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## POLARITY AND SELECTIVITY OF POLYOL- AND POLYPHENOL-DERIVATIZED SILICA GELS FOR NORMAL-PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY\*

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### SUMMARY

The selectivity, polarity and retentivity in normal-phase high-performance liquid chromatography of polyol- and polyphenol-derivatized silica gels were compared with those of non-derivatized silica gel and amino- and cyanopropyl-derivatized silica gels, using a polarity mixture containing anisole, nitrobenzene, diethyl phthalate, acetophenone, anthraquinone, benzhydrol, benzoic acid and benzanilide with a hexane-dioxane mobile phase. Both polyphenol-bonded silica gel and polyol-bonded silica gel were more polar than silica gel, as indicated by the higher retentivities and higher slopes of the graphs of the logarithm of  $k'$  against the logarithm of the molar fraction of the stronger solvent in a binary solvent system.

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### INTRODUCTION

Several groups have reported on the selectivity and polarity of silica gel and of aminopropyl- and cyanopropyl-derivatized silica gel in normal-phase high-performance liquid chromatography (HPLC)<sup>1,2,18,19</sup>. Hara and Ohnishi<sup>1</sup> compared polarities using steroids as samples. They concluded that the retentivity of a non-bonded silica gel was similar to that of an amino-bonded phase and was higher than that of a cyano-bonded phase. The selectivities of a silica gel phase and a cyano-bonded phase were very similar. The amino-bonded phase, however, possessed a different selectivity for phenolic and alcoholic solutes.

An advantage of normal-phase HPLC is the possibility of changing the mobile phase solvent to obtain different selectivities. With reversed-phase HPLC this is also possible, but not as extensively. Solvents have been classified in different types with respect to hydrogen bond-forming capacity<sup>3-5</sup>. Weak solvents (W) are divided into three classes: solvents with no hydrogen bonding capacity are classified as class 0 (aliphatic hydrocarbons), solvents possessing  $\pi$ -electrons as class P (aromatic hydrocarbons) and solvents with non-bonded electrons as class N (haloalkanes). Stronger

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eluting solvents (S) are classified as class B (basic) or class AB (acidic/basic); the former possess non-bonded electron-pair donors with heteroatoms such as oxygen and nitrogen (acetone, dioxane) and the latter consist of solvents with proton donor and acceptor character (acetic acid, alcohols).

There are different models for liquid-solid chromatography (LSC)<sup>6</sup>. Soczewinski's model IIa<sup>6-8</sup>, an adsorption model based on the mass action law and based on thin-layer chromatographic (TLC) data, explains solvent composition effects in adsorption chromatography on silica gel. From this model a theoretical linear relationship between the logarithm of the capacity factor ( $k'$ ) and the logarithm of the molar fraction of the stronger solvent in the binary system ( $X_S$ ) can be derived:

$$\log k' = C - n \log X_S \quad (1)$$

where  $c$  (intercept) and  $n$  (slope) are constants. This theoretical linear relationship, useful in the optimization of a chromatographic system, has been validated for steroids, protected oligopeptides and many other solutes by Hara and co-workers<sup>9-13</sup> with numerous mobile phase combinations. Not only silica gel but also amino- and cyanopropyl-derivatized silica gels follow this relationship (when the S concentration is higher than 5-10%).

Recently we developed two new polar derivatized silica gels, a polyphenol-bonded stationary phase (Polyphenol-RSiL)<sup>14</sup> and a polyol-bonded phase (Polyol-RSiL)<sup>15</sup>, both of which are capable of forming strong hydrogen bonds with polar solvents and solutes. In the adsorption-desorption equilibrium of LSC, hydrogen bonding is the major interaction<sup>6,7</sup>. For medium polar and polar compounds the new phases show higher  $k'$  values than those obtained on silica gel under analogous conditions.

In this work, polyol- and polyphenol-bonded phases were compared with silica, cyanopropylsilica and aminopropylsilica gels using a newly composed polarity mixture under normal-phase conditions with a hexane-dioxane mobile phase (class 0 and B solvents, respectively). We tried to compose a polarity mixture containing a UV-active representative of most fundamental homologous series. This turned out to be difficult because of excessive retention differences. The actual choice was therefore based on availability and on chromatographic suitability. The compounds selected were anisole, nitrobenzene, diethyl phthalate, acetophenone, anthraquinone, benzhydrol, benzoic acid and benzanilide.

The validity of eqn. 1 was tested for the new stationary phases with strong hydrogen bond-forming potential. Hopefully a polarity sequence for phases in normal-phase HPLC can be derived.

Phases more polar than silica gel are interesting for a number of reasons, as discussed earlier<sup>14,15</sup>.

## EXPERIMENTAL

The chromatographic system consisted of a 5020-LC pump, a UV-50 variable-wavelength detector (Varian, Walnut Creek, CA, U.S.A.) and a 9176 Linear recorder. A 7000 p.s.i., 15- $\mu$ l Valco sample loop injector was used. Columns were Li-chroma tubes (250  $\times$  4.1 mm I.D.) provided with Valco fittings. The packing ma-

terials were all based on 10  $\mu\text{m}$  RSiL (Alltech-RSL, Eke, Belgium). This irregularly shaped silica gel has a specific surface area of about 350  $\text{m}^2$  and a mean pore size of 8–10 nm. The amounts of organic material bonded to the silica gel in the different phases were determined by thermogravimetric analysis (TGA). The use of this procedure for this purpose can be criticized, but it gives a good indication and allows comparisons. The percentage of bonded organic material was measured as the weight loss between 200 and 900°C with 2% deducted as the loss inherent to the silica gel matrix (blank). The percentages of bonded organic material found in this way were RSiL-NH<sub>2</sub> 7%, RSiL-CN 8%, Polyol-RSiL 14% and Polyphenol-RSiL 15%.

The amino- and cyano-bonded phases require no further comment, but the polyol phase is special in that it is obtained by bonding an organic moiety bearing four hydroxy groups. This phase is therefore probably more "polar" than, *e.g.*, the known "diol" phases. Polyphenol-RSiL is obtained by bonding tannic acid to silica gel and is therefore both a polyphenol and also a polyester.

A slurry packing technique was used as the filling procedure. A 5-g amount of the stationary phase was suspended in 20 ml of methanol–water (90:10) and pumped into the columns downwards with a Haskel air-driven fluid pump at a pressure of 400 bar. The same solvent was used as the pressurizing liquid. After packing, the columns were rinsed with 100 column volumes of methanol, followed by hexane–dioxane (85:15, + 0.1% citric acid) until equilibration was attained. All experiments were carried out at ambient temperature. The column dead volume ( $t_0$ ) was determined by injection of nonane, producing a solvent negative dip. Capacity factors were calculated from the equation  $k' = t_R/t_0 - 1$ . The solvents hexane, methanol and dioxane were of UV-grade and were equilibrated with air moisture prior to use. The test compounds were obtained from various chemical suppliers and were used as received.

## RESULTS AND DISCUSSION

### *Elution order*

The results are shown in Figs. 1 and 2.

On all the columns anisole is eluted first. The ability to form hydrogen bonding with the non-bonded electron pairs on the oxygen atom leads to the retention of this compound. The more polarized N–O bonds are responsible for the longer retention time of nitrobenzene. Diethyl phthalate and anthraquinone have similar  $k'$  values on the various phases. Both possess a polarized carbonyl function. Only the silica gel and the cyano- and polyphenol phases are able to resolve this pair of solutes (hexane–dioxane, 85:15). The elution order is reversed on the cyanopropyl phase, anthraquinone being eluted first. The strong hydrogen bonding capacity of alcohols (benzhydrol) is manifested in a higher capacity factor. For this solute the highest  $k'$  is obtained on Polyol-RSiL. Benzoic acid is highly retained on polyol- and polyphenol-bonded phases and it did not elute from the amino-bonded phase, as would be expected. The amide function of benzanilide, however, is the most polarized of the compounds examined. Here hydrogen bonds are possible through the acceptor character on the highly polarized carbonyl oxygen and the donor character of the N–H bond.

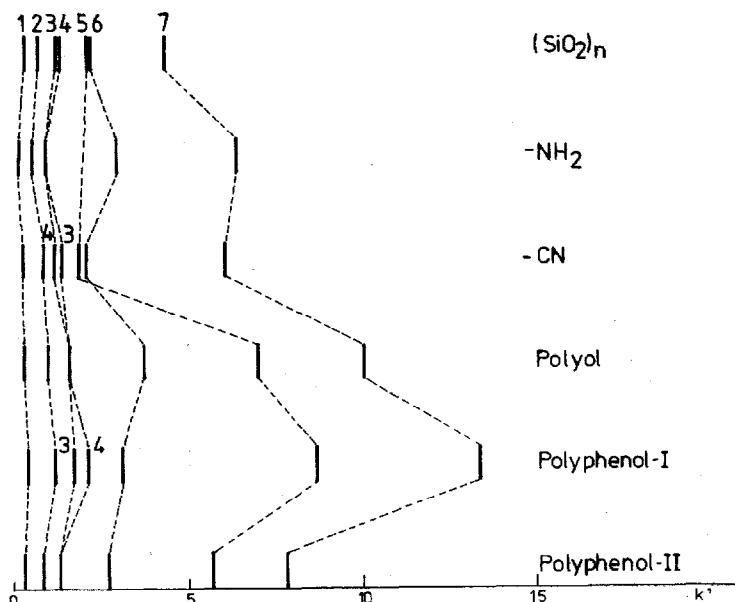


Fig. 1. Capacity factors ( $k'$ ) for anisole (1), nitrobenzene (2), diethyl phthalate (3), anthraquinone (4), benzhydrol (5), benzoic acid (6) and benzanilide (7) on six stationary phases.  $(\text{SiO}_2)_n$  = silica gel;  $-\text{NH}_2$  = aminopropylsilica gel;  $-\text{CN}$  = cyanopropylsilica gel; Polyol = polyol-bonded silica gel; Polyphenol-I = polyphenol-bonded silica gel; Polyphenol-II = cross-linked polyphenol phase. Columns:  $250 \times 4.1$  mm I.D. filled with the above materials of  $10 \mu\text{m}$  particle size. Mobile phase: hexane-dioxane (85:15, + 0.1% citric acid). Detection: UV (254 nm).

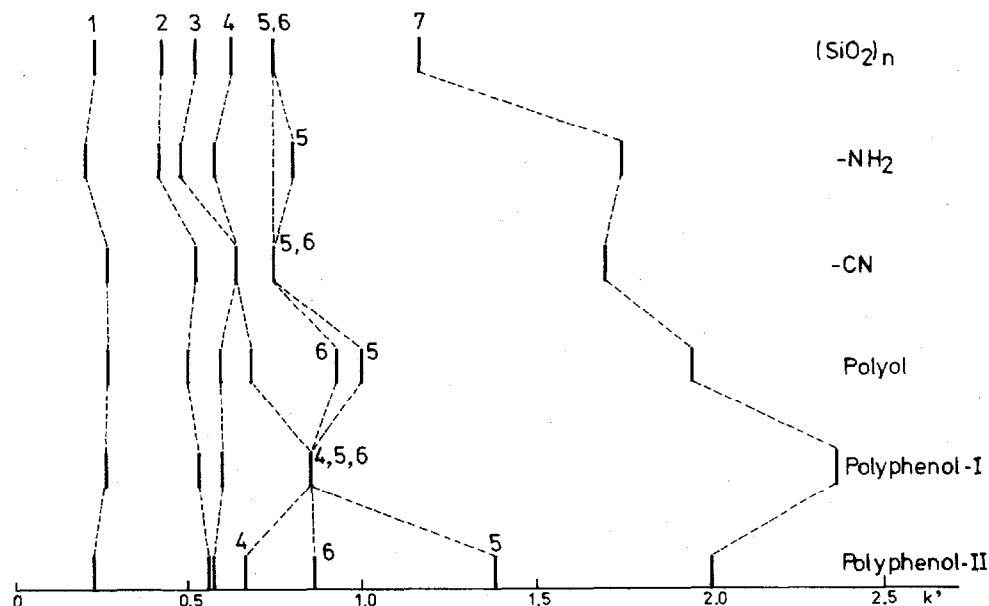


Fig. 2. As Fig. 1, with hexane-dioxane (70:30, + 0.1% citric acid) as the mobile phase.

The elution order, apparently following the hydrogen bond-forming capacity of the solutes, is therefore about the same on all the phases.

#### *Selectivity of the stationary phases*

Figs. 1 and 2 show the capacity factors of seven of the compounds, with hexane-dioxane compositions of 85:15 and 70:30, respectively, on six stationary phases. The separation of anthraquinone and diethyl phthalate is obviously the most difficult. This pair is not separated on the amino- and polyol phases. The resolution is best on Polyphenol-RSiL, which possesses phenol functions with a strong hydrogen bond-forming capacity. This results in a higher selectivity for the compound with the most polarized carbonyl function (anthraquinone). Strongly polar solutes such as benzoic acid and benzanilide are most retained on the polyol and polyphenol phases, which have the highest retention strength for polar solutes. The highest  $k'$  values are obtained on the phenolic phase, and this is therefore the most polar. As explained above, Polyphenol-RSiL is also a polyester and as such it is sensitive to low and high pH media and will even, in principle, hydrolyse or solvolyse in any medium containing OH or NH functions. In neutral media microgram amounts of gallic acid then appear in the eluent. Considering that a usual analytical column contains several hundred milligrams of bonded gallic acid, this does not influence noticeably the chromatographic retention, or only after a very long time. However, gallic acid is a strong UV absorber and may, under less favourable conditions, have a greater effect in gradient HPLC. To avoid this drawback of Polyphenol-RSiL two approaches were considered. The solvent can be composed with no or a minimum of solvolytically active components. This is what we have done here and is what is recommended by the manufacturer of Polyphenol-RSiL. Another approach we investigated is to cross-link the aromatic rings with terephthaldialdehyde. This does stabilize the phase against solvolysis, but unfortunately the polarity and hence the high selectivity for polar compounds are considerably decreased. The cross-linked phase was also included in this investigation.

With a mobile phase containing hexane-dioxane (70:30), complete resolution of the polarity mixture is achieved only on the polyol-bonded phase and on the cross-linked phenolic phase. The resolution of nitrobenzene and the phthalate is best on Polyol-RSiL. With this mobile phase combination the polyol-bonded phase is clearly the most powerful. This is an interesting example of solvent selectivity: with another mobile phase on the same stationary phase a completely new selectivity is introduced.

#### *Capacity factor ( $k'$ ) versus $X_S$*

For eight solutes  $\log k'$  was plotted against  $\log X_S$  for dioxane in the binary system hexane-dioxane. Slopes, intercepts and correlation coefficients of the best-fitting straight line were calculated using the method of least squares. The values of  $n$  (slope),  $c$  (intercept) and  $r$  (correlation coefficient) are presented in Table I. The graphical relationships for the different solutes are shown in Figs. 3-10. The low  $r$  value for anisole is caused by small retention time variations which, for this early eluting compound, lead to large errors in the  $k'$  values. This compound was therefore not used as a standard to determine the polarity of the different stationary phases. The correlation coefficients were in general higher than 0.98, except on the silica gel

TABLE I

SLOPES ( $n$ ), INTERCEPTS ( $c$ ) AND CORRELATION COEFFICIENTS ( $r$ ) FOR EIGHT SOLUTES ON THE DIFFERENT STATIONARY PHASES AS CALCULATED FROM THE FIGS. 3-10

Solute	Parameter	Stationary phase					
		(SiO <sub>2</sub> ) <sub>n</sub>	-NH <sub>2</sub>	-CN	Polyol	Polyphenol-I	Polyphenol-II
Anisole	$n$	0.84	0.14	0.69	0.60	0.58	0.70
	$c$	0.88	0.81	0.84	0.83	0.83	0.91
	$r$	0.74	0.38	0.81	0.87	0.94	0.95
Nitrobenzene	$n$	1.25	0.81	1.12	1.25	1.25	0.89
	$c$	0.81	0.74	0.72	0.79	0.74	0.62
	$r$	0.90	0.98	0.97	0.99	1.00	0.97
Diethyl phthalate	$n$	1.65	1.22	1.47	1.67	1.65	1.45
	$c$	0.88	0.82	0.77	0.88	0.85	0.81
	$r$	0.95	0.98	0.99	0.99	1.00	0.99
Anthraquinone	$n$	1.44	1.09	1.38	1.51	1.55	1.38
	$c$	0.72	0.70	0.73	0.76	0.67	0.76
	$r$	0.94	0.98	0.97	0.99	1.00	0.98
Acetophenone	$n$	1.20	0.69	1.16	1.16	1.21	1.02
	$c$	0.73	0.66	0.69	0.74	0.67	0.69
	$r$	0.90	0.96	0.98	0.99	1.00	0.98
Benzhydrol	$n$	1.88	0.49	1.95	2.14	1.98	1.85
	$c$	0.81	0.95	0.90	0.84	0.80	0.78
	$r$	0.97	0.99	0.99	1.00	0.99	1.00
Benzoic acid	$n$	1.88	—	1.72	2.63	3.79	2.29
	$c$	0.81	—	0.80	0.87	1.52	0.75
	$r$	0.97	—	0.99	0.98	1.00	1.00
Benzanilide	$n$	2.31	2.24	2.19	2.55	2.60	2.30
	$c$	0.77	0.66	0.61	0.64	0.59	0.60
	$r$	0.98	1.00	1.00	1.00	1.00	1.00

column for nitrobenzene (0.90), diethyl phthalate (0.95), anthraquinone (0.95) and acetophenone (0.90). A possible explanation is the more difficult equilibration of the silica gel column, which needs longer rinsing when changing to another solvent. Equilibration is generally quicker on chemically bonded phases, which makes them more attractive than silica gel itself for normal-phase chromatography.

The fact that the new bonded phases follow the same mathematical relationship as the other phases is an indication that the adsorption-desorption process is also the main retention mechanism for these phases. Because retention is related to hydrogen bonding capacity, this hydrogen bond must be a leading interaction in the adsorption-desorption equilibrium at the adsorbent surface. Competition between the polar solvent and the solute for the active sites on the stationary phase must be considered in this context.

To determine the polarity of the various stationary phases, the  $c$  and  $n$  values (intercept and slope in eqn. 1) for a given solute on the silica gel column are taken as standard reference values. The  $c$  and  $n$  values of a solute on a bonded phase are

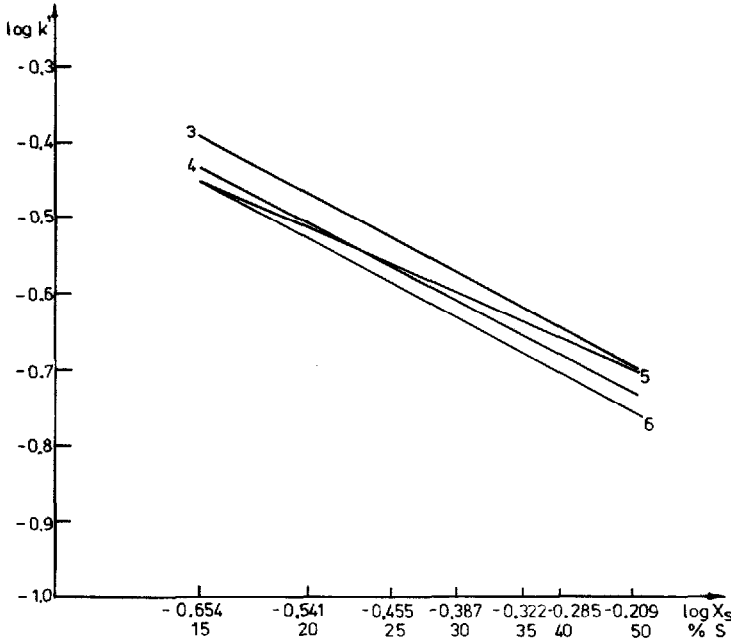


Fig. 3. Correlation between the logarithm of the capacity ratio ( $k'$ ) and the logarithm of the molar fraction of the stronger solvent ( $X_s$ ) in the binary system hexane-dioxane on the different stationary phases. 1 =  $(\text{SiO}_2)_n$ ; 2 =  $-\text{NH}_2$ ; 3 =  $-\text{CN}$ ; 4 = Polyol; 5 = Polyphenol; 6 = cross-linked polyphenol. Solute: anisole. Data for stationary phases 1 and 2 are not shown, because anisole is hardly retained on these phases.

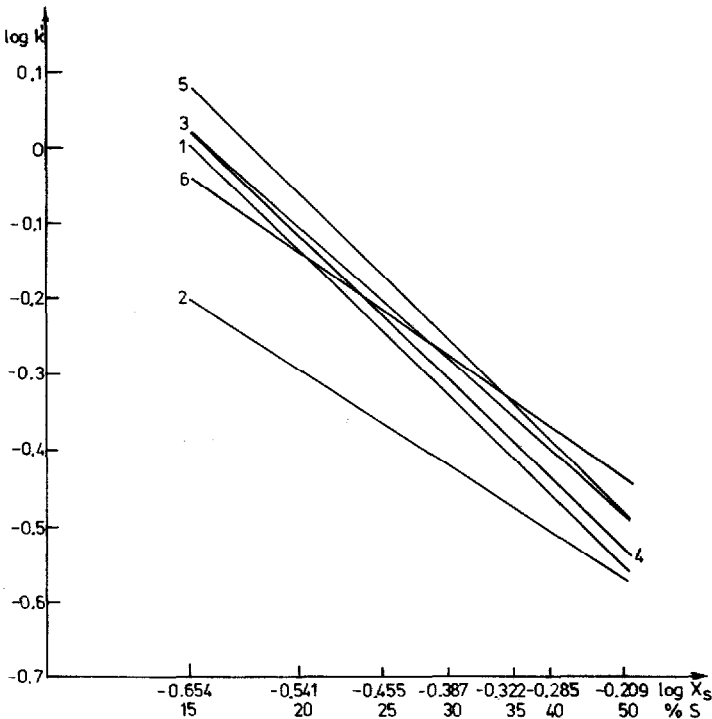
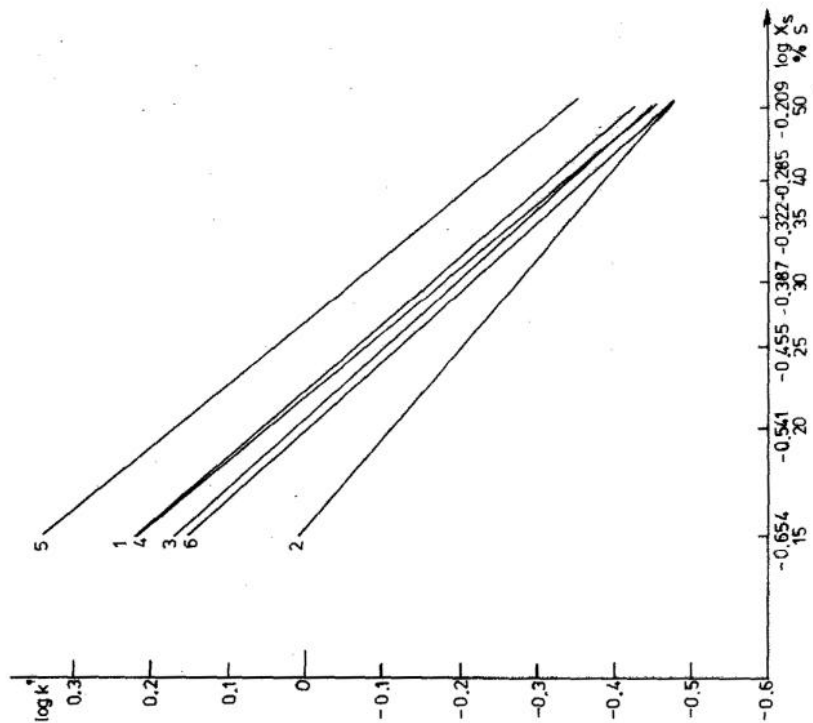
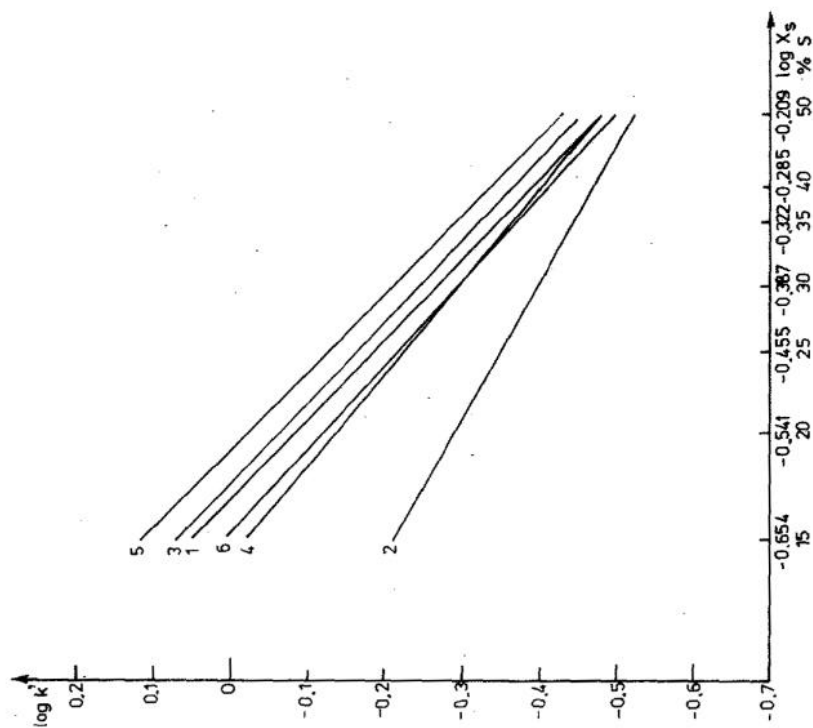


Fig. 4. Correlation of  $\log k'$  with  $\log X_s$  with nitrobenzene as solute. Stationary phases as in Fig. 3.

Fig. 5. Correlation of  $\log k'$  with  $\log X_s$  with acetophenone as solute. Stationary phases as in Fig. 3.Fig. 6. Correlation of  $\log k'$  with  $\log X_s$  with anthraquinone as solute. Stationary phases as in Fig. 3.



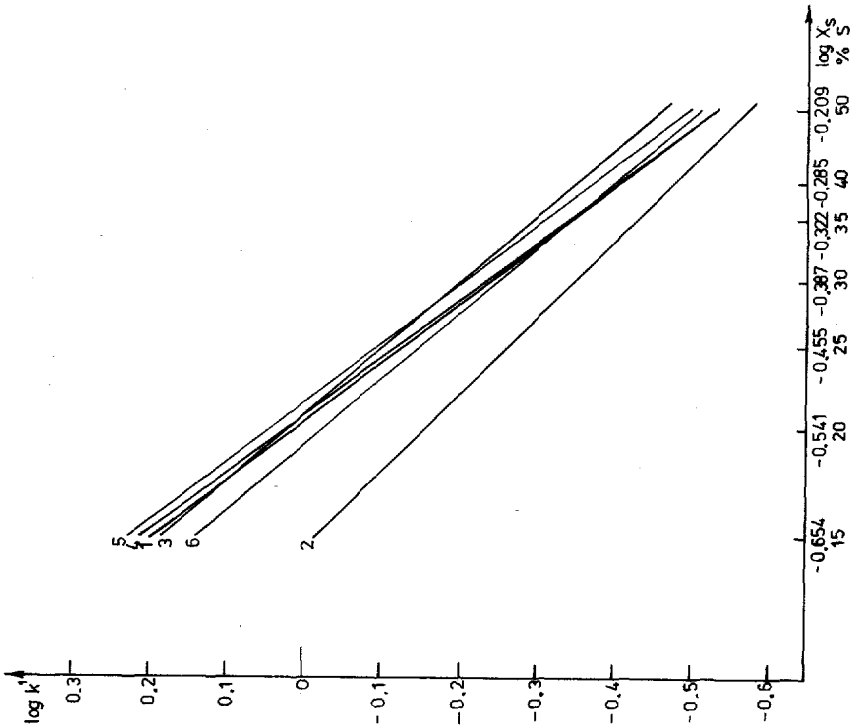
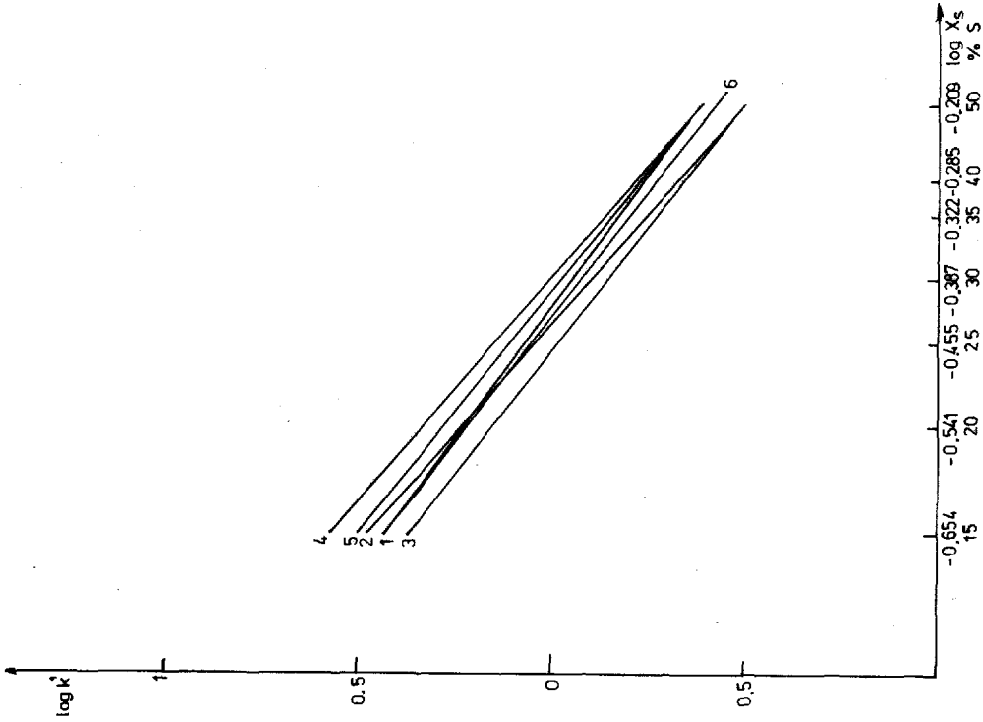


Fig. 7. Correlation of  $\log k'$  with  $\log X_s$  with diethyl phthalate as solute. Stationary phases as in Fig. 3.

Fig. 8. Correlation of  $\log k'$  with  $\log X_s$  with benzhydrol as solute. Stationary phases as in Fig. 3.

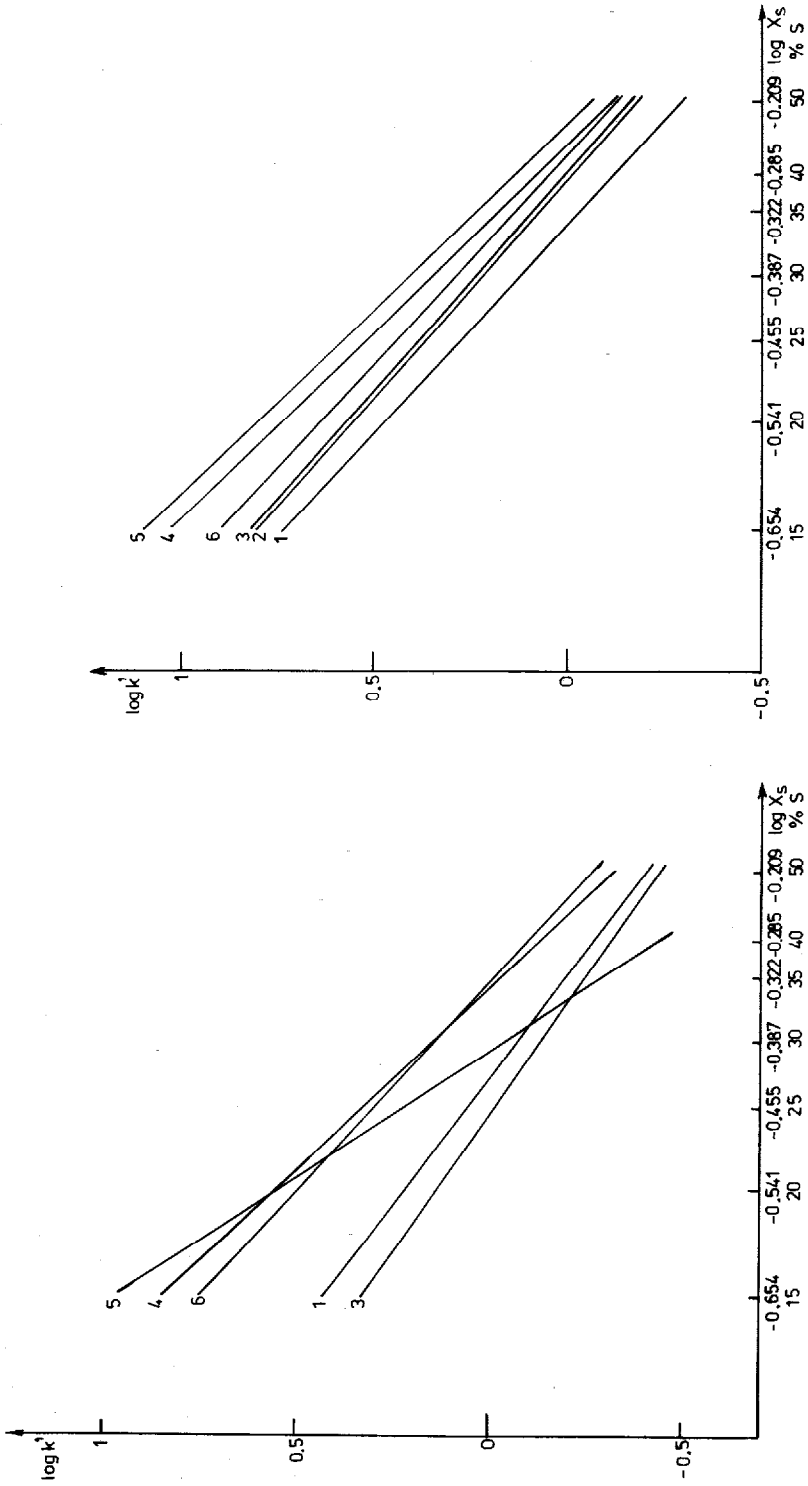


Fig. 9. Correlation of  $\log k'$  with  $\log X_s$  with benzoic acid as solute. Stationary phases as in Fig. 3.

Fig. 10. Correlation of  $\log k'$  with  $\log X_s$  with benzamide as solute. Stationary phases as in Fig. 3.

divided by the corresponding values of the silica column. These  $c$  and  $n$  ratios ( $c'$  and  $n'$ ) are given in Table II. Ratios larger than 1 are an indication of a higher polarity of the bonded phase for this compound, and a ratio smaller than 1 is an indication of a lower polarity (relative to silica gel). The mean values (Table II) of the ratios are a measure of the polarity of a phase relative to silica gel. The amino-bonded phase column ( $n' = 0.66$  and  $c' = 0.99$ ) is less polar than silica gel. The cyano-bonded phase column ( $n' = c' = 0.95$ ) is also less polar. Polyol-bonded silica gel ( $n' = 1.09$  and  $c' = 1.04$ ) is slightly more polar. The most polar, however, is the Polyphenol-RSiL ( $n' = 1.18$  and  $c' = 1.06$ ). The cyano-bonded phase has a selectivity closest to that of silica gel. This could be predicted from Fig. 1, in which corresponding  $k'$  values do not differ much. The  $c$  and  $n$  values of a stationary phase run parallel except for the amino-bonded phase, where  $n'$  (0.66) is much smaller than  $c'$  (0.99). Here the first four solutes (see Fig. 1) are eluted earlier than on other phases. In the system used, the amino function of the amino-bonded phase must be largely protonated (citric acid). How this affects hydrogen bond formation is not clear and anyway is outside the scope of this paper.

The polyphenol-bonded silica possesses the largest  $n'$  and  $c'$  values and is thus the most polar with the highest retentivity. After cross-linking the phase a decrease in polarity is induced, which results in a decrease in retentivity. The cross-linked polyphenol phase has a lower polarity than silica gel ( $n' = 0.94$  and  $c' = 0.90$ ).

#### *Values of $n$ and $c$*

In Soczewinski's Model II the slope  $n$  should be 1.0 for monofunctional solutes and solvents<sup>7,16</sup>. The value can nevertheless be higher if solute molecules are large enough to displace vicinal solvent molecules or if polar groups in the solute molecule are competitively solvated by a polar solvent. For bi- and polyfunctional solutes, higher  $n$  values can occur. Theoretically, a molecule possessing  $x$  substituents can simultaneously interact with  $x$  adsorption sites on the surface of the stationary phase. When a larger number of functional groups are present the scattering of the  $n$  and  $c$  values is possibly due to very specific solute-solvent interactions<sup>17</sup>.

For nitrobenzene the  $n$  values (for the different phases) are close to unity: one solvent molecule is exchanged by one solute molecule. Diethyl phthalate shows values of 1.4–1.6, except on amino-bonded silica gel (see Table I). This could mean that an average of three solvent molecules (dioxane) are exchanged by two phthalate molecules (bifunctional). The monofunctional acetophenone gives slope close to unity and the bifunctional ketone (anthraquinone) gives  $n$  values of 1.3–1.5, indicating a higher exchange ratio. Although benzhydrol is monofunctional, the  $n$  values vary around 2.0 with a maximum for polyol-bonded silica (2.1). A very low value is found for the amino bonded-phase column (also for other solutes). Benzoic acid is different. On silica gel (1.9) and on the cyano-bonded phase (1.7) an average of 1 for 2 to 2 for 3 (solute for solvent) exchange apparently occurs. On polyol-RSiL ( $n = 2.6$ ) a higher value is reached, indicating strong hydrogen bond(s) between the carboxylic acid function and the bonded phase. On the polyphenol phase an even higher value ( $n = 3.8$ ) is reached. Possibly adsorption occurs in the solvated state. For benzanilide all  $n$  values are higher than 2.0. This is expected because the amide function is known to behave as both a donor and an acceptor for hydrogen bonds and can therefore be considered as bifunctional.

TABLE II  
 CONSTANTS ( $c$  AND  $n$ ) AND CONSTANT RATIOS ( $c'$  AND  $n'$ ) OF THE LINEAR RELATIONSHIP BETWEEN RETENTION DATA AND SOLVENT  
 COMPOSITION OF DIOXANE IN HEXANE FOR SILICA GEL AND CHEMICALLY BONDED PHASES

$c$  and  $n$  = intercept and slope of eqn. 1;  $c'$  and  $n'$  =  $c$ - and  $n$ -ratios relative to non-bonded silica gel as standard.

Solute	Constants		Constant ratios						Polyphenol-I		Polyphenol-II		
	$(\text{SiO}_2)_n$	$c$	$-NH_2$	$n'$	$c'$	$-CN$	$n'$	$c'$	Polyol	$n'$	$c'$	$n'$	$c'$
Nitrobenzene	1.25	0.81	0.65	0.90	1.08	0.88	1.00	0.98	1.00	1.00	0.92	0.71	0.77
Diethyl phthalate	1.65	0.88	0.74	0.89	0.94	0.87	1.01	1.00	1.00	1.00	0.96	0.88	0.92
Anthraquinone	1.44	0.72	0.76	0.96	0.98	1.02	1.05	1.06	1.08	1.08	0.94	0.96	1.06
Acetophenone	1.20	0.73	0.58	0.97	0.91	0.95	0.96	1.02	1.01	0.92	0.92	0.86	0.95
Benzoic acid	1.88	0.81	—	0.92	—	0.99	1.40	1.07	2.01	1.89	1.22	0.99	0.93
Benzamide	2.31	0.77	0.97	0.95	0.86	0.79	1.10	0.84	1.12	0.77	0.99	0.78	0.78
Benzhydrol	1.88	0.81	0.26	1.04	1.17	1.12	1.14	1.04	1.05	0.99	0.99	0.98	0.96
Mean values			0.66	0.95	0.99	0.95	1.09	1.04	1.18	1.06	1.06	0.94	0.90
Standard deviation ( $\sigma_{r-1}$ )			0.24	0.051	0.12	0.11	0.15	0.078	0.37	0.37	0.37	0.16	0.11

The  $c$  values ( $\log k' = c$  for 100% stronger solvent) do not scatter much, except with benzoic acid on Polyphenol-RSiL, on which it is about double that on the other phases.

#### CONCLUSION

Retention of polar solutes in normal-phase chromatography on silica gel and on derivatized silica gels is mainly determined by hydrogen bond formation. The different polarities of the phases, evident from differences in retention times, can be expressed by the ratios of the  $n$  values of eqn. 1 relative to silica gel. Thus a polarity figure is obtained that is higher for more polar phases. Polyphenol-RSiL (polarity factor 1.18) and also to a lesser extent Polyol-RSiL (polarity factor 1.09) are more polar than the usual adsorption phases.

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