at 10% peak height.

3. Results and discussion

As mentioned in Section 2.3, chromatographic separation of cations has usually been accomplished on bonded-phase ion exchangers with either a styrene–divinylbenzene or silica gel support. An alternate, albeit less used, approach, is to employ a nonbonded exchanger, a so-called ion-pairing or ion-interaction reagent (IIR), in conjunction with a reversed-phase column, either as a mobile phase additive or as a coating on the nonpolar stationary phase [6–10]. Given that we wished to separate organic analytes (normally done on a reversed-phase column) simultaneously with metal ions we chose the IIR approach for this study.

Ion-interaction reagents incorporate the usual exchanger groups, sulfonic acid or quaternary ammonium moieties, as cation and anion exchanger, respectively, attached at the terminus of an alkyl chain. The nonpolar, hydrophobic portion of the exchanger may contain as many as thirty methylene units. Of course, the longer the alkyl chain, the more hydrophobic the entire unit becomes. The mechanism of retention of an analyte cation in the ion-interaction chromatographic mode, where the IIR is an additive in the mobile phase, has been under discussion for years. One model holds that the ion pair forms in the mobile phase and the neutral pair may subsequently interact with the nonpolar stationary phase, the so-called ion-pair model [11]. However, Bidlingmeyer et al., through a series of conductance measurements, has shown that ion pairs do not form in the mobile phase to any significant extent, so that ion interaction must occur on or near the stationary phase surface [12]. Another view is that the exchanger, which is dynamically distributed between the mobile and stationary phases, may interact with the analyte ions when it is in the immobilized state, the so-called ion-exchange model [13,14]. This model implies that the IIR–analyte ion interaction is essentially coulombic. In a series of papers [10,12,15] Bidlingmeyer and coworkers present a broader perspective, the ion-interaction model, which takes into account the electrical double-layer that exists when the IIR sorbs onto the nonpolar surface, but also the remaining reversed-phase character of the surface. This model is able to accomodate the coulombic interactions of analyte ions, either with a particular adsorbed IIR, or with the charged primary layer of IIR ions. But it also recognizes that organic ions may undergo entropic forces (hydrophobic interactions with the mobile phase) as well as enthalpic forces (both coulombic, with the oppositely charged IIRs, and dispersion interactions with the still mostly exposed C_{18} groups). Which forces predominate depends on the analyte and mobile phase, of course. For example, the more aqueous the mobile phase and the more hydrophobic the analyte ion, the less stable it will be in the mobile phase (large entropic force) and the more attracted it will be to the stationary phase (large enthalpic dispersion forces in addition to the coulombic interaction). Inorganic ions, especially those not very polarizable, probably interact primarily coulombically. Further refinements of the ion interaction model have focused on the electrical double layer and the ionic equilibria which occur there [16,17]. Whatever model is employed, increasing the exchanger concentration and chain length is expected to cause greater retention of analyte ions. Of course, if the exchanger is employed as a

"coating" on the nonpolar surface, and so is not a part of the mobile phase, the ion-pair model is not applicable and the ion exchange and ion interaction models better describe this situation.

As the present study involved separation of both neutral organic and ionic analytes it was decided to use the IIRs in conjunction with a C_{18} reversed-phase column. We believed that the neutral organics might behave optimally in this configuration. Cation-exchange behavior was achieved both by incorporating IIRs as part of the mobile phase and by coating the C_{18} stationary phase with the larger IIRs investigated.

As the mobile phase needs to be able to elute organics as well as metal ions, methanol was used as a cosolvent along with water. Typical mobile phase eluting agents for divalent cations are doubly charged competing ions such as ethylenediammonium as well as metal complexors such as oxalate, citrate or tartrate. We chose to use tartrate as the primary metal-ion eluter in this study. In essence, the tartrate competes with the IIR for the divalent cations. It was of interest here to investigate the effect of methanol concentration and of pH on metal-ion retention and column efficiency for metal ions. It was also desirable to examine the influence of the IIR concentration in the mobile phase, not only on metal-ion retention, but also on retention and column efficiency for the organic analytes. However, knowing the exact mechanism of cation retention with mobile phase IIRs is really not critical in an operational context. Whichever mechanism is operative, an increase in IIR concentration in the mobile phase can be shown to drive the equilibria toward greater analyte–ion retention. This was in fact observed in the present study, as discussed below, as well as in earlier work [10,12,18,19].

3.1. Coated columns

IIR-coated columns have been used for metal-ion separations in all-aqueous mobile phases [6,20]. The more hydrophobic ones (e.g., C_{18} and C_{20} sulfate) were found to be stable for a week or longer with no appreciable bleeding of the coating. Shorter chain IIRs, for example C_8 or C_{12}, were not hydrophobic enough to remain sorbed if they were not also contained in the mobile phase [6,20]. As we wanted to simultaneously determine both metal ions and neutral organic analytes it was necessary to incorporate an organic modifier in the eluent, as mentioned above. For a coated column to be useful in this context it must resist bleeding in these aqueous–organic mobile phases. We examined an octadecyl sulfate coated column in a methanol–aqueous tartrate (30:70) mobile phase. Retention times for both Pb(II) and Zn(II) decreased by about 7% after 6.5 l of mobile phase had been pumped through the column (equivalent to about 100 average runs). In a 50% methanol mobile phase k' for Pb(II) decreased from 8.8 at the first injection to 1.5 after 3.5 l of mobile phase had been used. Similar results were obtained with the sodium eicosyl sulfate (C_{20}) IIR. While chromatograms were of good quality on the coated columns (see Fig. 5 below), it appears that unacceptable bleeding of the IIR from the column occurs at about 30% methanol or higher. In situations in which low amounts of organic modifiers can be used with very hydrophobic IIRs, column stability may prove sufficient for coated columns to be useful. Indeed, no bleeding of eicosyl sulfate was observed in an all-aqueous mobile phase [6]. However, cetylpyridinium chloride (C_{16}) was found to have bled from the column after several days of use of an all-aqueous mobile phase [21]. Because of bleeding of the coated columns in the methanol–aqueous eluents used in this study most of the work described here was done with the IIRs incorporated in the mobile phase.

3.2. Mobile phase IIRs

As metal-ion elution was accomplished by competitive tartrate complexation in the mobile phase, it was expected that pH would affect retention. This weak base ligand serves to lower the positive charge on the metal and thus competes with the IIR for the cations. It is expected that, as complexation increases, with increasing pH, retention should decrease. This trend was in fact observed in this study, as shown in Fig. 1(top). Cd(II), the most retained of the four metals studied, eluted with a k' of about 25 at pH 3, and about 3 at pH 4. It should be noted that this study was done in 40% methanol, and the pH values are apparent pH values in this mobile phase [10]. In purely aqueous solution the fraction of the

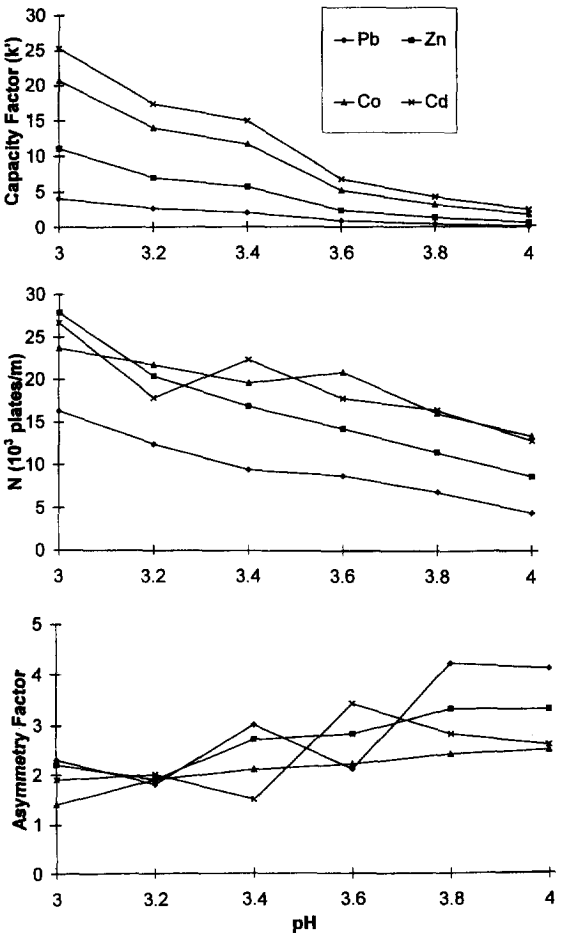

Fig. 1. Effect of pH on retention, column efficiency and peak symmetry of metal ions. Mobile phase: methanol–water (40:60), 45 m*M* tartrate, 0.45 m*M* sodium dodecyl sulfate; Top: capacity factor. Middle: theoretical plates. Bottom: asymmetry factor.

more highly complexing tartrate dianion can be calculated to be about 0.02 at pH 3 and 0.28 at pH 4. In 40% methanol solution tartaric acid dissociation is less, but the trend is the same, greater complexation of metals and so less retention at higher pH values. It appears that the presence of tartrate in the mobile phase serves only to lessen metal-ion retention by complexation of the metal ions. Neither the tartrate itself nor the tartrate–metal complexes appears to interact significantly with the stationary phase, either the C_{18} or the IIR-produced charged surface. The metal ion k' values drop essentially to 0 as the pH is raised and complexation becomes more complete [Fig. 1(top)], and as the IIR concentration drops to zero [Fig. 3(top, left); Fig. 4(top)]. Uncomplexed metal ions and metal–tartrate complexes that still retain some positive charge can interact (coulombically) with the IIR, but apparently not the fully complexed metal ions. Without the IIR no retention was observed. Thus it should be possible to manipulate k' by varying either pH or the amount of tartrate in addition to the amount of IIR. It should be noted in this context that the order of elution of the four test metals [Pb(II), Zn(II), Co(II), Cd(II)] follows the order of metal–tartrate formation constants, with Cd(II), the least strongly complexed, being the most retained [22].

Column efficiency and asymmetry factor for the test metal ions were also obtained as a function of pH. The general trend is that as retention decreases with increased pH so does column efficiency [Fig. 1(middle)]. For example, N decreased from about 28 000 plates/m at pH 3 to about 10 000 at pH 4 for Zn(II). Peak asymmetry increased also as pH increased [Fig. 1(bottom)], probably contributing to decreased efficiency. At this time reasons for this behavior are not known. In any case, a pH of 3.4 was chosen for most of this work, based on these results.

As the amount of organic modifier in the mobile phase is the main controller of organic analyte retention in a reversed-phase separation, it would presumably need to be varied to fit the nature of the organics in different mixed-class samples. Thus it was of interest to study the effect of methanol concentration on retention of metal ions in the mixed class sample. It has been known for some time that increased methanol will decrease cation retention in an ion-exchange situation, whether a conventional ion exchanger [22] or an ion-pairing approach [10,11] is used. Indeed, this was the result found in the present study, with metal-ion retention exhibiting typical reversed-phase behavior. For example, Cd(II) exhibits a k' of about 2 at 55%, about 8 at 45%, and about 22 at 35% methanol [Fig. 2(top)]. There are probably two main reasons for this behavior. First, the formation constants of the metal–tartrate complexes increase with added methanol, as the complex is more thermodynamically stable in the less polar (lower dielectric constant) aqueous–organic solvent than in its all-aqueous counterpart [22] which sol-

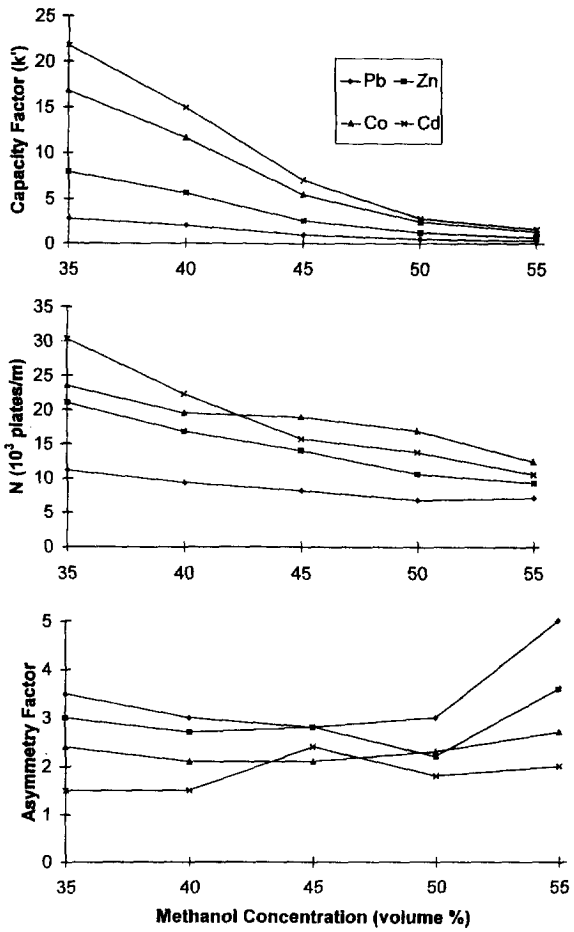

Fig. 2. Effect of methanol concentration on retention, column efficiency and peak symmetry of metal ions. Mobile phase: as in Fig. 1, except apparent pH 3.4, and changing methanol concentration. Top: capacity factor. Middle: theoretical plates. Bottom: asymmetry factor.

vates the reactant metal ions more highly than does the mixed solvent. This lower dielectric constant should lead to increased interaction of the ions with the IIR and result in greater retention; but in fact the opposite is observed, as noted above. Secondly, decreased dispersion interactions of the IIRs or the ion pairs with the nonpolar surface occurs in the mixed solvent compared to the all-aqueous solvent [10,11], as these species are more soluble the higher the content of methanol. As the observed behavior mimics typical reversed-phase behavior the latter reason may be the most important. No change in selectivity was observed here with increased methanol content (Fig. 2), only retention decreases for the test metal ions. Column efficiency also decreased as methanol amount increased, as seen by others [22]. For example, the efficiency for Cd(II) changed from 30 000 plates/m at 35% methanol to about 16 000 at 45% to about 12 000 at 55% methanol [Fig. 2 (middle)]. Peak symmetry was relatively unchanged from 30 to 50% methanol, but decreased rather substantially above 50% methanol [Fig. 2 (bottom)]. Again, the reasons for this peak degradation are not known.

All the organic analytes used as test compounds are neutral in the pH region studied here and so no pH effect on retention was observed. The analytes all behaved as expected with changing methanol concentration in the mobile phase, that is, decreased retention with increased amount of methanol, even in the presence of tartrate and various IIRs.

The last mobile phase additive for which the effect on both metal-ion and organic analyte retention and efficiency was sought was the nature and amount of IIR in the eluent. As expected [10,12,19], and as shown in Fig. 3 (C_{12} exchanger) and Fig. 4 (C_8 exchanger) metal-ion retention increased with increased IIR concentration. This increase was especially dramatic for the dodecyl sulfate IIR [Fig. 3(top left)] for which k' values increased 10–15 fold with an increase in C_{12} IIR mobile phase concentration from $3 \cdot 10^{-4}$ to $5 \cdot 10^{-4}$ M. Less dramatic increases in k' were observed for the octanesulfonate IIR, and over a much larger concentration range (Fig. 4). In both cases retention appeared to level off after the initial sharp increase [10,12], although k' values at $10 \cdot 10^{-4}$ M dodecyl sulfate were obtained only for Pb(II) and Zn(II). As mentioned above, increasing IIR concentration drives the equilibria for all retention models toward greater IIR–metal association with the bonded-phase surface. Efficiency values for metal-ion retention with both the C_{12} and C_8 IIR increased sharply with retention, tracking the increase in k' rather closely. N values also leveled off as k' leveled off (Figs. 3 and 4).

Retention and plate counts for the phenol test analytes were much less sensitive to changes in IIR concentration than were those of the metal-ion analytes. Except for an apparent slight maximum, where the C_{12} IIR increased from $3 \cdot 10^{-4}$ to $5 \cdot 10^{-4}$

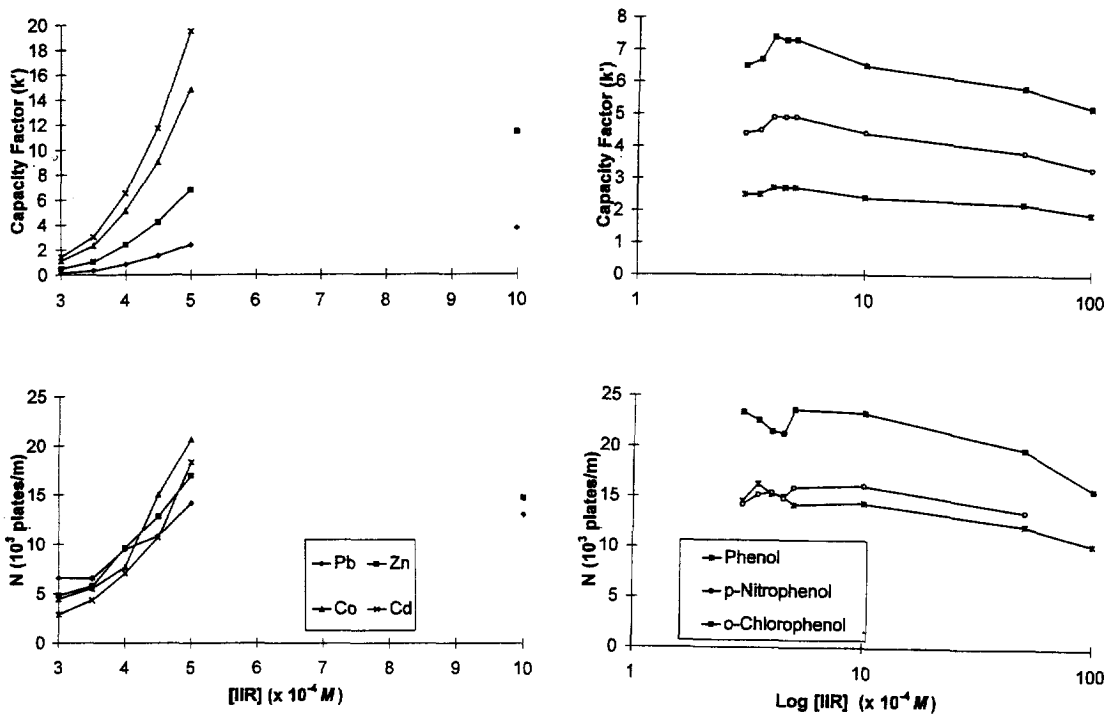

Fig. 3. Effect of mobile phase concentration of sodium dodecyl sulfate on retention and column efficiency for metal ions and organics. Mobile phase: as in Fig. 1, except apparent pH 3.4 and changing IIR concentration. Left top and bottom: capacity factor and theoretical plates for metal ions. Right top and bottom: capacity factor and theoretical plates for phenols. Note the two points at $10 \cdot 10^{-4}$ *M* IIR for the metal ion plots.

M, *k'* values for the three phenols tested changed by one *k'* unit or less over a 30-fold change in C_{12} sulfate concentration (Fig. 3). The phenol *k'* values in the C_8 sulfonate mobile phase were virtually unchanged when the IIR contentration changed from zero to $50 \cdot 10^{-3}$ *M* (Fig. 4). Similar data for neutral molecules have been described previously [10,12]. If there is a trend, it is to slightly lower retention of the phenols with increasing IIR concentration. The organic analytes may interact with the original bound C_{18} groups at low IIR concentration just as they would in the absence of any IIR, as the charged IIR moieties probably do not sorb on the surface too densely due to charge repulsion [10]. However, at higher IIR concentrations the greater density of sorbed charged groups may repel the somewhat polar phenol analytes resulting in slightly lowered retention. This effect seems to be larger for the C_{12} IIR than the C_8 IIR, as the greater hydrophobicity of the C_{12} allows greater extent of surface sorption. Efficiency (*N* values) of the organic analytes is also little affected by changing IIR contentration (Figs. 3 and 4), although there seems to be some anomolous distortion of the plots at the position of the apparent maxima in the *k'* vs. IIR concentration plot. There may be some sort of disturbance of the stationary phase surface in this concentration region, where the IIRs begin to sorb to an ever larger extent. While in some cases these anomolous regions were above the aqueous critical micelle concentrations, these mobile phases contained 40% methanol, so that micelle formation is not thought to be the cause of the *k'* maxima and *N* minima. Changing ionic strength due to the IIR itself could have an effect [15], but no studies were done to investigate this.

3.3. Metal-ion–organic simultaneous separation

The type of organic analytes in the mixture dictates not only the amount of methanol modifier in

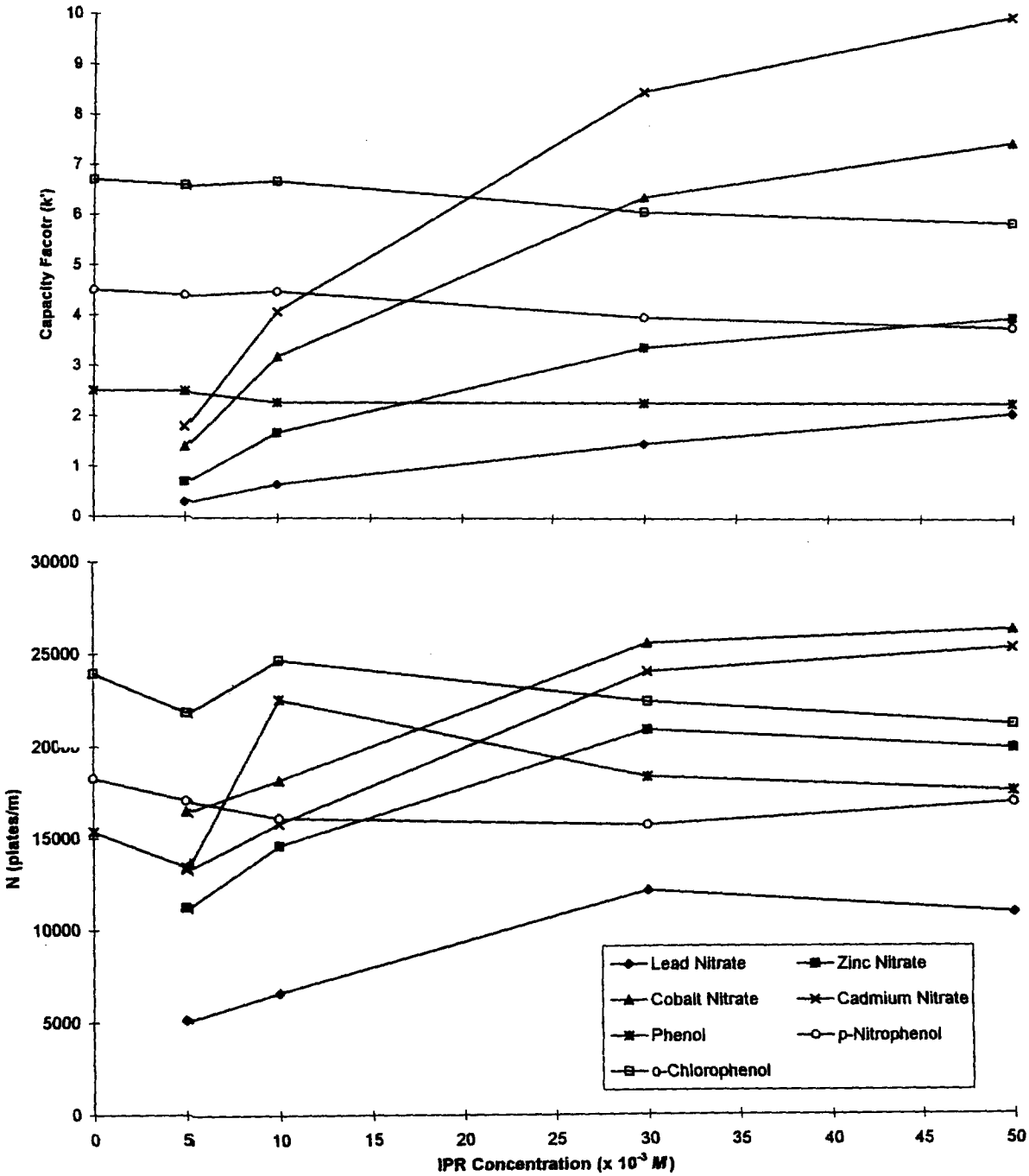

Fig. 4. The effect of mobile phase concentration of sodium octanesulfonate on retention and column efficiency of metal ions and organics. Mobile phase: methanol–water (40:60), 45 m*M* tartrate, apparent pH 3.4. Top: capacity factor. Bottom: theoretical plates.

the mobile phase but also, indirectly, the particular IIR that can be used for the metal-ion separation. If rather polar, water-soluble, organics were present, than a low methanol concentration (30% or less) might be called for. In this case the smaller IIRs (C_8 or C_{12}) could be used at relatively low concentrations in the mobile phase, or, alternatively, a C_{18} or C_{20} coated column could be used, even though gradual bleed of the sorbed IIR may occur if methanol is present in the mobile phase. Fig. 5 (left) illustrates a separation of some phenols and the test metal ions in a methanol–water (30:70) mobile phase (0.045 *M* in tartrate) at an apparent pH of 3.4 on a sodium eicosyl sulfate-coated C_{18} column. Fig. 5(right) shows the same separation using the octanesulfonate IIR in the mobile phase in 40% methanol. The C_8 IIR, due to its rather low hydrophobicity, is useful only at low organic modifier concentrations, probably less than 40% methanol. Otherwise, excessively high C_8 IIR concentrations are required. The C_{12} (dodecyl sulfate), however, is useful at higher organic modifier concentrations, perhaps up to 70 or 80% methanol. A mixed-class separation of the test metals and several phenols and anilines in 50% methanol using the C_{12} IIR is shown in Fig. 6(left) and one at 80% methanol, where the organics are rather hydrophobic polynuclear aromatic hydrocarbons, is seen in Fig. 6(middle). Note the much higher C_{12} concentration required in the 80% methanol mobile phase than the 50% (50 m*M* C_{12} vs. 1 m*M*) in order to compensate for the reduced metal-ion retention at the higher methanol concentration. The octadecyl sulfate (C_{18}) IIR is a better choice at higher methanol concentrations (above 70%), due to its greater hydrophobicity and consequent greater metal-ion retention [10]. Fig. 6(right) shows the same separation in 80% methanol using the C_{18} IIR, but with only a 1 m*M* concentration in the mobile phase instead of the 50 m*M* required with the C_{12} IIR. The C_{18} IIR (and other very hydrophobic additives) is not useful at low methanol concentrations due to the very long (several hours) equilibration times required. These IIRs have high affinities for the hydrophobic surface and consequently continue to adsorb over a long time period. Based on the above results, it would appear that useful simultaneous separations of metal ions and organic analytes is possible using conventional ion-pair chromatographic approaches, and that the choice of IIR depends on the concentration of organic modifier used in the mobile phase for the particular organic analytes in question.

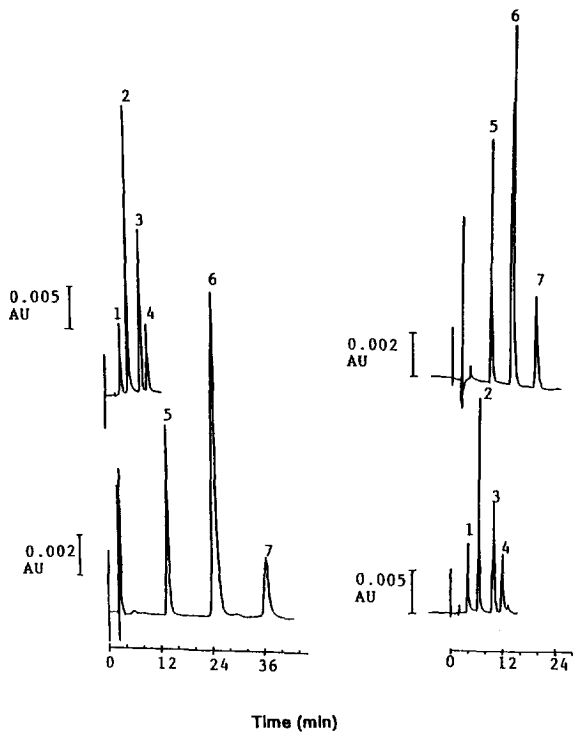

Fig. 5. Simultaneous determination of a mixture of metal ions and phenol both with a coated column and mobile phase IIR. Left: sodium eicosyl sulfate-coated column (67 μmoles); mobile phase was methanol–water (30:70), 45 m*M* tartrate, apparent pH 3.4. Right: sodium octanesulfonate mobile phase IIR; mobile phase was methanol–water (40:60), 45 m*M* tartrate, 10 m*M* IIR, apparent pH 3.4. Analytes: (1) lead(II), (2) zinc(II), (3) cobalt(II), (4) cadmium(II), (5) phenol, (6) *p*-nitrophenol, (7) *o*-chlorophenol.

3.4. Quantitation

It was of interest to determine if the test metals and organics followed typical linear behavior of peak area and height against concentration in the mixed mobile phases used for the simultaneous separations. Therefore the four test metals as well as *p*-nitroaniline and *p*-nitrophenol were tested for linear behavior. Table 1 gives the results for these quantitation studies in a mobile phase consisting of metha-

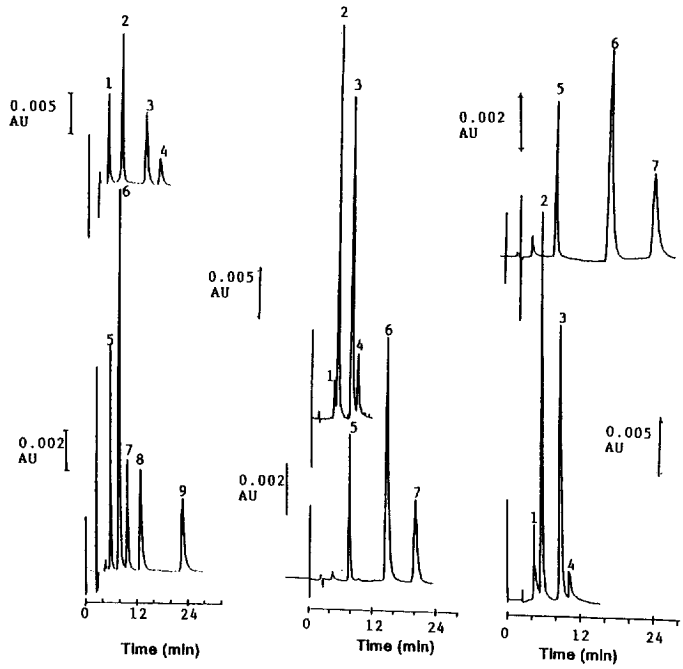

Fig. 6. Simultaneous determination of metal ions and organics using mobile phase IIRs. Left: sodium dodecyl sulfate IIR; mobile phase was methanol–water (50:50), 45 m*M* tartrate, 1 m*M* IIR, apparent pH 3.4. Middle: sodium dodecyl sulfate IIR; mobile phase was methanol–water (80:20), 45 m*M* tartrate, 50 m*M* IIR, apparent pH 3.4. Right: sodium octadecyl sulfate IIR: mobile phase was same as middle chromatogram except 1 m*M* in IIR. Analytes: all three chromatograms, (1) lead(II), (2) zinc(II), (3) cobalt(II), (4) cadmium(II); left, (5)phenol, (6) *p*-nitrophenol, (7) *o*-chlorophenol, (8) aniline, (9) *p*-chloroaniline; middle and right, (5) naphthalene, (6) anthracene, (7) pyrene.

nol–water (45:55) which was 45 m*M* in tartrate and 0.45 m*M* in SDS IIR at an apparent pH of 3.4. The concentration ranges tested were 10^{-2}–10^{-5} *M* for Pb(II) and Zn(II); 10^{-3}–10^{-6} *M* for Co(II) and Cd(II); and 10^{-1}–10^{-5} *M* for the two organic analytes. Relative standard deviations of the slopes were in the range of 1–8%, and all correlation coefficients were at least 0.99 with most being 0.999. Each injection was repeated three or more times. The data are comparable to, but a bit less precise than, previously reported results [21]. No real effort was made to optimize the method for sensitivity, but detection limits (2×noise) were less than a part per million for the metal ions [e.g., 0.3 for Pb(II) and 0.7 for Zn(II)] with the post-column derivatization system. These detection limits compare favorably with those reported for anions separated by ion-interaction methods [21]. It appears that reasonable quantitation can be obtained over three or four orders of magnitude for both metal ions and organic analytes in the typical mobile phases used for simultaneous separations.

Table 1
Calibration curve slopes by area and height methods[a]

Analyte	Slope (R.S.D., %)	
	Area Method	Height Method
Pb(II)	$2.7 \cdot 10^9$ (5.3)	–
Zn(II)	$8.5 \cdot 10^9$ (0.3)	$1.0 \cdot 10^5$ (8.2)
Co(II)	$8.1 \cdot 10^9$ (2.5)	$9.4 \cdot 10^4$ (7.2)
Cd(II)	$4.4 \cdot 10^9$ (1.7)	$4.3 \cdot 10^4$ (7.9)
p-Nitroaniline	$6.8 \cdot 10^8$ (7.8)	$2.6 \cdot 10^4$ (0.1)
p-Nitrophenol	$6.5 \cdot 10^8$ (0.5)	$8.5 \cdot 10^4$ (0.4)

[a] Mobile phase: methanol–water (45:55), 45 m*M* tartrate, 0.45 m*M* sodium dodecyl sulfate, apparent pH 3.4.

4. Conclusions

Simultaneous separations of metal ions and or-

ganics are easily done using the ion-pair chromatographic approach in aqueous–organic mobile phases. At low methanol content (30–40%) the C_8 IIR is useful in the 5–10 m*M* range; from 40–70% methanol the C_{12} IIR can be used in the 0.5–10 m*M* range; and in 70–80% methanol the C_{18} IIR is used around 1 m*M* or less. In the IIR–tartrate mobile phase metal-ion retention can be controlled not only with a change in IIR concentration but also by adjusting pH and the amount of methanol and/or tartrate competitive complexor.

Changes in the amount of IIR had only a slight effect on organic analyte retention. Therefore, the organic separation can be optimized in a mobile phase containing only water and methanol, and then the pH, and tartrate and IIR concentrations can be optimized for the metal-ion separation.

Useful quantitation of both metal-ion and organic analytes was accomplished over several orders of magnitude using both peak area and height. Retention reproducibility from one batch of mobile phase to another and one column equilibration to another was about the same as the precision of the quantitation studies, in the 1–10% relative range.

5. Notice

Although the information in this manuscript has been partially funded by the US Environmental Protection Agency, through its Office of Research and Development, under assistance agreement (CR-819041-01) to The University of North Carolina-Greensboro, it may not necessarily reflect the views of the Agency. Therefore, no official endorsement should be inferred. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

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