Chemically modified polymeric resins for high-performance liquid chromatography

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ABSTRACT

Chemical reactions were used to attach various hydrophilic functional groups and one hydrophobic group to cross-linked polystyrene resins. Those with hydrophilic groups had much better wettability by water. The capacity factors of a number of test compounds were compared on columns packed with derivatized and underivatized polymeric resins. The capacity factor of most test compounds were distinctly different on columns packed with the derivatized resins. In some cases the elution order of test compounds was different on the derivatized resin columns. The modified resins offer an additional selectivity parameter for liquid chromatographic separations.

INTRODUCTION

Reversed-phase liquid chromatography is generally performed on columns packed with C_{18}- or C_8-bonded phase silica [1]. Although such columns are very efficient, they do have several drawbacks. The most serious of these is the instability of silica in alkaline solutions or in highly acidic solutions. Silica also has residual silanol groups that can cause peak broadening or tailing by interaction with polar compounds such as amines and alcohols. After a gradient elution, silica-based columns may require some time to re-establish equilibrium for the next run [2].

In recent years there has been a growing interest in polymeric materials for use in reversed-phase high-performance liquid chromatography (HPLC). In particular, resins based on poly(styrene–divinylbenzene) (PS–DVB) are stable with eluents from pH 1–14 and give excellent separations [3–6]. Some difficulties have been associated with polymeric resins, such as swelling in the presence of organic solvents, which can be especially troublesome when solvent gradients are used [7]. However, rapid improvements are being made in stability and performance so that polymeric resins can now be considered to be very attractive for HPLC.

A considerable variety of polymeric resins have been prepared and used for HPLC [8–13]. There has been a remarkable tendency to bond C_{18}-hydrocarbon groups to the surface so that the retention behavior of the polymeric resins will closely approximate that of the popular C_{18} silica materials [11,13,14]. In fact, silica resins with a C_{18}- or C_8-bonded phase dominate the field of reversed-phase liquid

chromatography. Using these resin columns, necessary changes in selectivity are brought about by varying the solvents that make up the mobile phase. However, several investigators have used silica columns with more polar bonded phases to provide an additional parameter for varying selectivity in liquid chromatography [15–17].

In the present work cross-linked polystyrene resins have been modified by introduction of any of several hydrophilic functional groups, and in one case by introduction of a more hydrophobic group. The modified resins are easily prepared by the Friedel–Crafts reaction with the benzene ring of the polymer. It is shown that the derivatized resins can be used for practical liquid chromatographic separations and that the type of functional group incorporated in the resin has a major effect on the retention behavior of various analytes.

EXPERIMENTAL

Apparatus

A Gilson 302 HPLC system equipped with a microprocessor controller (Gilson Medical, Middleton, WI, U.S.A.), a 7125 Rheodyne injector (Rheodyne, Berkeley, CA, U.S.A.) equipped with a 20-μl loop, a Spectroflow 783 Kratos variable-wavelength UV–VIS detector (Kratos, Ramsey, NJ, U.S.A.), a Fisher Recordall series 5000 recorder (Fisher Scientific/Instrument Lab., Itasca, IL, U.S.A.) and a Hitachi D-2000 intergrator (EM Science, Cherry Hill, NJ, U.S.A.), were used for HPLC. A Shandon HPLC packing pump (Shandon Southern, Sewichley, PA, U.S.A.) was used for column packing. A Bruker FT-IR 98 instrument (USA Bruker Instruments, San Jose, CA, U.S.A.) was used for structure determination.

Reagents and chemicals

Two kinds of PS–DVB resins were used in this experiment; non-spherical XAD-4 resin (Rohm & Haas, Philadelphia, PA, U.S.A.) of 45–58 μm particle size, 50 Å pore size and 784 m^2/g surface area, and 10-μm spherical resin (Sarasep, Santa Clara, CA, U.S.A.) of 80 Å pore size and 415 m^2/g surface area. The XAD-4 resin was ground and then sieved with a Model L3P sonic sifter (Allen-Bradley, Milwaukee, WI, U.S.A.). The particles of size 45–58 μm (*ca.* 250–325 mesh) were chosen for derivatization. The ground resin was washed with water and acetonitrile, Soxhlet-extracted overnight with methanol, ether and acetonitrile, and then dried. The 10-μm spherical resin was cleaned by the same way.

The reagents and solvents used for the derivatization reactions were reagent grade and were dried by molecular sieves. Reagent grade test compounds were used for the HPLC experiments. HPLC-grade acetonitrile was used, and laboratory distilled water was further deionized by a Barnstead Nanopure II system (Sybron Barnstead, Boston, MA, U.S.A.).

Synthetic procedures

Different functional groups were introduced into the benzene ring of PS–DVB resins by the following procedures:

(1) –CH$_2$OH derivative. Add 1.2 g of paraformaldehyde, 16 ml of acetic acid and 4 ml of acetic anhydride to 5.2 g of PS–DVB resin. Stir for a few minutes, then add 6.0

g of anhydrous zinc chloride and keep at 60°C overnight. Filter the resin, rinse with methanol, then heat with a solution of methanol–conc. HCl (90:10) for 1 h. Wash the final product with methanol and dry. The concentration of –CH_2OH on the resin was 1.3 mmol/g. This was determined by a standard acetylation procedure using acetic anhydride in pyridine as the reagent.

(2) –$COCH_2CH_2CO_2H$ derivative. Mix 2.5 g of resin with 3.6 g of succinic anhydride, 30 ml of tetrachloroethane and 15 ml or nitrobenzene. Stir for a few minutes, then add 10.7 g of anhydrous aluminum chloride and keep at 45°C for 24 h. Pour the product into ice water. Isolate the resin, wash with acetone, methanol and water, then dry. The amount of derivatization was found to be 1.1 mmol/g by acid-base titration of the carboxylic acid.

(3) –$COCH_3$ derivative. To 5.1 g of resin add 30 ml of carbon disulfide, 9.5 g of anhydrous aluminum chloride, add 5.5 g of acetyl chloride, added dropwise. Keep at 50°C for 24 h. Pour the product into ice water. Isolate the resin, wash with acetone, methanol and water, then dry. The presence of a carbonyl group was proved by a good band at 1690 cm^{-1} on the spectrum obtained by Fourier transform infrared spectrometry (FT-IR). The concentration of –$COCH_3$ on the resin was determined as 1.2 mmol/g by derivatization of –CO– group.

(4) –$C(CH_3)_3$ derivative. Mix 4.6 g of resin, 60 ml of nitromethane, 8.0 g of tert.-butylchloride and 8.0 g of AlCl$_3$. Keep at 60°C for 24 h. Pour into ice water, wash with acetone, methanol and water then dry. The capacity of the tert.-butyl group is difficult to determine because of lack of suitable methods, though the chromatographic behavior is greatly different from that of the underivatized resin.

(5) –CH_2CN derivative. To 5.4 g of resin add 30 ml of tetrachloroethane and 20 ml of chloroacetonitrile. Keep at 85°C for 24 h. Pour the product into ice water, then wash and dry as above. The concentration of –CH_2CN on the resin was 0.9 mmol/g, determined by nitrogen analysis.

RESULT AND DISCUSSION

XAD-4 resin derivatives

Ground up and sieved XAD-4 resin was chosen for the first experiments because it is highly cross-linked and has a very high surface area (784 m^2/g), which makes for easy preparation of various derivatives. XAD-4 resins were prepared containing each of the following functional groups attached to the benzene ring: –$C(CH_3)_3$, –CH_2OH, –$COCH_3$, –$COCH_2CH_2CO_2H$ and –CH_2CN.

The parent XAD-4 resin and its derivatives were packed into 250 × 2.1 mm columns and the retention times of various organic compounds were measured by the UV-VIS detector at wavelength 270 nm, using acetonitrile–water (50:50) as the eluent. Capacity factors (k') of the analytes on the various resins are given in Table I. The results show some interesting trends:

(1) The less-polar analytes all have lower capacity factors on the resins containing polar functional groups than on the parent XAD-4 resin (R is the ratio of k' values of derivatized:parent resin). The opposite is true for the polar analytes; these have larger k' values on the polar derivatized resins.

(2) On the tert.-butyl resin the less polar analytes have significantly larger k' values than on the parent XAD-4 resin. However, the polar analytes are less attracted to the tert.-butyl resin and have lower k' values.

TABLE I

CAPACITY FACTORS (k') OF TEST COMPOUNDS ON XAD-4 RESIN AND ITS DERIVATIVES

R is the ratio of k' on the derivatized resin to k' on the XAD-4 resin. Chromatographic conditions: 250 × 2.1 mm column; acetonitrile–water (50:50) eluent, adjusted to pH 1.7 with HCl.

| Compounds	XAD-4, k'	Derivatives of XAD-4 resin									
		$-C(CH_3)_3$		$-CH_2OH$		$-COCH_3$		$-COCH_2CH_2COOH$		$-CH_2CN$	
		k'	R	k'	R	k'	R	k'	R	k'	R
Cumene	19.3	23.2	1.20	18.4	0.80	14.2	0.74	17.7	0.92	15.6	0.81
o-Dichlorobenzene	18.7	22.0	1.18	17.0	0.91	16.9	0.91	18.7	1.00	17.1	0.91
Toluene	9.83	11.5	1.17	8.83	0.90	8.50	0.86	8.83	0.90	8.78	0.89
Anisol	6.53	7.33	1.12	5.81	0.89	6.22	0.95	5.83	0.89	5.67	0.87
Diethylphthalate	6.25	7.30	1.19	4.83	0.77	4.58	0.73	5.17	0.83	3.89	0.78
Methylbenzoate	5.25	5.83	1.11	4.84	0.92	4.61	0.88	4.15	0.90	4.78	0.91
2,4-Dinitrofluorobenzene	4.92	4.41	0.89	4.50	0.92	4.28	0.87	4.83	0.98	4.08	0.83
Acetophenone	3.17	3.25	1.03	3.17	1.00	3.00	0.95	3.00	0.95	3.00	0.95
p-Cresol	2.17	1.92	0.88	2.25	1.04	2.69	1.24	2.08	0.96	2.11	0.97
Phenol	1.50	1.42	0.94	1.67	1.11	1.94	1.30	1.67	1.11	1.61	1.07
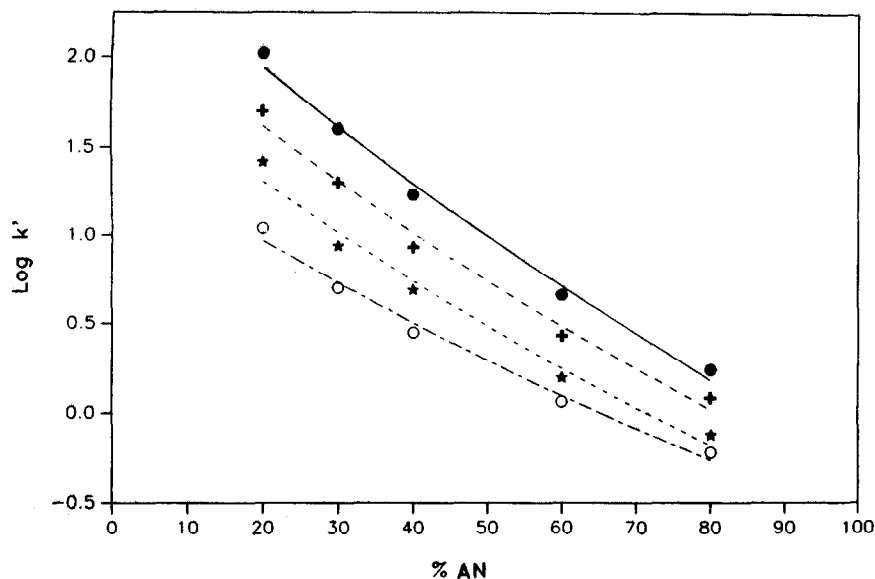
Fig. 1. Log k' on XAD-CH$_2$OH resin column as a function of the percentage of acetonitrile (AN) in the eluent. ● = Toluene; + = methylbenzoate; ★ = p-cresol; ○ = phenol.

Capacity factors for some of the analytes were measured as a function of the percentage acetonitrile in the eluent. Fig. 1 was typical of the results that were obtained. The plots are not quite linear and show better separation factors at lower concentrations of acetonitrile in the eluent.

Derivatives of small, spherical PS–DVB resins

The fairly large and somewhat irregular particle size of the XAD-4 resin limited their separation ability. For this reason a spherical polymeric resin of small, uniform particle size was sought. The material used was a spherical PS–DVB resin of 10 μm average diameter and a surface area of 415 m^2/g [18]. The coverage of the functional group was calculated to be 3.1 μmole/m^2 for the hydroxymethyl resin, which is similar to the coverage of typical silica resins reported by Antle *et al.* [16]. Portions of this resin were derivatized as before, packed into columns, and the k' values of several analytes measured using acetonitrile-water eluents.

Several test compounds were separated on each of the columns using a very fast chart speed so that column efficiency and peak shape could be observed. The theoretical plate numbers of the polymeric resin columns were rather low (*ca.* 10 000 plates/m), but this would be expected because the average resin diameter was around 10 μm. Peak asymmetry factors [19] for the four test compounds are given in Table II for the three polymeric resin columns and for a commercial 5-μm silica C$_{18}$ column. The *tert.*-butyl column shows the most tailing. The parent polymeric resin column shows essentially no tailing for three of the four test compounds. The acetyl column also compares favorably with the silica column.

In Table III the k' values for 20 aromatic compounds are compared to the k'

TABLE II

PEAK ASYMMETRY FACTORS OF FOUR TEST COMPOUNDS ON DIFFERENT RESIN COLUMNS

Resin	Peak asymmetry factor			
	Toluene	Anisol	Acetophenone	4-Nitrophenol
PS-C(CH$_3$)$_3$	1.97	1.43	1.67	1.86
PS	0.96	0.95	0.96	1.33
PS-COCH$_3$	1.46	1.48	1.44	1.50
Silica C$_{18}$	1.38	1.50	1.53	1.77

value of benzene on each of the three polymeric columns. In this way the effect of a single aromatic substituent can be measured.

The k' values relative to benzene in Table III are listed in decreasing order. Compared to the underivatized resin the relative k' values on the *tert.*-butyl resin are higher for the more hydrophobic test compounds and lower for the polar compounds.

TABLE III

CAPACITY FACTORS (k') AND CAPACITY FACTORS RELATIVE TO k' (BENZENE) FOR BENZENE DERIVATIVES ON THREE POLYMERIC RESIN COLUMNS

R is the ratio of k' on the derivatized resin compared to the underivatized resin. The eluent was acetonitrile–water (50:50), acidified with HCl. k'_x is the capacity factor of a benzene derivative.

Compounds	PS–DVB		PS–DVB–COCH$_3$			PS–DVB–C(CH$_3$)$_3$		
	k'	$k'_x/k'_{benzene}$	k'	$k'_x/k'_{benzene}$	R	k'	$k'_x/k'_{benzene}$	R
Benzene	6.59		4.82			7.21		
Biphenyl	41.28	6.27	28.46	5.90	0.69	50.63	7.02	1.23
Cumene	22.98	3.49	11.25	2.33	0.49	28.71	3.98	1.25
o-Dichlorobenzene	19.18	2.91	12.38	2.57	0.65	24.29	3.37	1.27
Iodobenzene	17.89	2.71	13.83	2.87	0.77	23.19	3.22	1.30
Bromobenzene	15.53	2.36	12.93	2.68	0.83	19.78	2.74	1.27
Chlorobenzene	11.98	1.82	10.18	2.11	0.85	15.05	2.09	1.26
Toluene	10.83	1.64	6.61	1.37	0.61	12.84	1.78	1.19
Anisol	6.93	1.05	4.50	0.93	0.65	7.73	1.07	1.12
Diethylphthalate	6.33	0.96	2.97	0.62	0.47	6.72	0.94	1.06
Fluorobenzene	5.99	0.91	5.17	1.07	0.86	6.85	0.95	1.14
Methylbenzoate	5.48	0.79	3.57	0.74	0.65	6.12	0.85	1.12
Nitrobenzene	5.24	0.80	4.70	0.98	0.90	5.99	0.83	1.14
2,4-Dinitrofluorobenzene	4.56	0.69	4.08	0.85	0.89	4.29	0.60	0.94
Acetophenone	3.06	0.46	2.05	0.43	0.67	3.25	0.45	1.06
p-Cresol	1.70	0.26	1.96	0.41	1.15	1.65	0.23	0.97
3-Nitrophenol	1.59	0.24	2.07	0.43	1.30	1.50	0.21	0.94
4-Nitrophenol	1.43	0.22	1.97	0.41	1.38	1.27	0.18	0.89
Phenol	1.23	0.19	1.42	0.29	1.15	1.11	0.15	0.90
Benzoic acid	0.78	0.12	2.55	0.53	3.27	0.75	0.10	0.96
Benzyl alcohol	0.76	0.12	0.93	0.19	1.22	0.71	0.10	0.93

However, the *order* of the relative k' values is the same for the *tert.*-butyl and the underivatized resin, with the minor exception of diethylbenzene and fluorobenzene.

The relative k' values of non-polar test compounds are lower on the resin derivatized with a polar acetyl group than on the underivatized resin. Exactly the opposite is true for the polar test compounds. More significantly, there are numerous changes in the elution *order* of the various test compounds on the acetyl resin.

It is interesting to compare the relative k' values for the halogenated benzenes on the three resin columns (Table IV). The relative k' values of the four simple halobenzenes are higher on both the butyl and acetyl columns than on the underivatized resin column. However, the ratio of relative k' (butyl) to relative k' (unsubstituted) increases in the order F, Cl, Br, I. The ratio of relative k' (acetyl) to relative k' (unsubstituted) decreases in the same order.

TABLE IV

RELATIVE k' VALUES ($k'_x/k'_{benzene}$) FOR HALOGENATED BENZENES (C_6H_5X) ON THREE RESIN COLUMNS

X	Relative k'			k' (*tert.*-butyl)	k' ($COCH_3$)
	tert.-Butyl	Unsubstituted	–$COCH_3$	k' (unsubstituted)	k' (unsubstituted)
I	3.22	2.71	2.87	1.19	1.06
Br	2.74	2.36	2.68	1.16	1.14
Cl	2.09	1.82	2.11	1.15	1.16
F	0.95	0.91	1.07	1.04	1.18

Following the treatment of Sadek and Carr [15], log k' was plotted against the number of carbon atoms in the side chain of alkyl benzenes and of alkyl phenols. Excellent linear plots were obtained for both classes of compounds (Fig. 2 and Table V). The slopes show the effect of a single –CH_2 group on the increased retention of a test compound. The higher slopes on the butyl resin indicate a greater affinity for a –CH_2 group than on the underivatized phenyl resin. The lower slopes on the more polar acetyl resin indicates a lower affinity for a –CH_2 group. The slopes of each of the three resins were higher for the alkyl benzenes than for the alkyl phenols.

The intercepts for the alkyl benzenes (Table V) are in the order of decreasing polarity of the resins acetyl < underivatized < *tert.*-butyl. The intercepts of the more polar alkyl phenols are in exactly the opposite order: *tert.*-butyl < underivatized < acetyl.

The capacity factors of several test compounds were measured on a column packed with polymer resin derivatized with –CH_2CN groups. In Table V, the results are compared with values obtained under identical conditions using underivatized resin. Polar test compounds are retained a little longer and non-polar compounds are less retained on the cyanomethyl column, although the differences are generally less than those between underivatized and acetyl resin columns.

It is interesting to compare the effects of introducing a cyanomethyl group into
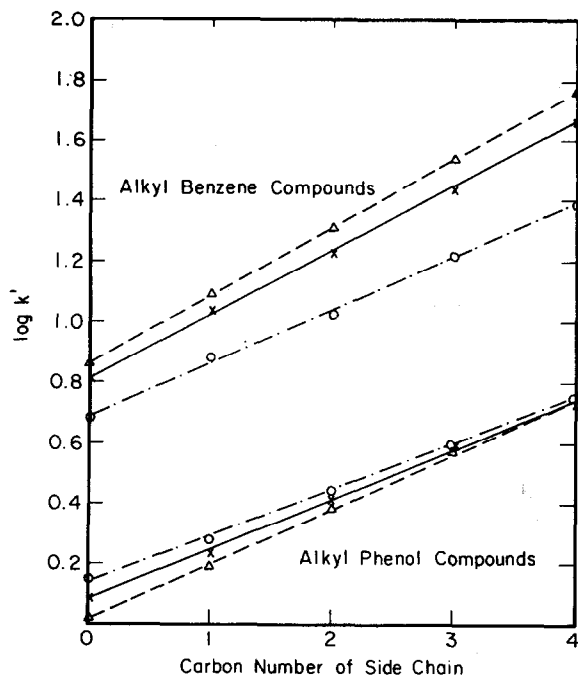
Fig. 2. Plot of log k' vs. carbon number of side chain of alkyl benzene and aklyl phenol compounds on diferent polystyrene resins. Chromatographic conditions as in Table I. $\triangle$---$\triangle$ = PS–DVB–C(CH$_3$)$_3$; ×—× = PS–DVB; ○—·—·—○ = PS-DVB-COCH$_3$.

TABLE V

LINEAR REGRESSION DATA FOR PLOTS OF LOG k' AGAINST THE NUMBER OF CARBON ATOMS IN THE R GROUP FOR C$_6$H$_5$R AND FOR HOC$_6$H$_4$R

Resin	Slope	y-intercept	Correlation coefficient
For C$_6$H$_5$R, where R = 0, 1, 2, 3, 4			
PS–C(CH$_3$)$_3$	0.23	0.86	0.9997
PS	0.21	0.81	0.9995
PS–COCH$_3$	0.18	0.69	0.9995
For HOC$_6$H$_5$R, where R = 0, 1, 2, 3, 4			
PS–C(CH$_3$)$_3$	0.185	0.016	0.9996
PS	0.165	0.081	0.9995
PS–COCH$_3$	0.15	0.140	0.9992

a polystyrene resin with changing from a C$_{18}$- to cyanoalkyl bonded-phase silica resin column. The results in Table VI show a much lower retention for cumene and *o*-dichlorobenzene on cyano silica than on C$_{18}$ silica. Polar test compounds are more strongly retained on the cyano silica column, but the overall range of k' values is much less on the cyano silica than on the C$_{18}$ silica. It is probably for this reason that cyano silica columns have generally been less used for reversed-phase LC than for normal-phase chromatography.

It is logical to expect a large change in capacity factors with cyanoalkyl silica column compared to an alkyl silica column because of the drastic difference in polarity of the bonded groups. The difference in polarity between a phenyl group and a cyanomethyl phenyl group in polystyrene resins is less drastic. Accordingly, smaller differences in capacity factors of test compounds would be expected.

Separations of mixtures by 10-μm spherical PS–DVB resins. Separations of several mixtures were compared on a parent 10 μm spherical resin and an acetyl-derivatized one. The column was 100 mm × 4.6 mm I.D. for practical applications. While columns packed with 10-μm resins are no longer state-of-the-art, the separations are still good enough to be of practical value and to show clearly the effects of derivatization.

Examination of the data in Table III shows several pairs of analytes for which the separation factor ($\alpha = k'_2/k'_1$) is larger on the acetyl resin. For several pairs of analytes the separation factors on the acetyl resin and the un-derivatized resin are as follows: anisol and diethylphthalate $\alpha = 1.52$ and 1.09, 3-nitrophenol and phenol $\alpha = 1.47$ and 1.29, 4-nitrophenol and phenol $\alpha = 1.40$ and 1.16. Figs. 3 and 4 show that better separations are indeed obtained on the acetyl resin under the same experimental conditions.

Several actual separations were performed to confirm the differences in elution orders predicted by the data in Table II. For example methyl benzoate elutes before diethyl phthalate on a column of underivatized resin, but the elution order is reversed on the acetyl resin column.
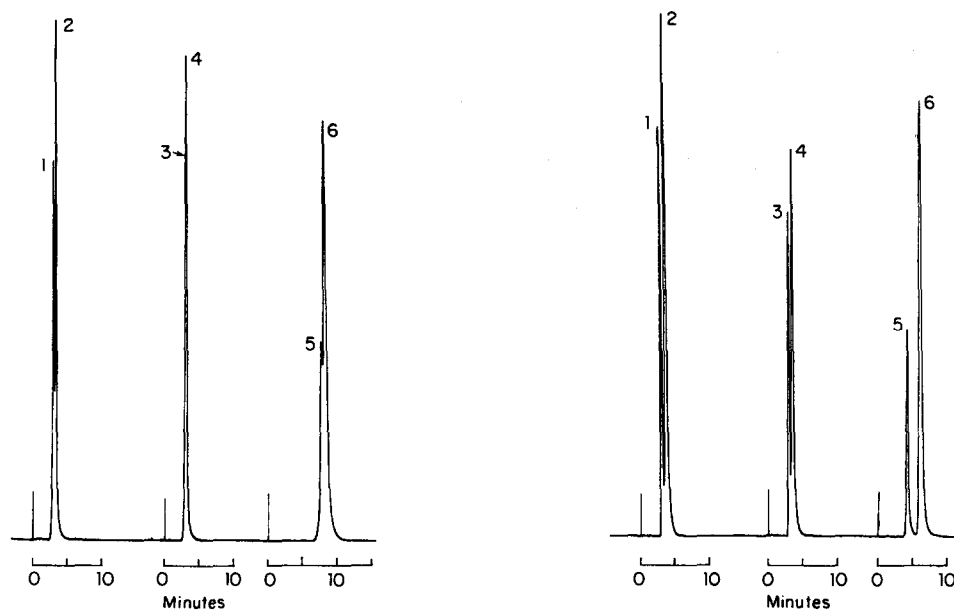

Fig. 3. Chromatographic separations on PS–DVB resin column (100 × 4.6 mm), using 60% aqueous acetonitrile (pH 1.7) eluent. Peaks: 1 = phenol; 2 = 3-nitrophenol; 3 = phenol; 4 = 4-nitrophenol; 5 = diethylphthalate; 6 = anisol.

Fig. 4. Chromatographic separations on an acetyl derivatized PS–DVB column. Conditions as in Fig. 3.

TABLE VI

COMPARISON OF CAPACITY FACTORS (k') ON CYANO SILICA TO C_{18} SILICA AND OF CYANOMETHYL POLYMERIC TO POLYMERIC RESIN

The eluent was acetonitrile–water (50:50), adjusted to an apparent pH of 1.7 with HCl.

Compound	Silica columns			Polystyrene columns		
	$k'(C_{18})$	$k'(CN)$	$\dfrac{k'(CN)}{k'(C_{18})}$	$k'(PS)$	$k'(CN)$	$\dfrac{k'(CN)}{k'(PS)}$
Cumene	3.51	1.33	0.38	23.0	20.6	0.90
o-Dichlorobenzene	2.40	1.17	0.49	19.2	16.4	0.85
Toluene	1.57	0.97	0.62	10.8	10.5	0.97
Anisol	0.92	0.80	0.87	6.93	6.32	0.91
Diethylphthalate	1.13	0.89	0.79	6.33	5.08	0.80
Methylbenzoate	0.82	0.75	0.91	5.48	4.79	0.87
2,4-Dinitrofluorobenzene	0.69	0.90	1.30	4.56	4.41	0.97
Acetophenone	0.53	0.64	1.21	3.06	3.08	1.01
p-Cresol	0.40	0.56	1.40	1.70	1.74	1.02
Phenol	0.29	0.49	1.69	1.23	1.35	1.10

CONCLUSION

Even with the limited number of test compounds studied, it is apparent that functional groups introduced into PS–DVB resins have an appreciable effect on the retention times and k' values obtained in reversed-phase liquid chromatography. The retention times of all compounds are distinctly different on the derivatized columns, and in several cases the relative capacity factors (R) of many of the test compounds are significantly different on the derivatized columns. Therefore, derivatized polymeric columns offer an additional selectivity parameter (*e.g.* interaction of analyte with the resin) over reversed-phase chromatography with C_{18}- or C_8-silica-bonded resin in which the selctivity effects are determined primarily by interaction between the analytes and the solvents in the mobile phase.

ACKNOWLEDGEMENTS

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