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Chromatography on Poly-RP and its cyano and diol derivatives using both polar and non-polar solvent systems

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ABSTRACT

A 4-μm polystyrene–divinylbenzene resin, Poly-RP CO, was synthesized for high-performance liquid chromatography. The surface of this resin was functionalized with cyano and diol groups that are capable of enhancing the selectivity and capacity of the base resin. Chromatography was performed with traditional polar solvent systems, such as acetonitrile–water, and also with non-polar solvent systems, such as dichloromethane–hexane; the latter solvent system can give separations that are otherwise difficult to achieve with acetonitrile–water. Good separations of simple isomer mixtures were achieved on these resins.

INTRODUCTION

Over the last decade, the use of polymeric resins in reversed-phase liquid chromatography has grown dramatically. In 1980, virtually all commercial reversed-phase columns were alkyl-bonded silicas [1], whereas now the list of commercial polymeric columns for reversed-phase high-performance liquid chromatography (HPLC) includes PRP-1 [2], PLRP-S [3] and ACT-1 [4], all of which are polystyrene–divinylbenzene-based resins, as well as Asahipak ODP-50 [5], a vinyl alcohol polymer gel with bonded octadecyl groups, and Hypercarb [6], a porous graphitic carbon stationary phase. One of the driving forces behind the development of these polymeric columns is the inherent pH instability of silica-based columns, especially above pH 7, which places severe limitations on their practical use [7]. Numerous attempts have been made to improve the pH stability of silica-based columns, including the use of bulky silane reagents [8] and the coating of polymers on the surface of silica [9], but these materials still do not have the pH stability associated with the polystyrene–divinylbenzene matrix (stability from pH 0 to 14). Although there have been many recent reports on the HPLC properties of more pH-stable inorganic supports, such as alumina [10], zirconia [11] and titania [12], these supports are not yet available in the same wide range of particle sizes and pore sizes as silica gel and polymer resins. Currently, commercial silica-based columns are still far superior to polymer-based columns in terms of column efficiency and mechanical rigidity.

One advantage of polystyrene-based resins in the ease with which the phenyl rings can be functionalized with a variety of different organic moieties, so that a wide range of sorbents with different properties and applications can be derived from a single base material. Polystyrene beads have been modified with a number of useful functional groups, including amino [13], nitro [14] and hydroxyl [15] groups. Although interesting HPLC separations on these resins have been reported, all of the chromatography was performed with methanol–water and/or acetonitrile–water mobile phases, which are the standard polar solvent systems used for reversed-phase chromatography.

This paper demonstrates that good separations can be achieved on polystyrene-based resins by using non-polar solvent systems, such as dichloromethane–hexane and tetrahydrofuran–hexane. A 4-μm macroporous polystyrene resin, Poly-RP CO, was used for the chromatography, together with two hydrophilic derivatives, Poly-RP CYANO and Poly-RP DIOL. It is demonstrated that the use of non-polar solvent systems allows separations that are difficult to achieve with acetonitrile–water, and that the addition of the cyano and diol functional groups to the base resin vastly improves the resolution of simple mixtures of isomers in non-polar solvent systems.

EXPERIMENTAL

Equipment

All polymer resins were packed into 15 × 0.46 cm I.D. stainless-steel columns for 20 min at 4000 p.s.i. using a Shandon HPLC packing apparatus (Keystone Scientific, State College, PA, USA). Valco end-fittings with 2-μm pressed-in frits were used to end-cap the columns (Valco Instruments, Houston, TX, USA). The chromatographic system consisted of two Hitachi (Danbury, CT, USA) Model 655A-12 pumps, a Hitachi L-5000 LC gradient controller, a Hitachi D-2000 ChromatoIntegrator, a Kratos (Ramsey, NJ, USA) Spectroflow 773 absorbance detector and a Rheodyne (Cotati, CA, USA) Model 7125 sample injector equipped with a 20-μl sample loop. Infrared spectra were recorded on a Perkin-Elmer (Norwalk, CT, USA) 1600 Series Fourier transform (FT) IR spectrometer equipped with a diffuse-reflectance sample accessory.

Materials and methods

All chromatographic solvents were purchased from EM Science (Cherry Hill, NJ, USA) and were of OmniSolve grade. Test solutes for HPLC were obtained from Aldrich (Milwaukee, WI, USA). Solute solutions were made up by dissolving 0.1–0.5 g of the compound in either hexane, acetonitrile, dichloromethane or the mobile phase and then diluting these stock solutions by a factor of 1:100 with the mobile phase or with pure hexane. Triton N-101 was purchased from Polysciences (Warrington, PA, USA). Capacity factors (k') and efficiencies (plates per meter; N/m) were determined according to the method described by Snyder and Kirkland [16]. All of the chromatographic runs were performed at ambient temperature.

The base polymer, Poly-RP CO, is a 4-μm, 300-Å, macroporous polystyrene–divinylbenzene resin available from Interaction Chemicals (Mountain View, CA, USA). Poly-RP CYANO and Poly-RP DIOL resins were obtained by chemical derivatization of the Poly-RP CO base resin. The success of the synthetic procedures and the presence of the functional groups was confirmed by FT-IR spectrometry; the Poly-RP CYANO resin showed a strong absorption at 2250 cm^{-1}, which is uniquely characteristic of the cyano moiety [17], while the Poly-RP DIOL resin displayed intense broad bands centered at 3400 and 1100 cm^{-1}, which confirms the presence of hydroxyl groups [17]. Elemental analysis (Galbraith Labs., Knoxville, TN, USA) was performed on the Poly-RP CYANO and Poly-RP DIOL resins, and gave levels of functionalization of 0.9 mmol/g for the cyano group and 1.1 mmol/g for the diol group.

RESULTS AND DISCUSSION

Comparison of the polarity of Poly-RP CO and its derivatives

Fig. 1 shows a standard test chromatogram of a Poly-RP CO column packed in acetonitrile–water (70:30) and then tested with the same mobile phase containing 0.01 M dibasic potassium phosphate buffer. The description of the solute mixture and the corresponding efficiencies, asymmetries and capacity factors are given in Table I, with the retention time of potassium nitrite being used as the t_0 value. The peak efficiencies are good relative to other polymer-based columns, with several peaks having efficiencies above 50 000 N/m, but are still much lower than those of commercial silica-based columns. This difference in efficiency between polymer- and silica-based columns has been reviewed by other workers [18,19] and is believed to be caused mainly by the microporous gel structure of polymer resins.

The unfunctionalized Poly-RP CO is by nature extremely hydrophobic. In contrast, the Poly-RP CYANO and the Poly-RP DIOL were easily dis-

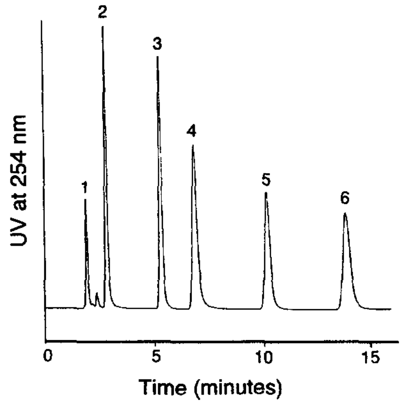

Fig. 1. Separation of a standard test mixture on a Poly-RP CO column. Mobile phase, acetonitrile–water (70:30) with 0.01 *M* dibasic potassium phosphate buffer; flow-rate, 0.75 ml/min. Peaks: 1 = potassium nitrite; 2 = physostigmine; 3 = acetophenone; 4 = diethyl phthalate; 5 = butyrophenone; 6 = valerophenone.

persed in water (*i.e.*, hydrophilic) and, in fact, we were able to pack both of these polymers at 4000 p.s.i. into 15 × 0.46 cm I.D. columns using water as both the slurry solvent and packing solvent. In order to compare all three resins closely, some Poly-RP CO was packed into a 15 × 0.46 cm I.D. column at 4000 p.s.i. using a 1% solution of Triton N-101 in water as the packing and slurry solvent. Triton N-101 is a non-ionic surfactant that enabled the hydrophobic Poly-RP CO to be dispersed and packed in aqueous media.

TABLE I

CHARACTERISTICS OF A POLY-RP CO COLUMN USING A STANDARD TEST MIXTURE

The peak asymmetry factor, A_s, was calculated at 10% of the peak height, as described in ref. 16, p. 222. Conditions: mobile phase, acetonitrile–water (70:30) with 0.01 *M* dibasic potassium phosphate buffer; flow-rate, 0.75 ml/min; back-pressure, 1400 p.s.i.

Component	Retention time (min)	k'	A_s	Efficiency (N/m)
Potassium nitrite[a]	1.93	0.00	2.7	16 000
Physostigmine	2.84	0.47	1.4	27 000
Acetophenone	5.34	1.77	1.3	56 000
Diethyl phthalate	6.89	2.57	1.6	33 000
Butyrophenone	10.21	4.29	1.4	57 000
Valerophenone	13.93	6.21	1.4	55 000

[a] The potassium nitrite peak is obscured by some oxidation products of physostigmine.

After packing, all three columns were equilibrated in water and then the mobile phase was changed to acetonitrile–water (70:30) by subjecting each column to a 30-min gradient at 0.75 ml/min. Each column was then tested and the N/m values for valerophenone were found to be 42 000, 25 000 and 32 000 for Poly-RP CO, Poly-RP CYANO and Poly-RP DIOL, respectively, as indicated in Table II. A second gradient from 100% acetonitrile to 100% water and then to acetonitrile–water (70:30) was performed on each column. While the hydrophilic Poly-RP CYANO and Poly-RP DIOL columns showed no decrease in efficiency after the gradient, the N/m value for valerophenone on the Poly-RP CO decreased from 42 000 to 27 000, probably because the hydrophobic Poly-RP CO partly collapsed when subjected to 100% water. In general, we have found that Poly-RP CO performs best in the range from acetonitrile–water (30:70) to 100% acetonitrile, whereas the Poly-RP CYANO and Poly-RP DIOL columns can operate anywhere from 100% water to 100% acetonitrile.

Conversion of the columns to non-polar solvent systems from polar solvent systems

To change from acetonitrile to hexane, a much more non-polar solvent, it was necessary to use an intermediate solvent because hexane and acetonitrile are not totally miscible. Acetone was chosen because it wets polystryrene resins very well, it is

TABLE II

WATER COMPATIBILITY OF POLY-RP CO AND ITS DERIVATIVES

Gradient 1 = 100% water to acetonitrile–water (70:30) at 0.75 ml/min over a 30-min period. Gradient 2 = 100% acetonitrile to 100% water to acetonitrile–water (70:30) at 0.75 ml/min over a 1-h period. Conditions: flow-rate, 0.75 ml/min; back-pressure, *ca.* 2000 p.s.i. for all three columns. k' = Capacity factor of valerophenone. Efficiency = N/m value of valerophenone.

Column	k'	Efficiency after gradient 1 (N/m)	Efficiency after gradient 2 (N/m)
Poly-RP CO	6.2	42 000	27 000
Poly-RP CYANO	4.7	25 000	25 000
Poly-RP DIOL	4.5	32 000	32 000

TABLE III

INTERCHANGE OF SOLVENTS ON A POLY-RP CYANO COLUMN

Conditions: 1-h linear gradients from 100% of the original solvent to 100% of the final solvent at 0.5 ml/min with UV detection at 350 nm; the time required for a baseline is the total time measured from the start of the gradient.

Original solvent	Final solvent	Time required for baseline (min)
Acetonitrile	Acetone	70
Acetone	Hexane	102
Hexane	Acetone	70
Acetone	Acetonitrile	88

miscible with both acetonitrile and hexane and it has a low viscosity, thereby leading to low column back-pressures. All columns were subjected to 1-h linear gradients from 100% acetonitrile to 100% acetone at 0.5 ml/min, followed by 1-h linear gradients from 100% acetone to 100% hexane; this is similar to a procedure used by other workers for changing mobile phases on a cyanopropylsilica column [20]. A reverse gradient, from hexane to acetone to acetonitrile, was run on the Poly-RP CYANO column and the efficiency was re-evaluated in acetonitrile–water (70:30) using valerophenone as the test solute. The efficiency was found to be 25 000 N/m, which is the same efficiency that this resin displayed before the gradient runs. These data show that the derivatized resins can be used in mobile phases of virtually any polarity. Table III summarizes the times required to achieve the baseline with these gradients on the Poly-RP CYANO column.

pH stability of Poly-RP CO, Poly-RP CYANO and Poly-RP DIOL

In order to demonstrate the pH stability of Poly-RP CO and its derivatives, the columns were tested under both strongly basic conditions [acetonitrile–water (70:30) containing 1% of triethylamine] and strongly acidic conditions [acetonitrile–water (70:30) containing 1% of phosphoric acid]; the results are reported in Table IV. Each column was equilibrated in the appropriate mobile phase and then the capacity factor (k'), efficiency (N/m) and peak asymmetry factor (A_s) were measured using valerophenone as a test solute. After pumping 1000 ml of mobile phase through each column, these parameters were remeasured and are reported in parentheses in Table IV next to the original values. The results show that all three resins suffered little or no deterioration in capacity factor, efficiency or peak asymmetry under conditions of strong base or strong acid.

Choice of non-polar mobile phase solvents

Most of the early HPLC work was carried out on polar silica gels using relatively non-polar mobile phases. Later, non-polar bonded-phase silica columns using polar mobile phases became more popular. The former type of chromatography is known as "normal-phase" and the latter as "reversed-phase" [21]. There have been many recent

TABLE IV

pH STABILITY OF POLY-RP CO AND ITS DERIVATIVES

Basic conditions: 0.75 ml/min of acetonitrile–water (70:30) containing 1% of triethylamine. Acidic conditions: 0.75 ml/min of acetonitrile–water (70:30) containing 1% of phosphoric acid. The initial values of k', N/m and A_s for the valerophenone test solute are listed, followed in parentheses by the values obtained after pumping 1000 ml of mobile phase through each column.

Conditions	Parameter	Poly-RP CO	Poly-RP CYANO	Poly-RP DIOL
Basic	k'	6.5 (6.6)	5.3 (5.2)	4.6 (4.5)
	N/m	45 000 (45 000)	35 000 (35 000)	25 000 (26 000)
	A_s	1.3 (1.2)	1.2 (1.2)	1.0 (0.9)
Acidic	k'	7.0 (7.1)	5.7 (5.6)	4.8 (4.9)
	N/m	45 000 (45 000)	34 000 (34 000)	26 000 (26 000)
	A_s	1.3 (1.3)	1.2 (1.1)	0.9 (1.0)

reports of separations on bonded-phase silicas using non-polar solvents because of the unique separation selectivities that these mobile phases can offer. Some examples include the separations of fat-soluble vitamins [22], polyhydroxysteroids [23], flavonoids [24] and metal–diethyldithiocarbamate chelates [25].

In most of these reports, hexane was chosen as the base or "weak" solvent and various percentage of "strong" solvent modifiers, such as dichloromethane, tetrahydrofuran, ethyl acetate or isopropanol, were added to adjust the retention times of the solutes to reasonable levels. For our purposes, isopropanol was rejected because its high viscosity leads to very high column back-pressures with small polymer particles. Ethyl acetate was rejected because it is not transparent in the UV range of interest for typical HPLC applications. Because initial testing showed that higher column efficiencies were obtained with the dichloromethane–hexane system than with the tetrahydrofuran–hexane system, all of the "normal-phase" chromatography reported in this paper was done in the former solvent system. An increase in the percentage of dichloromethane gave shorter retention times for all the solutes studied on both the base resin and its functionalized derivatives. These results are analogous to those found for solvent strength studies performed on silica columns and their polar bonded-phase derivatives. For example, the mobile phase strength on an aminosilica column was found to increase as the percentage of tetrahydrofuran in hexane was raised [26]. A decrease in retention time with an increase in the amount of polar modifier in a non-polar solvent is generally observed in normal-phase chromatography [27].

Separation of aniline derivatives

Fig. 2 shows the separation of N,N-diethylaniline (2.93 min), N,N-dimethylaniline (3.38 min) and aniline (5.75 min) on a Poly-RP CO column in a mobile phase consisting of dichloromethane–hexane (10:90) containing 0.1% of triethylamine. As has been noted in previous work on bonded-phase silica columns [28], the peak elution order is the opposite of that observed in reversed-phase chromatography with the most non-polar compound, N,N-diethylaniline, eluting first and the most polar compound, aniline, eluting last.

The effects of the cyano and diol functional groups can be seen in the separation of the isomers of dimethylaniline (Fig. 3); the corresponding numerical data for these separations and the others reported in this work are presented in Table V. A

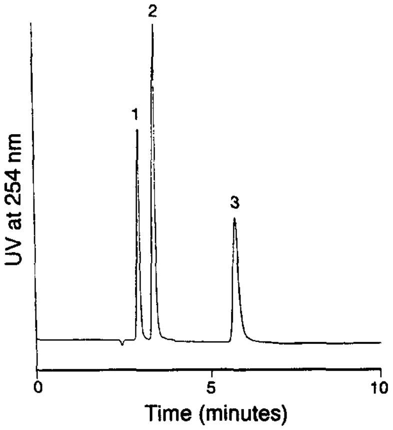

Fig. 2. Separation of anilines on a Poly-RP CO column using a non-polar mobile phase. Mobile phase, dichloromethane–hexane (10:90) containing 0.1% of triethylamine; flow-rate, 0.75 ml/min. Peaks: 1 = N,N-diethylaniline; 2 = N,N-dimethylaniline; 3 = aniline.

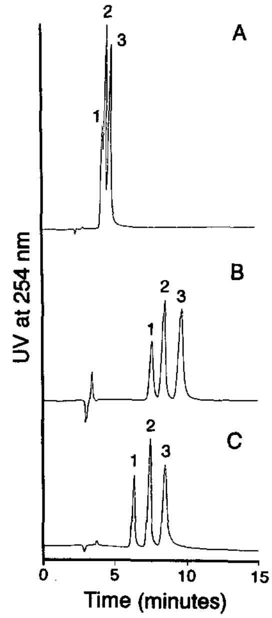

Fig. 3. Separation of dimethylanilines on (A) Poly-RP CO, (B) Poly-RP CYANO and (C) Poly-RP DIOL columns. Mobile phase, dichloromethane–hexane (10:90) containing 0.1% of triethylamine; flow-rate, 0.75 ml/min. Peaks: 1 = 2,6-dimethylaniline; 2 = 2,4-dimethylaniline; 3 = 3,5-dimethylaniline.

TABLE V

CAPACITY FACTORS (k') FOR VARIOUS SOLUTES ON POLY-RP CO AND ITS DERIVATIVES USING BOTH POLAR (P) AND NON-POLAR (NP) MOBILE PHASES

All experiments were done at a flow-rate of 0.75 ml/min.

Solute	k'					
	Poly-RP CO		Poly-RP CYANO		Poly-RP DIOL	
	NP	P	NP	P	NP	P
2,6-Dimethylaniline[a]	1.15	3.26	3.12	2.99	2.00	2.52
2,4-Dimethylaniline[a]	1.24	2.76	3.58	2.64	2.52	2.20
3,5-Dimethylaniline[a]	1.41	2.75	4.22	2.64	3.03	2.23
o-Xylene[a]	0.52		0.72		0.39	
m-Xylene[a]	0.49		0.64		0.35	
p-Xylene[a]	0.47		0.65		0.34	
2,6-Dimethylpyridine[b]	0.65	2.81	2.08	2.67	2.26	2.47
2,5-Dimethylpyridine[b]	0.69	3.34	2.79	3.27	3.23	3.08
2,4-Dimethylpyridine[b]	0.73	3.05	3.15	3.03	3.72	2.91
2,6-Dimethylphenol[c]	0.33	3.58	1.30	3.86	0.76	2.99
2,4-Dimethylphenol[c]	0.50	2.78	3.32	3.70	2.23	2.61
2,3-Dimethylphenol[c]	0.54	2.79	3.73	3.69	2.57	2.61
3,5-Dimethylphenol[c]	0.56	2.26	5.02	3.13	3.30	2.16
1-Naphthol[d]	0.97	8.43	9.60	16.30	7.24	10.97
2-Naphthol[d]	0.98	6.41	11.15	12.24	8.06	8.45

[a] NP = dichloromethane–hexane (10:90) with 0.1% triethylamine; P = acetonitrile–water (60:40) with 0.1% triethylamine.
[b] NP = dichloromethane–hexane (10:90) with 0.1% triethylamine; P = acetonitrile–water (30:70) with 0.1% triethylamine.
[c] NP = dichloromethane–hexane (50:50); P = acetonitrile–water (55:45).
[d] NP = dichloromethane–hexane (50:50); P = acetonitrile–water (45:55).

mixture of 2,6-, 2,4- and 3,5-dimethylaniline was injected on to each of the three columns under identical conditions of dichloromethane–hexane (10:90) containing 0.1% of triethylamine at a flow-rate of 0.75 ml/min. Under these conditions, the unfunctionalized Poly-RP CO showed a low capacity (k' = 1.1–1.4) and poor resolution of the isomer mixture, while the functionalized resins, Poly-RP CYANO and Poly-RP DIOL, showed much higher capacities (k' = 2.0–4.2) and better resolution of the isomer mixture. A possible explanation for this increase in retention and resolution is that the unfunctionalized Poly-RP CO is capable of interacting with solute molecules primarily through only dispersion and π–π forces, whereas the functionalized resins can interact with solute molecules through a much broader range of forces, including dispersion, π–π, dipole–dipole, acid–base and hydrogen-bonding interactions. For example, because of their polar cyano and hydroxyl functional groups, the Poly-RP CYANO and Poly-RP DIOL resins could undergo strong dipole–dipole and hydrogen-bonding interactions with the nitrogen-containing aniline solutes; such strong interactions would not be possible between the unfunctionalized Poly-RP CO and the aniline solutes. Also, the sample retention is expected to increase as the polarity of the stationary phase is increased because the driving force for retention in normal-phase chromatography is the attraction of polar solute molecules by a polar stationary phase.

To illustrate our point further, we injected some aromatic isomers without any polar functional substituents, namely a mixture of *o*-, *m*- and *p*-xylene, onto all three columns under the same experimental conditions as used for the aniline isomers; the corresponding numerical data are given in Table V. These three isomers could not be separated on any of the columns (they virtually co-eluted) and, in fact, they had very similar capacity factors on both the functionalized and unfunctionalized resins, with the Poly-RP DIOL actually giving lower capacity factors than the Poly-RP CO resin. The xylene isomers would only be able to interact with the three resins through dispersion and π–π forces and not through strong dipole–dipole and hydrogen-bonding interactions, and so one would expect the separations to be very similar on all three resins in this instance.

The order of elution of these dimethylaniline isomers (2,6- followed by 2,4- and 3,5-) is similar to that observed by Truedsson and Smith [29] using a cyanosilica column with normal-phase solvents. They postulated that the 2,6-isomer would elute first, partly because its amino nitrogen is the most sterically shielded from interaction with the nitrile group of the resin.

Comparison of the separation of pyridine derivatives in non-polar vs. polar solvent systems

A mixture of 2,6-, 2,5- and 2,4-dimethylpyridine was injected on to each of the three columns under the same conditions as used for the aniline derivatives; the results are reported in Fig. 4A and Table V. Once again, the unfunctionalized Poly-RP CO showed a much lower capacity (k' = 0.65–0.73) than the functionalized resins (k' = 2.08–3.72). The best separation of this mixture of isomers was obtained on the Poly-RP DIOL column (see Fig. 4A), which gave a broader range of capacity factors (k' = 2.26–3.72) than the Poly-RP CYANO column (k' = 2.08–3.15).

In order to demonstrate the usefulness of non-polar solvent systems with polymeric columns, the same separation was attempted on all three columns using a mobile phase of acetonitrile–water (30:70) containing 0.1% of triethylamine, which gave capacity factors similar to those obtained with dichloromethane–hexane (10:90). These results are reported in Fig. 4B and Table V. The data show that the Poly-RP DIOL column is capable of good resolution of all three isomers in dichloromethane–hexane, whereas the use of acetonitrile–water resulted in very poor resolution for the 2,5- and 2,4-dimethylpyridines. Research on bonded-phase silica columns has also shown that isomer selectivity is generally superior in normal-phase systems than reversed-phase systems, as was shown for the separation of retinol isomers on silica columns in both types of mobile phases [30].

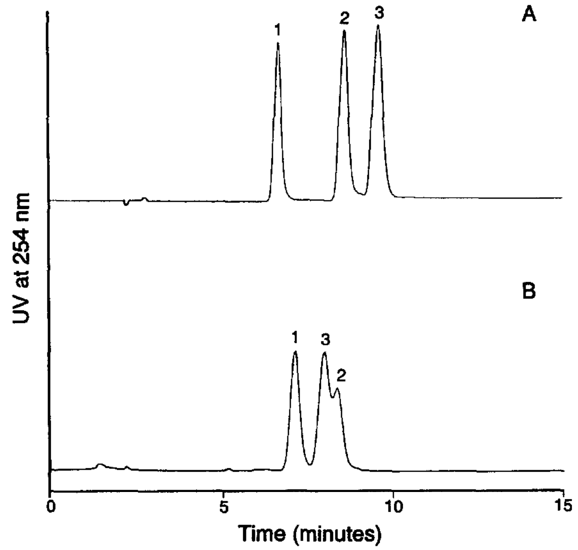

Fig. 4. Separation of dimethylpyridines on a Poly-RP DIOL column using both (A) non-polar and (B) polar mobile phases. Mobile phase, (A) dichloromethane–hexane (10:90) containing 0.1% of triethylamine and (B) acetonitrile–water (30:70) containing 0.1% of triethylamine; flow-rate, 0.75 ml/min. Peaks: 1 = 2,6-dimethylpyridine; 2 = 2,5-dimethylpyridine; 3 = 2,4-dimethylpyridine.

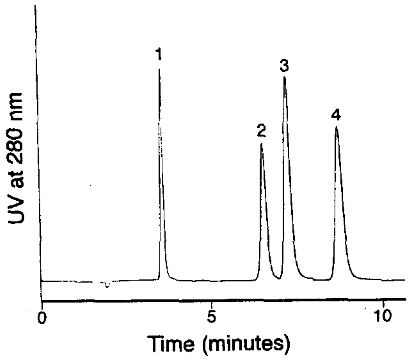

Fig. 5. Separation of dimethylphenols on a Poly-RP DIOL column using a non-polar mobile phase. Mobile phase, dichloromethane–hexane (50:50); flow-rate, 0.75 ml/min. Peaks: 1 = 2,6-dimethylphenol; 2 = 2,4-dimethylphenol; 3 = 2,3-dimethylphenol; 4 = 3,5-dimethylphenol.

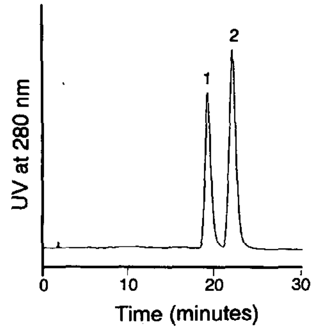

Fig. 6. Separation of naphthols on a Poly-RP CYANO column using the same conditions as in Fig. 5. Peaks: 1 = 1-naphthol; 2 = 2-naphthol.

Separation of phenolic compounds in non-polar solvent systems

The elution of the isomers of dimethylphenol and naphthol (hydroxynaphthalene) on the functionalized columns required a very strong solvent system, dichloromethane–hexane (50:50) (Figs. 5 and 6 and Table V). Fig. 5 shows the separation of four isomers of dimethylphenol on the Poly-RP DIOL column. Fig. 6 shows that baseline resolution of 1- and 2-naphthol can be achieved with the Poly-RP CYANO column. It is also interesting to note the large difference in capacity factors for these compounds on the unfunctionalized and on the functionalized resins (see Table V). This is probably caused by strong hydrogen-bonding interactions between the acidic phenolic hydrogens of the solutes and the polar functional groups on the cyano and diol resins, because we have found that the corresponding solutes without the phenolic groups, namely xylene and naphthalene, can easily be eluted from the cyano and diol resins with dichloromethane–hexane (10:90).

Most of the isomers in this study have been tested using both acetonitrile–water and dichloromethane–hexane (Table V). Generally, acetonitrile–water gave very similar separations and capacity factors on all three columns; the differences between the functionalized and unfunctionalized columns were much more exaggerated in dichloromethane–hexane. Unlike normal-phase chromatography, where the driving force for retention is the attraction of polar solutes to a polar stationary phase, the driving force for retention in reversed-phase chromatography is the rejection of less polar solutes by the highly polar mobile phase. When the polar Poly-RP CYANO and Poly-RP DIOL resins are used with the polar mobile phases employed in reversed-phase chromatography, the difference in polarity between the mobile phase and stationary phase relative to that of a polystyrene–divinylbenzene packing is much lower and the driving force for retention is much less, which explains the similarity in capacity factors among all three stationary phases in reversed-phase solvents. Similar results have been observed on bonded-phase silica columns in reversed-phase systems [31].

CONCLUSIONS

This work demonstrates that good separations can be achieved on polymeric separation media using non-polar solvent systems. The use of these non-polar solvents allows for separations that might be difficult to achieve using the traditional polar solvent systems that are based on mixtures of various organic solvents with water. By avoiding the use of viscous, aqueous-based mobile phases, low operating pressures can be achieved even with 4-μm polymeric particles. The functionalization of the polymer surface with diol and cyano moieties increases the capacity and resolving power of these columns in non-polar solvents.

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