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# Influence of the concentration of adsorbate and modifier in the mobile phase on retention in high-performance liquid chromatography

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## Abstract

A method for the determination of the monomolecular adsorption constants from binary (adsorbate–eluent) solution directly from chromatography data (retention volume and concentration) is suggested. The constants of the adsorption equation are calculated from an anisole chromatogram measured on hydroxylated silica using *n*-hexane in the low concentration region. The adsorption isotherm of anisole is constructed. Good agreement between isotherms calculated by the suggested and by Glueckauf's methods is shown. The influence of the modifier concentration on retention of benzene derivatives is studied for the chromatographic system: *n*-hexane–tetrahydrofuran–aminopropyl silica. The deviation from linear dependence of the reciprocal of the retention volume on mole fraction of the polar modifier is explained by molecular modifier–modifier interaction in eluent. Taking into account this interaction and using the quasi-chemical model of retention allows one to describe the dependence of chromatographic data of benzene derivatives on the modifier concentration in the eluent. © 1998 Published by Elsevier Science B.V.

**Keywords:** Adsorption; Mobile phase composition; Adsorption isotherms; Anisole

## 1. Introduction

The study of the influence of the adsorbate and modifier concentration in a mobile phase on the retention in HPLC is important for understanding the regularities of the retention and the mechanisms of separation of substances in the chromatographic process, as well as for measuring adsorption from a solution especially in the low concentration region. Many aspects of the theory of liquid–solid chromatography (LSC) still remain unclear. This is explained by the variety and complexity of molecular interactions between the components even in the simple chromatographic systems.

At present the theory of the retention and selectivity of retention in LSC with binary eluents is the most developed one. Changes of the nature and composition of a mobile phase enables one to regulate the retention parameters of substances under separation in a wide range and to optimise the chromatographic process.

The Snyder–Soczewinski [1–4] (Eq. (1)), and Scott–Kucera [5–9] (Eq. (2)) models are known and widely used.

$$\log k' = a - n \log M_m \quad (1)$$

$$1/V'_R = A + BC_m \quad (2)$$

where  $k'$  is the capacity factor;  $M_m$  is mole fraction of the modifier;  $V'_R$  is the corrected retention volume;

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$C_m$  (% v/v) is the concentration of the modifier in mobile phase;  $a$ ,  $n$ ,  $A$ ,  $B$  are factors.

These models adequately describe the experimental data as a direct linear dependence of retention values on the concentration of a polar additive.

However in many cases there are deviations of experimental data from data calculated from the linear dependence in Eqs. (1) and (2), especially in the region of low concentration (less than 1–2%) of the more polar component of the mobile phase. In Refs. [10–16] these deviations were explained by the influence of molecular interactions in the mobile phase. The authors suggested taking into account not only the competitive adsorption of adsorbate and modifier, but also the association of the adsorbate and modifier molecules in a solution and self-association of the modifier molecules. Equations that describe the retention of substances in a normal-phase (NP)- and reversed-phase (RP)-HPLC with a binary eluent were obtained for various types of molecular interaction in the chromatographic system (solute–mobile phase–stationary phase).

The influence of the adsorbate concentration on the retention and on the form of the chromatographic peak is usually observed for nonlinear equilibrium chromatography (for large samples of adsorbate in preparative chromatography). However even in analytical chromatography, it is not always possible to work in the linear region of the isotherm (Henry's law) and it shows up as strong asymmetry of the chromatographic peaks. Now HPLC is more often used for research of adsorption from solutions and measurements of adsorption isotherms, especially in the low concentration region [17–20]. For construction of adsorption isotherms from chromatograms, Glueckauf's method is widely used [21], which requires a precision apparatus for carrying out an experiment and for processing of results.

In our work, reasonably simple model chromatographic systems were used to obtain dependences that enable to quantitatively describe adsorbate retention in NP-HPLC. The dependence of the chromatographic behaviour of polar adsorbate on its concentration in the eluent (*n*-hexane) was investigated. In this case, it is possible to neglect the contribution of specific interactions of anisole–*n*-hexane and anisole–*n*-anisole types in the mobile phase. Molecular interactions of modifier–modifier

type have to be taken into account when studying the influence of the concentration of the modifier tetrahydrofuran (THF) in *n*-hexane on retention of aromatic substances in NP-HPLC.

## 2. Experimental

A microcolumn liquid chromatograph Milichrom with electromechanical syringe pump and UV spectrophotometer ( $\lambda = 254$  nm) was used. Steel columns (120 × 2 mm) are slurry-packed with hydroxylated silica Silasorb-300 (adsorbent mass in the column  $m = 0.202$  g) and silica Silasorb-NH<sub>2</sub> with a specific surface area about 300 m<sup>2</sup>/g ( $d_{\text{pore}} \sim 10$  nm,  $d_p \sim 10$  μm). Pure *n*-hexane (dried by zeolite NaA) and a mixture of *n*-hexane with polar modifier THF were used as a mobile phase. The mobile phases with the following THF concentrations in *n*-hexane: 0; 0.005; 0.01; 0.05; 0.075; 0.1; 0.15; 0.18; 0.2; 0.3% (v/v) were used. The dead volume of a column was calculated from the retention of the practically nonadsorbed substance—carbon tetrachloride.

For measurement of an adsorption isotherm, the injected volumes of the anisole solution were varied from 1 to 50 μl, and the concentration of the anisole solutions was 0.1837 and 1.837 mol/l.

## 3. Results and discussion

Earlier [14–16] we suggested the model of adsorption and retention of adsorbate from binary eluent. We took into account the following assumptions: the sorbent surface is chemically and geometrically homogeneous and therefore the sorption energy of the components of the mobile phase is constant on any part of the surface; the absence of molecular associations in the adsorption layer; the surface solution and bulk solution are ideal. On the basis of this model it is possible to obtain the following equation of adsorption isotherm from the ternary solution:

$$a = \frac{a_m K_S X}{1 + (K_S - 1)X + (K_M - 1)X_M} \quad (3)$$

where  $X$ ,  $X_M$  are mole fractions of the adsorbate and

modifier in the mobile phase, accordingly;  $a_m$  is the limiting surface concentration of adsorbate in dense adsorption monolayer ( $\mu\text{mol/g}$ );  $K_i$  is the factor of distribution of components between two (bulk and surface) ternary equilibrium solutions, that is equal to:

$$K_i = \frac{X_1^S X_2}{X_1 X_2^S} \quad (4)$$

where  $X_i$ ,  $X_i^S$  are mole fractions of the adsorbate (1) and solvent (2) in the mobile phase and the adsorption layer (S), accordingly.

### 3.1. Adsorption and retention of anisole from *n*-hexane

For a binary solution (when the modifier is absent) we obtain an equation of adsorption isotherm [Eq. (5)] from Eq. (3):

$$a = \frac{a_m K X}{1 + (K - 1)X} \quad (5)$$

where  $K$  is constant of adsorption equilibrium.

To determine the values of constants from Eq. (5) it is convenient to transform Eq. (5) to the linear form:

$$\frac{X}{a} = \frac{1}{a_m K} + \frac{K - 1}{a_m K} \cdot X \quad (6)$$

On the other hand, it is known from the theory of equilibrium chromatography, that:

$$V'_g = \frac{da}{dc} = \nu_m \cdot \frac{da}{dX} \quad (7)$$

where  $\nu_m$  is average mole volume of a binary solution (i.e. the mixture of solvent and adsorbate), and  $V'_g$  is the corrected retention volume per gram of adsorbent mass.

Differentiation of Eq. (5) yields:

$$\frac{da}{dX} = \frac{a_m K}{[1 + (K - 1)X]^2} \quad (8)$$

After substitution from Eq. (8) into Eq. (7) and rearrangement, we obtain an equation of dependence of reciprocal of the corrected specific retention volume on the mole fraction of adsorbate in the mobile phase:

$$\frac{1}{V'_g} = \frac{[1 + (K - 1)X]^2}{a_m \nu_m K} \quad (9)$$

or in a linear form:

$$\sqrt{\frac{\nu_m}{V'_g}} = \frac{1}{\sqrt{a_m K}} + \frac{K - 1}{\sqrt{a_m K}} \cdot X \quad (10)$$

The adsorption isotherm of anisole was calculated by Glueckauf's method from equilibrium chromatograms (Fig. 1). Reasonably good agreement between the back borders of chromatographic peaks for the different samples of anisole shows that adsorption equilibrium was established in the column. Calculated values of adsorption ( $a$ ) and equilibrium concentration ( $c$ ) of adsorbate are presented in Table 1.

We can see from the above that there are two ways to use the results of chromatographic measurements for the description of the adsorption isotherm from a binary solution and to check the validity of the adsorption equation used. It is possible using Glueckauf's method to calculate the values of equilibrium adsorption and concentration from each chromatogram (Table 1) and to construct adsorption isotherm  $a = f(X)$  (see Fig. 2), as well as to calculate constants  $K$  and  $a_m$  in Eq. (6). But it is possible on the other hand using Eq. (10) to determine constants in adsorption Eq. (5) directly from chromatographic data  $1/V'_R = f(X)$ , and then to construct an adsorption isotherm using Eq. (5) (Fig. 2).

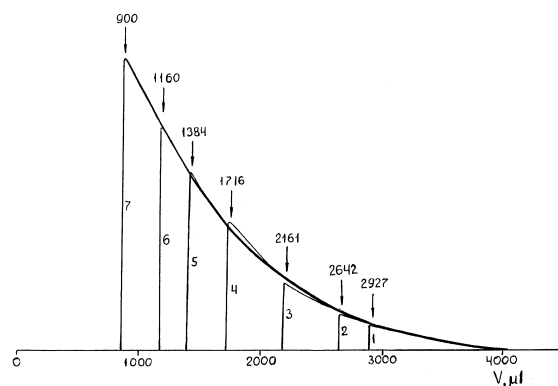


Fig. 1. Anisole chromatograms for sample volumes of solution with concentration 0.1837 mol/l: 1=5; 2=10; 3=25; 4=50 and with concentration 1.837 mol/l: 5=7; 6=10; 7=15  $\mu\text{l}$ . Adsorbent, Silasorb-300; mobile phase, *n*-hexane.

Table 1

Values of mass of anisole ( $m$ ) in sample, corrected retention volume ( $V'_R$ ), mole fraction ( $X$ ) and adsorption ( $a$ ) for various volumes of the sample solution ( $v_s$ )

$v_s$ (ml)	$m_{\text{ads}}$ ( $\mu\text{mol}$ )	$V'_R$ <sup>b</sup> ( $\mu\text{l}$ )	$X \cdot 10^4$	$a$ ( $\mu\text{mol/g}$ )
1	0.184	3044	1.031	12.64
2	0.368	2871	1.778	21.10
5	0.919	2582	2.884	31.57
10	1.837	2297	4.346	46.64
15	2.756	2072	5.951	51.91
20	3.675	1857	7.334	69.86
25	4.593	1816	8.522	81.44
50	9.187	1371	16.075	125.90
7 <sup>a</sup>	12.862	1039	21.358	146.60
10 <sup>a</sup>	18.374	824	28.896	179.55
15 <sup>a</sup>	27.561	555	39.833	217.02

The mobile phase is dried *n*-hexane, room temperature. The concentrations of anisole in the solutions of injected samples are 0.1837 and 1.837 mol/l.

<sup>a</sup> The concentration of anisole solution is equal 1.837 mol/l.

<sup>b</sup> Retention volume, relating to weight of adsorbent in a column.

From Fig. 3 it can be seen that Eq. (10) really represents linear dependence while dependence  $1/V'_R = f(X)$  in Eq. (9) is nonlinear. It specifies the adsorption retention mechanism and the absence of appreciable influence of molecular sorbate–solvent and sorbate–sorbate interactions on the retention.

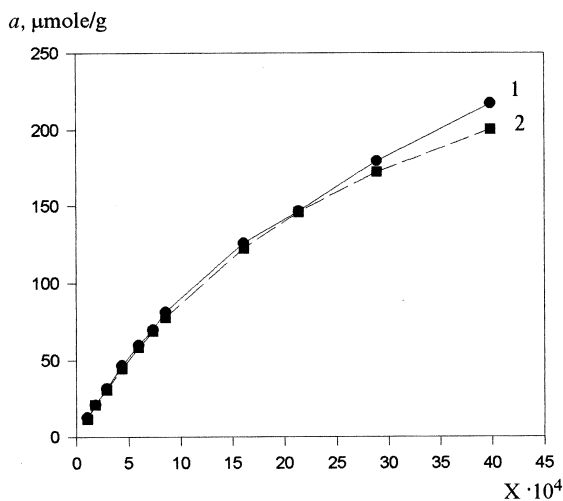


Fig. 2. Dependence adsorption of anisole ( $a$ ) on its equilibrium concentration ( $X$ ) in solution. Adsorbent, Silasorb-300, mobile phase, *n*-hexane. 1 is calculated by Glueckauf's method, 2 is calculated by Eq. (5) with constants  $K$  and  $a_m$  found by Eq. (10).

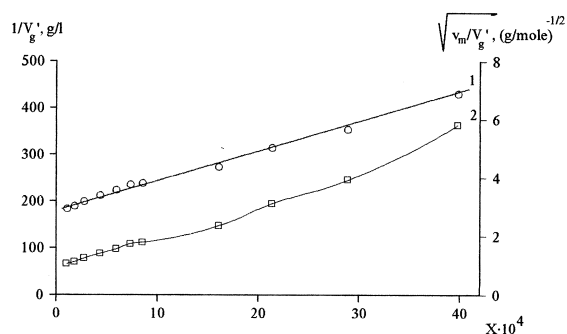


Fig. 3. Dependence of anisole retention on its mole fraction ( $X$ ) in the mobile phase in co-ordinates of Eq. (10), 1 and Eq. (2), 2. Adsorbent, Silasorb-300, mobile phase, *n*-hexane.

The constants of adsorption equilibrium  $K$  and  $a_m$  calculated from linear dependences are indicated in Table 2.

Thus it can be seen from Fig. 2 that isotherms obtained from Glueckauf's method and from Eq. (5) (with factors  $K$  and  $a_m$  found from Eq. (10)) are in good agreement. However the method of calculation of isotherms from chromatograms offered by the authors is considerably simpler than Glueckauf's method.

### 3.2. Influence of concentration of the polar additive on retention

At small concentration of adsorbate in eluent ( $S \ll M$ ) Eq. (3) is transformed into Eq. (11):

$$a = \frac{a_m K_S X}{1 + (K_M - 1)X_M} \quad (11)$$

or in view of Eq. (7):

$$\frac{1}{V'_g} = \frac{1}{a_m v_m K_S} + \frac{(K_M - 1)X_M}{a_m v_m K_S} \quad (12)$$

Table 2

Values of distribution constants ( $K$ ) and limiting surface concentration ( $a_m$ ) of anisole in dense adsorption monolayer for the system: Silasorb-300-*n*-hexane-adsorbate

Calculation from	$K$	$a_m$ ( $\mu\text{mol/g}$ )
Eq. (6) (Glueckauf)	304.7	394.0
Eq. (10) (from retention parameters)	336.5	349.0

Table 3

Factors of capacity ( $k' \times 10^3$ ) of benzene and its derivatives at the various concentrations of THF in the mobile phase

Substance	THF mole fraction in the mobile phase ( $X \cdot 10^3$ )									
	0	0.08	0.16	0.81	1.21	1.61	2.42	2.89	3.21	4.81
Benzene	11.7	10.7	9.9	8.4	7.7	6.9	5.9	5.7	5.5	3.7
Toluene	10.5	9.0	8.5	7.3	6.8	5.4	5.0	4.4	4.1	
Ethylbenzene	9.7	7.8	7.3	6.8	6.2	4.5	4.4	3.7	3.4	
Amylbenzene	7.9	6.9	5.8	5.0	4.3	3.9	2.5	2.2	1.6	
Hexylbenzene	7.6	6.7	5.3	4.4	3.5	3.1	2.2	1.7	1.3	
<i>o</i> -Xylene	10.2	9.9	9.1	6.6	5.6	3.9	3.7	4.3	3.4	
<i>p</i> -Xylene	8.2	7.5	7.0	4.7	4.1	5.4	5.0	3.6	3.8	
1,2,4,5-Tetramethylbenzene	13.5	11.3	9.0	5.6	4.3	3.6	2.8	2.5	2.2	
Pentamethylbenzene	16.7	13.6	11.7	7.3	6.2	5.1	3.7	3.1	3.0	
Hexamethylbenzene	19.6	15.7	14.6	9.4	8.2	6.5	5.0	4.0	3.7	
Anisole	55.6	46.7	42.6	34.5	26.5	23.6	22.0	20.5	20.3	18.9
Nitrobenzene	192.7	179.4	162.4	122.3	99.4	91.3	84.8	80.1	77.0	73.6
Phenyl acetate	367.8	346.4	297.1	164.2	118.2	119.1	88.8	87.8	81.9	74.9
Naphthalene	41.7	43.8	40.2	32.8	26.8	25.3	21.1	18.6	18.1	16.5
Phenanthrene	156.2	136.8	121.0	91.8	65.3	58.8	48.5	44.7	41.1	37.7

Values of capacity factors benzene and its derivatives are indicated in Table 3.

As can be seen from Fig. 4, the equation of linear dependence (Eq. (12)) describes the retention of polymethylbenzenes well. However for the majority of polar aromatic substances, the linear dependence  $1/V_R' = f(X)$  is not followed (Fig. 5). Such distinction in the dependence of sorbate (AHs and anisole) retention on the concentration of modifier in the

mobile phase may be explained by the influence of molecular interactions in the mobile phase. In earlier work [16] the equation taking into account molecular interactions in the mobile phase was considered.

$$1/k' = (1/\Phi K_S)(1 + K_{SM}M_m)[1 + (K_M - 1)M_m - K_{SM}S_mM_m/L^2 - K_{MM}M_m(1 - L^2)/L^2] \quad (13)$$

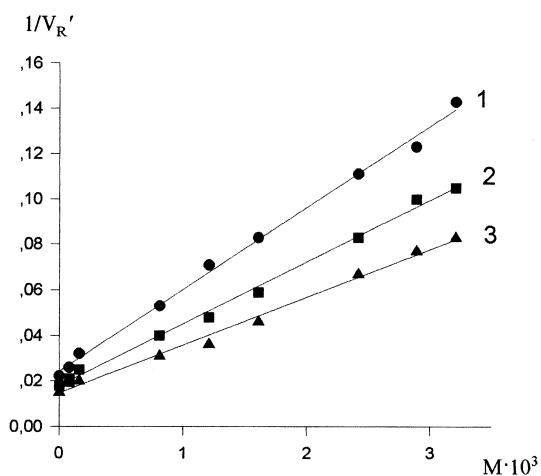


Fig. 4. Dependence of the reciprocal of the corrected retention volume ( $1/V_R'$ ) on mole fraction ( $M$ ) of modifier (THF) in *n*-hexane. Adsorbent, Silasorb-NH<sub>2</sub>. 1=1,2,4,5-tetramethylbenzene, 2=pentamethylbenzene, 3=hexamethylbenzene.

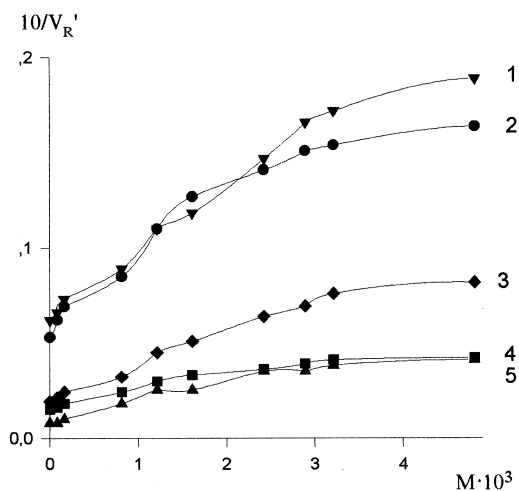


Fig. 5. Dependence of the reciprocal of the corrected retention volume ( $1/V_R'$ ) on mole fraction ( $M$ ) of modifier (THF) in *n*-hexane: 1=naphthalene, 2=anisole, 3=phenanthrene, 4=nitrobenzene, 5=phenyl acetate. Adsorbent, Silasorb-NH<sub>2</sub>.

where  $K_S$ ,  $K_M$ ,  $K_{SM}$ ,  $K_{MM}$  are equilibrium constants of quasi-chemical reactions of adsorption–desorption of the adsorbate ( $K_S$ ) and modifier ( $K_M$ ) and their association in a mobile phase ( $K_{SM}$ ,  $K_{MM}$ );  $S_m$ ,  $M_m$  and  $L$  are mole fractions of the adsorbate, modifier and main component in a mobile phase;  $\Phi$  is the phase ratio of a column.

In the general case, Eq. 13 represents a complex curvilinear dependence of solute retention on the modifier concentration. Only in the absence of associates in the solution, i.e.,  $K_{SM}=0$  and  $K_{MM}=0$ , do we obtain the linear dependence of  $1/k'$  on  $M_m$ .

In the case when the adsorbate–modifier interactions are commensurable with specific interactions of adsorbate with a polar sorbent surface and in the absence of association of modifier molecules  $K_{MM}=0$ , the deviation of experimental data (dependence of  $1/k'$  on  $M_m$ ) from linearity towards ordinate axis ( $1/k'$ ) is observed. If the process of formation of modifier associates (interaction of modifier–modifier type) dominates in a mobile phase the deviation towards the abscissa axis ( $M_m$ ) is observed, the latter was observed in our experimental data (Fig. 5). In this case we have equations:

$$1/k' = (1/\Phi K_S)[1 + (K_M - 1)M_m - K_{MM}M_m(1 - L^2)/L^2] \quad (14)$$

or

$$1/V_R' = (1/V_a K_S)[1 + (K_M - 1)M_m - K_{MM}M_m(1 - L^2)/L^2] \quad (15)$$

since  $k' = V_R'/V_0$ ,  $\Phi = V_a/V_0$ , where  $V_R'$ , and  $V_0$  and  $V_a$  are corrected retention volume, dead volume and volume of the sorption space in a column accordingly.

For treatment of the experimental data, we used Eq. (14) taking into account the interaction of a modifier–modifier type and ignoring adsorbate–modifier type. Calculations were done for phenanthrene, naphthalene and some derivatives of benzene on the basis of retention data for Silasorb-NH<sub>2</sub>. The constants  $K_M$  and  $K_{MM}$  and product  $V_a K_S$  (Table 4) were calculated by the regression analysis. With the calculated values of these constants for anisole and phenanthrene the dependences of  $1/V_R'$  on mole fraction of THF in *n*-hexane have been constructed

Table 4

Values of constants of Eq. (15)

Substance	$V_a K_S$	$K_M$	$K_{MM}$
Benzene	40	549	30939
Toluene	36	615	22582
Anisole	190	1028	75896
Nitrobenzene	659	846	60442
Naphthalene	161	573	10324
Phenanthrene	535	1080	35015

(Fig. 6). It can be seen from these dependences that the calculated and experimental data are in good agreement.

Thus in the case of weak molecular interactions in a solution the influence of concentration of adsorbate on its retention can be described by the equations, based on the theory of equilibrium adsorption. And the adsorption isotherm can be obtained directly from chromatographic data (from values of retention and peaks heights, i.e., the concentration of adsorbate).

At the introduction of modifier into an eluent, molecular interactions in a solution cannot be neglected. To describe the dependence of retention on modifier concentration it is necessary to use equations that take into account the molecular interactions in the mobile phase and the influence of the nature and composition of the mobile phase on retention.

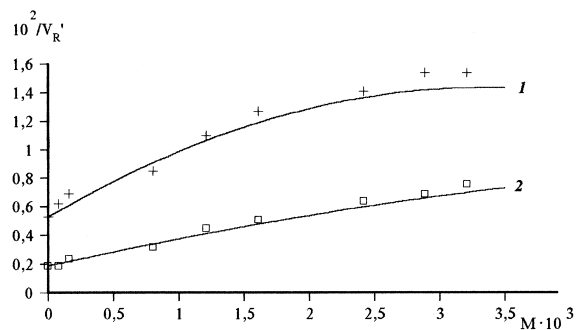


Fig. 6. Dependence of the reciprocal of the retention volume ( $1/V_R'$ ) on mole fraction ( $M$ ) of modifier (THF) in *n*-hexane: 1 = anisole, 2 = phenanthrene. 1, 2 are calculated data, the points are experimental data. Adsorbent, Silasorb-NH<sub>2</sub>.

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