228 Russian Chemical Bulletin, International Edition, Vol. 50, No. 2, pp. 228—232, February, 2001

Chromatographic study of adsorption from binary and ternary solutions

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Adsorption of benzene, anisole, and nitrobenzene on hydroxylated silica gel from binary and ternary solutions (adsorbate—*n*-hexane and adsorbate—*n*-hexane—terahydrofuran) was studied by HPLC. The equation that describes the adsorbate retention as a function of its concentration in binary and ternary solutions was proposed. The equation makes it possible to calculate the adsorption isotherms and adsorption equilibrium constants directly from chromatograms using the retention parameters and equilibrium concentrations of the adsorbate in the mobile phase.

Key words: adsorption, silica gel, chromatography, calculation method, adsorption isotherm, binary and ternary solutions.

The composition of a multicomponent mobile phase is usually changed to regulate retention and selectivity of separation of liquid chromatography. It is of interest to study adsorption from multicomponent solutions because at least three components are present in the adsorbed solution even when a binary mixed mobile phase is used.

Previously[1] we have studied retention of several benzene derivatives in the normal-phase variant of high-performance liquid chromatography (NP HPLC) with the binary mobile phase (*n*-hexane—tetrahydrofuran) on aminated silica gel. We found that the relationship of retention to the concentration of the modifier (tetrahydrofuran) in the mobile phase is complicated, and to describe it, one should take into account intermolecular interactions in the mobile phase along with adsorption equilibria. We used the previously proposed equation[2] containing the equilibrium constants of quasi-chemical reactions describing adsorption—desorption of the adsorbate (K_S) and modifier (K_M) and their association in the mobile phase adsorbate—modifier (K_{SM}) and modifier—modifier (K_{MM}). Successful application of this equation requires an independent determination of constants of adsorption equilibrium from binary and ternary solutions using isotherms that describe adsorption at low concentrations.

The purpose of this work is to develop the liquid-chromatographic method of measurement and calculation of isotherms of adsorption from binary and three-component solutions.

Experimental

Measurements were carried out in the normal-phase elution variant of HPLC on a Milikhrom-1 microcolumn chromatograph with a syringe-type pump (the flow rate of the mobile phase was 2—600 μL min^{-1}) and a UV-spectrophotometric detector (spectral range 190—360 nm). Analyzed substances (benzene, anisole, and nitrobenzene) were introduced into the chromatographic column using an injection needle by the stop-flow method. A steel column (120×2 mm) was filled with hydroxylated silica gel Silasorb-300 with a specific surface area of ~300 m^2 g^{-1} and an average particle diameter of ~5 μm (the adsorbent weight in the column was 0.202±0.008 g) using the suspension method. *n*-Hexane (analytical purity grade) dehydrated for 2—3 days above zeolite NaA, which was pre-heated for 4 h at 450 °C, and *n*-hexane modified with THF were used as the mobile phase. Experimental conditions: flow rate of the mobile phase w = 100 μL min^{-1}, ~20 °C. Detection was carried out at 254 and 300 nm. The volume of introduced samples was varied from 1 to 25 μL.

Results and Discussion

Earlier[3] we have proposed the displacement model of retention of substances in HPLC on the chemically and geometrically uniform adsorbent surface. The model assumes that molecules of the adsorbate and components of the mobile phase do not associate and the surface and bulk solutions are ideal.

If the system consists of the adsorbate (S) and two-component mobile phase "solvent (L)—modifying additive (M)," adsorption can be considered as the reversible quasi-chemical exchange reaction

$$\mathrm{S} + \mathrm{L^s} = \mathrm{S^s} + \mathrm{L}, \quad (1)$$

$$K_S = (X_S{}^s X_L)/(X_S X_L{}^s), \quad (2)$$

$$\mathrm{M} + \mathrm{L^s} = \mathrm{M^s} + \mathrm{L}, \quad (3)$$

$$K_M = (X_M{}^s X_L)/(X_M X_L{}^s), \quad (4)$$

where K_S and K_M are the constants of equilibrium of reactions (1) and (3), and $X_S{}^s$, X_S, $X_M{}^s$, X_M, $X_L{}^s$, and X_L

Published in Russian in *Izvestiya Akademii Nauk. Seriya Khimicheskaya,* No. 2, pp. 220—224, February, 2001.

are the molar fractions of the adsorbate (S), modifier (M), and solvent (L) in the stationary (superscript s) and mobile phases. The equation of adsorption for the adsorbate and modifier has previously been derived.[3]

Let us consider this equation for the adsorbate. It follows from Eqs. (2) and (4) that

$$X_M{}^s/X_S{}^s = K_MX_M/K_SX_S. \tag{5}$$

Taking that the sum of molar fractions of all components of the adsorption layer and mobile phase is equal to 1

$$X_M{}^s + X_S{}^s + X_L{}^s = 1, \tag{6}$$

$$X_M + X_S + X_L = 1 \tag{7}$$

and inserting the $X_S{}^s$ and $X_M{}^s$ values from Eqs. (4) and (5) into Eq. (6), we obtain

$$X_S{}^sX_MK_M + X_SK_SX_S{}^s + X_S{}^sX_L = X_SK_S. \tag{8}$$

It is known that

$$X_S{}^s = a/a_m,$$

where a_m/μmol g^{-1} is the surface concentration of the adsorbate in the compact adsorption monolayer of a three-component solution, and a/μmol g^{-1} is the amount of the substance adsorbed (total content). Then the adsorption isotherm of the adsorbate from a three-component solution is described by the following equation:

$$a = \frac{a_mK_SX_S}{1 + (K_S - 1)X_S + (K_M - 1)X_M}. \tag{9}$$

To calculate the a_m and K_S constants, it is convenient to use Eq. (9) in the linear form

$$\frac{X_S}{a} = \frac{1 + (K_M - 1)X_M}{a_mK_S} + \frac{K_S - 1}{a_mK_S}X_S. \tag{10}$$

Adsorption isotherms are determined by the HPLC method in two stages: (a) detection of chromatograms of adsorbates and (b) calculation of the adsorption values and equilibrium concentrations of the adsorbate in the mobile phase from the chromatograms obtained. The Glueckauf method is most widely used for calculation of adsorption isotherms.[4,5]

At the same time, it is known[6] from the theory of equilibrium chromatography that

$$V'_{R,g} = \left(\frac{\partial a}{\partial C}\right)_T = v_m\left(\frac{\partial a}{\partial X_S}\right)_T, \tag{11}$$

where c/mol L^{-1} is the equilibrium concentration, v_m is the molar mean volume of the binary solution of the mobile phase, and $V'_{R,g}$ is the corrected retention volume related to the adsorbent weight.

Differentiating Eq. (9), we obtain

$$\left(\frac{\partial a}{\partial X_S}\right)_T = \frac{a_mK_S[1 + (K_M - 1)X_M]}{[1 + (K_M - 1)X_M + (K_S - 1)X_S]^2}. \tag{12}$$

Then it follows from Eqs. (11) and (12) that $v_m/V'_{R,g}$ as a function of the molar fraction of the adsorbate X_S can be presented as

$$v_m/V'_{R,g} = \frac{[1 + (K_M - 1)X_M + (K_S - 1)X_S]^2}{a_mK_S[1 + (K_M - 1)X_M]} \tag{13}$$

or in the linear form

$$\sqrt{\frac{v_m}{V'_{R,g}}} = \frac{\sqrt{1 + (K_M - 1)X_M}}{\sqrt{a_mK_S}} + \frac{K_S - 1}{\sqrt{a_mK_S[1 + (K_M - 1)X_M]}}X_S. \tag{14}$$

The derived equations can be simplified for the cases where the mobile phase is a dilute solution.

1. When the concentrations of the adsorbate in the mobile phase are low and much lower than those of the modifier ($X_S \ll X_M$, $X_S \to 0$), Eq. (9) transforms into Eq. (15)

$$a = \frac{a_mK_S}{1 + (K_M - 1)X_M}X_S, \tag{15}$$

i.e., a depends linearly on X_S at X_M = const.

2. In the case of low concentrations of the modifier, $X_M \to 0$; therefore, in the denominator of Eq. (9) the term $(K_M - 1)X_M \to 0$, and Eq. (9) takes the form of the isotherm describing adsorption from binary solutions:

$$a = \frac{a_mK_SX_S}{1 + (K_S - 1)X_S}, \tag{16}$$

and, correspondingly, we obtain from Eq. (14)

$$\sqrt{\frac{v_m}{V'_{R,g}}} = \frac{1}{\sqrt{a_mK_S}} + \frac{K_S - 1}{\sqrt{a_mK_S}}X_S. \tag{17}$$

From this relationship we can calculate the a_m and K_S constants.

To determine the limiting adsorption values a_m and adsorption constants of the adsorbate K_S from Eq. (14), in the case of three-component solutions, the value of the adsorption constant of the modifier K_M are needed.

Equations (14) and (17) can be presented in the form

$$\sqrt{v_m/V'_{R,g}} = A + BX_S, \tag{18}$$

where the A and B coefficients for two- and three-component solutions are given by

Three-component solution	Binary solution
$A = \sqrt{1+(K_M-1)X_M}\Big/\sqrt{a_m K_S}$	$A = 1\Big/\sqrt{a_m K_S}$
$B = (K_S-1)\Big/\sqrt{a_m K_S[1+(K_M-1)X_M]}$	$B = (K_S-1)\Big/\sqrt{a_m K_S}$
$AB = (K_S - 1)/(a_m K_S)$	$AB = (K_S - 1)/(a_m K_S)$
$A^2 = [1 + (K_M - 1)X_M]/(a_m K_S)$	$A^2 = 1/(a_m K_S)$

A comparison of the expressions presented above with Eq. (10) gives the following equations:

$$X_S/a = A^2 + AB \cdot X_S \tag{19}$$

and

$$a = X_S/(AB \cdot X_S + A^2). \tag{20}$$

Formula (20) is convenient for the calculation of adsorption of substances from a three-component solution using retention volumes. With this purpose, the A and B coefficients can be found from Eq. (18), and then the adsorption isotherm can be calculated from Eq. (20).

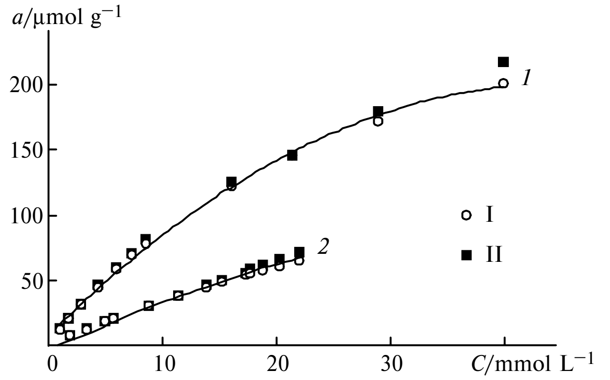

Fig. 1. Isotherms of adsorption of anisole from n-hexane (*1*) and an n-hexane—0.005 vol.% THF solution (*2*). I, calculation from the retention parameters (Eq. (20)); II, calculation by the Glueckauf method. Hydroxylated silica gel Silasorb-300 was used as the adsorbent.

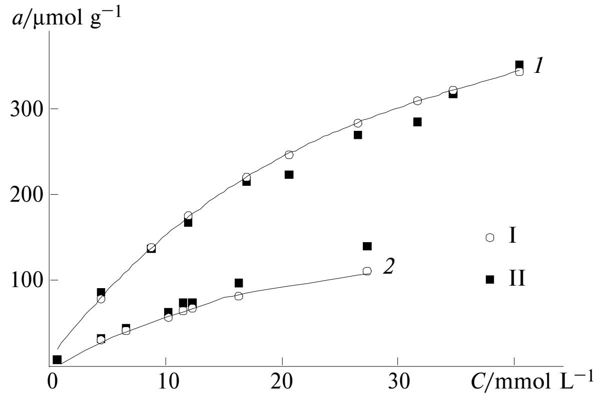

Fig. 2. Isotherm of adsorption of nitrobenzene from n-hexane (*1*) and an n-hexane—0.01 vol.% THF solution (*2*). I, calculation from the retention parameters (Eq. (20)); II, calculation by the Glueckauf method. Hydroxylated silica gel Silasorb-300 was used as the adsorbent.

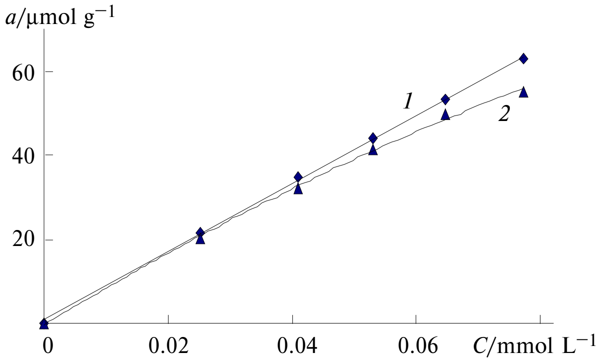

Fig. 3. Isotherm of adsorption of benzene from n-hexane on hydroxylated silica gel Silasorb-300: *1*, calculation by the Glueckauf method; *2*, calculation by Eq. (20).

The isotherms of adsorption of benzene, anisole, and nitrobenzene on hydroxylated silica gel, which were calculated by the method described above, are shown in Figs. 1—3.

Consider the results of the calculation of adsorption of anisole from a three-component solution (anisole—n-hexane—0.005 vol.% THF) and of nitrobenzene from the three-component solution (nitrobenzene—n-hexane—0.01 vol.% THF) on hydroxylated silica gel Silasorb-300. The experimental data on retention of anisole and nitrobenzene for different equilibrium concentrations X_S are presented in Table 1. Processing these data (Fig. 4) by the method of regression analysis gives the A and B coefficients in Eq. (18) for anisole

$$\sqrt{v_m/V'_{R,g}} = 5.8079 + 644.08\ X_S$$

and for nitrobenzene

$$\sqrt{v_m/V'_{R,g}} = 4.0639 + 1096.6\ X_S.$$

Then, using the A and B coefficients and Eq. (20), we calculated the adsorption values for anisole and nitrobenzene from solutions with different equilibrium concentrations of the adsorbate (see Table 2).

The experimental chromatograms show that anisole and nitrobenzene exhibit asymmetrical chromatographic peaks with the extended tail at the rear boundary and a considerable decrease in the retention with an increase in the sample, which corresponds to the adsorption isotherm convex toward the adsorption axis.[6] This agrees well with the data in Figs. 1 and 2.

A comparison of the isotherms of adsorption of anisole (see Fig. 1) and nitrobenzene (see Fig. 2) from n-hexane with the adsorption isotherms of the same substances from the three-component solution (adsorbate—n-hexane—THF) shows that the introduction of even very small amount of THF as a modifying additive into the mobile phase (see Table 2) decreases the adsorption capacity more than twofold. This is related to the competitive mechanism of adsorption of the anisole

Table 1. Corrected retention volumes (V'_R), equilibrium concentrations (X_S), and adsorption values (a) of anisole and nitrobenzene from three-component solutions: sorbate—*n*-hexane—tetrahydrofuran (0.01 vol.% THF)

V'_R/μL	$X_S \cdot 10^3$	a/μmol g^{-1}	
		I*	II**
Anisole—*n*-hexane—tetrahydrofuran (0.005 vol.%)			
760	0.249	7.54	7.18
724	0.429	12.49	12.14
669	0.652	18.07	18.03
656	0.745	20.49	20.40
609	1.147	30.47	32.46
571	1.485	38.22	37.80
557	1.806	46.21	44.61
524	1.987	49.75	48.27
501	2.263	55.65	53.63
494	2.303	57.91	54.38
483	2.458	61.95	57.26
466	2.641	65.68	60.56
456	2.895	71.17	64.97
Nitrobenzene—*n*-hexane—tetrahydrofuran (0.01 vol.%)			
1356	0.130	7.58	7.60
1243	0.571	31.64	29.96
1087	0.857	44.87	42.14
922	1.272	63.44	57.34
869	1.503	73.60	64.47
753	1.607	74.60	67.87
609	2.125	97.21	81.78
414	3.572	140.38	110.13

* I, calculated by the Glueckauf method. ** II, calculated by Eq. (20).

and THF molecules on the surface of hydroxylated silica gel. However, at a low THF concentration in the mobile phase, intermolecular interactions in a solution can be ignored, and Eqs. (18) and (20) can be used to determine the adsorption isotherms.

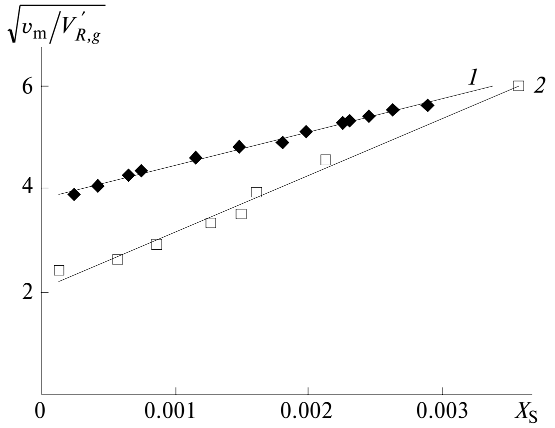

Fig. 4. Specific corrected retention volumes of anisole (*1*) and nitrobenzene (*2*) as a function of the molar fraction of the adsorbate in the coordinates of Eq. (17). Adsorbent, hydroxylated silica gel Silasorb-300; mobile phase, *n*-hexane with an additive of 0.005 (*1*) and 0.01 vol.% (*2*) THF. Points, experiment; curves (*1*) and (*2*), approximation by Eq. (17).

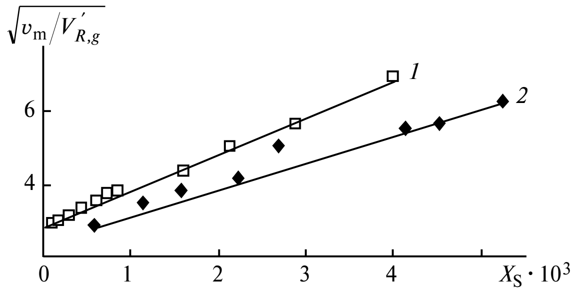

Fig. 5. Specific corrected retention volumes of anisole (*1*) and nitrobenzene (*2*) as functions of the molar fraction of the adsorbate in the coordinates of Eq. (17). Adsorbent, hydroxylated silica gel Silasorb-300; mobile phase, *n*-hexane. Points, experiment; curves (*1*) and (*2*), approximation by Eq. (17).

The chromatographic peaks of benzene are weakly asymmetrical, and the retention volumes V'_R decrease slightly with an increase in the amount of the adsorbate. As a result, the isotherm of adsorption of benzene is an almost straight line (see Fig. 3).

The plots obtained from both the chromatographic data (see Figs. 4 and 5) and adsorption isotherms calculated by the Glueckauf method (see Fig. 6) are presented in Figs. 4 and 5 in the coordinates of Eqs. (14) and (17) and in Fig. 6 in the coordinates of Eq. (19). It is seen that Eqs. (14), (17), and (19) rather well express the character of the relationships, which indicates that the equations of adsorption isotherms (9) and (16) can be applied for the description of the chromatographic systems under study. When we use the retention parameters $V'_{R,g}$ and the equilibrium concentration of the adsorbate in the mobile phase (19) measured directly in the chromatographic experiment, the linearity is better expressed than for the adsorption isotherms calculated by the Glueckauf method. In the latter case, additional errors due to a possible diffusion broadening of chromatographic zones are introduced into the calculation.[7]

The K_S constants of the adsorption equation and a_m values were calculated for adsorption from *n*-hexane by the proposed method (see Eq. (17)) and Glueckauf method. The K_S and a_m values calculated by the method of regression analysis from Eqs. (16) and (17) are rather

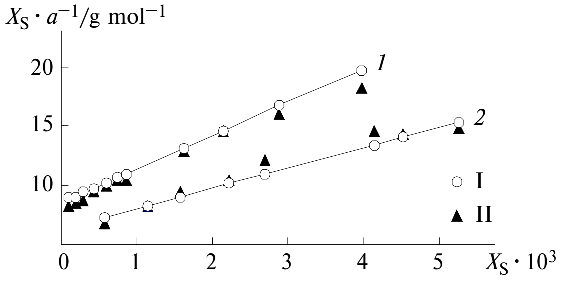

Fig. 6. Isotherms of adsorption of anisole (*1*) and nitrobenzene (*2*) from *n*-hexane on hydroxylated silica gel Silasorb-300 in the linear coordinates of Eq. (19). I, calculation from the retention parameters (Eq. (19)); II, calculation by the Glueckauf method.

Table 2. Equilibrium constants for the adsorbate (K_S) and limiting surface concentrations (a_m) in the compact adsorption monolayer calculated by Eqs. (16) and (17)

System	K_S		a_m/μmol g^{-1}		a_mK_S/mmol g^{-1}	
	(16)	(17)	(16)	(17)	(16)	(17)
Anisole—*n*-hexane	304.7	336.5	394±26	349±8	120.0±4.5	117.4±4.6
Nitrobenzene—*n*-hexane	275.3	259.7	580±67	551±35	159.6±16.0	143.1±7.2
Benzene—*n*-hexane	—	9.9	—	642±32	—	6.4±0.3

close (see Table 2), and their difference is most likely associated with the above mentioned diffusion broadening of the peaks, which is ignored in the Glueckauf method.[4,5]

Thus, in the case of weak intermolecular interactions in a solution, the influence of the concentration of the adsorbate in the mobile phase on its chromatographic retention can be described by the equations based on the theory of equilibrium adsorption. Within the experimental error, the adsorption isotherm can sufficiently exactly be calculated directly from the chromatographic data using the retention values and heights of the peaks, *i.e.*, the equilibrium concentration of the adsorbate. At an elevated concentration of the modifier, the interactions in the mobile phase should be taken into account because they result in the deviation from linearity of the plot of the inverse retention volume *vs.* concentration of the modifier.[1,2]

As compared to the Glueckauf method, the proposed method of calculation of adsorption isotherms is more accurate because the values of retention volumes ($V'_{R,g}$) can be determined with better reliability and more easily than the "adsorption area" and area of chromatographic peak even if these surface areas are found by integrators or on a computer.

Further in the method proposed by us, for the calculation of sorption isotherms from retention volumes and equilibrium concentrations, as in the known method,[7] the influence of diffusion broadening of chromatographic peaks is minimized. The curve drawn through the peak tops approaches the elution curve for the case of ideal equilibrium chromatography, and the sorption isotherm calculated from it is close to the equilibrium isotherm.[7]

Thus, the proposed method allows one to calculate the adsorption isotherms and K_S constants and and a_m values for the studied adsorbates directly from the chromatographic data (retention parameters and equilibrium concentrations of the adsorbate in the mobile phase).

This work was financially supported by the Russian Foundation for Basic Research (Project Nos. 98-03-32690 and 00-15-97346).

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Received December 30, 1999;
in revised form July 12, 2000