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Simultaneous separation of inorganic ions and neutral organics on ion-exchange stationary phases

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Abstract

Mixtures of inorganic ions and neutral organics (polars, polynuclear aromatics) were simultaneously separated on several ion-exchange stationary phases that contained both exchange- as well as reversed-phase functionality. An 8-quinolinol silica gel column and a commercial C_8/anion exchange phase were used for inorganic anion/neutral organic mixtures. Commercial C_8/weak-acid and benzene sulfonate strong-acid cation-exchange columns were employed for metal cation/neutral organic mixtures. Mobile phases were a blend of those normally used for ion-exchange separation with those generally used for reversed-phase high-performance liquid chromatography (HPLC). Both UV and conductivity detectors were used, in series. Indirect UV detection of anions on the C_8/anion column was effected with a phthalate eluent. The indirect detection could be switched off by operating at 306 nm, just above the range where phthalate absorbs; it could be switched back on again at 299 nm. Retention of metal ions on the C_8/cation column decreased with increasing mobile-phase concentration of ethylenediammonium and tartrate, as well as with increasing amounts of methanol in the mobile phase. A study of retention factor versus pH indicated that the pK_a of the bound carboxylic acid is about 6.3. Efficiency was found to be better on the strong acid column than on the weak-acid C_8/cation phase. Quantitation of cations and organics was acceptable on both cation-exchange phases. Overall, this work shows the viability of simultaneous ion/neutral separation both on phases designed for mixed-mode work as well as on those intended only for ion-exchange separations. These phases permit good separation of neutral organics at lower methanol concentrations than on conventional reversed-phase columns.

Keywords: Stationary phases, LC; Ion exchangers; Inorganic ions; Polynuclear aromatic compounds; Neutral organic compounds

1. Introduction

High-performance liquid chromatography (HPLC) is generally used to separate analytes of a single class, e.g. nonpolar organics, or inorganic cations [1]. However, there is no fundamental reason why more than one class of analyte cannot be separated simultaneously, both from other classes and within each class. Many situations exist where this might save considerable time rather than doing a different chromatographic run for each class of analyte present in the sample, or even using methodologies other than chromatography for one or more classes. While it is an easy matter to separate one class from another in a mixed-class sample, e.g., inorganic cations are not retained on a nonpolar reversed-phase

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column, it is not so straightforward to separate all analytes of more than one class simultaneously, which was the purpose of the present work. As described below, several workers have begun to address this problem.

Simultaneous separation of mixed-class analytes requires that two or more columns be used or that the stationary phase–mobile phase combination in a single column facilitate the chromatographic phase transfer of the different types of analytes. For example, it has been noted that the organic character of a polystyrene–divinylbenzene (SDVB) support for a cation exchange phase would provide another interaction possibility besides coulombic for organic ions [2] and also should be suitable for neutral organic separations [3]. If the different interactions are to be exhibited by silica-based bonded-phase functionalities, they may be produced in one column by mixed-bed, mixed-ligand (two or more different functionalized ligands on the same substrate particle) or mixed-mode packings (two or more types of interaction sites or modes on the same surface-attached moiety). The silica support itself does not contribute significantly to neutral organic separations.

Several workers have designed systems to simultaneously separate anions and cations using a series arrangement of anion- and cation-exchange columns [4–7] or mixed beds of cation- and anion-exchange particles [3,8,9]. Another approach is to use the oppositely charged sites of zwitterionic ligands, either covalently bound to support particles [10] or dynamically sorbed from the mobile phase onto a C_{18} stationary phase [11,12]. Much of this work has involved organic ions, for which dispersion as well as coulombic interactions come into play [2].

Some work has also been done to simultaneously separate ionic and neutral organic analytes. Serial columns, mixed-bed [13] and mixed-ligand approaches were used [10,14], with the mixed-ligand column (C_8-cation exchange) [14] proving most efficient. Recent work in this laboratory addressed the separation of metal ions and neutral organics on a C_{18} column with an ion-interaction reagent in the mobile phase [15].

A few groups have deliberately designed multi-site or mixed-mode columns to separate charged and uncharged analytes in one run [10,11,16]. Wongyai's phenylpropanol amine phase displayed both anion-exchange and reversed-phase/polar character [17]. Some commercial columns have recently been introduced which were also designed to provide both reversed-phase and ion-exchange character, for example, a latex-coated SDVB support [18]. This and other so-called mixed-mode phases have not been used much to separate both ionic and neutral analytes and, when they have, the compounds usually belonged to one class, e.g. protonated and deprotonated organic acids [10,19] or pharmaceutical compounds [18].

While other commercial ion-exchange phases are not deliberately designed for both neutral and ionic analytes, they also seem to offer the possibility of mixed-class separations. The purpose of the present study was to develop both mobile phases and detection schemes to effect ionic-neutral analyte chromatography in one run using these silica-based mixed-mode or multi-site phases, especially for inorganic ions and neutral organics. This is an extension of the earlier work using ion-interaction reagents [15]. We present results on a phase prepared in our laboratory, 8-quinolinol silica gel (QSG), [20,21], as well as on several commercial phases, a C_8/anion-exchange, C_8/cation-exchange and a benzene sulfonate cation-exchange phase. Hopefully, these results will suggest useful and general approaches for performing mixed-class separations on systems containing both ionic and neutral analytes, for example, environmental samples and foods and beverages.

2. Experimental

2.1. Apparatus

Several combinations of pumping system and detectors were used in this work. Studies on the QSG column utilized a Spectra-Physics Model 8000B pump, a Valco model CV-6-4HPa-N60 injection valve and the conductivity mode of a Perkin-Elmer TriDet detector in series with a Schoeffel Model 770 detector set at 254 nm. The cation/organic work employed a Kratos model 400 pump equipped with a Rheodyne 7010 injection valve; detection was performed on an Alltech model 320 conductivity

monitor in series with an ISCO model V^4 variable wavelength detector. The anion/organic studies used an SSI model 300 pump, a Rheodyne 7010 injector and the same Model $320/V^4$ series combination of detectors as used in the cation work. All injectors were equipped with 10 μl sample loops.

The QSG column (250×4.6 mm) was packed using the upward slurry-packing method [22]. Anion/organic separations were done on an Alltech C_8/weak anion-exchange mixed-mode column. Cation/organic separations employed both an Alltech C_8/weak cation exchange and a Nucleosil SA column (from Machery-Nagel, packed by Alltech Associates). All three commercial columns had dimensions of 150×4.6 mm.

Water used for samples and mobile phases was purified with a Barnstead Nanopure II system, or equivalent, using deionized feed water.

2.2. Reagents and solutions

The QSG stationary phase was supported on Polygosil 100-7 silica gel from Machery-Nagel. This is an irregular, 7-μm-diameter material with 100-Å pores. The synthesis was accomplished using triethoxysilylpropyl-*p*-nitrobenzamide silylating agent to produce a nitrobenzamide parent material. The initial silylation and subsequent reactions have been described previously [20,21]. The Alltech C_8/anion and C_8/cation phases and Nucleosil strong-acid cation phase were supported on 5-μm, spherical silica gel with 100-Å pores. The initial silylating reagent for both the Alltech phases was a trifunctional reagent producing a 'polymeric' phase. The nature of the original silylating reagent for the strong-acid cation-exchange phase is not known. The QSG and Nucleosil phases were both end-capped, while the two Alltech mixed-mode phases were not.

All mobile phases and analyte solutions were made up in water purified with the Nanopure II system. Acetonitrile and methyl alcohol were either of ACS- or HPLC grade. Ionic analytes were generally in the 0.1–1 m*M* range and organic analytes were in the 0.01–1 m*M* range. Mobile phases and analyte solutions were filtered through 0.45 μm membrane filters. The pH meter was calibrated with standard aqueous buffers so that reported pH values in methanol- or acetonitrile-containing mobile phases are apparent only. All reagents were of ACS grade or better. The pH of the mobile phases was generally adjusted with sodium hydroxide, aqueous ammonia, nitric or acetic acid, depending on the situation.

2.3. Chromatographic procedures

All chromatography was performed at ambient temperature under isocratic conditions. Flow-rates were generally 1.0 ml/min. The QSG column was flushed for 15–30 min with deionized water, then for at least 30 min with mobile phase before analytes were injected. The other columns were flushed with mobile phase for 30–45 min before injection. All columns were flushed with water and methanol or acetonitrile at the end of each day to wash out residual salts and organics.

3. Results and discussion

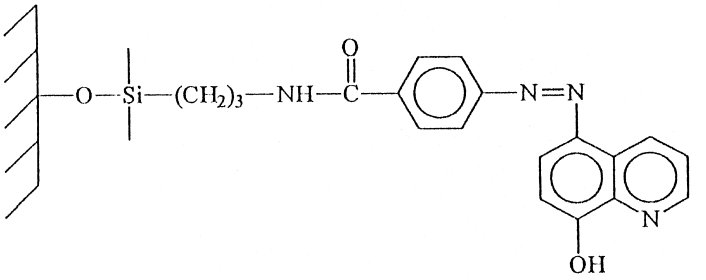
In trying to simultaneously separate multiple classes of analytes, not only must appropriate stationary-phase sites be present (e.g. ionic, dispersion) but the mobile phase must also be designed to accommodate all analyte species and to get reasonable k' values. It would seem from the several studies of mixed-class separations referenced above, as well as the results described below, that the different interparticle interactions available on mixed-mode packings operate essentially independently of each other. Likewise, the mobile-phase conditions that promote one type of interaction, e.g., ion-exchange or dispersion, do not preclude other types. So, it turns out to be reasonably straightforward to design mobile phases for mixtures of inorganic ions and neutral organic analytes despite their different interparticle interactions. As both ion and reversed-phase organic analyte separations use water-based mobile phases, all that is required is that appropriate ion-eluting agents, pH buffers and organic modifiers be present in an aqueous phase at concentrations required for each analyte class.

This report describes inorganic ion-neutral organic molecule mixed-class separations both on stationary phases that are deliberately designed to have mixed-mode character and, in one case, on a phase intended

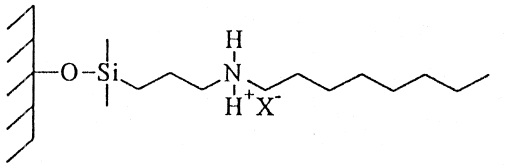
to interact only with cationic analytes. It should be noted that the mixed-class analytes employed, e.g., inorganic anions and polynuclear aromatic hydrocarbons (PNAs), are not meant to imply that such mixtures are important in real situations but are used here as extremes of polar character, not only to illustrate their separation but also to show that mobile phases can be designed to accommodate these very different analytes.

3.1. 8-Quinolinol silica gel phase

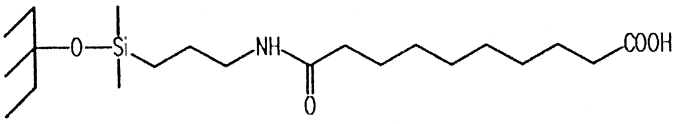
The first phase considered here is 8-quinolinol silica gel (QSG) (Fig. 1), a noncommercial material prepared in our laboratory [20,21]. QSG has several types of organic analyte interaction sites (hydrogen bonding, polar, dispersion) as well as the metal-complexing 8-quinolinol moiety. When the heterocyclic nitrogen atom is protonated ($pK_a \approx 3.3$



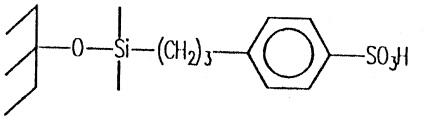
8-Quinolinol Silica Gel (QSG)



C_8/Anion Phase



C_8/Weak Acid Cation Exchange Phase



Strong Acid Cation Exchange Phase

Fig. 1. Stationary phases employed in this work.

[23]), an anion-exchange site is also present. If the silica support is trimethylsilyl end-capped, additional dispersion interaction is possible, and the column would then be classified as a mixed-ligand–mixed-mode combination phase. QSG has been used for a variety of organic analyte separations [22], for metal-ion complexation chromatography [24] and for the single-injection separation of organics, metal ions and anions [25]. The anion separation reported [25] probably occurred mostly on protonated residual aminopropyl groups (from the original silylation reaction) [26]. In this case, the phase again was of the mixed-ligand–mixed-mode type.

This report describes the simultaneous separation of four inorganic anions and three PNAs on QSG and illustrates the possibilities of this phase for mixed-class work (Fig. 2). The very hydrophobic PNAs and the hydrophilic anions are quite distinct in their interaction with both an aqueous-based mobile phase and an organic charged-site stationary phase. The anions interact with the protonated heterocyclic-nitrogen exchange site while the PNAs interact dispersively with the organic portions of the quinolinol appendage and the trimethylsilyl end-capping groups. In this case, a water–acetonitrile mobile phase was used to elute the PNAs. The mobile phase also contained tartrate at pH 2.8 as the negatively charged anion eluter. The ions were detected conductimetrically and the PNAs spectroscopically at 254 nm. The end-capped QSG phase contained about 70 μmol/g of 8-quinolinol groups; this falls in the low-capacity-exchanger category. Uncapped versions of this phase exhibit lower, but still useful, retention of the PNAs. Studies with capped and uncapped QSG phases reveal that small inorganic anions exhibit somewhat lower retention on the capped than the uncapped phase, while larger ('softer') anions, e.g., ClO_4^-, exhibit higher retention. The result is better anion resolution on end-capped than on non-capped phases.. These results will be described more fully in a later publication.

In the example given in Fig. 2, all anions have eluted from the column before the first PNA does, but this is not always the case, as will be seen below. Coelution and intermixing of ion and neutral peaks often occurs, and selective detection is then useful to monitor individual analyte classes.

While reasonable anion–organic separation can be accomplished on QSG and similar custom-synthesized, mixed-mode columns, methods to perform these separations on commercially available columns are probably of more interest to the chromatographic community. The rest of this report describes work done with off-the-shelf columns.

3.2. Commercial columns

As indicated above, most neutral analytes can be separated on an alkyl or phenyl phase using water-based mobile-phase manipulation; and ions require

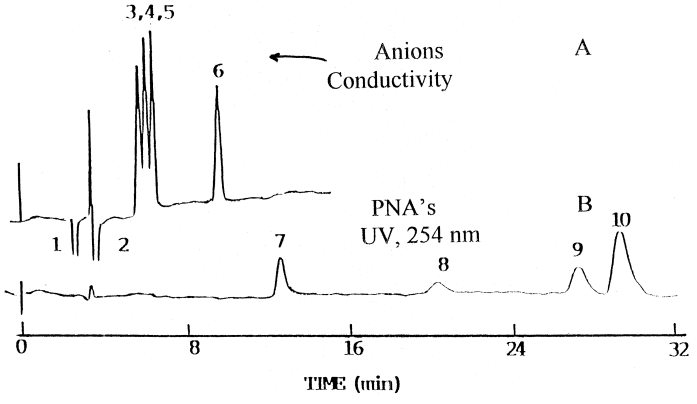


Fig. 2. Simultaneous determination of anions and PNAs on a capped 8-quinolinol silica gel column, with an exchange capacity of 70 μmol/g. Mobile phase: water–acetonitrile (60:40, v/v), 10 m*M* tartrate, with an apparent pH of 2.80. (A) Conductivity detection. (B) UV detection, 254 nm. Analytes: (1) Na^+, (2) system peak, (3) Cl^-, (4) Br^-, (5) NO_3^-, (6) ClO_4^-, (7) naphthalene, (8) unknown, (9) phenanthrene and (10) anthracene.

only an oppositely charged site and aqueous charged eluents. The C_8/anion and C_8/cation phases illustrate this idea of combining both alkyl and ion-exchange sites on one silica-bonded ligand (Fig. 1). Both of these are weak exchangers. On the other hand, the strong-acid exchanger, a conventional benzene sulfonic acid, is a commercial example of a phase designed for a single purpose, i.e., cation exchange. However, this material also has possibilities for interactions with neutral organic species, as the charged site is coupled to the silica support through an organic appendage, the propyl benzene moiety. In addition, the phase is trimethylsilyl end-capped. This is another example, like the end-capped QSG, of a combination mixed-ligand–mixed-mode stationary phase. The C_8/cation and C_8/anion phases, however, are not end-capped and, therefore, fall into the simple mixed-mode category. Both are made with trifunctional silanes and so may have two links to the silica surface [27] and crosslinks to neighboring appendages [28] (i.e., these are so-called polymeric phases [29]).

3.2.1. The C_8/anion column

Fig. 3A illustrates the simultaneous separation on the C_8/anion column of five inorganic anions and three PNAs in a methanol–water (70:30, v/v) mobile phase, which was 2 m*M* in potassium hydrogen phthalate (KHP). As with the QSG work (Fig. 2), the anions are detected conductimetrically and the organics spectroscopically, here at 306 nm. The methanol is present to solubilize the PNAs and to adjust their k' values. The HP^- anion can serve double duty. First, it is the eluting agent that advances the analyte anions through the column. However, because it absorbs UV radiation below about 300 nm, it can also be used to indirectly detect nonabsorbing anions [30], adding versatility to the detection possibilities where a conductivity detector is not available. This is illustrated in Fig. 3B, where the anion's are seen as

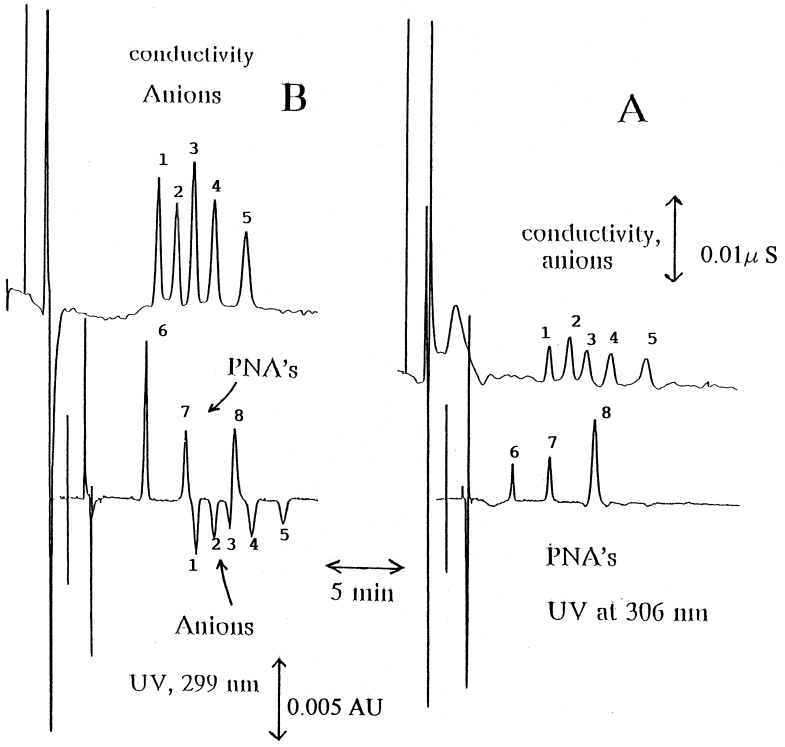


Fig. 3. Simultaneous determination of anions and PNAs on an Alltech mixed-mode C_8/anion-exchange column. Mobile phase: methanol–water (70:30, v/v), potassium hydrogen phthalate (A: 2 m*M*, B: 3 m*M*). Detection: top traces, conductivity; bottom traces, UV. Analytes: (1) Cl^-, (2) NO_2^-, (3) Br^-, (4) NO_3^-, (5) I^-, (6) phenanthrene, (7) pyrene and (8) chrysene.

negative and the PNAs as positive UV peaks at a detector wavelength of 299 nm. The anions are also detected conductimetrically. Conditions are virtually the same as in Fig. 3A except that phthalate is at a concentration of 3 m*M* and the wavelength was changed from 306 nm, where phthalate does not absorb, to 299, where it does. The indirect UV detection, then, can be turned on and off with a change in wavelength of just a few nm. Some additional mobile phase and detection fine-tuning, namely, reducing the methanol concentration to 50% and monitoring at 260 nm (the valley between the E_2 and B bands of phthalate), allows several small organic analytes to be eluted, with positive UV detection, before the negative UV peaks of the five anions. These are then followed by the larger PNAs, again with positive peaks (Fig. 4).

3.2.2. The C_8/cation column

The C_8/cation column is the companion to the C_8/anion. While the manufacturer will not reveal the exact structure of the stationary phase, it is known that it is 'polymeric', the original silica derivatizing reagent being a trifunctional aminopropylsilane [31]. Further chemistry on the original silylated silica yields an amide functionality and, eventually, an 8-carbon chain terminating in a carboxylic acid group (Fig.1). This is the cation-exchange site when it is deprotonated.

As the exchange site is a weak carboxylic acid, it was of interest to determine cation retention as a function of pH. Fig. 5 shows the capacity factors for three metal-ion analytes from pH 4 to 7 in an all-aqueous mobile phase consisting of 1.5 m*M* tartrate, 1.0 m*M* ethylenediamine (en) and 20 m*M* acetate. Retention begins to occur at about pH 5 and levels off at about pH 6.5. As the tartrate ($pK_{a_2}=4.2$) and acetate ($pK_a=4.8$) are fully ionized at pH 6, the increasing k' values above that pH imply a pK_a for the bonded carboxylic acid group of about 6.3. This is the pH at about 50% of the rise in k', where the carboxyl group is presumably half protonated and half deprotonated and where the pH is equal to the pK_a.

The tartrate–en mobile phase used in this study has been employed in all-aqueous systems by other workers [1,32]. The en is a charged eluting species at

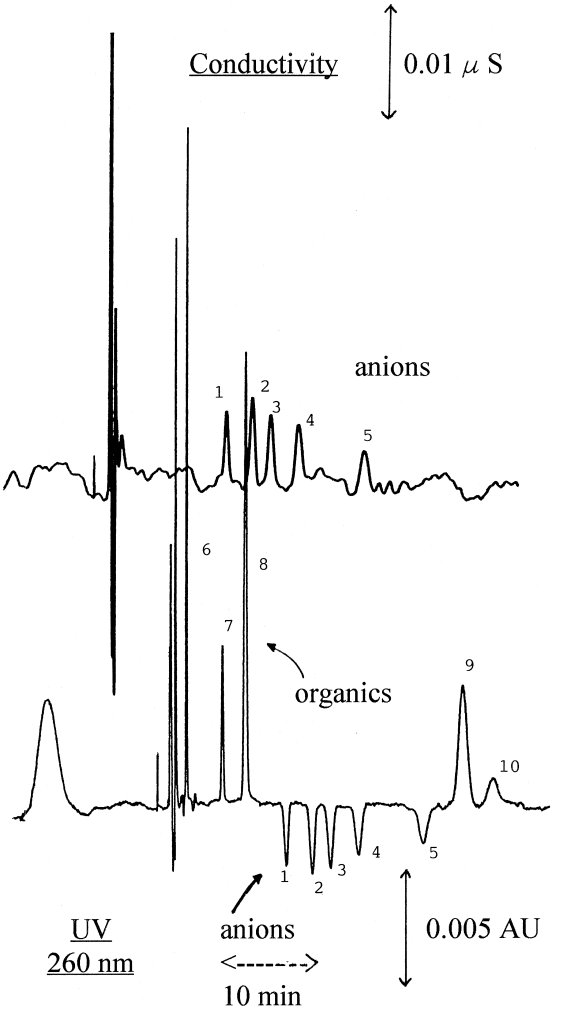


Fig. 4. Simultaneous determination of anions and organics on an Alltech mixed-mode C_8/anion-exchange column. Mobile phase: methanol–water (50:50, v/v), 1.0 m*M* potassium hydrogen phthalate. Detection: top, conductivity; bottom, UV at 260 nm. Analytes: (1) Cl^-, (2) NO_2^-, (3) Br^-, (4) NO_3^-, (5) I^-, (6) phenethyl alcohol, (7) chloroethylbenzene, (8)naphthalene, (9) phenanthrene and (10) pyrene.

the pH values below 6 used in this work and does not complex the metal ions [33] but rather competes for exchange sites [1]. The tartrate is a weak cation complexor ($pK_{a_1}=2.9$ and $pK_{a_2}=4.2$), which interacts with metal ions in the mobile phase, reducing the effective metal-ion charge and allowing k' to be manipulated [1,32]. As has been previously observed in all-aqueous systems [1,32–34], we found metal-

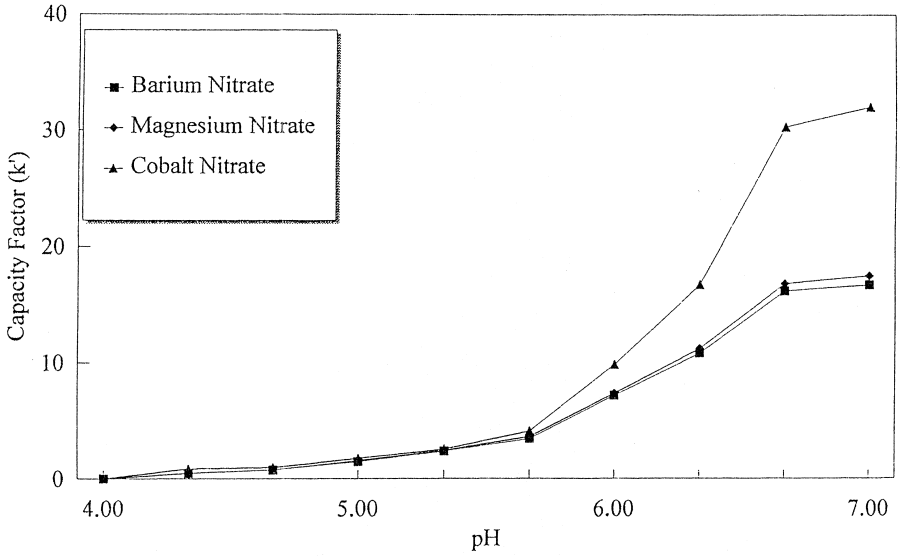


Fig. 5. Capacity factor of metals as a function of pH on the Alltech C_8/cation exchange column. Mobile phase: all aqueous, 1.5 m*M* tartrate, 1.0 m*M* ethylenediamine, 20 m*M* sodium acetate.

ion retention to decrease with increases in both tartrate and en concentrations when the mobile-phase pH was held constant in water–methanol mobile phases. Values of k' for several metals are given in Tables 1 and 2 as a function of the tartrate and en concentration at various water–methanol ratios. Retention of both organics and metal ions also decreased with increasing methanol concentration (Table 3), as has been observed by others in metal-ion exchange work [15,35].

Fig. 6 shows a typical separation of six metal ions and seven organic compounds in a water–methanol

Table 1
Retention factor as a function of tartrate concentration[a] on the C_8/cation column

Analyte	Tartrate concentration (m*M*)		
	1.0	1.5	2.0
		k'	
Mn (II)	11.4	8.6	5.9
Mg (II)	7.3	6.1	3.9
Ca (II)	6.0	4.9	3.2
Ni (II)	5.2	4.0	2.7

[a] Mobile phase: methanol–water (20:80, v/v), 1.0 m*M* en, varying tartrate, with an apparent pH of 5.3.

Table 2
Retention factor as a function of ethylenediamine concentration[a] on the C_8/cation column

Analyte	Ethylenediamine concentration (m*M*)			
	0.50	1.0	2.0	3.0
		k'		
Mg(II)	11.8	8.1	6.4	4.7
Ba (II)	13.0	8.8	6.8	5.0
Co (II)	13.9	10.0	8.9	6.8

[a] Mobile phase: methanol–water (40:60, v/v), 1.5 m*M* tartrate, varying en, 5 m*M* sodium acetate, with an apparent pH of 6.30.

Table 3
Retention factor as a function of methanol concentration[a] on the C_8/cation column

Analyte	% MeOH		
	20	40	60
		k'	
Mn (II)	10.1	5.7	3.6
Mg (II)	6.6	3.1	1.7
Ni (II)	4.7	1.7	0.0
p-Nitroaniline	2.1	1.3	0.5
2-Chloroethylbenzene	8.7	3.2	1.1

[a] Mobile phase: methanol–water, 1.5 m*M* tartrate, 1.0 m*M* ethylenediamine, with an apparent pH of 5.3.

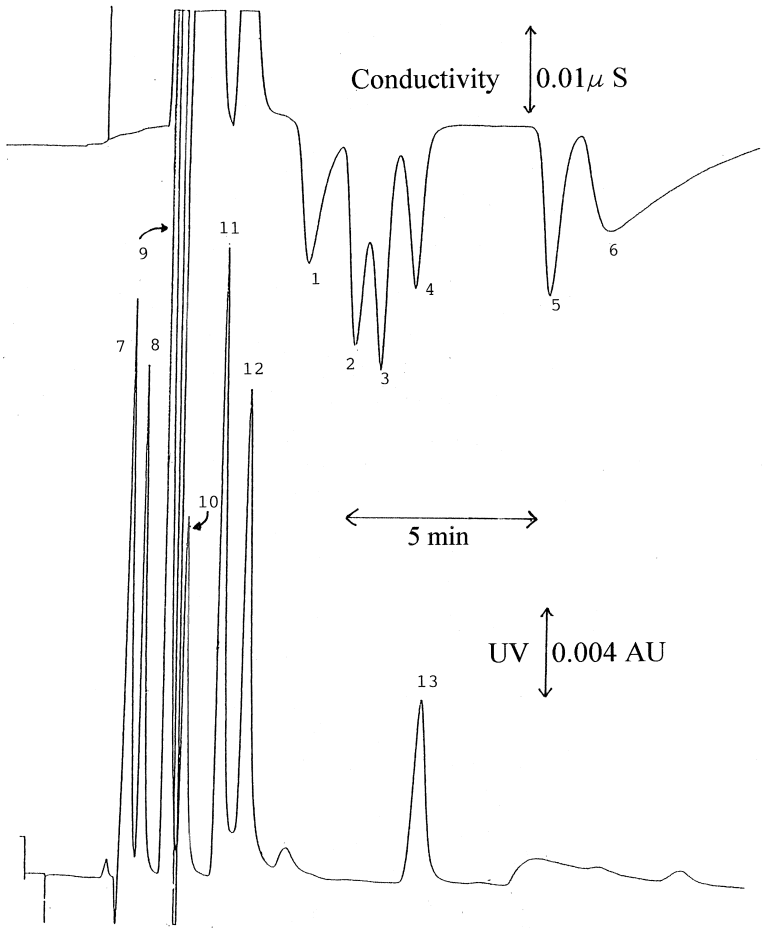


Fig. 6. Simultaneous determination of metal ions and neutral organics on an Alltech C_8/cation-exchange column. Mobile phase: methanol–water (40:60, v/v), 1.5 m*M* tartrate, 1.0 m*M* ethylenediamine, apparent pH 5.3. Detection: top, conductivity; bottom, UV at 254 nm. Analytes: (1) Ni^{2+}, (2) Co^{2+}, (3) Ba^{2+}, (4) Mg^{2+}, (5) Mn^{2+}, (6) Zn^{2+}, (7) *o*-hydroxybenzoic acid, (8) benzoic acid, (9) aniline, (10) phenethyl alcohol, (11) *p*-nitroaniline, (12) *p*-nitrophenol and (13) 2-chloroethylbenzene.

(60:40, v/v) mobile phase containing tartrate and en. The cations were detected conductimetrically and the organics were detected spectroscopically at 254 nm. The negative (indirect) peaks for the cations result because the eluted analytes have a lower conductance than the chromatographic displacing species, in this case, the tartrate and charged en [32]. As this C_8 mixed-mode phase is not end-capped, the organics can be eluted with a relatively low methanol content in the mobile phase. Even the fairly hydrophobic 2-chloroethylbenzene elutes in less than 10 min in the 40% methanol mobile phase.

3.2.3. The benzene sulfonate column

The C_8/cation mixed-mode phase was deliberately designed for either cation exchange or organic analyte separation and so was a good prospect for the simultaneous separation of ions and neutral organic analytes. However, we wanted to test a conventional strong-acid cation-exchange phase to see if similar simultaneous separations were possible (Fig. 7). Apparently, the end-capped propylbenzene sulfonate exchanger used here exhibits only moderately strong dispersion interactions, as relatively low methanol concentrations in the mobile phase yielded useful k'

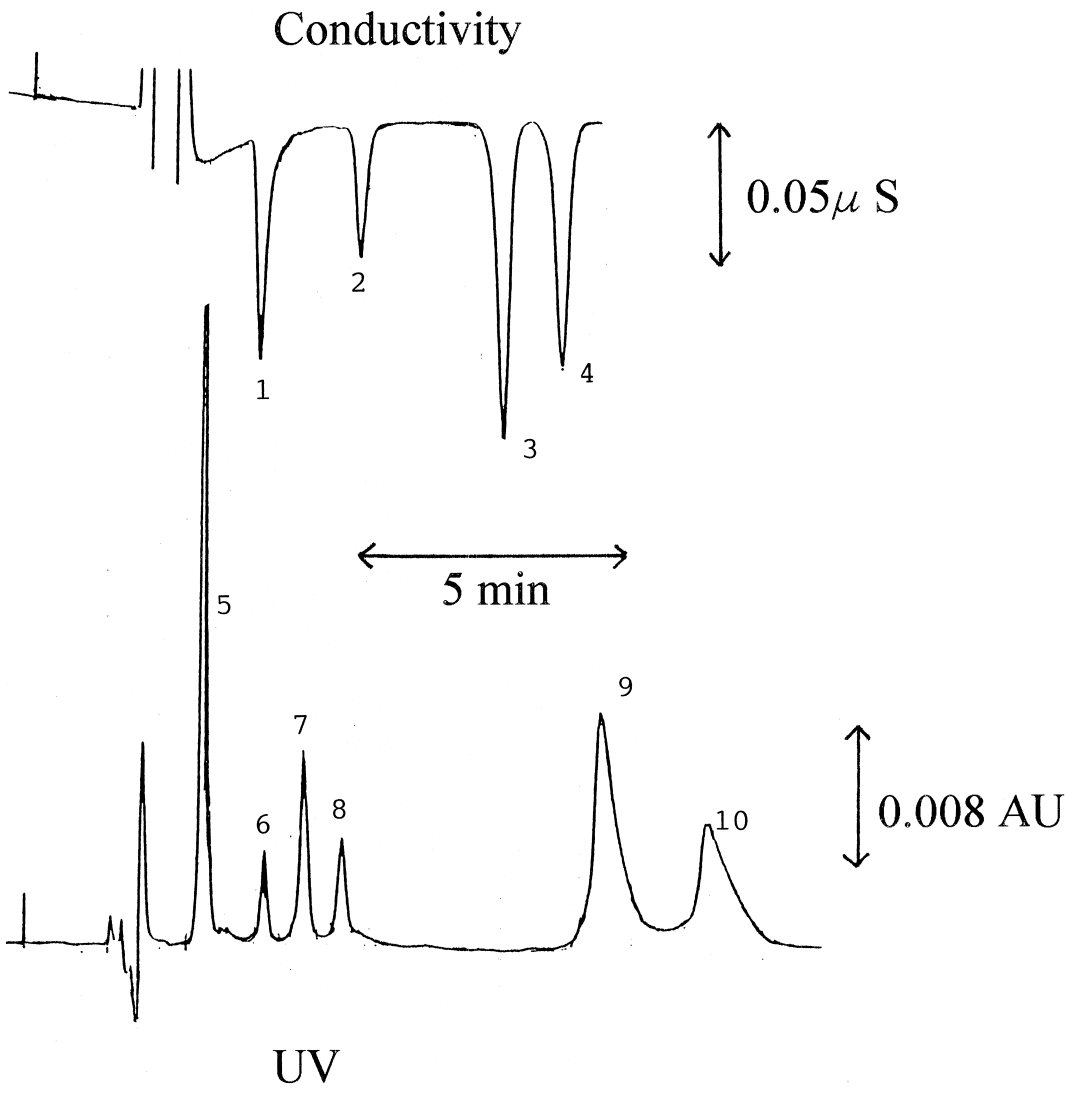


Fig. 7. Simultaneous determination of metal ions and neutral organics on a Machery-Nagel Nucleosil strong-acid cation-exchange column. Mobile phase: methanol–water (15:85, v/v), 10 m*M* tartrate, 5 m*M* ethylenediamine, apparent pH 3.35. Detection: top, conductivity; bottom, UV at 254nm. Analytes: (1) Pb^{2+}, (2) Zn^{2+}, (3) Ca^{2+}, (4) Mg^{2+}, (5) phenol, (6) *o*-chlorophenol, (7) aniline, (8) *p*-chloroaniline, (9) *p*-nitroaniline and (10) 2-chloroethylbenzene.

values for organics. Note that 2-chloroethylbenzene elutes at about 12.5 min with only 15% methanol. Likewise, benzo[*k*]fluoranthene, a five-ring PNA, elutes in 23 min with only 55% methanol (not shown). The ability to use relatively low concentrations of organic modifier to elute large organic analytes simultaneously with cations is a useful, if unintended, benefit of these ion-exchange phases.

3.3. Retention order and efficiency

It is interesting to examine both the retention order of the cations as well as peak shape on the two cation-exchange phases. The order of elution on the strong-acid exchanger, which primarily interacts coulombically with metal ions, was Pb(II) <Zn(II)< Co(II)<Cd(II). This order is consistent with metal–

tartrate formation constants. The most-strongly complexed Pb(II) elutes first; the least-strongly complexed Cd(II) interacts most strongly with the bound sulfonate and so elutes last. On the C_8/weak-acid phase, the elution order was Ni(II)<Co(II)<Ba(II)≈Mg(II)<Mn(II)<Zn(II). The Ba(II) and Mg(II) have very similar k' values and sometimes exchange positions. The Zn(II) and Co(II), which eluted very close to each other on the strong-acid column (not shown), have very different k' values on the weak-acid exchanger. In addition, the Zn(II) and, to a degree, the Ni(II) have tailed peaks on the weak-acid phase. These differences may result from some complexation interaction of the bound weak-acid carboxyl group that is absent with the strong-acid phase. We have observed this sort of behavior previously with the chelating QSG stationary phase [25], for which Zn(II) peaks were seriously tailed in an aqueous tartrate–acetonitrile mobile phase. Indeed, efficiency comparison of the weak-acid and strong-acid exchangers (Table 4) reveals that generally the strong-acid phase is more efficient, exhibiting larger plate counts and better peak symmetry for both metals and PNAs. Further studies are underway to confirm these initial findings.

3.4. Quantitation

Quantitation studies were run for several metals and organics on both the weak- and strong-acid columns. Linearity of response was generally found over three or four orders of magnitude, generally from 10^{-2}–10^{-5} *M*. Some representative data are given in Table 5. In general, better precision of the slope and intercept was found for peak area than peak height. No noticeable difference in quantitation was observed between the weak- and strong-acid phases. The reason for the large deviation from the expected slope of unity for Ca^{2+} is not known.

Table 4
Efficiency (*N* plates/m) and asymmetry factors (A_s) on the weak- and strong cation-exchange columns

Analyte	C_8/weak acid[a] *N*	A_s	strong acid[b] *N*	A_s
Zn (II)	3×10^2	6.8[c]	3×10^4	0.7
Cd (II)	8×10^3	4.2	3×10^4	0.7
Pyrene	2×10^3	2.5	3×10^4	1.2

[a] Mobile phase: methanol–water (60:40, v/v), 1.5 m*M* tartrate, 1.0 m*M* ethylenediamine, apparent pH 5.3.
[b] Mobile phase: methanol–water (55:45, v/v), 10 m*M* tartrate, 5.0 m*M* ethylenediamine, apparent pH 3.4.
[c] Mobile phase: methanol–water (40:60, v/v), other concentrations were the same as for a.

Table 5
Calibration curve data (1og area−1og concentration) on the weak- and strong cation-exchange columns[a]

Analyte	Slope	Intercept	Correlation coefficient
Weak-acid column			
Ca (II)[b]	0.78 (2.0)	9.2 (3.7)	0.984
Zn (II)[b]	1.02 (0.5)	10.1 (0.2)	1.00
p-Nitroaniline[b]	0.98 (3.7)	9.4 (1.4)	0.998
Anthracene[c]	0.91 (5.8)	10.5 (2.5)	0.997
Strong-acid column			
Ca (II)[d]	0.76 (2.5)	7.7 (0.7)	0.999
p-Nitroaniline[d]	0.87 (8.6)	8.9 (2.8)	0.986
Anthracene[e]	0.94 (8.6)	10.6 (3.0)	0.993

[a] Tartrate and ethylenediamine concentrations are as given in Table 4. Uncertainties (shown in parentheses) are percent relative standard deviation.
[b] Methanol–water, 40:60 (v/v).
[c] Methanol–water, 60:40 (v/v).
[d] Methanol–water, 15:85 (v/v).
[e] Methanol–water, 55:45 (v/v).

4. Conclusions

The above studies have shown that it is possible to use silica-based ion-exchange packings in the reversed-phase mode for neutral organic analyte separations. Indeed, these phases can be used to simultaneously separate and quantitate inorganic ions and neutral organic species not only on columns that are designed for both coulombic and dispersive interactions, but also on those, for example the strong-acid cation-exchange column, that are intended for ion-exchange only. Further work is underway in our laboratory to exploit these types of phases for simultaneous ion–neutral separations. The major findings of the present study can be summarized as

follows:

- Commercial mixed-mode weak exchangers and conventional strong exchangers have the ionic sites and dispersion interactions needed for simultaneous ion-neutral separations.
- The mobile-phase requires an ionic eluting agent for exchange interactions and an organic modifier to elute neutral organic analytes.
- Neutral organics elute at relatively low methanol concentrations (10–50%), especially on the strong cation-exchanger.
- Cation retention decreases with increasing concentration of both ionic eluting agents and metal complexors in the mobile phase.
- Increasing organic modifier concentration decreases retention of both neutral organic and metal cation analytes.
- Cations elute from the strong benzene sulfonate exchanger in the order of their metal-complex formation constants (most stable complex first) when a complexing eluent is used.
- Chromatographic efficiency appears to be better on the strong cation-exchanger than the weak-acid mixed-mode phase.
- Linear calibration curves were found for both neutral organics and cations from about 10^{-2}–10^{-5} *M*.

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 at 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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