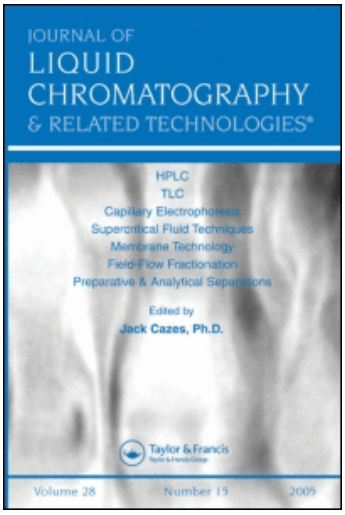

Journal of Liquid Chromatography & Related Technologies

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Effect of the Mobile Phase on Solute Retention in Liquid-Solid Chromatography

S. M. Petrović[a]; M. Špika[a]

[a] Institute of Microbiological Processes and Applied Chemistry University of Novi Sad Bul, Novi Sad, Yugoslavia

To cite this Article Petrović, S. M. and Špika, M.(1991) 'Effect of the Mobile Phase on Solute Retention in Liquid-Solid Chromatography', Journal of Liquid Chromatography & Related Technologies, 14: 16, 3061 — 3076

To link to this Article: DOI: 10.1080/01483919108049376

URL: http://dx.doi.org/10.1080/01483919108049376

JOURNAL OF LIQUID CHROMATOGRAPHY, 14(16&17), 3061-3076 (1991)

EFFECT OF THE MOBILE PHASE ON SOLUTE RETENTION IN LIQUID-SOLID CHROMATOGRAPHY

S. M. PETROVIĆ AND M. ŠPIKA
Institute of Microbiological Processes and Applied Chemistry
University of Novi Sad
Bul. AVNOJa 1
21000 Novi Sad, Yugoslavia

ABSTRACT

Utilizing retention data estimated by HPTLC on silica and activity coefficients in the non-aqueous mobile phase determined on the basis of saturation solubility of solutes, the effect of the mobile phase on retention of a set of structurally different solutes was studied. A quadratic relationship between the logarithm of retention factors or activity coefficients and the volume fraction of ethyl acetate in heptane - ethyl acetate solution was observed, suggesting a common retention machanism in liquid chromatography. The retention and/or relative retention of a solute was affected by both the mobile and the stationary phase. The magnitude of these effects depended merely on the molecular structure of a solute.

INTRODUCTION

The retention in liquid chromatography (LC) is determined by the interactions of a solute with both mobile and stationary phase of the system. A limiting model, usually employed to describe the experimental retention data of liquid-solid chromatography (LSC) and widely known in the chromatographic literature as the displacement model, was proposed by Snyder (1) and Soczewinski (2). According to this model, based on the law of mass action, a solute is distributed between a mobile and a stationary phase as a result of a competitive solute and solvent adsorption. In LSC with mixed mobile phases this competitive adsorption can be expressed by the exponential equation (2,3):

$$\ln k' = \ln k'_o - n \ln \varphi \qquad (1)$$

where $k' = (1/R_f) - 1$ is the capacity factor, φ is the volume fraction of the polar moderator in a non-aqueous binary solution, and k'_o and n are constants.

Let us consider that processes occur on non-adsorbents (cellulose, polyamid, alkyl-aryl silica derivatives etc.) also belong to LSC, then a second limiting model of LSC is that assuming distribution of a solute between a mobile and a stationary phase as a consequence of classical partitioning. Equations describing partitioning of a solute between both phases are quite analogous to those used in liquid-liquid chromatography. These equations, derived from the solubility parameter theory (4), can be written in the following

forms (5,6):

$$\ln k' = m\varphi^2 + p\varphi + q \quad (2)$$

or simpler:

$$\ln k' = q - p\varphi \quad (3)$$

Here, φ is the volume fraction of organic modifier in an aqueous mobile phase and m, p and q are constants.

Any of these models can not distinguish interactions of a solute with a mobile and a stationary phase separately. However, the solution of this problem offers an equation which correlates the chromatographic retention factor k' and the thermodynamic equilibrium constant K_{th} by:

$$k' = K_{th}\Phi = \frac{\gamma^m}{\gamma^s}\Phi \quad (4)$$

where γ is the Raoult-law activity coefficient of the solute in the mobile (m) and the stationary (s) phase, and Φ is the so-called phase ratio. It was argued previously (7) that the best correlation between the experimental and calculated γ^m values and φ for non-aqueous mobile phases was obtained when they are related by the equation:

$$\ln \gamma^m = m'\varphi^2 + p'\varphi + q' \quad (5)$$

where m', p' and q' are constants.

In this paper retention data measured by HPTLC on silica were correlated with the activity coefficients based on the saturation solubility of a set of structurally different solutes in the non-aqueous mobile phase, in order to examine the effect of the mobile phase on a solute retention and relative retention in LSC.

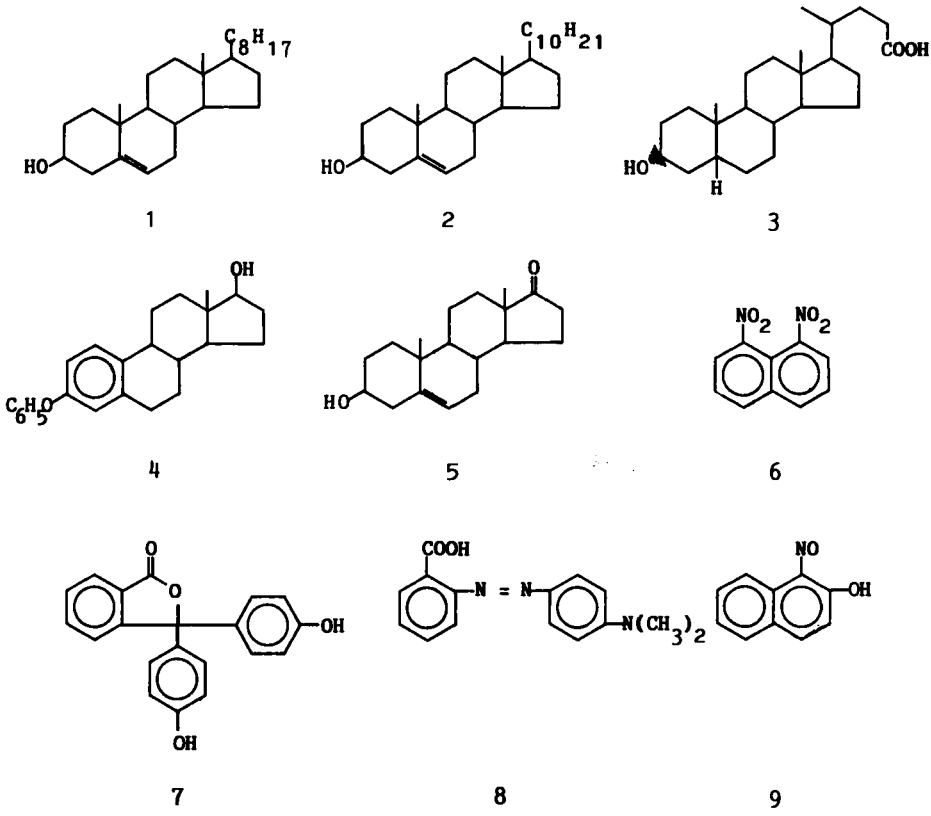

FIGURE 1. Structural formulas of the compounds examined. 1- Cholesterol, 2- β-Sitosterol, 3- Lithocholic acid, 4- Estradiolbenzoate, 5- Dehydroepiandrosterone, 6- 1,8-Dinitronaphthalene, 7- Phenolphthalein, 8- Methyl Red, 9- 1-Nitroso-2-naphthol.

EXPERIMENTAL

Determination of activity coefficients in the mobile phase

Appropriate quantity of a compound examined (Fig. 1) was dissolved to saturation in volumetrically prepared heptane - - ethyl acetate mixtures, containing 0, 20, 40, 60, 80, and

100% (v/v) of ethyl acetate, by shake-flask method. The saturation concentration of a compound was determined gravimetrically in an aliquot of saturated solution after the removal of solvent by vacuum evaporation. At least three determinations for each compound and solvent composition were made. Heptane and ethyl acetate were from E. Merck (Darmstadt, F.R.G.) and solutes examined from various sources. All chemicals were of analytical grade purity.

The activity coefficients of solutes in the mobile phase, γ^m, were calculated using the following expression:

$$\gamma^m = \frac{n_m}{c_m} \qquad (6)$$

where n_m is the total solvent concentration, i.e. the total number of heptane and ethyl acetate moles per liter of solvent mixture, and c_m is the solute concentration in mole/l (Table 1).

Chromatographic procedure

Silica 60 HPTLC glass plates (E. Merck) were used. Chromatograms were run by ascending technique in heptane - ethyl acetate mobile phase of various compositions (Table 2). At least three chromatograms of each composition were made.

The spots of steroids were visualized in usual way by sulphuric acid - methanol solution, and phenolphthalein with aqueous ammonia.

RESULTS AND DISCUSSION

The experimental retention and activity coefficients data were correlated using eqns. 2, 4 and 5. The saturation con-

centrations of examined solutes in heptane - ethyl acetate solution of various compositions and respective ln γ^m values, calculated according to eqn. 6, are presented in Table 1. The retention data of solutes are shown in Table 2. No separation of cholesterol and β-sitosterol was obtained. In Tables 1 and 2 and eqns. 2 and 5 φ denotes the volume fraction of ethyl acetate in binary mixtures.

As it has been expected, ln γ^m values from Table 1 fit very well eqn. 5 (Figs. 2 and 3). Mean relative deviation* between experimental and calculated ln γ^m values using eqn. 5 was 1.09 ± 1.07%. However, data from Table 2 fit well both eqns. 1 and 2 (Figs. 2 and 3), at least in the φ region between 0.1 and 0.9, when the silica surface is completely covered by the ethyl acetate molecules (8). Mean relative deviation of eqns. 1 and 2 was 4.53 ± 8.84 and 5.56 ± 6.55%, respectively. The fact that retention data also fit eqn. 2 indicates that the saturation with moderator molecules quantitatively changes the properties of the silica surface, suggesting a common retention machanism in LC. If this is thrue, then eqn. 1 is a special case in LC.

The effect of each phase on retention can be distinguished by combining eqns. 2, 4 and 5. The following equation is obtained:

$$\ln(\gamma^S/\Phi) = (m'-m)\varphi^2 + (p'-p)\varphi + q'-q = \Delta m\varphi^2 + \Delta p\varphi + \Delta q \qquad (7)$$

---

*Relative deviation = $\frac{\ln \gamma^m_{ex} - \ln \gamma^m_{calc}}{\ln \gamma^m_{ex}} \times 100$

TABLE 1

Saturation Concentrations, c_m (mole/l), of the Test Solutes in Heptane - Ethyl Acetate Solutions and Respective ln γ^m Values. φ ▫ Volume Fraction of Ethyl Acetate.

φ	$c_m \times 10^4$	ln γ^m	$c_m \times 10^4$	ln γ^m	$c_m \times 10^4$	ln γ^m
	CHOLESTEROL		β-SITOSTEROL		LITHOCHOLIC ACID	
0	279.0	5.4994	455.7	5.0086	19.4	8.1653
0.2	1176.5	4.1552	1651.6	3.8161	42.5	7.4761
0.4	2580.0	3.4567	3693.4	3.0980	58.4	7.2450
0.6	2660.0	3.5060	3259.1	3.3029	95.6	6.8319
0.8	2012.0	3.8591	2129.8	3.8022	119.5	6.6827
1.0	1054.0	4.5745	846.6	4.7936	132.8	6.6460
	ESTRADIOL-BENZOATE		DEHYDROEPI-ANDROSTERONE		1,8-DINITRO-NAPHTHALENE	
0	15.1	8.4159	34.5	7.5893	4.6	9.6080
0.2	55.8	7.2038	203.8	5.9084	18.3	8.3170
0.4	177.0	6.1361	737.9	4.7085	71.4	7.0440
0.6	274.0	5.7789	1386.8	4.1573	210.0	6.0450
0.8	419.0	5.4281	1754.3	3.9962	451.0	5.3545
1.0	486.0	5.3486	2229.3	3.8254	715.0	4.9625
	PHENOLPHTHALEIN		METHYL RED		1-NITROSO-2-NAPHTHOL	
0	1.8	10.5495	14.9	8.4326	86.6	6.6689
0.2	8.4	9.0938	22.3	8.1219	320.0	5.4572
0.4	34.6	7.7696	29.7	7.9208	1005.0	4.3995
0.6	119.6	6.6079	37.1	7.7777	2636.0	3.5150
0.8	333.5	5.6563	44.6	7.6692	5219.0	2.9059
1.0	628.3	5.0918	48.2	7.6605	7318.0	2.6467

TABLE 2

R_f and ln k' Values of the Test Solutes in Heptane - Ethyl Acetate Mobile Phase. φ = Volume Fraction of Ethyl Acetate.

φ	R_f	ln k'	R_f	ln k'	R_f	ln k'	R_f	ln k'
	CHOLESTEROL+ β-SITOSTEROL		LITHOCHOLIC ACID		ESTRADIOL-BENZOATE		DEHYDROEPI-ANDROSTERONE	
0.1	0.09	2.314						
0.2	0.28	0.944			0.13	1.902	0.16	1.658
0.3	0.46	0.161	0.10	2.197	0.25	1.098	0.33	0.709
0.4	0.59	-0.363	0.21	1.324	0.42	0.322	0.50	0
0.5	0.70	-0.847	0.34	0.663	0.55	-0.200	0.64	-0.576
0.6	0.77	-1.209	0.47	0.120	0.62	-0.490	0.75	-1.098
0.7			0.58	-0.322	0.67	-0.709	0.80	-1.386
0.8			0.68	-0.753				
0.9			0.75	-1.098				
	1,8-DINITRO-NAPHTHALENE		PHENOLPHTHALEIN		METHYL RED		1-NITROSO-2-NAPHTHOL	
0.1							0.16	1.658
0.2	0.04	3.178					0.27	0.995
0.3	0.13	1.902	0.06	2.752			0.40	0.405
0.4	0.25	1.098	0.16	1.658	0.15	1.734	0.54	-0.161
0.5	0.40	0.405	0.30	0.847	0.24	1.154	0.65	-0.619
0.6	0.55	-0.200	0.47	0.120	0.33	0.709	0.73	-0.995
0.7	0.66	-0.663	0.63	-0.532	0.43	0.281	0.79	-1.323
0.8	0.74	-1.045	0.73	-0.995	0.51	-0.039	0.83	-1.586
0.9			0.81	-1.450	0.59	-0.364		

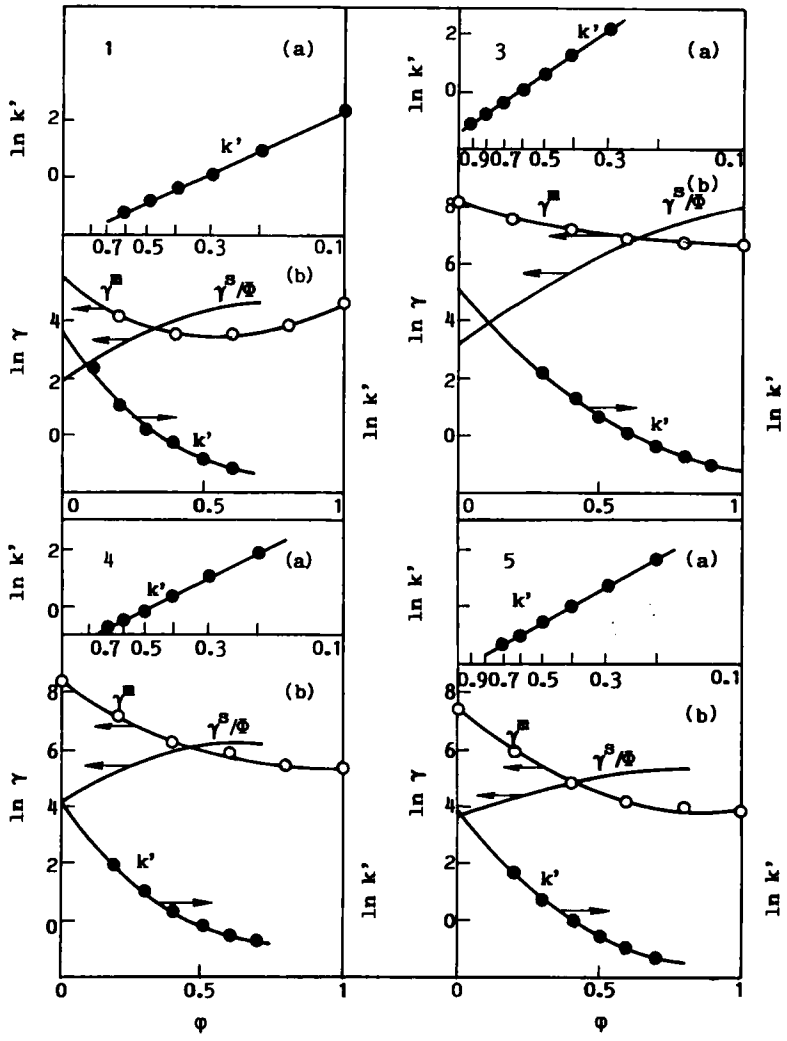

FIGURE 2. Plots of ln k' vs. ln φ (eqn. 1) (a) and ln k', ln γ^m and ln(γ^S/Φ) vs. φ (eqns. 2, 5 and 7) (b) for examined solutes. Designation of solutes is as in Fig. 1.

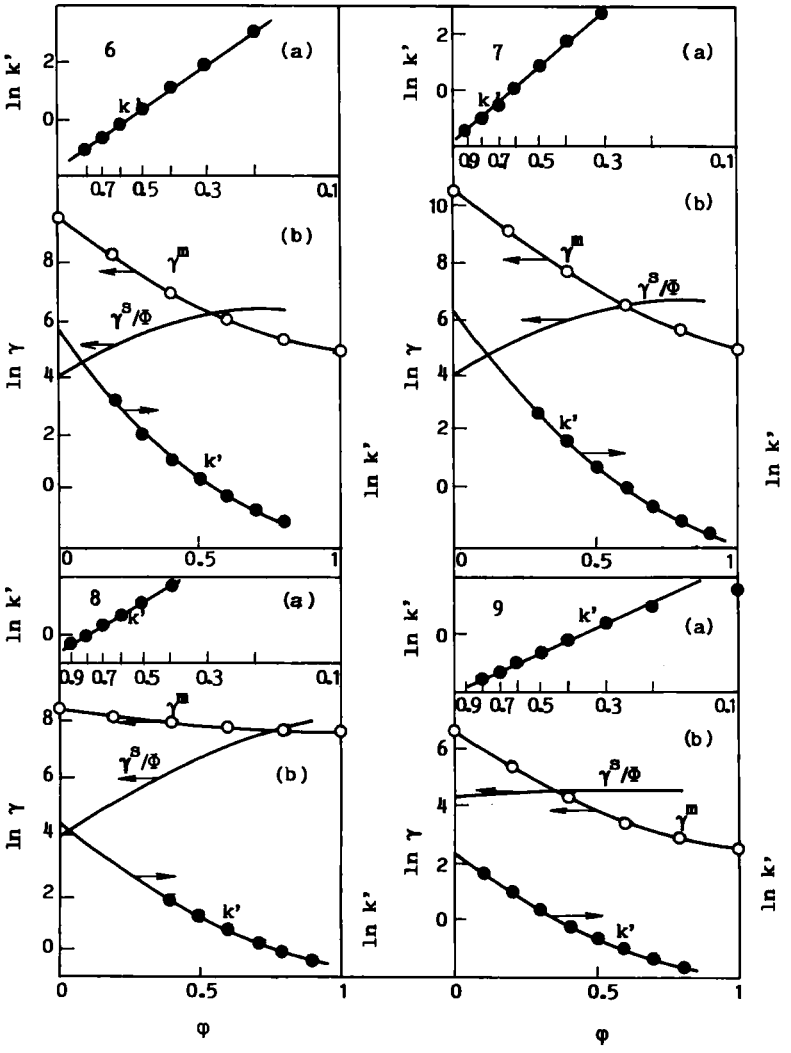

FIGURE 3. Plots as in Fig. 2. Designation of solutes is as in Fig. 1.

TABLE 3

Constants of Eqns. 2, 5 and 7 for the Test Compounds.

Solute	m	p	q	m'	p'	q'	Δm	Δp	Δq
Cholesterol	11.136	-14.513	3.557	6.475	-7.256	5.429	-4.661	7.257	1.872
β-Sitosterol	"	"	"	7.049	-7.179	4.975	-4.087	7.334	1.418
Lithocholic acid	4.752	-11.069	5.045	1.603	-3.087	8.130	-3.150	7.982	3.086
Estradiol-benzoate	8.695	-13.066	4.186	3.808	-6.811	8.395	-4.886	6.254	4.209
Dehydroepi-androsterone	7.338	-12.609	3.870	5.226	-8.813	7.521	-2.107	3.797	3.651
1,8-Dinitro-naphthalene	7.561	-14.381	5.666	3.047	-7.777	9.660	-4.514	6.604	3.994
Phenol-phthalein	6.054	-14.153	6.413	2.655	-8.192	10.584	-3,489	5.961	4.171
Methyl Red	3.170	-8.263	4.518	0.840	-1.605	8.425	-2.330	6.637	3.907
1-Nitroso-2-naphthol	3.476	-7.765	2.405	2.905	-7.005	6.701	-0.571	0.760	4.297

Constants of eqns. 2, 5 and 7 are presented in Table 3. As constant p determines the slope and constant m the curvature of a parabola, it seems that constant p is a good measure of the mobile and the stationary phase effects on retention. Thus, constants p' and Δp in Table 3 will be considered.

From data presented in Table 3 it can be seen that the retention and/or relative retention of a solute in LSC is affected by both the mobile and the stationary phase. The magnitude of these effects, under the same conditions, depends merely on the molecular structure of a solute. For example, constants p' and Δp for cholesterol, β-sitosterol

and estradiolbenzoate are approximately equal, indicating the same effect of both phases on the retention, if we assume that the phase ratio, Φ, for φ values above 0.1 is constant. For dehydroepiandrosterone, 1,8-dinitronaphthalene, phenolphthalein, and especially for 1-nitroso-2-naphthol the effect of the mobile phase on retention is more expressive in comparison with the effect of the stationary phase. For instance, $\ln(\gamma^S/\Phi)$ values of 1-nitroso-2-naphthol are approximately constant for φ between 0.1 and 0.8 (Fig. 4). On the other hand, for lithocholic asid and methyl red constant Δp is significantly higher than constant p', i.e. the effect of stationary phase predominates the effect of mobile phase for these solutes.

A good example for the effect of mobile phase on retention is the resolution of methyl red and phenolphthalein as a function of φ. The $\ln \gamma^m$ functions (eqn. 5) of methyl red and phenolphthalein intersect each other approximately at $\varphi = 0.4$ (Fig. 4). At this φ no resolution of methyl red and phenolphthalein was obtained, i.e. the selectivity factor α for the pair methyl red/phenolphthalein is equal to 1.08. Similarly, the selectivity factor in the mobile phase α^m for the same solute pair is equal to 1.16. As α and α^m are related to each other by (9,10):

$$\alpha = \frac{k'_2}{k'_1} = \frac{\gamma_2^m \gamma_1^s}{\gamma_1^m \gamma_2^s} = \frac{\alpha^m}{\alpha^s} \qquad (8)$$

where, by definition, subscript 2 denotes more retained solute, the selectivity factor in the stationary phase α^s for

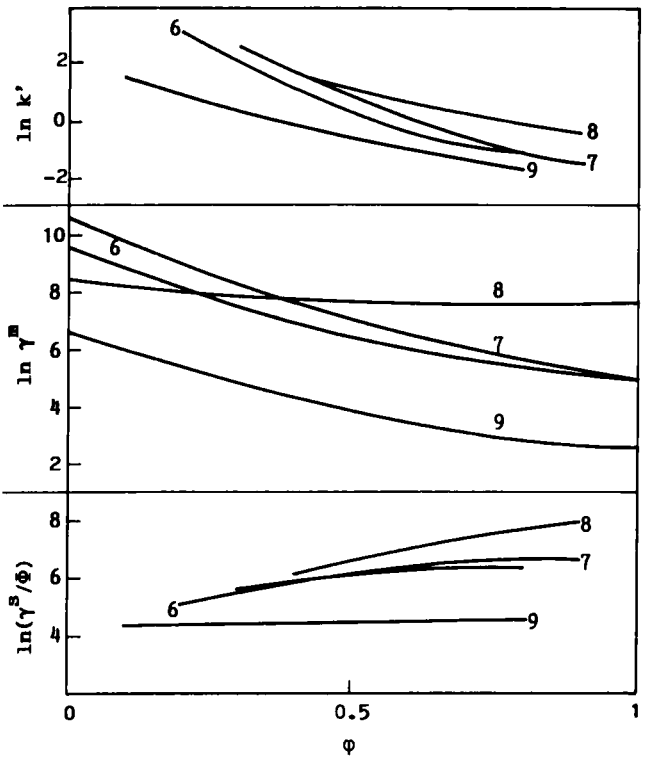

FIGURE 4. Plots of ln k', ln γ^m and ln(γ^S/Φ) vs. φ for examined solutes. Designation of solutes is as in Fig. 1.

the solute pair methyl red/phenolphthalein at φ = 0.4 is equal to 1.07. However, for example, at φ = 0.8 these two solutes are clearly resolved, and α, α^m and α^S are equal to 2.60, 7.48 and 2.88, respectively. The effect of the mobile phase on the separation of methyl red and phenolphthalein is evident. Generally, for most of the solute pairs and φ values α^m is higher than α^S.

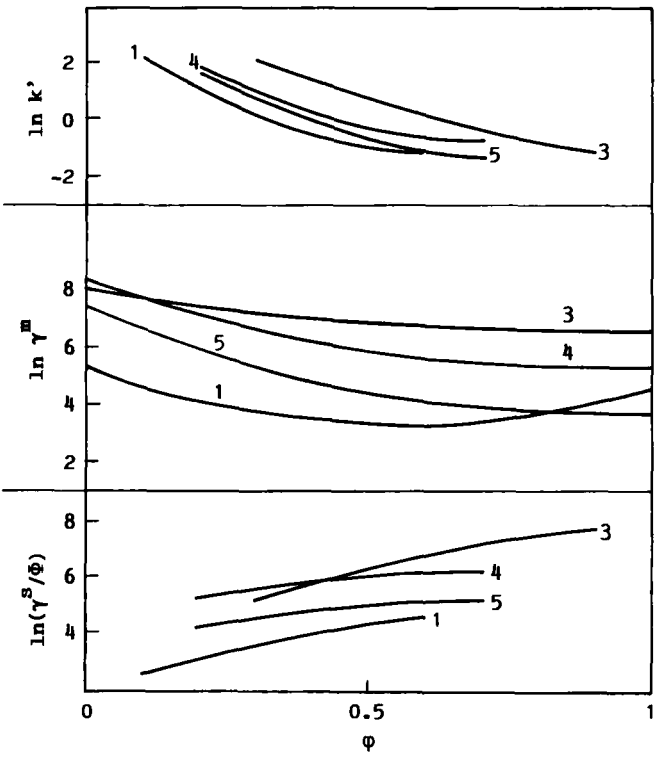

FIGURE 5. Plots as in Fig. 4. Designation of solutes is as in Fig. 1.

According to the displacement theory of Snyder and Soczewinski (1,2) more polar solutes are more retained in LSC in comparison with less polar solutes due to their higher affinity toward the adsorbent surface. Thus, for example, more polar lithocholic acid is more retained at any φ than less polar cholesterol, due to its higher affinity to the silica surface. On the basis of data presented here, cholesterol, for instance, is more soluble in the heptan - ethyl

acetate mobile phase than lithocholic acid at any φ. However, at the same time less polar cholesterol demonstrates higher affinity to the stationary phase than more polar lithcholic acid, i.e. $\ln(\gamma^S/\Phi)$ values for lithocholic acid are higher than those for cholesterol. Such a behavior is common for all examined solutes (Figs. 4 and 5). The oposite fenomenon has been noticed in reversed phase LC (10), i.e. more polar solutes showed a higher affinity toward both phases in comparison with less polar solutes. Thus, the total effect of the mobile and the stationary phase on retention is summarized in the distribution constant of a solute, K_{th}, which is the ratio of the magnitude of these two effects and represents the only measure of a solute retention in LC.

CONCLUSIONS

The solute retention in LSC on silica, using mixed non-aqueous mobile phase, is affected by bothe the mobile and the stationary phase. These effects are significant and under the same conditions are merely determined by the solute molecular structure. Increased polarity of a solute decreases the affinity of its molecules to both phases. Thus, the only measure of a solute retention in LC is its distribution constant.

REFERENCES

1. Snyder, L.R., Principles of Adsorption Chromatography, M. Dekker, New York, 1968.
2. Soczewinski, E., Anal. Chem., **46**, 179 (1969).

3. Jandera, P., Churaček, J., J. Chromatogr., **93**, 17 (1974).
4. Hildebrand, J.H., Prausnitz, J.M., Scott, R.L., Regular and Related Solutions, Van Nostrand-Reinhold, New York, 1970.
5. Jandera, P., Churaček, J., J. Chromatogr., **91**, 207 (1974).
6. Schoenmakers, P.J., Billiet, H.A.H., Tijssen, R., de Galan, L., J. Chromatogr., **149**, 519 (1978).
7. Petrović, S.M., Lomić, S., Šefer, I., Chromatographia, **23**, 915 (1987).
8. Scott, R.P.W., Kucera, P., J. Chromatogr., **149**, 93 (1978).
9. Petrović, S.M., Lomić, S., Šefer, I., Chromatographia, **24**, 800 (1987).
10. Petrović, S.M., Lomić, S., J. Liq. Chromatogr., **12**, 59 (1989).