

## Retention of benzene derivatives on the aminopropyl stationary phase in high-performance liquid chromatography

S. N. Lanin,\* M. Yu. Ledenkova, and Yu. S. Nikitin

Department of Chemistry, M. V. Lomonosov Moscow State University,  
Vorob'evy Gory, 119899 Moscow, Russian Federation.  
Fax: 007 (095) 932 8846. E-mail: lanin@achr.chem.msu.ru

The retention of benzene derivatives in the normal-phase mode of high-performance liquid chromatography (HPLC) using a binary mobile phase (*n*-hexane—THF) on aminopropyl silica gel was considered. The possibility of describing the retention of the sorbates by known models was evaluated. It was established that molecular interactions between a sorbate and components of the mobile phase in solution can significantly affect the retention of the sorbates and the character of the dependence of the retention on the concentration of a modifier in the mobile phase. A quantitative description of this dependence was proposed.

**Key words:** liquid adsorption chromatography, aminopropyl stationary phase; quasi-chemical model of retention; benzene derivatives.

The theory of liquid adsorption chromatography is not well developed, although it is significant for practice. This is because of the complex character of the chromatographic system and the necessity of taking into account interactions of all of the components of the solution not only with the adsorbent but also with one another both in the adsorbed state and in solution. Great attention is presently being given to the behavior of diverse chromatographic systems, on the basis of which the theory of chromatographic retention and selectivity can be developed using the theory of adsorption from solutions.

One of the most important and commonly used methods of optimization of the chromatographic process is related to changing the nature and composition of the mobile phase. In particular, in the normal-phase mode of HPLC, the addition of small amounts of a polar modifier to a nonpolar eluent is widely used.<sup>1–4</sup>

In the present work, the retention of aromatic compounds in the normal-phase mode of HPLC on an aminopropyl grafted phase as well as the effect of the content of a modifier in the mobile phase (THF) on the retention of the sorbates mentioned were studied.

### Experimental

The study was carried out in the normal-phase developing mode of HPLC on a Milikhrom microcolumn liquid chromatograph with an electromechanical syringe pump and a UV-spectrometric detector (detection at  $\lambda = 254$  nm). A steel column 120×2 mm in size was packed with Silasorb-NH<sub>2</sub> silica gel with a specific surface of 300 m<sup>2</sup> g<sup>-1</sup> (pore diameter ~10 nm, average particle diameter ~10  $\mu$ ) using the suspension method. Mixtures of *n*-hexane (dried on zeolite NaA) with the polar modifier, THF, were used as the mobile phase. The

concentrations of THF in hexane were 0, 0.005, 0.01, 0.05, 0.075, 0.1, 0.15, 0.18, 0.2, and 0.30 vol.%. On going from one composition of the mobile phase to another, the chromatographic column was conditioned by passing the new phase through it for 6–18 h. The duration of conditioning was determined by the concentration of THF in the mobile phase and its flow rate. The reproducibility of retention volumes of sorbates served as the criterion of stabilization of the column. The "dead" volume of the column was calculated from the retention of the almost unsorbed compound CCl<sub>4</sub>. The volume of the introduced sample of a solution of the sorbate in *n*-hexane was 2  $\mu$ L.

Benzene, toluene, ethylbenzene, *n*-pentylbenzene, *n*-hexylbenzene, 1,2,4,5-tetramethylbenzene, pentamethylbenzene, hexamethylbenzene, anisole, nitrobenzene, phenyl acetate, naphthalene, and phenanthrene were used as sorbates.

### Results and Discussion

In the case of binary mobile phases in HPLC,<sup>1–12</sup> the Snyder—Soczewinski<sup>1–4</sup> (Eq. (1)) and Scott—Kucera<sup>5–9</sup> (Eq. (2)) models are usually used.

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

$$1/V_R' = A + BC_m, \quad (2)$$

where  $k'$  is the capacity coefficient;  $M_m$  is the mole fraction of the modifier;  $V_R'$  is the corrected retention volume;  $C_m$  (vol.%) is the concentration of the modifier;  $a$ ,  $n$ ,  $A$ , and  $B$  are coefficients.

These models satisfactorily describe experimental data as a linear dependence of retention values on the concentration of a polar additive (for the Snyder—Soczewinski model, in the region of modifier concentrations corresponding to the coverages close to a monolayer of modifier molecules on the adsorbent surface).

**Table 1.** Capacity coefficients ( $k'$ ) of benzene and its derivatives for different contents of THF (0–0.3 vol.%) in the mobile phase

Compound	$k' \cdot 10^2$									
	0*	0.005*	0.01*	0.05*	0.075*	0.10*	0.15*	0.18*	0.20*	0.30*
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	
<i>n</i> -Pentylbenzene	7.9	6.9	5.8	5.0	4.3	3.9	2.5	2.2	1.6	
<i>n</i> -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

\* Content of THF (vol.%).

In the present work, the retention of benzene derivatives was studied for small concentrations of a polar additive in the mobile phase (THF in *n*-hexane). Tetrahydrofuran was chosen as the polar solvent because it is strongly sorbed on the adsorbent surface, which corresponds to the requirements of the aforementioned models, and does not form associates with aromatic hydrocarbons.

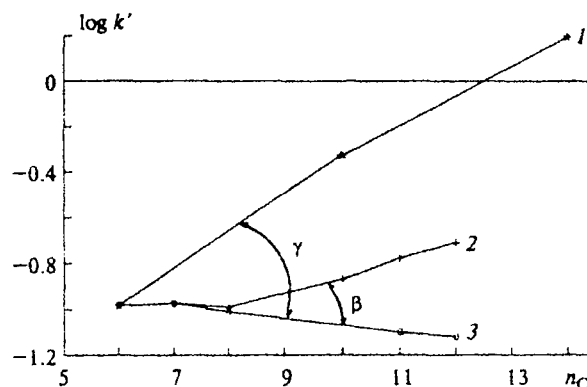
The aminopropyl stationary phase was chosen because retention on this sorbent has been studied to a lesser extent<sup>13–15</sup> than retention on hydroxylated silica gel. In addition, it was of interest to compare the chromatographic behavior of aromatic hydrocarbons on the aminopropyl stationary phase and on silica gel and to compare the selectivity of these sorbents.

#### Effect of a polar additive on the retention

The obtained values of the retention parameters (capacity coefficient  $k'$ ) for monoalkyl- and polymethyl-substituted benzenes (Table 1) are comparatively low even in the case of elution with pure *n*-hexane ( $k' = 0.076–0.196$ ). This testifies, first of all, that these sorbates interact to a smaller extent with the aminopropyl phase than with hydroxylated silica gel, where  $k'$  varies from 0.79 (ethylbenzene) to 1.35 (1,2,4,5-tetramethylbenzene).<sup>16</sup> The introduction of a methyl group to the benzene ring increases the electron density in the cycle, which increases the retention of methyl-substituted benzenes: polymethylbenzenes (PMB) are eluted in order of increasing molecular weight (Fig. 1). When a polar additive (THF) is introduced to the mobile phase, the retention of PMB decreases sharply (see Table 1), since

low-polar PMB have less competitive sorption ability than THF molecules. When the concentration of THF in the mobile phase increases, the coverage of the aminopropyl silica gel surface with THF molecules increases. This likely results in a relative increase in the interaction of PMB with the mobile phase compared to their interaction with the adsorbent.

In the case of mono-*n*-alkyl-substituted benzenes, a change in the length of the alkyl chain has a weak effect on the distribution of the electron density in the benzene cycles. Nevertheless, as the length of the alkyl chain increases, the retention of mono-*n*-alkylbenzenes



**Fig. 1.** Dependence of the logarithm of the capacity coefficient ( $\log k'$ ) on the number of carbon atoms ( $n_C$ ) in the adsorbate molecule: 1, nonsubstituted aromatic hydrocarbons (benzene, naphthalene, phenanthrene); 2, polymethylbenzenes (1,2,4,5-tetramethylbenzene, pentamethylbenzene, and hexamethylbenzene); 3, mono-*n*-alkylbenzenes (toluene, ethylbenzene, *n*-pentylbenzene, *n*-hexylbenzene).

on aminopropylated silica gel decreases gradually, since the hydrophobicity of the sorbate increases and the nonspecific adsorbate—eluent interaction increases. A similar dependence has been previously observed on hydroxylated silica gel.<sup>17,18</sup> In the case of aminopropylated silica gel, the straight line of the dependence of  $\log k'$  on the number of C atoms in the sorbate molecule for PMB is considerably lower than that for polycyclic aromatic hydrocarbons (PAH) and is close to the similar dependence for monoalkylbenzenes (MAB), i.e., the angle  $\beta$  between the dependences of  $\log k'$  on  $n_C$  for PMB and MAB is less than the angle  $\gamma$  formed by the curves for PAH and MAB ( $\beta < \gamma$ , see Fig. 1). In the case of hydroxylated silica gel, these dependences for *ortho*-substituted PMB and PAH almost coincide<sup>17,18</sup> ( $\beta = \gamma$ ). It follows that the use of the aminopropyl phase is preferable for the separation of aromatic hydrocarbons (mono- from bicyclic aromatic hydrocarbons). It can be seen in Fig. 1 that even hexamethylbenzene, which is the most strongly sorbed of the PMB studied, is eluted before naphthalene.

In the case of PAH, the retention increases as the number of aromatic rings increases. The substantial difference between the retention of MAB, PMB, and PAH even at a high concentration of THF (see Table 1) is important from the viewpoint of practical use, because it extends the capabilities of the method in the group separation of complex mixtures of aromatic hydrocarbons.

The introduction of a polar additive (THF) into the mobile phase not only noticeably decreases the retention, but also changes the order of elution of some mono-*n*-alkyl- and polymethyl-substituted benzenes. For example, when the modifier is absent, the following order of elution is observed: hexamethylbenzene > pentamethylbenzene > 1,2,4,5-tetramethylbenzene > benzene > *o*-xylene. When the concentration of THF in the mobile phase is equal to 0.15 vol.%, the elution order is the following: benzene > hexamethylbenzene > pentamethylbenzene > *o*-xylene > 1,2,4,5-tetramethylbenzene. Thus, the effect of the polar additive on the selectivity ( $\alpha$ ) of the retention in the chromatographic system aminopropyl phase—(*n*-hexane + THF) is explicit. The selectivity of a chromatographic system is quantitatively determined as the ratio of the retention values of the sorbate and a reference substance. For benzene derivatives, benzene itself is often chosen as the reference substance. It can be seen in Fig. 2 that the values of  $\log \alpha$  change sign as the concentration of THF increases, which is evidence for the inversion of the elution order of PMB with respect to benzene. When the concentration of the polar additive in the mobile phase is increased to the inversion point, the selectivity of the retention of the sorbates studied decreases with respect to benzene. The stronger the adsorption, the greater the amount of modifier needed to reach the inversion point. For aromatic compounds (naphthalene,

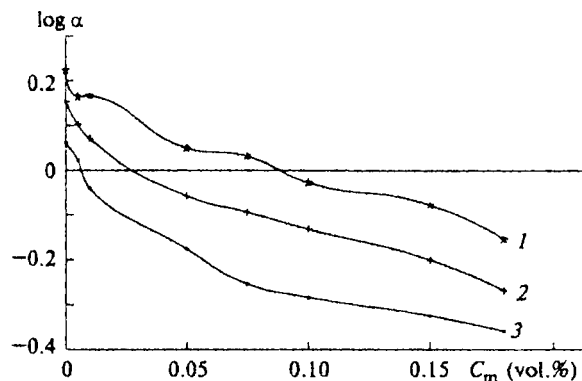


Fig. 2. Dependence of the logarithm of selectivity (with respect to benzene) on the concentration of the modifier (THF) in the mobile phase for PMB: 1, hexamethylbenzene; 2, pentamethylbenzene; 3, 1,2,4,5-tetramethylbenzene.

phenanthrene, anisole, nitrobenzene, and phenyl acetate) that are retained more strongly than PMB, the selectivity of the retention decreases in the whole range of concentrations of the modifier studied.

As can be seen in Fig. 1, PAH (naphthalene, phenanthrene) are retained much more strongly than polymethyl- and monoalkyl-substituted benzenes.

The introduction of polar substituents to the benzene molecule considerably increases their retention over that of nonsubstituted aromatic hydrocarbons in the whole range of concentrations of the modifier. For example, polar compounds (anisole, nitrobenzene, and phenyl acetate) are retained (from pure *n*-hexane) more strongly than benzene by 4.8, 16.5, and 31.4 times, respectively.

#### Use of retention models for describing experimental data

The dependences of the logarithm of the capacity coefficient ( $k'$ ) on the logarithm of the molar ratio of the modifier ( $M_m$ ) and of the inverse value of the corrected retention volume ( $1/V_R'$ ) on the concentration of the modifier ( $C_m$ ) were plotted to estimate the possibility of describing the experimental data by Eqs. (1) and (2).

Noticeable deviations from the linear dependence are observed almost in all cases (Figs. 3–6). It can be seen in Fig. 3 that for the Snyder—Soczewinski model the deviation of the dependences from linearity decreases gradually as the retention of aromatic hydrocarbons increases. However, a small deviation is retained even for phenanthrene. In the case of polar sorbates (phenyl acetate and nitrobenzene), the nonlinearity is less pronounced than for aromatic hydrocarbons.

In the coordinates of the Scott—Kucera equation (see Fig. 4), the maximum deviation from a linear dependence is also observed for weakly retained com-

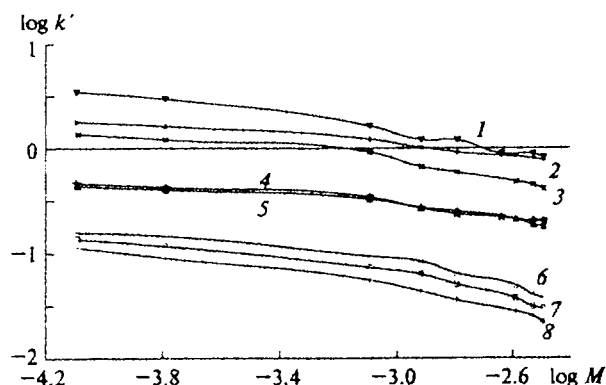


Fig. 3. Dependence of the logarithm of the capacity coefficient ( $\log k'$ ) on the logarithm of the mole fraction of the modifier (THF) for PAH and PMB: 1, phenyl acetate; 2, nitrobenzene; 3, phenanthrene; 4, anisole; 5, naphthalene; 6, hexamethylbenzene; 7, pentamethylbenzene; 8, 1,2,4,5-tetramethylbenzene.

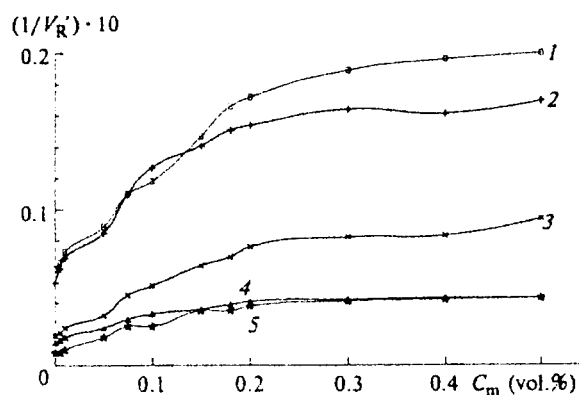


Fig. 4. Dependence of the inverse retention volume ( $1/V_R'$ ) on the concentration of the modifier (THF) for PAH and benzene derivatives with polar substituents: 1, naphthalene; 2, anisole; 3, phenanthrene; 4, nitrobenzene; 5, phenyl acetate.

pounds (naphthalene and anisole). Processing of the experimental data by the least-squares method, using anisole and phenanthrene as examples, showed that (at the confidence level  $p = 0.95$ ) the confidence intervals of the values of the coefficients  $A$  and  $B$  in Eq. (2) for anisole ( $A = 0.008 \pm 0.0022$ ,  $B = 0.023 \pm 0.0096$ , correlation coefficient  $r = 0.8590$ ) and phenanthrene ( $A = 0.0030 \pm 0.00098$ ,  $B = 0.015 \pm 0.0043$ ,  $r = 0.9268$ ) are rather large ( $\sim 25$ – $35\%$ ) and considerably exceed the errors of the experimentally measured retention ( $< 2\%$ ). A reasonably good approximation of a linear dependence is observed only for 1,2,4,5-tetramethylbenzene, pentamethylbenzene, and hexamethylbenzene (see Fig. 5).

Thus, in the majority of cases, the data obtained cannot be described by the classical Snyder–Soczewinski and Scott–Kucera models, which is explained first of

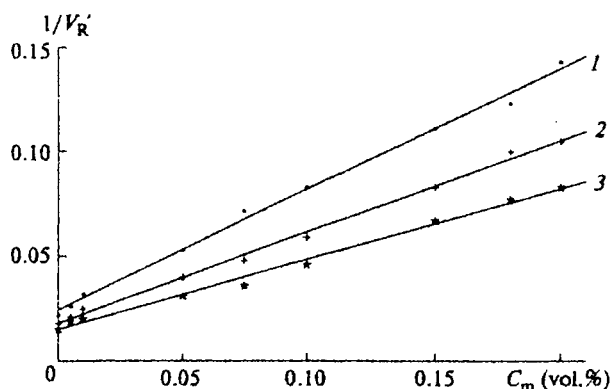


Fig. 5. Dependence of the inverse retention volume ( $1/V_R'$ ) on the concentration of the modifier (THF) for PMB: 1, 1,2,4,5-tetramethylbenzene; 2, pentamethylbenzene; 3, hexamethylbenzene.

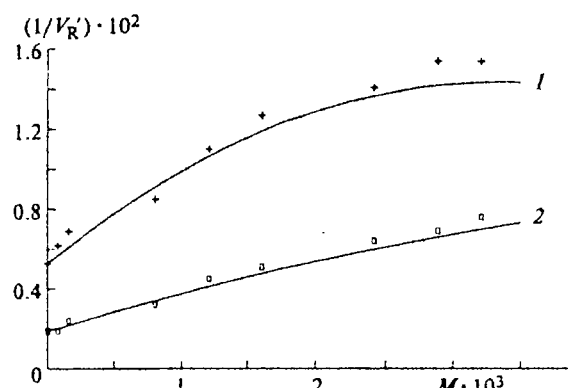


Fig. 6. Dependence of the inverse retention volume ( $1/V_R'$ ) on the mole fraction of the modifier (THF) ( $M$ ) in the mobile phase. Curves indicate the calculation from Eq. (4'); 1, anisole and 2, phenanthrene; points show experimental data.

all, in our opinion, by the fact that these models do not take into account molecular interactions of the modifier molecules with each other and interactions between the modifier and sorbate molecules in the mobile phase.

These molecular interactions in the mobile phase were taken into account by the model of monomolecular adsorption,<sup>19</sup> and the following equation was derived using the quasi-chemical equilibria of the components of the chromatographic system:

$$1/k' = (1/\Phi K_S)(1 + K_{SM}M_m/L)[1 + (K_M - 1)M_m - K_{SM}S_mM_m/L^2 - K_{MM}M_m(1 - L^2)/L^2], \quad (3)$$

where  $K_S$ ,  $K_M$ ,  $K_{SM}$ ,  $K_{MM}$  are the equilibrium constants of the quasi-chemical adsorption–desorption reactions of the sorbate ( $K_S$ ) and modifier ( $K_M$ ) and of their association in the mobile phase ( $K_{SM}$ ,  $K_{MM}$ , respectively);  $S_m$ ,  $M_m$ , and  $L$  are the mole fractions of the sorbate, modifier, and main components in the mobile

**Table 2.** Parameters of Eq. (4') for benzene derivatives

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

phase, respectively; and  $\Phi$  is the phase ratio of the column. Without taking into account the sorbate—modifier (SM) interactions, we obtain

$$1/k' = (1/\Phi K_S)[1 + (K_M - 1)M_m - K_{MM}M_m(1 - L^2)/L^2] \quad (4)$$

or

$$1/V_R' = (1/V_a K_S)[1 + (K_M - 1)M_m - K_{MM}M_m(1 - L^2)/L^2], \quad (4')$$

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

Without taking into account the modifier—modifier (MM) interactions, Eq. (3) takes the following form:

$$1/k' = (1/\Phi K_S)(1 + K_{SM}M_m/L)[1 + (K_M - 1)M_m - K_{SM}S_mM_m/L^2]. \quad (5)$$

When the sorbate—modifier interactions are comparable with the specific interactions of the sorbate with the polar sorbent surface, deviation of the experimental data (dependences  $1/k'$  on  $M_m$ ) from linearity toward the ordinate axis ( $1/k'$ ) is observed. When the formation of associates of the modifier (modifier—modifier interaction) predominates in the mobile phase, deviation toward the abscissa axis ( $M_m$ ) takes place, as was observed in our experiments.

Therefore, for processing the experimental data, we used Eq. (4'), which takes into account the modifier—modifier interactions and ignores the sorbate—modifier interaction. The calculations were performed for phenanthrene, naphthalene, and some benzene derivatives from the data for their retention on Silasorb-NH<sub>2</sub>. The constants  $K_M$  and  $K_{MM}$  and the product  $V_a K_S$  were calculated by the regression analysis method (Table 2). The dependences of the  $1/V_R'$  value on the mole fraction of THF in *n*-hexane were plotted using the calculated

values of these constants for anisole and phenanthrene (see Fig. 6). It can be seen from these dependences that the quasi-chemical model of retention corresponding to Eq. (4') describes the experimental data on retention of the benzene derivatives better than the Snyder—Soczewinski and Scott—Kucera models.

Thus, when describing processes in chromatographic systems, intermolecular interactions in the mobile phase and the effect of the nature and composition of the mobile phase on the chromatographic retention of substances must be taken into account.

This work was financially supported by the Russian Foundation for Basic Research (Project No. 95-03-09105).

## References

1. L. R. Snyder, *Principles of Adsorption Chromatography*, Marcel Dekker, New York, 1968, 413 pp.
2. L. R. Snyder, *Anal. Chem.*, 1974, **46**, 1384.
3. E. Soczewinski, *Anal. Chem.*, 1969, **41**, 179.
4. E. Soczewinski and W. Golkiewicz, *Chromatographia*, 1973, **6**, 269.
5. R. P. W. Scott and P. Kucera, *Anal. Chem.*, 1973, **45**, 749.
6. R. P. W. Scott and P. Kucera, *J. Chromatogr.*, 1975, **112**, 425.
7. R. P. W. Scott, *J. Chromatogr.*, 1976, **122**, 35.
8. R. P. W. Scott and P. Kucera, *J. Chromatogr.*, 1979, **171**, 37.
9. R. P. W. Scott, *J. Chromatogr. Sci.*, 1980, **18**, 297.
10. M. Jaroniec and A. Patrykiewicz, *J. Chem. Soc., Faraday Trans. 1*, 1980, **76**, 2486.
11. M. Jaroniec and J. Piotrowska, *J. High Resol. Chromatogr., Chromatogr. Commun.*, 1980, **3**, 257.
12. A. M. Tolmachev, *Zh. Fiz. Khim.*, 1973, **47**, 2629 [*Sov. J. Phys. Chem.*, 1973, **47** (Engl. Transl.)].
13. L. D. Olsen and R. J. Hurtubise, *J. Chromatogr.*, 1989, **479**, 5.
14. R. J. Hurtubise, A. Hussian, and H. F. Silver, *Anal. Chem.*, 1981, **53**, 1993.
15. M. Waksmundzka and M. L. Bieganska, *Chem. Anal. (Warsaw)*, 1988, **33**, 305.
16. Al'-Akhamad Akram, Ph.D. (Chem.) Thesis, M. V. Lomonosov Moscow State Univ., Moscow, 1989, 187 pp. (in Russian).
17. A. N. Ageev, A. V. Kiselev, and Y. I. Yashin, *Chromatographia*, 1980, **13**, 669.
18. Be En Il, Ph. D. (Chem.) Thesis, M. V. Lomonosov Moscow State Univ., Moscow, 1992, 227 pp. (in Russian).
19. S. N. Lanin and Yu. S. Nikitin, *Zh. Anal. Khim.*, 1991, **46**, 1971 [*Sov. J. Anal. Chem.*, 1991, **46** (Engl. Transl.)].

Received July 9, 1996;  
in revised form October 16, 1996