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INVESTIGATIONS ON THE ADSORPTION PROCESS IN THIN-LAYER CHROMATOGRAPHY BY USING TWO-COMPONENT MOBILE PHASES*

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SUMMARY

Earlier investigations of the adsorption process in multicomponent solutions led to the derivation of an equation that describes the simultaneous relationship beween the R_M value of a given substance and the composition of the binary mobile phase and the R_M values of the given substance when the solvents are the pure components of the binary mobile phase. Good agreement between the function $R_{M_{1,2}} = f(\varphi_1)$ (where φ_1 is the volume fraction of component 1 in the mobile phase) obtained experimentally and calculated by the above equation confirms the suitability of the equation for use in the study of the mechanism of the chromatographic process.

INTRODUCTION

In thin-layer adsorption chromatography, multicomponent mobile phases are frequently used and in complex systems the prediction of the optimal conditions for chromatographic separations is difficult. The knowledge of the basic process, adsorption from multicomponent solutions, can make the management of the chromatographic process easier.

When analyzing adsorption from multicomponent solvent mixtures, Ościk[1–4] derived an equation that gives the relationship between the data of an adsorption process from ideal and conformal multicomponent solutions and those of adsorption from the respective binary solvents. A simple relationship between the adsorption affinity of a given substance and its R_M value can be written for liquid adsorption chromatography:

$$R_{M_{\Sigma i}} = \sum_{i}^{n} x_i R_{M_i} + \sum_{i}^{n-1} (x_i^s - x_i)\,(\log {}^x k_{i,n}^{\infty} + R_{M_i} - R_{M_n}) + Y \qquad (1)$$

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In eqn. 1, Y is a constant for a given substance and for a given chromatographic system. For multicomponent ideal solvent systems, $Y = 0$. The values of this constant are not high for regular solvents and it can therefore be neglected in the calculation of $R_{M_{\Sigma i}}$ values.

In the case of a two-component mobile phase, eqn. 1 can be written as

$$R_{M_{1,2}} = x_1 R_{M_1} + x_2 R_{M_2} + (x_1^s - x_1)(\log {}^{x}k_{1,2}^{\infty} + R_{M_1} - R_{M_2}) \qquad (2)$$

or, after modification

$$R_{M_{1,2}} = x_1 \Delta R_{M_{1,2}} + (x_1^s - x_1)(\Delta R_{M_{1,2}} + A_z) + R_{M_2} \qquad (3)$$

where

$$\Delta R_{M_{1,2}} = R_{M_1} - R_{M_2} \text{ and } A_z = \log {}^{x}k_{1,2}^{\infty}$$

Eqn. 2 or 3 describes the simultaneous relationships between the $R_{M_{1,2}}$ value of a given substance z and the composition of the two-component mobile phase and the R_M values of this substance when the solvents are the pure components of the mobile phase. The difference $x_1^s - x_1$ represents the excess adsorption of component 1 of the mobile phase[5]. The coefficient ${}^{x}k_{1,2}^{\infty}$ is used to estimate the intermolecular interactions between substance z and the components of the mobile phase[6]. Instead of molar fractions (x), volume fractions (φ) can be used in the above equations. Then, instead of the rational coefficient ${}^{x}k_{1,2}^{\infty}$, we have the coefficient $k_{1,2}^{\infty}$ (the concentration of substance z in both solvents is expressed in moles per litre).

In a previous paper[7], eqn. 3 was checked experimentally by calculating $\varphi_1^s - \varphi_1$ values on the basis of excess isotherms of adsorption on alumina of component 1 from the mixtures (1) toluene–(2) benzene, (1) benzene–(2) carbon tetrachloride and (1) chloroform–(2) carbon tetrachloride, and the coefficients $k_{1,2}^{\infty}$ of some nitroanilines and nitrotoluidines in the systems examined. The course of the function $R_{M_{1,2}} = f(\varphi_1)$, obtained experimentally, showed good agreement with that calculated from eqn. 3.

In later papers[8,9], the possibility of calculating the function $R_{M_{1,2}} = f(x_1)$ theoretically was analyzed for the substance being chromatographed by using ideal or regular (conformal) two-component mobile phases.

Values $x_1^s - x_1$ for ideal mobile phases were calculated from the adsorption isotherm given by Dzhigit *et al.*[10] and Everett[5]:

$$x_1^s = \frac{K_1 x_1}{1 + (K_1 - 1) x_1} \qquad (4)$$

where K_1 is the adsorption equilibrium constant of component 1.

In the case of regular mobile phases, $x_1^s - x_1$ values are calculated from the equation derived by Everett[11]:

$$\ln \left(\frac{x_1^s x_2}{x_1 x_2^s} \right) = \ln K_1 + (l + m) \frac{\alpha}{RT} (x_2 - x_1) + l \frac{\alpha}{RT} (x_1^s - x_2^s) \qquad (5)$$

the values of l, m and α/RT being as defined by Everett[11].

Theoretical functions $R_{M_{1,2}} = f(x_1)$ were obtained for the K_1 data and various A_z values (from -1.0 to $+2.0$), which were compared quantitatively with the experimental data for thin-layer adsorption chromatography. It was found that the shape of the curves of the dependence of $R_{M_{1,2}}$ on the composition of the mobile phase corresponds to that of the curves obtained by means of the equation.

By choosing suitable values of K_1 and A_z, it is possible to obtain the curves of the relationships between $R_{M_{1,2}}$ and the composition of the mobile phase, calculated from eqn. 3, which will be identical or nearly identical with the curves obtained experimentally. For ideal or regular mixtures as mobile phases, one may take into consideration that an average $\Delta R_{M_{1,2}}$ value of the substances being chromatographed is approximately equal to $-\log K_1$.

As mentioned above, in previous papers it was shown that eqn. 3 described well the relationships between the $R_{M_{1,2}}$ values of the substances being chromatographed and the composition of the mobile phase. It can be postulated that the adjustment of the curves calculated by this equation to those obtained experimentally will permit the molecular mechanism of the chromatographic process in the systems described to be analyzed. This paper describes an attempt to carry out such an analysis for a number of substances and some two-component mobile phases in thin-layer adsorption chromatography.

EXPERIMENTAL

R_F values were obtained for a series of heterocyclic bases (Table I) using alumina G as the adsorbent and the binary solvent systems chloroform–benzene, butyl acetate–benzene, benzene–cyclohexane and chloroform–carbon tetrachloride as mobile phases. The adsorbent layers, 0.3 mm in thickness, were activated for 2 h at 135°. The solutions of the substances were spotted on the plates in volumes of 4 μl with calibrated pipettes. The chromatograms were developed by the ascending technique to a distance of 16 cm and the chromatographed substances were located with Dragendorff's reagent.

TABLE I

SOLUTES USED

Solute	*Concentration (mole/l)*
2-Aminopyridine	0.20
3-Aminopyridine	0.20
2-Amino-3-methylpyridine	0.20
2-Amino-6-methylpyridine	0.20
2-Amino-5-nitropyridine	0.20
Quinoline	0.20
Isoquinoline	0.20
6-Methylquinoline	0.20
2,6-Dimethylquinoline	0.20
Acridine	0.15
5,6-Benzoquinoline	0.16

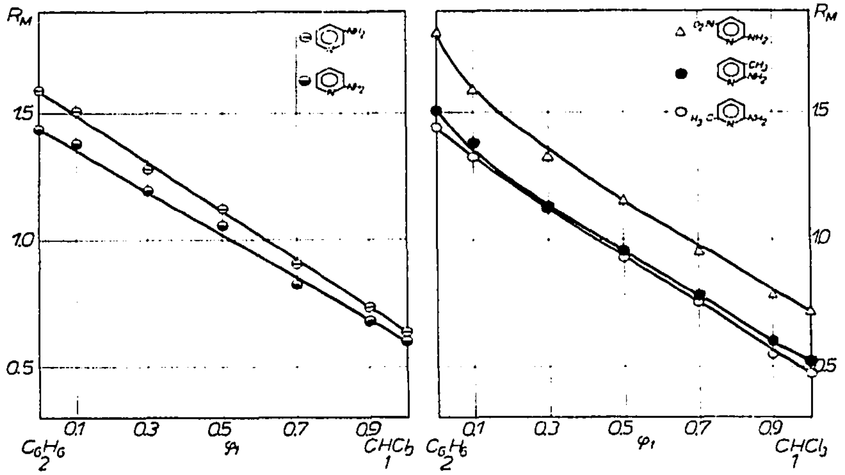

Fig. 1. Relationships $R_{M_{1,2}} = f(\varphi_1)$ for derivatives of aminopyridines calculated by eqn. 3 (lines) and obtained experimentally (points). Mobile phase, $CHCl_3$–C_6H_6; adsorbent, alumina G.

The R_F values were converted into R_M values by using Bate-Smith and Westall's equation[12].

The results obtained for individual substances were expressed as graphs of the function $R_{M_{1,2}} = f(\varphi_1)$ (Figs. 1–4). On these graphs, the lines show the course of the dependence of $R_{M_{1,2}}$ on the composition of the mobile phase, calculated by eqn. 3, while the individual points correspond to $R_{M_{1,2}}$ values obtained experimentally.

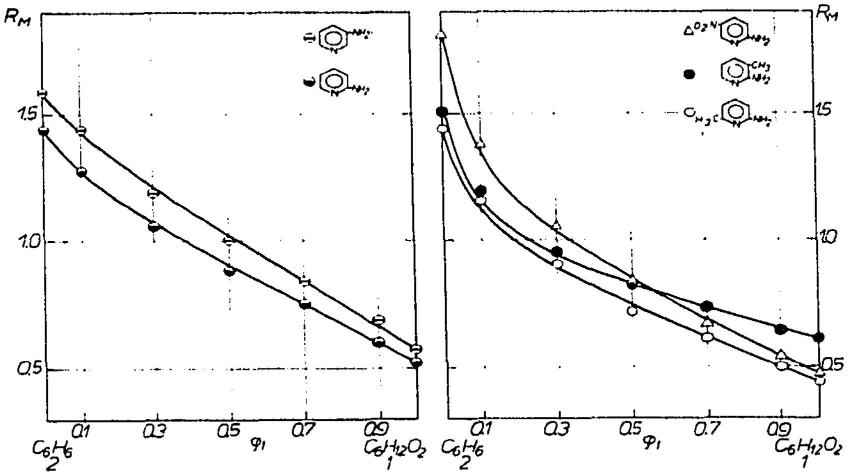

Fig. 2. Relationships $R_{M_{1,2}} = f(\varphi_1)$ for derivatives of aminopyridines calculated by eqn. 3 (lines) and obtained experimentally (points). Mobile phase, $C_6H_{12}O_2$–C_6H_6; adsorbent, alumina G.

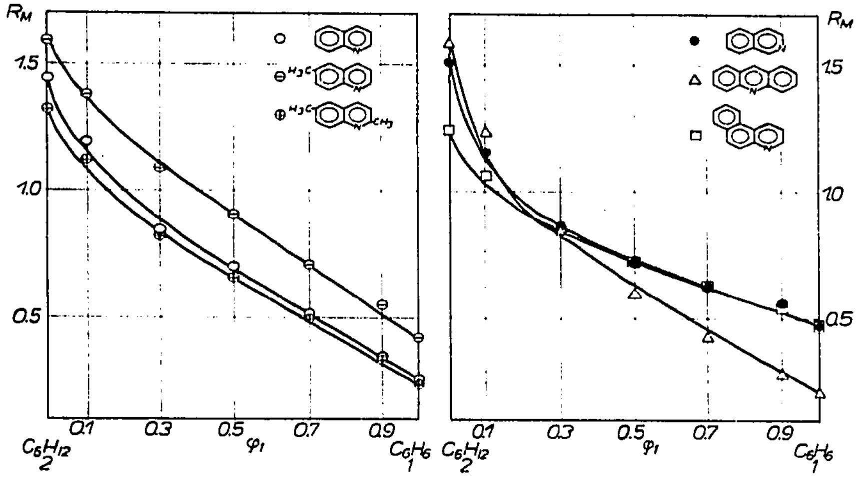

Fig. 3. Relationships $R_{M_{1,2}} = f(\varphi_1)$ for derivatives of quinoline calculated by eqn. 3 (lines) and obtained experimentally (points). Mobile phase, C_6H_6–C_6H_{12}; adsorbent, alumina G.

RESULTS AND DISCUSSION

Figs. 1–4 show that in case of the chromatographic systems examined, there is good agreement between the functions $R_{M_{1,2}} = f(\varphi_1)$ obtained experimentally and those calculated by eqn. 3. The chosen parameters in eqn. 3 (K_1 and $k_{1,2}^{\infty}$) offer the possibility of making an approximate analysis of the true mechanism of the chromatographic process.

The data concerning the chromatography of derivatives of aminopyridines

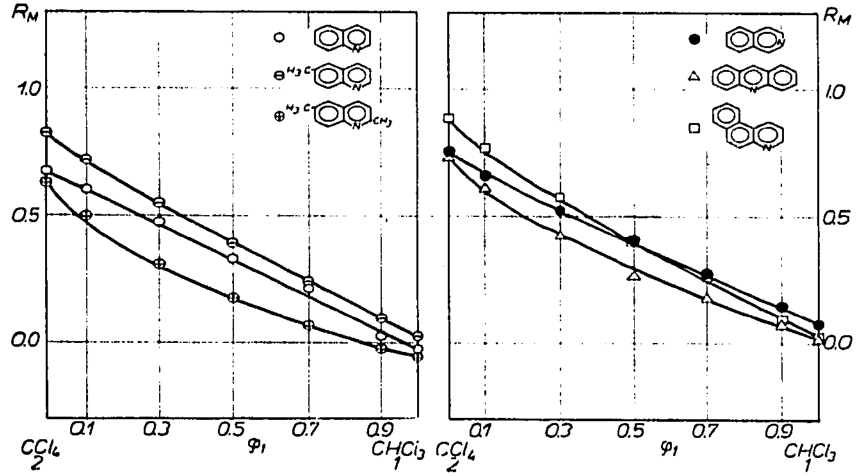

Fig. 4. Relationships $R_{M_{1,2}} = f(\varphi_1)$ for derivatives of quinoline calculated by eqn. 3 (lines) and obtained experimentally (points). Mobile phase, $CHCl_3$–CCl_4; adsorbent, alumina G.

TABLE II

PARAMETERS K_1, $k_{1,2}^{\infty}$ AND A_z OF EQN. 3 AND R_F AND R_M VALUES FOR AMINOPYRIDINE DERIVATIVES USING $CHCl_3$–C_6H_6 AS MOBILE PHASE AND ALUMINA G AS ADSORBENT

Solute	K_1	$k_{1,2}^{\infty}$	A_z	*Volume fraction of* $CHCl_3$	R_F	R_M	
						Exptl.	*Calc.*
2-Aminopyridine	8.65	6.88	0.838	0.0	0.035	1.440	—
				0.1	0.04	1.380	1.356
				0.3	0.06	1.195	1.188
				0.5	0.08	1.061	1.021
				0.7	0.13	0.826	0.853
				0.9	0.17	0.689	0.685
				1.0	0.20	0.602	—
3-Aminopyridine	8.65	8.97	0.953	0.0	0.025	1.591	—
				0.1	0.03	1.510	1.496
				0.3	0.05	1.279	1.305
				0.5	0.07	1.123	1.124
				0.7	0.11	0.908	0.924
				0.9	0.155	0.736	0.733
				1.0	0.19	0.630	—
2-Amino-3-methylpyridine	8.65	6.69	0.825	0.0	0.03	1.510	—
				0.1	0.04	1.380	1.350
				0.3	0.07	1.123	1.137
				0.5	0.10	0.954	0.956
				0.7	0.14	0.788	0.788
				0.9	0.20	0.602	0.602
				1.0	0.23	0.525	—
2-Amino-6-methylpyridine	8.65	8.18	0.913	0.0	0.035	1.440	—
				0.1	0.045	1.327	1.324
				0.3	0.07	1.123	1.127
				0.5	0.105	0.931	0.940
				0.7	0.15	0.753	0.753
				0.9	0.22	0.550	0.568
				1.0	0.25	0.477	—
2-Amino-5-nitropyridine	8.65	6.44	0.808	0.0	0.015	1.817	—
				0.1	0.025	1.591	1.595
				0.3	0.045	1.327	1.348
				0.5	0.065	1.158	1.157
				0.7	0.10	0.954	0.975
				0.9	0.14	0.788	0.803
				1.0	0.16	0.720	—

for the two mobile phases chloroform–benzene and butyl acetate benzene (Tables II and III) indicate a relatively small difference in the adsorption of the components of the mobile phases. One can, however, observe characteristic changes in the $k_{1,2}^{\infty}$ coefficients of the substances chromatographed, including changes in their molecules. The change in position of the $-NH_2$ group in 2- and 3-aminopyridine causes the $k_{1,2}^{\infty}$ to change by about 2 in both systems. There is also an interest-

TABLE III

PARAMETERS K_1, $k_{1,2}^{\infty}$ AND A_z OF EQN. 3 AND R_F AND R_M VALUES FOR AMINOPYRIDINE DERIVATES USING $C_6H_{12}O_2$-C_6H_6 AS MOBILE PHASE AND ALUMINA G AS ADSORBENT

Solute	K_1	$k_{1,2}^{\infty}$	A_z	*Volume fraction of* $C_6H_{12}O_2$	R_F	R_M *Exptl.*	R_M *Calc.*
2-Aminopyridine	11.64	5.47	0.738	0.0	0.035	1.440	—
				0.1	0.05	1.279	1.267
				0.3	0.08	1.061	1.070
				0.5	0.115	0.886	0.908
				0.7	0.15	0.753	0.753
				0.9	0.20	0.602	0.600
				1.0	0.23	0.525	—
3-Aminopyridine	11.64	7.39	0.880	0.0	0.025	1.591	—
				0.1	0.035	1.440	1.426
				0.3	0.06	1.195	1.213
				0.5	0.09	1.005	1.026
				0.7	0.125	0.845	0.844
				0.9	0.17	0.689	0.664
				1.0	0.21	0.575	—
2-Amino-3-methylpyridine	11.64	2.12	0.327	0.0	0.03	1.510	—
				0.1	0.06	1.195	1.158
				0.3	0.10	0.954	0.938
				0.5	0.13	0.826	0.826
				0.7	0.155	0.736	0.736
				0.9	0.185	0.644	0.654
				1.0	0.195	0.616	—
2-Amino-6-methylpyridine	11.64	3.40	0.531	0.0	0.035	1.440	—
				0.1	0.065	1.158	1.126
				0.3	0.11	0.908	0.891
				0.5	0.16	0.720	0.746
				0.7	0.195	0.616	0.621
				0.9	0.24	0.501	0.500
				1.0	0.265	0.443	—
2-Amino-5-nitropyridine	11.64	4.17	0.620	0.0	0.015	1.817	—
				0.1	0.04	1.380	1.352
				0.3	0.08	1.061	1.030
				0.5	0.125	0.845	0.846
				0.7	0.175	0.673	0.691
				0.9	0.22	0.550	0.547
				1.0	0.25	0.477	—

ing small change in $k_{1,2}^{\infty}$ caused by the introduction of an $-NO_2$ group into the 5-position in a molecule of 2-aminopyridine.

Results for the chromatography of derivatives of quinoline using as mobile phases the systems benzene–cyclohexane and chloroform–carbon tetrachloride are given in Tables IV and V. High values of the constant K_1 in case of one system (high adsorption of benzene from cyclohexane) influence the course of the relationship between $R_{M_{1,2}}$ and the composition of the mobile phase. In both systems, distinct

TABLE IV

PARAMETERS K_1, $k_{1,2}^{\infty}$ AND A_z OF EQN. 3 and R_F AND R_M VALUES FOR DERIVATIVES OF QUINOLINE USING C_6H_6–C_6H_{12} AS MOBILE PHASE AND ALUMINA G AS ADSORBENT

Solute	K_1	$k_{1,2}^{\infty}$	A_z	*Volume fraction of* C_6H_6	R_F	R_M	
						Exptl.	*Calc.*
Quinoline	12.60	6.47	0.811	0.0	0.035	1.440	—
				0.1	0.06	1.195	1.152
				0.3	0.125	0.845	0.880
				0.5	0.165	0.704	0.693
				0.7	0.235	0.513	0.516
				0.9	0.31	0.347	0.345
				1.0	0.355	0.259	—
6-Methylquinoline	12.60	8.71	0.940	0.0	0.025	1.591	—
				0.1	0.04	1.380	1.367
				0.3	0.075	1.091	1.111
				0.5	0.11	0.908	0.908
				0.7	0.16	0.720	0.720
				0.9	0.22	0.550	0.517
				1.0	0.275	0.421	—
2,6-Dimethylquinoline	12.60	6.31	0.800	0.0	0.045	1.327	—
				0.1	0.07	1.123	1.082
				0.3	0.13	0.826	0.839
				0.5	0.18	0.659	0.661
				0.7	0.24	0.501	0.490
				0.9	0.315	0.337	0.322
				1.0	0.365	0.240	—
Isoquinoline	12.60	2.75	0.440	0.0	0.03	1.510	—
				0.1	0.065	1.158	1.131
				0.3	0.12	0.865	0.868
				0.5	0.155	0.736	0.740
				0.7	0.19	0.630	0.630
				0.9	0.215	0.562	0.526
				1.0	0.25	0.477	—
Acridine	12.60	5.73	0.758	0.0	0.025	1.591	—
				0.1	0.055	1.235	1.165
				0.3	0.125	0.845	0.830
				0.5	0.20	0.602	0.638
				0.7	0.27	0.432	0.461
				0.9	0.335	0.298	0.294
				1.0	0.38	0.213	—
5,6-Benzoquinoline	12.60	2.99	0.475	0.0	0.055	1.235	—
				0.1	0.08	1.061	1.030
				0.3	0.125	0.845	0.849
				0.5	0.155	0.736	0.735
				0.7	0.19	0.630	0.629
				0.9	0.225	0.537	0.527
				1.0	0.25	0.477	—

changes of $k_{1,2}^{\infty}$ parallel to the change in the structure of the chromatographed molecules were observed. For example, in benzene–cyclohexane, $k_{1,2}^{\infty}$ varies considerably in the case of quinoline and isoquinoline.

TABLE V

PARAMETERS K_1, $k^{\infty}_{1,2}$ AND A_z OF EQN. 3 AND R_F AND R_M VALUES FOR DERIVATIVES OF QUINOLINE USING $CHCl_3$-CCl_4 AS MOBILE PHASE AND ALUMINA G AS ADSORBENT

Solute	K_1	$k^{\infty}_{1,2}$	A_z	*Volume fraction of $CHCl_3$*	R_F	R_M	
						Exptl.	*Calc.*
Quinoline	5.15	5.00	0.699	0.0	0.175	0.673	—
				0.1	0.20	0.602	0.603
				0.3	0.25	0.477	0.463
				0.5	0.32	0.327	0.323
				0.7	0.38	0.213	0.184
				0.9	0.485	0.026	0.043
				1.0	0.515	−0.026	—
6-Methylquinoline	5.15	5.02	0.700	0.0	0.13	0.826	—
				0.1	0.16	0.720	0.719
				0.3	0.22	0.550	0.547
				0.5	0.29	0.389	0.392
				0.7	0.365	0.240	0.244
				0.9	0.445	0.096	0.098
				1.0	0.485	0.026	—
2,6-Dimethylquinoline	5.15	2.19	0.340	0.0	0.19	0.630	—
				0.1	0.24	0.501	0.470
				0.3	0.33	0.308	0.294
				0.5	0.40	0.176	0.174
				0.7	0.46	0.070	0.076
				0.9	0.515	−0.026	−0.030
				1.0	0.53	−0.052	—
Isoquinoline	5.15	3.98	0.600	0.0	0.15	0.753	—
				0.1	0.18	0.659	0.665
				0.3	0.23	0.525	0.521
				0.5	0.28	0.410	0.390
				0.7	0.345	0.278	0.264
				0.9	0.415	0.149	0.139
				1.0	0.455	0.078	—
Acridine	5.15	2.94	0.469	0.0	0.155	0.736	—
				0.1	0.195	0.616	0.596
				0.3	0.27	0.432	0.434
				0.5	0.35	0.269	0.292
				0.7	0.395	0.185	0.177
				0.9	0.455	0.078	0.069
				1.0	0.49	0.017	—
5,6-Benzoquinoline	5.15	4.69	0.671	0.0	0.115	0.886	—
				0.1	0.145	0.770	0.752
				0.3	0.21	0.575	0.561
				0.5	0.285	0.399	0.400
				0.7	0.355	0.259	0.250
				0.9	0.445	0.096	0.105
				1.0	0.48	0.035	—

The results obtained confirm the possibility of examining the mechanism of the chromatographic process by using mobile phases that comprise two-component systems with the properties of ideal and regular (conformal) solutions.

LIST OF SYMBOLS

R_M $= \log\left(\frac{1 - R_F}{R_F}\right)$ (after Bate-Smith and Westall).

$R_{M_{\Sigma i}}$ $=$ R_M for substance z when using an n-component solvent mixture as the mobile phase.

R_{M_i} $=$ R_M for substance z when using pure solvent i.

R_{M_n} $=$ R_M for substance z when using pure solvent n.

R_{M_1} $=$ R_M for substance z when using pure solvent 1.

R_{M_2} $=$ R_M for substance z when using pure solvent 2.

$R_{M_{1.2}}$ $=$ R_M for substance z when using the two-component solvent system 1 + 2

${}^{x}k^{\infty}_{i,n}$ $=$ hypothetical rational partition coefficient for substance z relating to its partition between components i and n of the n-component solvent.

${}^{x}k^{\infty}_{1\ 2}$ $=$ hypothetical rational partition coefficient for substance z between components 1 and 2 of the binary solvent 1 + 2.

$k^{\infty}_{1,2}$ $=$ hypothetical partition coefficient for substance z between components 1 and 2 of the binary solvent 1 + 2.

A_z $= \log {}^{x}k^{\infty}_{1,2}$ (or $\log k^{\infty}_{1,2}$).

x_i and x_n $=$ molar fractions of components i and n of the multicomponent solvent in the volume phase (mobile phase).

x_1 and x_2 $=$ molar fractions of components 1 and 2 of the binary solvent 1 + 2 in the volume phase (mobile phase).

x_i^s and x_n^s $=$ molar fractions of components i and n of the multicomponent solvent the surface phase.

K_1 $=$ adsorption equilibrium constant of component 1 adsorbed from the ideal binary mixture 1 + 2.

l, m and α $=$ constant in Everett's eqn. 6.

Y $=$ a constant in eqn. 1 connected with the excess free enthalpies of the solvent mixture in the volume and surface phases.

φ $=$ volume fraction of the components of the solvent mixture (mobile phase).

φ^s $=$ volume fraction of the components in the surface phase.

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