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RETENTION PARAMETERS OF SOME ORGANIC SUBSTANCES AND PHYSICO-CHEMICAL PROPERTIES OF CHROMATOGRAPHIC SYSTEMS WITH TWO-COMPONENT MOBILE PHASES

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SUMMARY

　　Following work on the influence of the nature of the mobile phase on chromatographic parameters, the effects of sample concentration and the specific surface area of the adsorbent on the value of individual parameters in Ościk's equation were determined. It is concluded that adsorption of the chromatographed substances does not play a significant role in decreasing the K_{12} value. However, the specific surface area influences the retention parameters significantly.

INTRODUCTION

　　In physico-chemical investigations, the selection of optimal conditions for the separation of mixtures by liquid adsorption chromatography requires a knowledge and description of the underlying principles of this method. During the past 20 years significant progress has been made in this respect in the field of adsorption on solid surfaces from solutions, as is evident from numerous papers involving the use of a modified Snyder–Soczewiński equation[1-4].

　　In the theory of adsorption chromatography, Ościk's equation permits the determination of the parameters that influence the R_M values of substances chromatographed in systems containing a binary mobile phase[5-7]. A term describing adsorption interactions is used to evaluate the effects of adsorption phenomena on the mechanism of the chromatographic separation.

　　For a binary mobile phase, Ościk's equation has the following form:

$$R_{M12} = x_1 R_{M1} + x_2 R_{M2} + (y_1 - x_1)(\Delta R_{M1,2} + A_{12}) \tag{1}$$

where R_{M12}, R_{M1} and R_{M2} are the R_M values of the chromatographed substance in the binary mobile phase and in pure solvents 1 and 2, respectively (1 being the more polar solvent); x_1 and x_2 are the molar fractions of the mobile phase components; y_1 is the molar fraction of the more polar solvent in the surface phase; A_{12} is a constant characterizing interactions between the chromatographed substance and the mobile phase components; $\Delta R_{M1,2} = R_{M1} - R_{M2}$; and $y_1 - x_1$ is the excess adsorption of more polar component of the mobile phase.

For a given chromatographic system consisting of the adsorbent and a binary mobile phase, the expression $\Delta R_{M1,2} + A_{12} = C$ for an individual test substance has a constant value. Assuming ideality of the bulk and surface phases and homogeneity of the adsorbent surface, we can calculate the molar fraction of the more polar component in the surface phase by using the Everett equation[8]:

$$y_1 = \frac{K_{12}x_1}{x_2 + K_{12}x_1} \tag{2}$$

where K_{12} denotes the adsorption equilibrium constant in a solid binary solution system. After simple transformations[9], eqn. 1 assumes a linear form, in which a function on the left-hand side, denoted by G, is

$$G = \frac{x_1 x_2}{R_{M12} - x_1 R_{M1} - x_2 R_{M2}} = \frac{1}{C}\left(\frac{1}{K_{12}-1} + x_1\right) = ax_1 + b \tag{3}$$

where

$$a = \frac{1}{C} \text{ and } b = \frac{1}{C(K_{12}-1)}$$

Hence

$$K_{12} = \frac{a}{b} + 1 \tag{4}$$

From the boundary conditions of eqn. 3, K_{12} values can be calculated, because for $x_1 = 0$, $G = b$ and for $x_1 = 1$, $G = a + b$. K_{12} values determined from chromatographic data according to eqn. 4 will subsequently be designated K_{12G} in this paper[9].

In another method for the determination of K_{12} from the data obtained by liquid adsorption chromatography, the following equation is used[10]:

$$K_{12} = 10^{-\Delta R_{M1,2}} \tag{5}$$

K_{12} values determined according to eqn. 5 will be designated $K_{12\Delta}$[9].

Eqns. 3 and 5 contain the equilibrium constant values K_{12G} and $K_{12\Delta}$, characterizing adsorption interactions in the chromatographic process. Because in this process other effects apart from adsorption phenomena can also take place, it was necessary to examine what relationships exist between K_{12G} and $K_{12\Delta}$ on the one hand and the K_{12} value, characterizing adsorption of mobile phase components in the absence of chromatographed substances, on the other. In previous work, the influence of the nature of the mobile phase on chromatographic parameters was investigated[9]. Summarizing the results obtained previously, we can conclude that[9]:
(1) K_{12G} values for individual substances are greater than $K_{12\Delta}$ in many instances;
(2) K_{12G} and $K_{12\Delta}$ values are very small for substances with low adsorption affinities and significantly greater for strongly adsorbed substances; (3) significantly

different K_{12G} values are observed for various isomers of the same substance; and (4) K_{12G} and K_{12A} values are significantly smaller than K_{12} values obtained from adsorption data.

The next stage in the investigation of chromatographic mechanisms is the determination of the effect of the concentration of the sample and of the specific surface area of the adsorbent on the value of the individual parameters in eqn. 3.

EXPERIMENTAL AND RESULTS

Effect of the amount of sample

In theoretical descriptions of thin-layer adsorption chromatography, it is often assumed that the concentration of the substances being separated is negligibly small in comparison with the concentration of the mobile phase components. However, this assumption is a great simplification of the process, because the sample plays a significant role. In order to evaluate the direct effect of this substance on the adsorption of mobile phase components, adsorption measurements were caried out in the following systems:

(1) ethylene chloride–acetone–SiO$_2$;

(2) ethylene chloride–acetone–4% (v/v) *o*-nitrotoluene–SiO$_2$;

(3) ethylene chloride–acetone–14.3% (v/v) *o*-nitrotoluene–SiO$_2$;

(4) *o*-nitrotoluene–acetone–SiO$_2$.

The composition of the chromatographic systems was defined by the technical limitations of the apparatus. In many systems, conditions for the isothermal separation of ternary systems (binary mobile phase + chromatographed substance) cannot be obtained when gas chromatography is used. For systems 2 and 3, a satisfactory separation was obtained on a chromatographic column filled with Chromosorb 101 covered with 10% silicone oil at 453 K. K_{12G} and K_{12A} values of 2.77 and 1.58, respectively previously determined[9] for nitrotoluene chromatographed in an binary ethylene chloride–acetone mobile phase were also taken into account. For the above four systems the K_{12} values were determined and were as follows: (1) 84.3 when $n^s = 1.74$, (2) 41.7 when $n^s = 1.65$, (3) 38.5 when $n^s = 1.67$ and (4) 36.9 when n^s
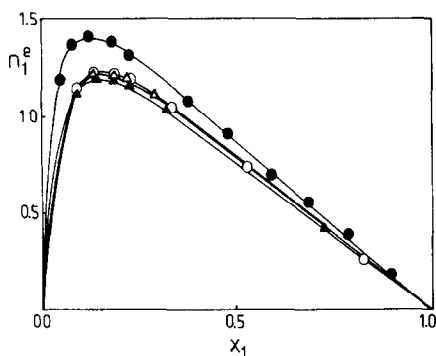

Fig. 1. Excess adsorption isotherms on SiO$_2$ for (●) ethylene chloride–acetone; (○) ethylene chloride–acetone–4% (v/v) *o*-nitrotoluene; (△) ethylene chloride–acetone–14.3% (v/v) *o*-nitrotoluene; and (▲) *o*-nitrotoluene–acetone.

$= 1.64$, where n^s denotes the maximum number of milimoles of acetone adsorbed on the surface of 1 g of adsorbent.

Acetone excess adsorption isotherms for systems 2 and 3, presented in Fig. 1, were plotted neglecting the amount of nitrotoluene added, because its addition did not cause significant changes in the K_{12} values. The adsorption data led to the conclusion that when 4% of nitrotoluene is added, the adsorbent surface is sufficiently covered by this substance, and a further increase in its concentration will not cause a significant decrease in the K_{12} value. A lower limit of the K_{12} value, which may be obtained by addition of nitrotoluene to the system investigated, is that obtained for nitrotoluene–acetone–SiO_2.

Comparing the values of K_{12} obtained with K_{12G} and K_{124}, determined for nitrotoluene in the ethylene chloride–acetone–SiO_2 system, it can be concluded that adsorption of the chromatographed substance does not play a significant role in the decrease in the K_{12} value. It can also be assumed that the K_{12G} and K_{124} values are parameters characterizing the mechanism of the entire liquid adsorption chromatographic process, because in this process its dynamics cannot be neglected. In deriving eqn. 4 we have assumed an ideal chromatographic process (and in this connection the Everett equation was used), in which adsorption processes predominate. Adsorption data for K_{12} are obtained by a static method, whereas during chromatographic measurements we deal with a complex dynamic state. In adsorption measurements the substance added to the system is distributed uniformly in the entire volume of equilibrium solution, whereas in TLC the substances are concentrated in the form of small spots. During development of the chromatogram, changes in the concentration of the sample occur, which are characterized by significant fluctuations that must be taken into account. Concentration distributions in the spot and mobile phase can be presented in the form of a Gaussian distribution curve. The adsorbent surface is covered by the chromatographed substance not only owing to surface interactions. In this connection, during chromatographic development the adsorption of components on the chromatographic system is only one among many factors that determine the course of this process.

Effect of specific surface area of the adsorbent

For the accurate investigation of the mechanism of the process underlying chromatographic separations, examination of the effect of the specific surface area of the adsorbent on the chromatographic process appears to be very important. In this connection, measurements were carried out by thin-layer chromatography. Kieselgel samples (Merck, Darmstadt, F.R.G.) with four different specific surface areas, i.e., 50, 150, 400 and 500 m²/g, were used as the adsorbent and the mobile phases were hexane, methylcyclohexane, benzene, ethanol, methanol, hexane–benzene, methylcyclohexane–benzene, benzene–methanol and benzene–ethanol. In this earlier work, Różyło and co-workers[11–13] investigated the effect of the specific surface area of the adsorbent on the R_M values of chromatographed substances. It became apparent that this effect can be described by the linear relationship

$$R_M = ms + n \tag{6}$$

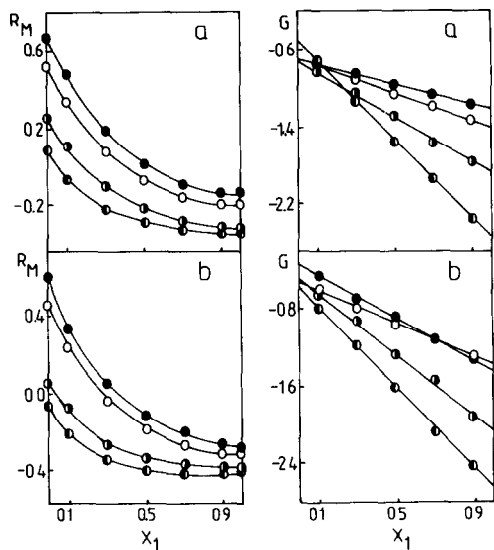where s is the specific surface area of the adsorbent and m and n are constants that

Fig. 2. R_M vs. x_1 and G vs. x_1 with hexane–benzene as the mobile phase. Samples: (a) anthracene; (b) dimethylnaphthalene. Adsorbent, SiO_2: (●) 500 m²/g; (○) 400 m²/g; (◑) 150 m²/g; (◐) 50 m²/g.
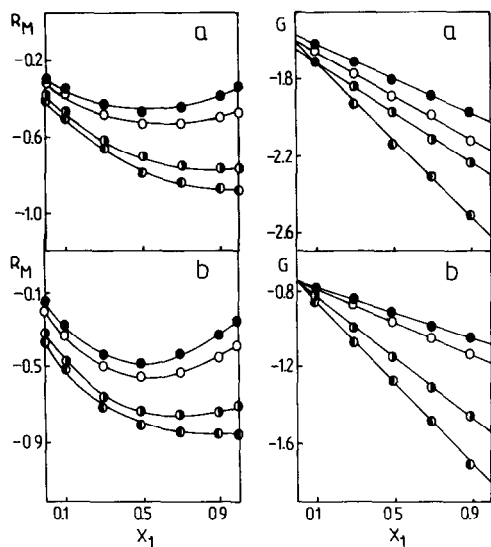
Fig. 3. R_M vs. x_1 and G vs. x_1 with benzene–methanol as the mobile phase. Samples: (a) methylnaphthalene; (b) fluoranthene. Adsorbent, SiO_2: (●) 500 m²/g; (○) 400 m²/g; (◑) 150 m²/g; (◐) 50 m²/g.

depend on the nature of the mobile phase and the chromatographed substances.

Figs. 2 and 3 show the R_M vs. s relationships for a few selected substances. These relationships were obtained for adsorbents of various specific surface areas. In each instance an analogous effect of the specific surface area, s, on the R_M values of test substances is observed, i.e., an increase in the specific surface area causes an increase in the R_M value. Simultaneously, the R_M vs. s function changes. For

TABLE I

VALUES OF PARAMETERS *a* AND *b* IN EQN. 3

Mobile phase: I = hexane–benzene; II = methylcyclohexane–benzene; III = benzene–methanol; IV = benzene–ethanol.

Substance	s (m^2/g)	I		II		III		IV	
		a	b	a	b	a	b	a	b
Diphenyl	50	−1.40	−0.54	−1.39	−1.24	−1.01	−0.99	−1.37	−0.73
	150	−1.24	−0.42	−1.34	−2.67	−0.79	−1.17	−0.98	−0.79
	400	−1.06	−0.53	−1.14	−0.89	−0.77	−0.33	−0.75	
	500	−0.91	−0.55	−1.45	−0.93	−1.01	−0.56	−0.20	−0.69
Pyrene	50	−0.81	−0.54	−1.59	−1.68	−0.63	−0.88	−0.67	−0.57
	150	−0.73	−0.35	−1.59	−1.19	−0.42	−0.85	−0.67	−0.52
	400	−0.62	−0.48	−1.37	−0.25	−0.32	−0.69	−0.51	−0.55
	500	−0.43	−0.51	−1.41	−0.40	−0.31	−0.66	−0.41	−0.54
Chrysene	50	−1.07	−0.37	−1.47	−0.30	−	−	−0.46	−0.83
	150	−1.19	−0.43	−1.62	−0.36	−	−	−0.37	−0.80
	400	−0.94	−0.31	−0.68	−0.52	−	−	−0.37	−0.69
	500	−1.08	−0.21	−1.01	−0.48	−	−	−0.28	−0.69
Fluoranthene	50	−0.98	−0.82	−1.04	−0.95	−1.14	−0.75	−	−
	150	−1.07	−0.47	−1.23	−0.99	−0.78	−0.75	−	−
	400	−0.75	−0.43	−1.33	−0.49	−0.43	−0.75	−	−
	500	−0.33	−0.61	−1.68	−0.18	−0.33	−0.75	−	−
Anthracene	50	−2.05	−0.51	−1.66	−1.7	−	−	−	−
	150	−1.16	−0.72	−1.88	−1.07	−	−	−	−
	400	−0.76	−0.69	−1.64	−1.44	−	−	−	−
	500	−0.51	−0.71	−1.17	−1.32	−	−	−	−
Naphthalene	50	−0.57	−1.20	−2.19	−0.81	−0.81	−1.05	−	−
	150	−1.40	−1.44	−1.83	−1.00	−0.80	−0.91	−	−
	400	−1.70	−1.03	−0.44	−1.14	−0.55	−0.89	−	−
	500	−1.70	−0.61	−0.72	−1.00	−0.27	−0.94	−	−
2,6-Dimethyl- naphthalene	50	−2.06	−0.35	−	−	−1.01	−1.61	−	−
	150	−1.97	−0.48	−	−	−0.66	−1.64	−	−
	400	−0.86	−0.51	−	−	−0.57	−1.60	−	−
	500	−1.08	−0.35	−	−	−0.45	−1.57	−	−

hexane–benzne and methylcyclohexane–benzene systems, the differences between the R_M values of the substances chromatographed in pure mobile phase components increase with increase in the specific surface area of the adsorbent (the $\Delta R_{M1,2}$ values increase).

Hence it can be concluded that for these mobile phases an increase in the specific surface area of the adsorbent causes an increase in the differences in the elution powers between individual components of the mobile phase. With benzene–methanol and benzene–ethanol as mobile phases, the situation is slightly different, as the differences between the R_{M1} and R_{M2} values of the test substance decrease with increases in the specific surface area of the adsorbent. Hence in this instance, with
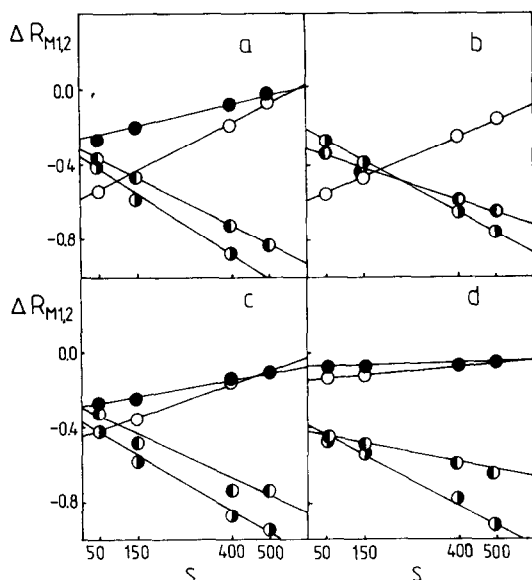
Fig. 4. $\Delta R_{M1,2}$ vs. s. Adsorbent: SiO_2. Samples: (a) diphenyl; (b) naphthalene; (c) pyrene; (d) chrysene. Mobile phase: (●) benzene–ethanol; (○) benzene–methanol; (◑) hexane–benzene; (◐) methylcyclohexane–benzene.

increasing specific surface surface area of the adsorbent, the differences between the elution powers of the pure mobile phase components decrease. These effects are shown in Fig. 4 as $\Delta R_{M1,2}$ vs. s.

On the basis of these investigations, efforts were made to define the effect of surface area on the G function (see Introduction). Figs. 2 and 3 show the results obtained for a few selected substances for all of the mobile phases investigated. Analysis of these figures permits an interesting relationship between the G function and specific surface area to be established. In all instances the increase in surface area is accompanied by an increase in the slope of the G vs. x_1 straight line (the parameter a increases, whereas b remains almost constant). Numerical data are presented in Table I. It should be noted that in hexane–benzene and methylcyclohexane–benzene systems the G functions for adsorbents with specific surface areas of 50 and 150 m^2/g and of 400 and 500 m^2/g are very similar in many instances.

Completely different regularities are observed with benzene–methanol and benzene–ethanol systems (Fig. 3). Very significant changes in the G function are observed, even for adsorbents differing slightly in specific surface area. Hence in this instance the specific surface area of the adsorbent influences the chromatographic process very significantly.

We next calculated K_{12G} and K_{12A} values for model substances from eqns. 3 and 5 and the results are summarized in Table II. The dependence of the K_{12G} and K_{12A} values on changes in the specific surface area is very interesting. In all instances it was found that the K_{12A} values are greater than the corresponding K_{12G} values. It should be noted also that, for hexane–benzene and methylcyclohexane–benzene systems, the K_{12A} values increase with increasing specific surface area of the adsor-

TABLE II

K_{12G} AND K_{12A} VALUES FOR CHROMATOGRAPHED SUBSTANCES

Mobile phase: I = hexane–benzene; II = methylcyclohexane–benzene; III = benzene–methanol; IV = benzene–ethanol.

Substance	s (m^2/g)	I		II		III		IV	
		K_{12A}	K_{12G}	K_{12A}	K_{12G}	K_{12A}	K_{12G}	K_{12A}	K_{12G}
Diphenyl	50	2.51	3.59	2.29	2.12	3.44	1.91	1.82	2.88
	150	3.80	3.95	2.88	1.13	2.85	1.67	1.55	2.24
	400	7.24	3.00	5.25	2.28	1.51	1.75	1.17	1.44
	500	6.61	2.65	6.53	2.56	1.17	1.55	1.07	1.29
Pyrene	50	2.63	2.50	2.04	1.94	2.64	1.72	1.86	1.85
	150	3.89	3.03	3.09	2.34	2.30	1.49	1.78	1.77
	400	7.41	2.29	5.50	6.46	1.48	1.46	1.41	2.08
	500	9.12	1.84	5.50	4.53	1.26	1.94	1.26	1.77
Chrysene	50	4.79	3.89	4.57	5.88	–	–	1.20	1.55
	150	5.62	3.77	5.01	2.32	–	–	1.20	1.46
	400	9.55	4.03	6.31	2.32	–	–	1.17	1.54
	500	13.18	6.14	7.08	3.09	–	–	1.15	1.41
Fluoranthene	50	2.69	2.20	3.02	2.10	3.20	2.52	–	–
	150	4.27	3.28	3.31	2.24	2.51	2.04	–	–
	400	10.00	2.74	5.49	3.71	1.58	1.57	–	–
	500	12.58	1.54	6.46	10.33	1.30	1.47	–	–
Anthracene	50	2.88	5.04	2.74	2.43	–	–	–	–
	150	4.07	2.61	3.09	2.76	–	–	–	–
	400	5.37	2.04	3.47	2.14	–	–	–	–
	500	6.61	1.72	3.80	1.89	–	–	–	–
Naphthalene	50	1.82	1.28	2.14	3.70	3.63	1.77	–	–
	150	2.45	1.97	2.75	2.83	2.88	1.88	–	–
	400	4.47	2.65	3.89	1.38	1.78	1.62	–	–
	500	5.75	4.24	4.47	1.72	1.45	1.29	–	–
2,6-Dimethyl- naphthalene	50	2.34	4.55	–	–	2.95	1.63	–	–
	150	3.31	4.27	–	–	2.37	1.40	–	–
	400	6.30	2.69	–	–	1.70	1.36	–	–
	500	8.51	4.07	–	–	1.10	1.29	–	–

bent, whereas for benzene–methanol and benzene–ethanol they decrease (Table II). This is a consequence of the previously considered dependence of the $\Delta R_{M1,2} = R_{M1} - R_{M2}$ value on the specific surface area, because changes in K_{12G} with changes in s (m²/g) are less significant than changes in the corresponding K_{12A} values. In some instances (especially for the benzene–methanol and benzene–ethanol systems) they are even negligibly small. In this connection, it can be assumed that for systems of this type the K_{12G} values are constant.

The considerations described here are intended to elucidate the effect of the specific surface area of adsorbents on chromatographic processes. It appears that this quantity significiantly influences the retention parameters of the chromatographed substance: intramolecular interactions originating at the adsorbent surface greatly

alter the intramolecular interactions taking place in the bulk phase. It should pointed out also that the effect of the specific surface area on the chromatographic process is strictly related to the type of mobile phase involved.

Analysis of the straight-line parameters in eqn. 3 does not explain fully the mechanism of the chromatographic process. However, it can facilitate the prediction of optimal chromatographic systems for the separation of various mixtures. Further investigations in this respect are continuing.

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