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# NEW LATEX-BONDED PELLICULAR ANION EXCHANGERS WITH MULTI-PHASE SELECTIVITY FOR HIGH-PERFORMANCE CHROMATOGRAPHIC SEPARATIONS

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## SUMMARY

A new polymeric "multi-phase" chromatographic packing material that combines ion exchange in a pellicular format with adsorption and ion-pair retention on a neutral macroporous core bead is described. The material is stable from pH 0 to 14 and from 1 to 100% (v/v) of common reversed-phase solvents in aqueous mixtures. The ability to independently control ion exchange and adsorption or ion-pair retention is demonstrated on a variety of inorganic and organic analytes.

## INTRODUCTION

In 1973 Horváth wrote "Ion-exchange resins are particularly suitable stationary phases because of their versatility and stability. Although their application has been mostly aqueous systems, it is not so restricted, and it is safe to say that ion-exchange resins represent the most universal class of stationary phases in liquid chromatography. The favorable chromatographic properties of ion-exchange resins are enhanced by the pellicular structure, although at the cost of reduced column loading capacity"[1]. Some of the most successful ion chromatography columns today are pellicular. To realize their full potential for liquid chromatography, they lack only solvent compatibility. This limitation of current pellicular ion-exchange materials is related to the low cross-link of the core bead polymer. Traditionally ion chromatography applications were entirely aqueous and low cross-linked materials were adequate. As ion chromatography progressed to organic ion problem solving, the need for a solvent amenable ion-exchange packing material became obvious. In the process of developing solvent-stable pellicular ion-exchange materials a new type of "multi-phase" material, that combines the features of a pellicular ion-exchange phase with the features of high-adsorption surface area of reversed phase on the same packing material, was developed. A multi-phase material provides multiple modes of retention during the separation process, including ion exchange, ion pair when a "pairing agent" is added, and adsorption retention. This paper will describe the new multi-phase material, will describe some of the effects of different solvents on ion-exchange selectivity and will postulate on mechanisms of retention during multi-mode separations.

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## BACKGROUND

Pellicular ion-exchange materials have come to be recognized as materials that have a relatively inert core with a thin layer or "skin" of active ion-exchange material applied to the outer surface. This ion-exchange layer is fully functionalized and is applied either as an encapsulating layer, where there is no physical attachment of the active layer to the core, or as a layer that is electrostatically bonded to the core and usually takes the form of latex beads. Pellicular materials are desirable over fully functionalized gel ion-exchange materials because capacity is controlled allowing reasonable eluent ionic concentrations. They are also chromatographically more desirable because the pellicular layer guarantees a much shorter analyte diffusion path allowing column efficiencies approaching that of reversed-phase columns.

Until Small and Stevens[2] made the first electrostatically agglomerated pellicular ion-exchange materials in the 1970s[2], encapsulation techniques developed in the 1960s[3] were the only method known for making pellicular ion-exchange materials. Encapsulation techniques, however, suffer from non-reproducible thickness of the pellicular layer and delamination of the pellicular layer as a result of shrinking and swelling of the pellicular layer with use. Encapsulation is nonetheless a viable pellicular ion-exchange packing. Recently a pellicular cation-exchange column, based on encapsulation, for the analytical determination of alkali metals and alkaline earths, was introduced[4]. Fig. 1a is a schematic of an encapsulated pellicular ion exchanger. Methods for making surface functionalized anion and cation polystyrene–divinylbenzene (PS–DVB) "pseudo" pellicular ion exchangers have also been known for some time, but these had poor analytical performance, because swelling, due to hydration of the sulfonated or aminated sites in the surface functionalized layer, was non-uniform. This non-uniform swelling was a result of a high to low gradient of functional sites with respect to depth.

### *Electrostatic agglomeration*

Most of the ion chromatography columns in use today are electrostatically latex-coated pellicular ion exchangers. These materials consist of three regions: (1) an inert PS–DVB core, (2) a shallow sulfonated layer on the surface of the inert core, (3) a mono-layer coating of colloidal ion-exchange particles which are permanently attached to the oppositely charged, functionalized surface of the inert core. The latex coating consists of fully functionalized anion-exchange latex particles made from vinylbenzylchloride (VBC) polymer cross-linked with DVB and fully functionalized with an appropriate tertiary amine for desired anion-exchange selectivity[5,6]. Fig. 1b is a schematic representation of an electrostatically agglomerated ion-exchange packing.

Ion-exchange materials prepared by electrostatic agglomeration have several distinct advantages over the encapsulated materials. First, agglomerates are easily prevented during the attachment process using electrostatic agglomeration since the core beads and the latex beads are polymerized independently and any agglomerates can be removed by filtration before the attachment step. Phase thickness is also easily controlled because only a mono-layer of essentially mono-disperse latex beads can be attached. Therefore phase thickness and uniformity is controlled by the latex bead diameter. Because the latex is fully functionalized, column capacity increases as latex

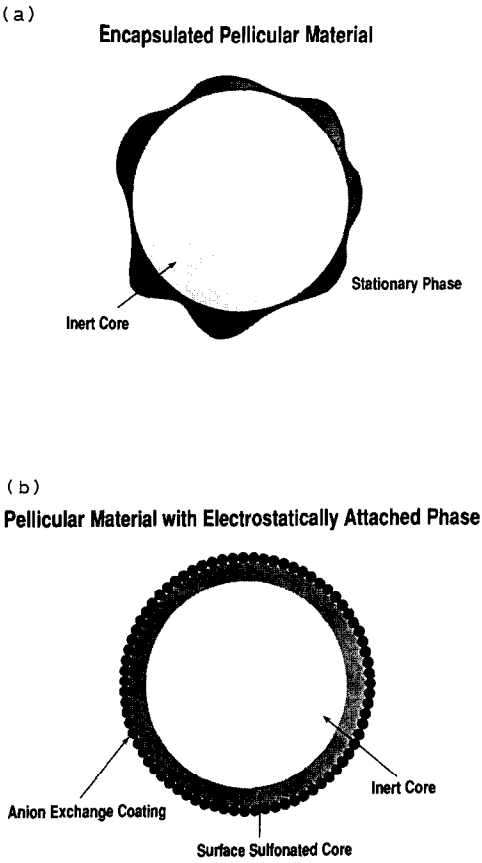


Fig. 1. (a) Schematic of an encapsulated pellicular ion-exchange packing. (b) Schematic of an electrostatically agglomerated latex-coated pellicular ion exchanger.

diameter increases, so latex diameter is also used to control column capacity. The diameters of the different latexes that are commonly used range from 50 to 500 nm and column capacity ranges from 5 to 150 $\mu$equiv./column. Because the core beads and latex beads are independently manufactured and brought together only at the last steps of making a column, each can be subjected to independent quality control in their manufacture. This provides very reproducible pellicular ion-exchange materials, with respect to capacity and selectivity, and greatly improves manufacturing efficiency. The electrostatic forces of adhesion are so strong that it is virtually impossible to separate the two phases. Theoretically there can be as many as several hundred electrostatic bonds holding a single aminated latex bead to the surface sulfonated core bead. Finally, the surface of the functionalized core has the same charge as that of the analyte. This means the analyte is effectively excluded from the core, thus concentrating analytes in the active pellicular layer, maintaining chromatographically favorable short diffusion paths into and out of the stationary phase. This is analogous to the very hydrophilic core of $C_{18}$ derivatized silica which excludes hydrophobic analytes from the core in reversed-phase high-performance liquid chromatography (HPLC).

Electrostatic agglomeration has two major disadvantages. The latex functionalization chemistry must be aqueous compatible to maintain the aqueous latex suspension prior to agglomeration. This effectively limits available chemistries to rather hydrophilic reagents. Also the functional groups on the surface of the inert core can also interact with sample components causing chromatographic anomalies. For example, transition metals in the sample matrix can concentrate on the residual cation-exchange sites and result in the complexation or precipitation of analyte ions such as phosphate and sulfate on subsequent injections.

## EXPERIMENTAL

### *Instrumentation*

The chromatographic system used for all chromatograms shown was a Dionex 4500i gradient ion chromatograph (Dionex, Sunnyvale, CA, U.S.A.) equipped with 0.010 in. I.D., 0.063 in. O.D. polyetherether ketone (PEEK) tubing on the high-pressure side of the analytical pump. The instrument was equipped with a conductivity detector (Dionex CDM-II) and a multi-wavelength UV–VIS detector (Dionex CDM-II). A helium headspace was maintained on the eluents with a Dionex eluent degas module (EDM-II). The ion chromatograph was interfaced to a Dionex AutoIon 450 data system. The AutoIon 450 is an IBM-compatible computer system with software for complete system control, data collection and data reprocess that is written within the Microsoft Windows operating environment.

### *Columns*

The ion-exchange columns used are the Dionex OmniPac™ PAX-100, OmniPac PAX-500 and the Hamilton PRP™-X100 (Hamilton, Reno, NV, U.S.A.). Both Dionex columns are 250 mm × 4 mm I.D. These columns are latex-coated pellicular anion-exchange columns. The substrate is 8-$\mu$m diameter particles made from ethylvinylbenzene (EVB) cross-linked with >50% DVB. The PAX-100 is made with a microporous substrate with no significant adsorption surface area and is strictly a solvent compatible anion-exchange column. The substrate for the PAX-500 is macroporous with approximately 300 $m^2/g$ of adsorption surface area and 60-Å pores. The latex for both columns is approximately 60 nm in diameter, fully quaternized, polyVBC, cross-linked with 4% DVB. Both column types have the same ion-exchange selectivity and average 40 $\mu$equiv./column ion-exchange capacity. Because it is macroporous the PAX-500 packing is a less dense material and measures approximately 25 $\mu$equiv./g whereas the microporous PAX-100 packing has approximately 16 $\mu$equiv./g ion-exchange capacity.

The Hamilton PRP-X100 is a surfaced quaternized (trimethylamine) macroporous PS–DVB 10 $\mu$m packing in 250 mm × 4.6 mm I.D. column and has approximately 200 $\mu$equiv./g ion-exchange capacity[7]. The column used measured 580 $\mu$equiv./column ion-exchange capacity.

Ion-pair chromatography was performed on a 250 × 4 mm I.D. Dionex Ionpac™ NS1 column packed with 10 $\mu$m neutral, macroporous EVB–DVB with an average of 300 $m^2/g$ surface area and an average pore size of 60 Å.

For conductivity detection the system was equipped with a Dionex micromembrane suppressor (AMMS)[8]. A Dionex micromembrane suppressor for Mobile Phase

Ion Chromatography (MPIC™) (AMMS-MPIC) was used if an ion-pair reagent is used in the eluent since the membranes in this suppressor are optimized for transport of the large hydrophobic alkyl quaternary ammonium ion-pair reagents used in ion-pair chromatography. Both the AMMS and the AMMS-MPIC suppressors are compatible with any concentration of acetonitrile, methanol, ethanol, isopropanol and other common reversed-phase HPLC solvents. In all cases the suppressor regenerant solution was 0.02 *N* sulfuric acid, flowing at 10 ml/min using a Dionex AutoRegen system.

*Reagents*

Sodium hydroxide eluents were made from 50% analytical grade (J. T. Baker, Phillipsburg, NJ, U.S.A.). Sulfuric acid was of J. T. Baker Instra-analyzed grade. Sodium chloride and sodium carbonate were Mallinckrodt (Mallinckrodt, Paris, KY, U.S.A.) analytical-reagent grade. Tetrabutylammonium hydroxide (TBAOH) was MPIC grade 0.1 *M* concentrate from Dionex. Acetonitrile, isopropanol and methanol were Fisher brand "Optima" grade solvents for HPLC (Fisher Scientific, Fair Lawn, NJ, U.S.A.07410).

Substrate resin and latex were polymerized from EVB–DVB and VBC–DVB respectively (Dow, Midland, MI, U.S.A.).

## RESULTS AND DISCUSSION

*Synthesis*

In this paper we are presenting a new method for latex attachment in order to generate a pellicular ion-exchange material and a new multi-phase column packing made possible by the new latex attachment method. The new pellicular material consists of three regions: (1) a highly cross-linked (> 50%) EVB–DVB polymer, microporous substrate bead which is compatible with all common reversed-phase HPLC solvents, (2) a highly reactive surface on the substrate bead created at the time of polymerization, and (3) an ion-exchange pellicular phase comprised of a polymeric VBC latex cross-linked with DVB that is electrostatically or covalently attached to the inert substrate bead. During manufacture, neutral latex can either be covalently attached to the substrate bead via the reactive surface and subsequently functionalized, or functionalized prior to electrostatic attachment to the substrate bead. As in traditional electrostatic attachment the inert core and the latex are independently polymerized regardless of the attachment mechanism.

The reactive surface on the substrate bead is generated by a proprietary method. During polymerization another polymer is added to the polymerizing solution, and is ultimately incorporated only onto the exterior surface of the substrate bead polymer. The concentration of polymer added controls the density of reactive sites on the surface of the core bead and a very low density of "reactive sites" can be achieved. For example, the added polymer can be chosen that has sulfonate, carboxylate or amine functional groups. These functional groups then allow electrostatic agglomeration of previously functionalized latex beads of opposite charge to the substrate. However, contrary to traditional electrostatic agglomeration, after agglomeration there is no measurable residual ion-exchange capacity on the surface of the substrate bead. This was verified by measuring breakthrough capacity for cation exchange on a

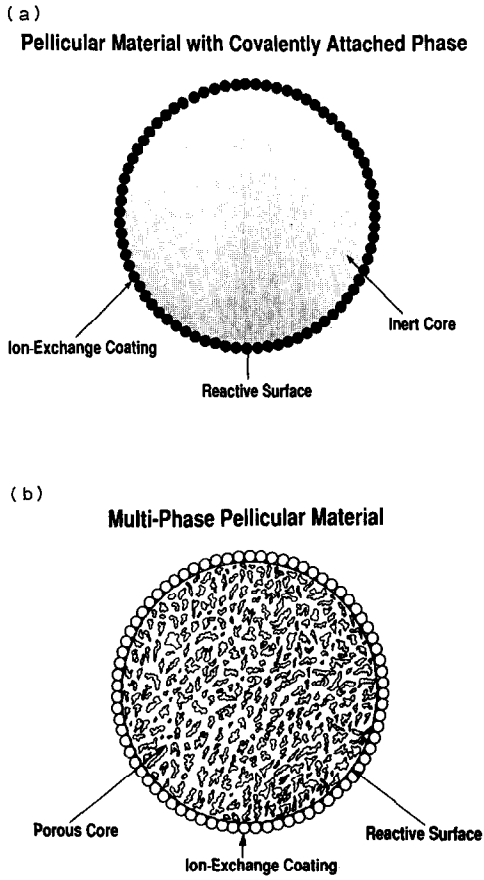


Fig. 2. (a) Schematic of a covalently attached, latex-coated pellicular ion exchanger. (b) Schematic of a multi-phase pellicular material with a macroporous substrate and latex ion-exchange coating.

column agglomerated as described. The column was treated with 0.001 *M* LiCl (pH 8 with LiOH) at 1 ml/min for 30 min. Then the column was connected to a pump primed with 0.001 *M* KCl (pH 8 with KOH) and KCl was pumped at 1 ml/min. The effluent was monitored, using direct conductance, until breakthrough of the KCl was indicated by the increase in the effluent conductance. The milliliters of column void volume (previously determined) is subtracted from the total milliliters of KCl to breakthrough. The net milliliters of KCl times the normality of KCl in $\mu$equiv./ml gives $\mu$equiv./column cation-exchange capacity. Using this method the residual cation-exchange capacity was measured for three columns and all three measured less than 1 $\mu$equiv./column capacity, below the detection limits of the technique. A column of the same dimensions, made by traditional electrostatic agglomeration, measured 11 $\mu$equiv./column residual cation-exchange capacity by the same method. The method as described was used so that there was no interference from anion-exchange selectivity and so that weak and strong acid cation-exchange capacity both were measured.

In a second possibility, the added polymer can be chosen with reactive functional groups allowing covalent attachment of neutral latex beads for agglomeration. The neutral, covalently agglomerated latex beads can then be functionalized after agglomeration allowing non-aqueous functionalization chemistries. Latex can be selectively functionalized in the presence of the core bead polymer by judiciously choosing the latex polymer and functionalization chemistry. For example, if an EVB–DVB substrate is covalently agglomerated with DVB cross-linked polyVBC latex, then the latex can be selectively aminated via traditional amination chemistry. Alternatively the latex can be selectively sulfonated, for example, by first reacting VBC with thiourea in alcohol to generate a thiouronium quaternary amine site, followed by hydrogen peroxide oxidation to a benzyl sulfonate cation-exchange functional group. Neither of these routes generates anion-exchange or cation-exchange groups on the core bead surface because the chemistries are not reactive with EVB–DVB.

This new attachment method has the advantages that no measurable residual ion-exchange sites exist on the substrate bead after agglomeration in either of the new attachment schemes, and latex is attached prior to functionalization in the covalent scheme of latex attachment and therefore functionalization is not limited to aqueous chemistries to maintain a latex suspension as in the traditional electrostatic attachment method. This allows for the possibility of amination with amines with low water solubility and for functionalization with chelating groups where functionalization chemistry is non-aqueous[9]. A schematic of a latex coated, highly cross-linked, microporous resin bead made by the new attachment process is shown in Fig. 2a.

*Effect of solvent on ion-exchange selectivity*

The ability to add significant concentrations of solvents, *e.g.* 1–100% acetonitrile or methanol, to ion-exchange eluents gives the added selectivity control of the influence of solvent on ion exchange. The degree of hydration of the ions in the bulk eluent, and the enthalpy of hydration of a hydrated ion in solution, which determines how easily an ion sheds waters of hydration to become associated with an ion-exchange site and *vice versa*, is a major determining factor in ion-exchange selectivity[10]. Ions that are highly hydrated tend to have shorter retention times than ions of lower hydration. This is generally the argument given for the order of elution of halides by anion exchange[5,6]. Divalent and polyvalent selectivity is generated by the three-dimensional orientation of adjacent ion-exchange sites combined with the degree of hydration of the gel polymer phase[11]. For example, for a particular divalent ion to be retained as a divalent it must find two adjacent fixed ion-exchange sites that are appropriately spaced. Also, these sites must have the right degree of hydration to make it thermodynamically favorable for the divalent ion to shed waters of hydration and become associated with both sites, displacing the co-ions (other analyte ions or eluent ions) which then become fully hydrated in the bulk eluent. Latex cross-linking is one of the three major parameters about the stationary phase that controls ion-exchange selectivity[6], the other two being the nature of the amine used to generate the quaternary anion-exchange site[6] and the nature of the base polymer of the active ion-exchange layer. Higher latex cross-linking results in higher density of ion-exchange sites (because of the higher polymer density) and therefore, statistically a higher number of sites that will thermodynamically "fit" a particular divalent ion over another divalent ion. Just about everything that affects ion-exchange selectivity is related to the enthalpy of hydration of ions and ion-exchange sites.

Adding a solvent to an ionic eluent disrupts the water structure in the bulk solution and changes the degree of hydration of the ions in solution and the ion-exchange sites in the polymer. The degree to which hydration changes is probably dependent on the degree of hydration in the 100% aqueous solution. Ions that are highly hydrated, *e.g.* have high enthalpies of hydration, tend to hold on to waters of hydration more strongly and will lose fewer waters of hydration than ions that are less hydrated and tend to give up waters of hydration more readily. Protic solvents like alcohols can also affect ionic eluents containing hydroxide by the formation of alkoxide ions.

Fig. 3a, b and c indicate an unexpected affect on the selectivity of ion exchange on a latex-coated pellicular ion exchanger with the addition of solvent. Fig. 3a shows the selectivity of a pellicular ion-exchange material with 4% cross-linked latex with no solvent in the eluent. Fig. 3b shows that by adding 20% (v/v) acetonitrile without changing the ionic concentration of the eluent, the selectivity has greatly changed. This chromatogram is approximately the same as would be seen on a 1% cross-linked latex anion exchanger with a 100% aqueous eluent. The chromatogram in Fig. 3c, generated with 40% acetonitrile in the eluent is equivalent to approximately a 0.25% cross-linked latex with a 100% aqueous eluent. In general, the addition of acetonitrile to the eluent tends to decrease the retention of analytes, apparently by changing (lowering) the "effective" cross-linking of the latex.

Table I is a summary of experiments where the concentration of the ionic component of the eluent (25 m*M* sodium hydroxide) is kept constant while the nature and concentration of the solvent is varied. The trend for eluent systems in Table I containing increasing amounts of methanol is for steadily increasing the capacity factor, $k'$. It might be predicted that adding solvent to a hydroxide eluent would tend to cause increasing retention since hydroxide is a highly hydrated ion and would tend to shed fewer waters of hydration on the addition of a solvent, as compared to other ions in solution or the ion-exchange sites in the polymer. This should tend to decrease the selectivity of the stationary phase ion-exchange sites for the hydroxide eluent ion

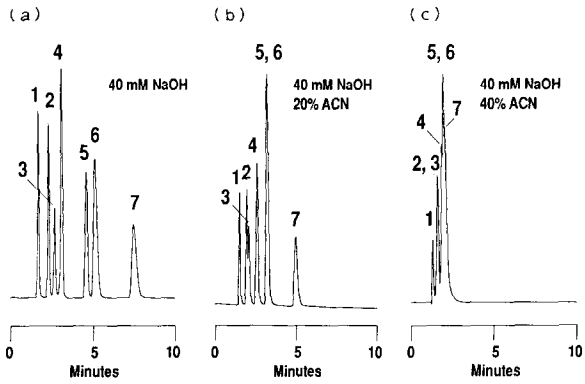


Fig. 3. Effect of acetonitrile on retention with OmniPac PAX-100 multi-phase column. Analytes are: 1 = 1 ppm fluoride; 2 = 2 ppm chloride; 3 = 3 ppm nitrite; 4 = 5 ppm sulfate; 5 = 5 ppm bromide; 6 = 8 ppm nitrate; 7 = 8 ppm phosphate. Flow-rate 1 ml/min; 10-$\mu$l injection loop; suppressed conductivity 10 $\mu$S/cm full scale. Eluents as indicated on figures. ACN = Acetonitrile.

TABLE I

ANALYTE $k'$ *VS.* SOLVENT TYPE AND CONCENTRATION ON OMNIPAC PAX-100

| *Analyte* | *Solvent* | *Solvent concentration in 0.025 M sodium hydroxide* | | | | |
|---|---|---|---|---|---|---|
| | | *0%* | *5%* | *30%* | *50%* | *80%* |
| Fluoride | Acetonitrile | 0.05 | 0.05 | 0.01 | 0.01 | 0.0 |
| | Methanol | 0.05 | 0.03 | 0.07 | 0.09 | 0.15 |
| | Isopropanol | 0.05 | 0.13 | 0.18 | nd | nd |
| Chloride | Acetonitrile | 0.55 | 0.71 | 0.38 | 0.24 | 0.0 |
| | Methanol | 0.55 | 0.37 | 0.62 | 0.79 | 0.15 |
| | Isopropanol | 0.55 | 1.4 | 1.1 | nd | nd |
| Nitrate | Acetonitrile | 1.1 | 1.4 | 1.2 | 0.56 | 1.9 |
| | Methanol | 1.1 | 1.2 | 2.4 | 5.9 | 8.4 |
| | Isopropanol | 1.1 | 7.0 | 3.4 | nd | nd |
| Sulfate | Acetonitrile | 2.6 | 2.4 | 1.7 | 1.0 | 1.9 |
| | Methanol | 2.6 | 2.5 | 2.4 | 2.8 | 2.5 |
| | Isopropanol | 2.6 | 8.8 | 8.0 | nd | nd |
| Phosphate | Acetonitrile | 4.0 | 3.7 | 6.0 | 1.9 | 1.9 |
| | Methanol | 4.0 | 1.9 | 5.2 | 5.9 | 8.4 |
| | Isopropanol | 4.0 | >15 | >15 | nd | nd |

causing a general increasing of retention for other ions. Methanol is also a protic solvent and capable of reacting with hydroxide to form a methoxide ion which is generally a weaker eluting ion than hydroxide. Although significant numbers of methoxide ions are usually only formed at high methanol concentrations, the hydrophobic polymer backbone of the ion-exchange latex will tend to concentrate methanol causing higher methanol concentrations in the stationary phase compared to the bulk eluent. The affect of a solvent on the hydration of ions, combined with methoxide formation, probably accounts for methanol containing eluents causing an increase in the $k'$ of analytes with increasing methanol concentration. Methanol does not appear to change the effective cross-linking of the latex like acetonitrile.

The data in Table I indicate that, of the solvents tested, acetonitrile has the greatest influence on decreasing the $k'$ values of the test analytes. With 30% acetonitrile added to the eluent there is a significant decrease in $k'$ values of all the analytes except phosphate, which has increased. The $k'$ of phosphate ion has probably increased due to the pH change as a result of the addition of acetonitrile to the eluent. The pH of 0.025 *M* NaOH is 12.4, the "apparent" pH of 0.025 *M* NaOH in 30% acetonitrile in water measures approximately 12.8, and the last pH of phosphate is 12.36 (at 25°C). By the addition 50% acetonitrile to the eluent, the influence of pH on phosphate is overcome by the selectivity influence of the solvent on the ion-exchange phase and phosphate has a much shorter $k'$ along with the rest of the test ions. It's interesting that at 80% acetonitrile the $k'$ actually begins to increase again for nitrate and sulfate.

We hypothesize that the pellicular ion-exchange phase formed by a latex with low cross-linking is probably swollen to a greater extent in an acetonitrile-water

mixture than in water alone. The result of this swelling is lower effective cross-linking since there is just less polymer, and therefore fewer ion-exchange sites, per unit volume of latex. Acetonitrile probably does cause a change in hydration of ions like other solvents but this effect is over-ridden by the swelling effect until high acetonitrile concentrations where the polymer is swollen to its maximum. This may explain the increase in retention for nitrate and sulfate at 80% acetonitrile in Table I.

Isopropanol seems to have the similar effect of reducing retention times for some ions but not for all ions tested. It certainly seems reasonable that the more hydrophobic alcohol isopropanol is capable of swelling the ion-exchange gel polymer to some extent while the more hydrophilic alcohol methanol can not. 5% Isopropanol causes an increase in $k'$ for all ions tested probably due to the effect of the low dielectric constant of isopropanol on ion exchange[10]. It is also possible that even at only 5% isopropanol there is sufficient isopropanol concentrated in the stationary phase polymer to cause a significant concentration of alkoxide ion formation. With the addition of 30% isopropanol, chloride, nitrate and sulfate all have a lower $k'$ value than with 5% isopropanol, nitrate showing the greatest change. Fluoride and phosphate, however have increased in $k'$ at 30% isopropanol. The fact that some ions have increased $k'$ and some decreased $k'$ suggests that the effect of isopropanol is not simply swelling but also the competing effect of a solvent on ion hydration and therefore ion-exchange selectivity.

Before the advent of these solvent amenable pellicular ion-exchange packings the only way to change the effective cross-link of a material was to change the synthesis parameters. The ability to control active phase cross-link with the addition of acetonitrile, and ion-exchange selectivity with the addition of other solvents means that separations that previously required several different ion-exchange columns can be performed on one ion-exchange column. Although other types of non-latex-coated ion-exchange columns may be solvent compatible, the only solvent selectivity effect that can be achieved is the influence on the degree of hydration of the ion-exchange site and the analyte, which tends to increase retention.

Table II is a tabulation of $k'$ values from the Hamilton PRP-X100 anion-exchange column with 0, 5 and 50% acetonitrile, and 0, 5, 50 and 80% methanol in a sodium carbonate eluent using chemically suppressed conductance detection. Note that the retention of ions on the PRP-X100 is generally increasing with increasing acetonitrile concentration. This is different from the effect of acetonitrile on the pellicular latex-coated ion-exchange column where $k'$ first decreases as the solvent concentration is increased, due to solvent swelling effects on cross-linking, and then $k'$ tends to increase due to solvent effects on the ion-exchange process directly. Because methanol is a poorer latex swelling solvent, the trend for methanol on the PAX-100 column is the same as the PRP-X100 where the retention of the ions tends to increase due to direct effects of solvent on ion exchange as described earlier.

Carbonate was used as the eluent for the PRP-X100 because this column does not have the hydroxide selectivity the PAX-100 does. The PRP-X100 is designed for use with ionic aromatic eluents commonly used in single column ion chromatography such as potassium phthalate and is therefore too hydrophobic for hydroxide to be an efficient eluent. For this reason $k'$ changes for analytes on each column cannot be directly compared but trends in $k'$ values due to solvents used on each column can. 80% acetonitrile selectivity could not be determined on the PRP-X100 because 0.010

TABLE II

ANALYTE *k' VS.* SOLVENT TYPE AND CONCENTRATION ON HAMILTON PRP-X100

ND = Not determined due to insolubility of 0.010 *M* $Na_2CO_3$ in acetonitrile.

| *Analyte* | *Solvent* | *Solvent concentration in 0.010 M $Na_2CO_3$* | | | |
|---|---|---|---|---|---|
| | | *0%* | *5%* | *50%* | *80%* |
| Fluoride | Acetonitrile | 0.65 | 0.63 | 0.68 | ND |
| | Methanol | 0.65 | 0.66 | 0.90 | 1.5 |
| Chloride | Acetonitrile | 2.4 | 2.6 | 3.2 | ND |
| | Methanol | 2.4 | 2.4 | 2.7 | 4.3 |
| Nitrate | Acetonitrile | 15 | 16 | 19 | ND |
| | Methanol | 15 | 9.6 | 13 | 8.0 |
| Sulfate | Acetonitrile | 6.0 | 7.2 | 9.3 | ND |
| | Methanol | 6.0 | 6.2 | 6.5 | 8.0 |
| Phosphate | Acetonitrile | 4.6 | 5.9 | 7.8 | ND |
| | Methanol | 4.6 | 4.6 | 7.3 | 17 |

*M* carbonate is not soluble in 80% acetonitrile. To allow the comparison of at least two analytes on both columns under similar eluent conditions fluoride and chloride were chromatographed on the PRP-X100 using 0.040 *M* sodium hydroxide with 30% (v/v) methanol and 0.040 *M* sodium hydroxide with 30% (v/v) acetonitrile. Retention times for fluoride were 5.5 and 6.0 min and for chloride 24.5 and 32.0 min, respectively. Acetonitrile actually seemed to cause slightly longer retention times than methanol on the PRP-X100. Although the PRP-X100 is not a true pellicular ion exchanger as defined earlier, the surface of the resin is fully functionalized and should have a gel-like nature similar to a latex coating. Because the bead is made up of a polymer with very high cross-linking, however, the fully functionalized ion-exchange gel-like surface is resistant to swelling in acetonitrile, unlike the latex coating.

Fig. 4 demonstrates another example of the advantage of using solvent in conjunction with pellicular latex-coated ion-exchange packing. By adding 20% acetonitrile to the eluent, enough to prevent any significant interfering adsorption retention, a mixture of mono- and polyvalent aromatic carboxylates and sulfonates are separated with a simple salt gradient. Sodium hydroxide is added (0.0002 *M* initially) to ensure all the acids are ionized. Sodium hydroxide is increased linearly, along with sodium chloride, to 0.0016 *M* to maintain a constant chloride–hydroxide ratio in the eluent which facilitates a more rapid equilibration of the ion-exchange phase back to initial conditions for the next gradient. When the ratios of different ions of the same valency in the gradient eluent are kept constant throughout the gradient, typical reequilibration times are 5 to 10 min. This is a difficult separation for reversed-phase HPLC and is usually done in the ion-suppression or ion-pair mode, as shown on a NovaPak $C_{18}$ column in Fig. 5. The peaks have poorer symmetry in the ion-suppression mode and peak resolution in either chromatogram is not as good as the ion-exchange separation for the same analysis time. Also notice there is a logical order to the elution by ion exchange; monovalents elute first followed by divalents followed by trivalents.

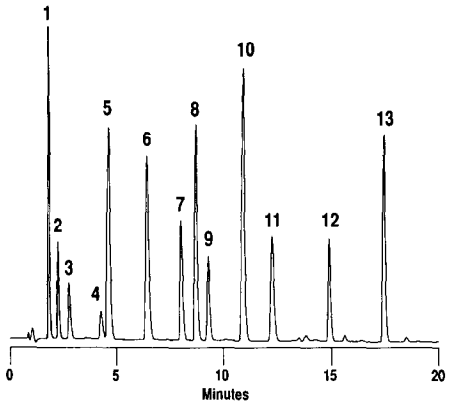


Fig. 4. Separation of aromatic acids on OmniPac PAX-100. Eluent: 20% (v/v) acetonitrile isocratic, 0.050 to 0.40 *M* NaCl gradient in 20 min, 0.0002 to 0.0016 *M* NaOH gradient in 20 min simultaneous with NaCl gradient. Detection: absorbance 254 nm 0.1 AUFS. Peaks: 1 = benzoate; 2 = benzenesulfonate; 3 = toluenesulfonate; 4 = *p*-chlorobenzenesulfonate; 5 = *p*-bromobenzoate; 6 = 3,4-dinitrobenzoate; 7 = phthalate; 8 = terephthalate; 9 = *p*-hydroxybenzoate; 10 = *p*-hydroxy-benzenesulfonate; 11 = gentisate; 12 = trimesate; 13 = pyromellitate.

*Multi-phase packing*

In addition to forming conventional pellicular ion-exchange materials, the new covalent attachment method allows the production of an entirely new class of multi-phase materials combining ion-exchange, ion-pair and reversed-phase retention mechanisms. The multi-phase material is comprised of the same three regions as described earlier. In the case of the multi-phase materials, the core bead is macro-porous with an average surface area of about 300 $m^2/g$ and an average pore size of approximately 60 Å. Latex is attached only to the exterior surface of the macro-

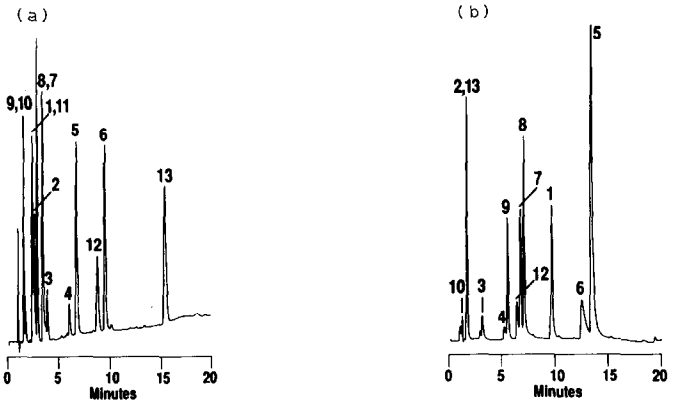


Fig. 5. HPLC separation of aromatic organic acids, Column: NovaPak $C_{18}$, 5 μm. Flow-rate: 1.0 ml/min. UV absorbance detection 0.1 AUFS. (a) Ion-pair separation. Peaks as in Fig. 4. Eluent: 0.010 *M* tetrabutyl ammonium phosphate, pH 6.5; 25–50% (v/v) acetonitrile gradient in 20 min. (b) Ion-suppression separation. Peaks as in Fig. 4. Eluent: 0.020 *M* phosphoric acid, 0.020 *M* ammonium sulfate buffer; 10–80% (v/v) acetonitrile gradient in 20 min.

porous bead, using less than 1% of the total surface area. The latex beads are too large to enter the pores, defining two distinctly different areas for retention, ion exchange in the latex coating and adsorption on the surface area of the interior of the pores. Fig. 2b is a schematic representation of a multi-phase bead. The new latex attachment route (covalent or electrostatic) allows the production of the multi-phase packing since the method generates no significant residual ion-exchange sites on the high surface area that would otherwise interfere with reversed-phase or ion-pair retention processes.

Pinkerton and Hagestam[27] describe a material that has two distinct and separate phases in one packing material. The external of the macroporous bead defines one phase that is neutral and very hydrophilic, designed to prevent retention of proteins. The internal phase in the interstices of the macroporous bead is functionalized with peptides containing hydrophobic moieties. This packing material is designed to allow sample preparation or direct injection of physiological, high protein containing samples for drug analysis with minimal protein interference or fouling since proteins or other macromolecules are too large to enter the pores and the external surface is too hydrophilic for their retention. Although this material has two distinct phases, only one of the phases is designed for analyte retention, the other is intended to prevent retention.

This multi-phase material is also distinctly different from the mixed-mode supports described by Hartwick and co-workers[12–14]. Specifically, the mixed-mode supports, besides being silica based, are a heterogeneous mixture of ion-exchange and hydrophobic ligands in a single active layer. Because they are mixed together it is not possible to take full advantage of the properties of ion-exchange selectivity which rely on a three dimensional relationship of ion-exchange sites. To understand the properties of the new multi-phase material it is important to understand the difference between ion-exchange and ion-pair selectivity.

*Mixed ion-exchange/ion-pair selectivity*

The literature suggests that for the most part, ion-exchange selectivity is an enthalpy driven process[6,11,15]. A net free energy decrease for an ion from the bulk solution associating with an ion-exchange site in the active phase and displacing a previously associated co-ion, is due to a favorable enthalpy change in the hydration energy difference of the eluent ion, the analyte ion and the ion-exchange site. Ion-exchange selectivity is due to differences in the net change in free energy of the ion-exchange process for different analyte ions resulting from differences in the free energy of analytes and the free energy of fixed ion-exchange sites. The free energies of analytes are relatively constant in the bulk solution but are usually different for each analyte due to differences in hydration energies. Ion-exchange site free energies vary as a result of the degree of hydration of the ion-exchange site, which is determined by its environment in the polymer. Polymers do not have uniform density throughout the latex bead. Areas of higher density (higher cross-linking) are less hydrated and have less hydrated ion-exchange sites. Areas of lower density (lower cross-linking) are more hydrated and have somewhat more hydrated ion-exchange sites. This heterogeneity in the degree of hydration of the fixed ion-exchange sites creates good selectivity for either ions of high hydration energy, or ions of low hydration energy but not both on the same ion-exchange phase. It is not possible to affect the heterogeneity of a

single ion-exchange phase dramatically enough to provide optimum selectivity for very high and very low hydration energy ionic analytes.

Ion exchange has good selectivity for polyvalent ions particularly polyvalent ions of the same charge because ion-exchange site density is high in the gel structure of a fully functionalized ion exchanger such as the latex coating. Polyvalent ions can more often be retained with some or all charges satisfied by the charges of the fixed ion-exchange sites as discussed earlier.

Ion exchange is rather narrow in the scope of ions that can be separated on one ion-exchange phase of a given selectivity. This limitation results in the requirement for several ion-exchange columns with different selectivities to separate a broad spectrum of analytes.

Ion-pair chromatography, originally described by Haney and Wittmer[16], is for the most part an entropy driven system and, whether one subscribes to the ion-pair formation theory[17,18], the dynamic ion-exchange theory[19–21] or the ion-interaction theory[22–24] of retention, is a surface phenomenon. As with adsorption retention the entropy of the system has to be thermodynamically favorable for "ion-pair" retention to take place[19,25]. That is the hydrated analyte ion is driven from the bulk solution onto the stationary phase to form an ion pair because the analyte ion pair imparts less order to the water already ordered in the vicinity of the stationary phase as compared to the much less ordered water in the bulk solution. Contrast this to ion exchange which is a three dimensional phenomenon where ions penetrate into the gel network of the active ion-exchange phase, as described earlier. The resulting ion pair is not very useful for divalent ions because retention is essentially only in two dimensions and divalent ions are not likely to be retained in a unique environment where they "fit" better thermodynamically than other divalent ions because the fit for any analyte ion only has two degrees of freedom.

Ion-pair chromatography has quite different strengths and weakness compared to ion exchange. Selectivity strengths include (1) good resolution of monovalent ions particularly when monovalent ions have low and very similar hydration energies (*e.g.* nitrate and chlorate) because "absorption" based ion-pair retention is mostly entropy driven and is not dependent on enthalpies of hydration as is ion exchange. This is why fluoride, a highly hydrated ion, is unretained by ion exchange but is retained by ion pair; (2) good resolution and selectivity for surface active, or large hydrophobic ions because the technique allows the hydrophobic tail to enhance the retention mechanism; (3) good selectivity for large polarizable ions like iodide since they readily form ion pairs in the solvent rich environment near the hydrophobic surface of the substrate; (4) a broad scope of ions can be eluted and resolved due to the generally lower overall selectivity of the method as compared to ion exchange. The weaknesses of ion-pair chromatography are mainly polyvalent ions. In particular, polyvalent ions of the same valency (*e.g.* sulfate and many divalent organic acids) are difficult or impossible to resolve by ion pair.

Obviously the advantages of the two techniques are complimentary. The new multi-phase material takes advantage of this by providing the selectivity of both retention mechanisms on the same column at the same time. Ion exchange takes place in the pellicular layer, ion pair on the high surface area of the rest of the macroporous core bead, when an ion-pair reagent is added to the eluent. Since the latex is agglomerated only to the exterior surface of the macroporous bead, it takes up less than

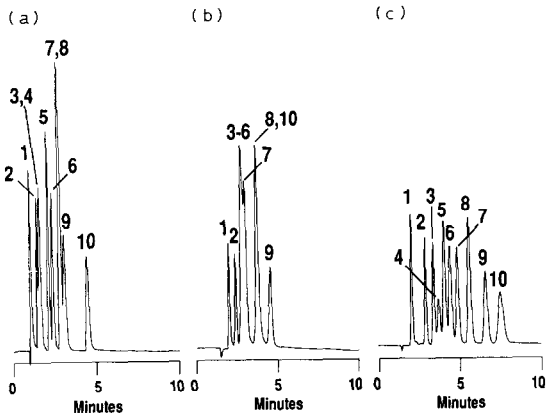


Fig. 6. Comparison of ion-exchange (a), ion-pair (b) and multi-phase (ion-exchange and reversed-phase, (c) selectivity. Peaks: 1 = fluoride 1 ppm; 2 = chloride 2 ppm; 3 = nitrite 3 ppm; 4 = succinate 10 ppm; 5 = sulfate 10 ppm; 6 = oxalate 10 ppm; 7 = bromide 5 ppm; 8 = nitrate 8 ppm; 9 = chlorate 10 ppm; 10 = phosphate 8 ppm. Flow-rate: 1 ml/min; suppressed conductivity 10 $\mu$S/cm full scale; 10-$\mu$l injection loop. Eluent: 17% acetonitrile, 42 m*M* NaOH, 1 m*M* TBAOH. Column: (a) OmniPac PAX-100; (b) IonPac NS1; (c) OmniPac PAX-500.

0.1% of the total available surface area in the bead. Fig. 6 illustrates that multi-mode ion pair/ion exchange is a combination of the selectivities of the two retention mechanisms. Fig. 6a shows only ion-exchange selectivity on a pellicular latex agglomerated phase that is agglomerated on a highly cross-linked but microporous core bead so there is no neutral surface area available for adsorption retention. This shows the strengths of ion exchange, with the divalent components sulfate, oxalate and succinate are separated from each other, but bromide, nitrate and chlorate, however, are poorly resolved and fluoride is in the void. Note that there is no evidence of ion-pair selectivity with 1 m*M* TBAOH in the eluent. Fig. 6b is strictly an ion-pair separation of the same set of analytes with the same eluent on a neutral macroporous stationary phase. The ion-pair separation displays good resolution of bromide, nitrate and chlorate and fluoride is retained, but the divalent ions are co-eluting. Actually, with ion-pair there are about two dozen divalent inorganic anions and divalent organic acids that all have the same selectivity and all co-elute under any eluent conditions. Fig. 6c shows the same analytes with the same eluent on the multi-phase material. The multi-phase material is actually the sum of the two selectivities. Notice that to a first approximation the $k'$ values for the multi-phase separation are the sum of the $k'$ values of ion exchange and ion pair. The divalent ions are resolved, and bromide, nitrate and chlorate are resolved. Fluoride is retained from the void.

*Ion exchange/adsorption*

If no ion-pair reagent is added to the eluent the high, neutral hydrophobic surface area of the substrate in the multi-phase column is available for adsorption retention. The separation shown in Fig. 7 demonstrates the differences between ion-exchange selectivity with the added advantage of solvent selectivity control and multi-phase selectivity this time combining ion-exchange and adsorption retention.

Fig. 7a shows a chromatogram generated on the Omnipac PAX-100 with sul-

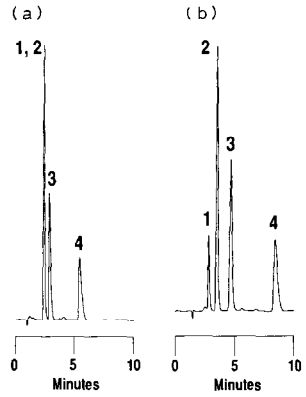


Fig. 7. Control of selectivity with the OmniPac PAX-100 (a) and PAX-500 (b) columns. Eluent: 40 m*M* NaOH, 20% acetonitrile. Suppressed conductivity, 10 μS/cm full scale; flow-rate = 1 ml/min; 10 μl injection loop. Peaks: 1 = $SO_4^{2-}$; 2 = benzenesulfonate; 3 = *p*-toluenesulfonate; 4 = *p*-chlorobenzenesulfonate.

fate and benzene sulfonate co-eluting. Using the PAX-500 (Fig. 7b) with the same eluent conditions sulfate and benzene sulfonate are resolved. Benzene sulfonate is retained longer on this column due to the combination of ion-exchange and adsorption retention. The concept of combining ion-exchange and adsorption retention selectivities on multi-phase materials can be extended to combinations of neutral and ionic analytes as shown in Table III.

Table III shows retention times of 2-aminopyrimidine, a neutral compound in the pH of the eluent throughout the gradient, and 2-thiouracil and 5-carboxy-2-thiouracil which are anionic over the full pH range of the gradient. Note that the anionic analytes, 2-thiouracil and 5-carboxy-2-thiouracil decrease in retention as the ionic portion of the gradient is made steeper, whereas 2-aminopyrimidine retention stays constant. The retention of 2-aminopyrimidine changes only as a result of a change in the solvent gradient from 1–23% acetonitrile to 1–27% acetonitrile in 10 min. This is because 2-aminopyrimidine is the only one of the three to be retained only by adsorption retention.

TABLE III

EFFECT OF IONIC AND SOLVENT GRADIENT CHANGE ON THE RETENTION TIME OF NEUTRAL AND IONIC ANALYTES ON A MULTI-PHASE COLUMN

Retention time R.S.D. < 1%.

| *Analyte* | *Gradient conditions* | | |
|---|---|---|---|
| | *1 to 30 mM Sodium carbonate, 1 to 27% acetonitrile* | *1 to 50 mM Sodium carbonate, 1 to 23% acetonitrile* | *1 to 75 mM Sodium carbonate, 1 to 23% acetonitrile* |
| 2-Thiouracil | 8.15 min | 7.85 min | 7.57 min |
| 2-Aminopyrimidine | 8.65 min | 8.90 min | 8.90 min |
| 5-Carboxy-2-thiouracil | 17.4 min | 15.4 min | 13.9 min |

2-Thiouracil, a monovalent anion, was affected to a lesser extent than 5-carboxy-2-thiouracil, a divalent anion, as a result of the change in ionic strength because the change in retention of a monovalent analyte due to a change in the concentration of a divalent eluent ion ($CO_2^-$) is one-half that of a divalent analyte[26]. Table III indicates that during methods development on a multi-phase column adsorption retention selectivity for neutral compounds and ion-exchange selectivity can be manipulated independently providing relatively easy control over the selectivity power of the column. Carrying this to a logical extreme, Fig. 8 shows it is possible to elute analytes by class. Neutral analytes are eluted first with an eluent containing solvent and water only, with no buffer added. After all neutral compounds are eluted the eluent is changed to lower solvent and a gradient of ionic strength to elute the anionic analytes. This capability has implications for "on-column" sample clean-up as well as two dimensional selectivity control on one column. We have already begun demonstrating in our lab the feasibility of selectively eluting otherwise interfering neutral compounds prior to elution of ionic compounds of interest, or *vice versa*, within one injection.

## CONCLUSIONS

A new type of multi-phase material that combines strong base anion-exchange retention and adsorption retention has been developed. The construction of this multi-phase column allows simultaneous ion-exchange and ion-pair or adsorption retention combining the positive attributes of both retention mechanisms. The ability to use solvents in conjunction with ion-exchange was discussed and the significant improvement of gradient focusing power (efficiency) of an ion-exchange salt gradient for some organic ions, over reversed-phase ion pair or ion suppression was demonstrated. Pellicular ion-exchangers built on a highly cross-linked, neutral, hydrophobic

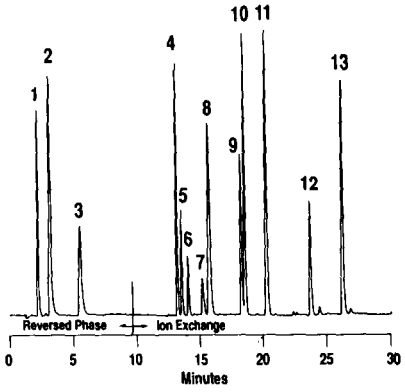


Fig. 8. Two-dimensional chromatography with a single column. OmniPac PAX-500 column, 1 ml/min. Absorbance detection 254 nm, 0.1 AUFS. 10-$\mu$l injection loop. Gradient: 80% (v/v) acetonitrile isocratic for 10 min; at 10.1 min: 20% (v/v) acetonitrile, 0.050 *M* NaCl, 0.0002 *M* NaOH, which is ramped to 20% (v/v) acetonitrile, 0.4 *M* NaCl, 0.0016 *M* NaOH in 10 min and held isocratic for 10 min. Peaks: 1 = benzylalcohol; 2 = diethyltoluamide; 3 = benzene; 4 = benzoic acid; 5 = benzenesulfonic acid; 6 = toluenesulfonic acid; 7 = *p*-chlorobenzenesulfonic acid; 8 = *p*-bromobenzoic acid; 9 = phthalic acid; 10 = terephthalic acid; 11 = *p*-hydroxybenzenesulfonic acid; 12 = 1,3,5-benzenetricarboxylic acid; 13 = 1,2,4,5-benzenetetracarboxylic acid.

substrate allow a new dimension in selectivity control for ion exchange, by allowing control of the ion-exchange gel phase cross-link with solvent concentration as well as changing ion-exchange selectivity by the nature of the solvent. Because ion exchange and adsorption are independent retention mechanisms they can be independently controlled on the multi-phase column providing two-dimensional selectivity control that has on-column sample preparation implication as well as analyte selectivity control. Ion-pair and ion-suppression techniques practiced on reversed-phase systems are a means for dealing with ionic organic analytes with a technique that doesn't handle ionic analytes well. The new multi-phase columns could well eventually replace reversed-phase ion-pair and ion-suppression techniques for many applications, and realize the potential of ion exchange alluded to by Horváth.

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