CHREV. 161

SELECTIVITY IN LIQUID ADSORPTION CHROMATOGRAPHY

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(Received March 15th, 1982)

CONTENTS

1. INTRODUCTION

Liquid column chromatography (LC) has been intensively developed in recent years and has become one of the most important separation and analytical methods for multi-component mixtures of high-boiling and unstable compounds[1-6]. LC is particularly useful for studies in high-molecular compound chemistry, petroleum chemistry, biochemistry and medicine.

In addition to achievements in high-speed separation theory and in instrumentation, the advances in LC have been the result of its high selectivity and high efficiency. Nowadays columns with efficiencies of 50,000–100,000 plates per metre are available[4-6], and efficiencies up to 250,000[7] or even 750,000 theoretical plates[8] can be obtained.

The selectivity of columns in liquid adsorption chromatography (LAC) is often higher than that in gas chromatography (GC) as separations are usually performed at lower temperatures (often close to room temperature) and on more active adsorbents.

LC has exeptional potential for the control of the selectivity of separation. In GC, substances are separated on the basis of differences in non-specific (mainly dispersive) interactions only, or the sum of specific and non-specific intermolecular interactions of the substance–adsorbent type (according to Kiselev's classification[9]). Retention and selectivity in LC may be determined by a number of different intermolecular interactions due to the active role of the mobile phase. In LC the retention may be determined mainly by specific interactions (normal-phase chromatography) or by non-specific substance–adsorbent interactions (reversed-phase chromatography), and also by their combinations.

In this paper the attempt is made to classify LAC variants according to the main types of intermolecular interactions that determine retention.

2. RESULTS AND DISCUSSION

In molecular LAC the retention of substances and the selectivity of their separation even in the simplest cases are determined by three types of intermolecular interactions: substance–adsorbent, eluent–adsorbent and substance–eluent[4-6].

TABLE I

CLASSIFICATION OF LAC VARIANTS ACCORDING TO THE MAIN TYPES OF INTER-
MOLECULAR INTERACTION

SI = specific interaction; NI = non-specific interaction.

Variant No.	Interaction determining substance retention	Retention characteristics
1	Substance–adsorbent SI	(1) High selectivity towards compounds differing in polarity; (2) high selectivity towards compounds differing in geometrical structure (isomers and others); (3) group separation of homologous series is possible
2	Substance–adsorbent SI and substance–eluent NI	Variation of retention order of aromatic alkyl derivatives
3	Substance–adsorbent SI and substance–eluent SI	Variation of retention order of polar compounds
4	Substance–adsorbent NI	Separation of homologues
5	Substance–adsorbent NI and substance–eluent SI	Polar substances elute earlier than non-polar substances

When a mixture is used as the eluent the number of possible interactions increases. However, in certain instances particular types of interaction predominate[10]. In general it may be stated that the total retention in LAC is determined by specific or non-specific intermolecular interactions both on the adsorbent surface and within the liquid mobile phase, the latter decreasing the total substance retention. In Table I variants of LAC are given where one or two types of interactions predominate. This classification is supported by some experimental results.

Selectivity towards various classes of compounds in the different variants will be determined by changes in the free energy of sorption, $\Delta(\Delta F)$, according to the following relationship:

$$\Delta(\Delta F) = -RT \ln t_R/t_{st} \tag{1}$$

where t_R is the retention time of the substance under study and t_{st} is the retention time of a standard substance, usually benzene. Values of $\Delta(\Delta F)$ are determined from the retention ratio of the compounds obtained under identical conditions and often from the same chromatogram, and therefore can be calculated with good accuracy.

According to changes in the $\Delta(\Delta F)$ values of benzene derivatives (relative to benzene) the selectivity of a chromatographic system towards a given class of compounds can be estimated. As the eluent usually plays an active role in LC, it is necessary to consider the selectivity of the system as a whole and not only that of the adsorbent.

Variant 1

For the first case of chromatography on a polar adsorbent (for example, hydroxylated silica gel) with a non-polar or weakly polar eluent, the retention of
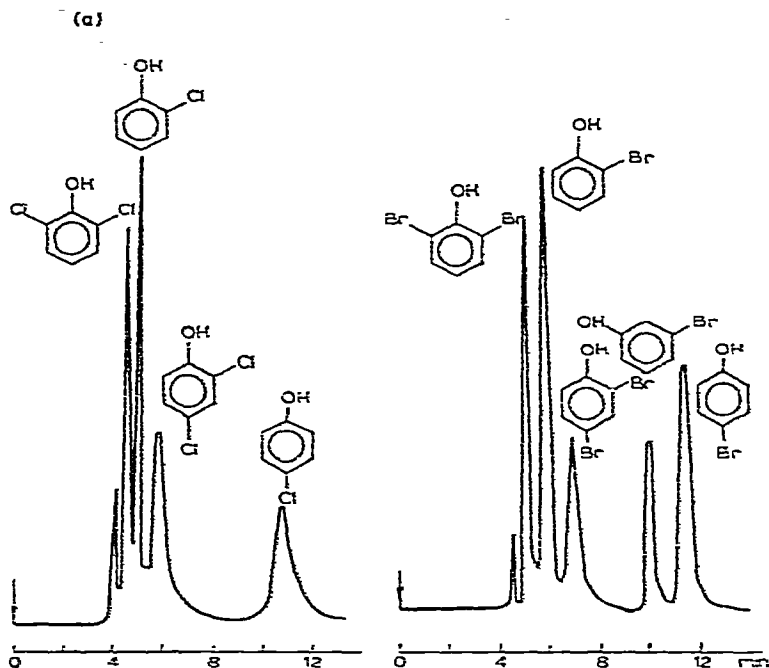
Fig. 1. (a) Chromatogram of chlorophenols on a 20 × 0.4 cm I.D. column of C-3 silica gel (s ≈ 260 m²/g), particle size 10–15 μm. Eluent: hexane–chloroform–isopropanol (78:20:2). (b) Chromatogram of bromophenols. Conditions as in (a).

polar substances without long hydrocarbon chains is mainly determined by specific substance–adsorbent intermolecular interactions.

The non-specific interaction of the methyl and methylene groups of the adsorbate with the adsorbent does not play an important role as these groups are also available in hydrocarbon eluents. The contribution of non-specific substance–eluent interactions in this instance is also small compared with specific substance–adsorbent interactions.

As the specific interactions are of orientational character, the retention is determined not only by dipole moments but also to a great extent by the accessibility of polar groups and by the orientation of molecules relative to polar groups on the adsorbent surface. A high selectivity of separation of isomers and other compounds having a spacious structure is observed in this variant. For example, LAC on a polar adsorbent and in particular on hydroxylated silica gel when using a non-polar or weakly polar eluent provides a good separation of *o*-, *m*- and *p*-isomers of aromatic compounds[11].

The selectivity towards such isomers in LC is much higher than in GC with the same silica gel because in LC the separation is due only to differences in specific interactions between the sample substance and polar groups of the adsorbent[11]. Here the screening of polar functional groups plays an active role, the retention of *ortho*-substituted derivatives being usually considerably smaller (Fig. 1).

If two polar functional groups in *ortho*-positions are able to participate in
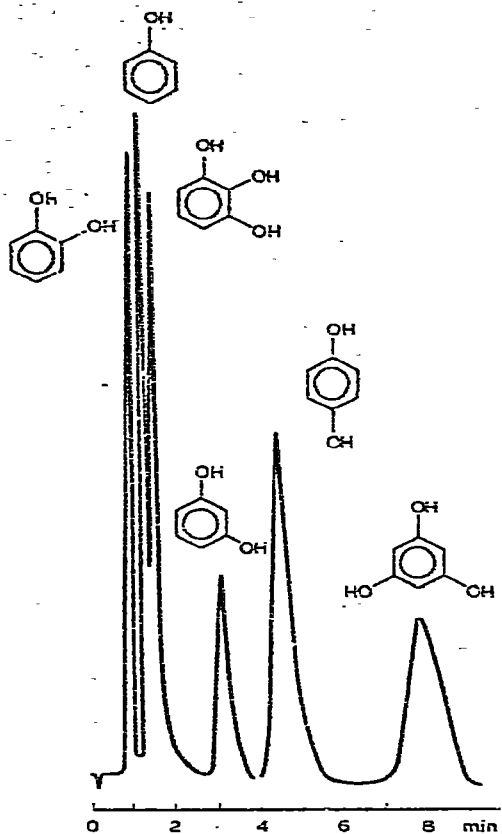
Fig. 2. Chromatogram of polyhydroxybenzenes on a 20 × 0.3 cm I.D. column of C-3 silica gel. Eluent: hexane–chloroform–isopropanol (78:20:2).

intramolecular bonds (hydrogen bonds in particular), the retention is also decreased (Fig. 2) as specific substance–adsorbent interactions are weakened. The $\Delta(\Delta F)$ values for phenol derivatives are pyrocatechol +530, pyrogallol −640, resorcinol −3800, hydroquinone −4940 and phloroglucinol −8660 J/mole. The influence of intramolecular hydrogen bonding on the decrease in $\Delta(\Delta F)$ values may be estimated by the difference of the values for hydroquinone and pyrocatechol, phloroglucinol and pyrogallol. Benzene derivatives on the same silica gel elute in the following sequence: benzene, toluene, chlorobenzene, anisole, nitrobenzene, methyl benzoate, benzonitrile, acetophenone, benzilic alcohol and phenol.

This variant is characterized by the following features:

(a) high selectivity of separation of the compounds according to their electronic structure, i.e., the separation of compounds that differ in the nature of their polar functional groups is marked by high selectivity; gradient elution is often necessary for the separation of compounds that differ considerably in polarity[12];

(b) high selectivity of separation of molecules that differ in geometrical structure (including isomers);

(c) alkyl derivatives differing in their methyl or methylene group content only

are not usually separated in this variant; this applies particularly to homologues with only slight changes in the electron density distribution; this drawback becomes an advantage as it allows a group separation of alkanes, naphthenes and different aromatic hydrocarbons in heavy hydrocarbon fractions to be achieved[13];

(d) in this variant the adsorption properies of polar adsorbents may vary with time owing to adsorption of water or other strongly polar substances present in the eluent. Under constant conditions, equilibrium is usually reached between the eluent and the adsorbent and the column is stable; when the temperature and the eluent composition are changed this equilibrium is upset[14]; the column should be regenerated if contamination is observed.

Variant 2

For the separation of alkyl derivatives when the methyl and methylene substituents change, the molecular electron density (in particular for benzene and naphthalene derivatives when eluting with n-hexane on a silica gel column) the retention may be determined by both specific substance–adsorbent and non-specific substance–eluent interactions[15,16].
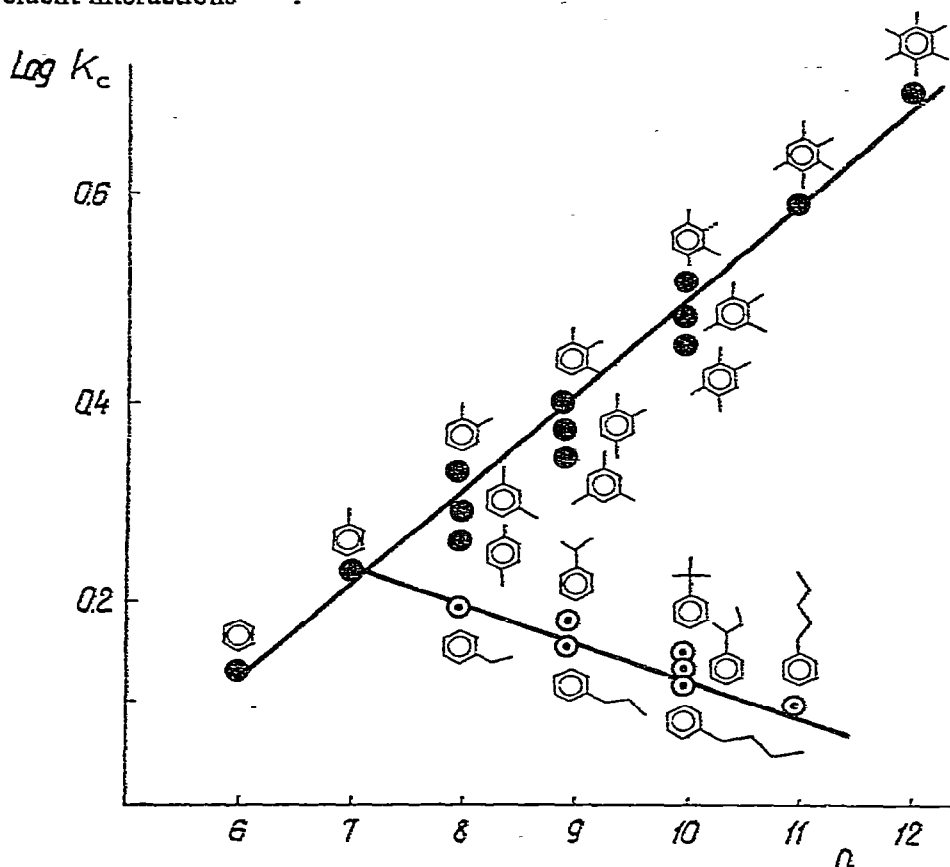

Fig. 3. Dependence of logarithm of capacity ratio (k_c) on the number of carbon atoms (n) in methyl-substituted benzene molecules. Column, 30 × 0.6 cm I.D., KCC-4 silica gel ($s \approx 560$ m^2/g), particle size 5–8 μm; temperature, 25°C; eluent, hexane; flow-rate, 3.5 cm^3/min; detector, UV.

Alkyl substituents in aromatic hydrocarbons (alkylbenzenes, alkylnaphtha-
lenes) influence the electron density distribution in the benzene ring; although this
influence is not strong it acts in different ways and, consequently, may change the
specific intermolecular interaction of the benzene ring with the hydroxyl groups of the
silica gel surface. In n-alkylbenzenes the influence of lengthening of the alkyl chain on
the electron density distribution in the benzene ring becomes negligible. However, in
this instance an increase in the contribution of the substance-eluent intermolecular
interaction, i.e., between the alkyl chain of the substituted aromatic hydrocarbon
molecules and the n-hexane molecules, would be expected. This also influences the
orientation of such molecules on the surface. These effects are small and an efficient
column is required in order to observe them.

The influence of the number of substituted methyl groups in the benzene ring
(n) on the strengthening of the specific adsorption of polymethylbenzenes on silica gel
from solutions can be seen from the approximately linear increase in log k_c with n for
this series of compounds (Fig. 3).

A different dependence of log k_c on n is observed for monoalkylbenzene ad-
sorption (starting with ethylbenzene). For ethylbenzene the second group in the alkyl
chain, CH_3, has only a very small influence on the electron density distribution in the
benzene ring (the negligible decrease in dipole moment of the ethylbenzene molecule
compared with that of toluene illustrates this effect). For propyl-, butyl- and amyl-
benzenes the electron density distribution in the benzene ring remains almost un-
altered; therefore, the contributions of specific intermolecular interactions of these
compounds (starting with toluene) with the silica gel surface should be similar. How-
ever, with lengthening of the alkyl chain the contribution of specific substance–eluent
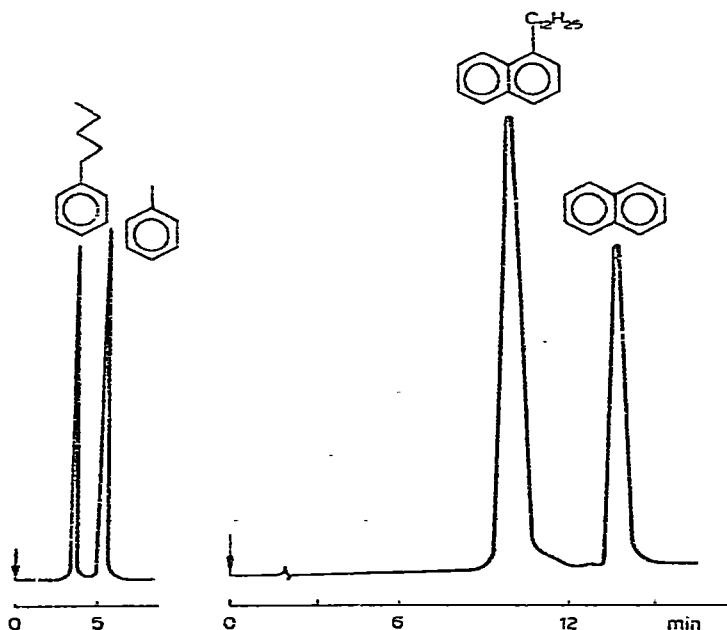interactions increases. As a result, the total energy of adsorption on silica gel from the

Fig. 4. Separation of alkyl derivatives of benzene and naphthalene. Conditions as in Fig. 3.

solutions becomes smaller. In accordance with this, the experimental data show that, starting with ethylbenzene, the retention of mono-n-alkylbenzenes decreases monotonously, which provides an unusual retention order of alkyl derivatives with long chains (Fig. 4).

We can determine the differences in the variations of the free energy of adsorption from n-hexane solutions on silica gel, $\Delta(\Delta F)$, for various polymethylbenzenes relative to benzene. The $\Delta(\Delta F)$ versus n relationship is close to linear, i.e., the contributions of CH_2 groups are additive. Variation of the $\Delta(\Delta F)$ value for one CH_3 group replacing a hydrogen atom in the ring is, on average, 550 J/mole. This value corresponds to the effect of CH_3 substitution on the electron density distribution in the benzene ring, increasing its capability for specific intermolecular interactions with silanol groups on the silica gel surface. The influence of the variation in the substance–eluent intermolecular interactions (per CH_2 group) on the decrease in retention in the mono-n-alkylbenzene series may be estimated in an analogous way. The corresponding decrease in the $\Delta(\Delta F)$ value due to the increase in the non-specific adsorbate–eluent intermolecular interaction and the possible weakening of the specific adsorbate–adsorbent intermolecular interaction per CH_2 group is 190 J/mole.

Variant 3

When in the normal-phase variant a polar eluent is used (which is usually a mixture of a non-polar or weakly polar eluent with a strongly polar eluent), the retention may be determined by specific substance–adsorbent and specific substance–eluent interactions, especially when high concentrations of polar eluent are added.

Scott and Kucera[17] showed that with low concentrations of a polar eluent an adsorbed monolayer of the eluent molecules is formed on the adsorbent surface, and with high concentrations several monolayers may be formed[18]. In this instance the retention of substances is considerably weakened owing to the adsorption of polar eluent molecules on the surface of the polar adsorbent. Adsorbate molecules may force polar molecules out of the adsorbent surface (some energy is consumed in this process and therefore the retention decreases) or they may adsorb on the monolayer of these polar molecules. If the concentration of polar molecules in the eluent is high, variant 3 may be expected.

Fig. 5 shows that the retention order of phenol and aniline on hydroxylated silica gel may change with variation in the concentration of the polar eluent (in particular an alcohol) added. With low alcohol concentrations (1 %), the specific substance–eluent interaction is small and phenol is retained more strongly than aniline. With high isopropanol concentrations (5 %) the specific substance–eluent interaction (hydrogen bond) plays an important role; the latter appears to be stronger for phenol and consequently the retention order changes. With intermediate concentrations (3 %) aniline and phenol are not separated.

With non-polar adsorbents and strongly polar eluents (especially water–alcohol mixtures) reversed-phase chromatography takes place[5]. In this instance the compounds with non-polar (methyl and methylene) functional groups are more strongly sorbed by the non-polar sorbent surface from a polar eluent. The compounds are mostly retained on the surface owing to adsorption by non-polar groups. Polar groups merely decrease the retention as they interact with polar groups of the eluent molecules and this interaction tends to carry the molecule from the adsorbent
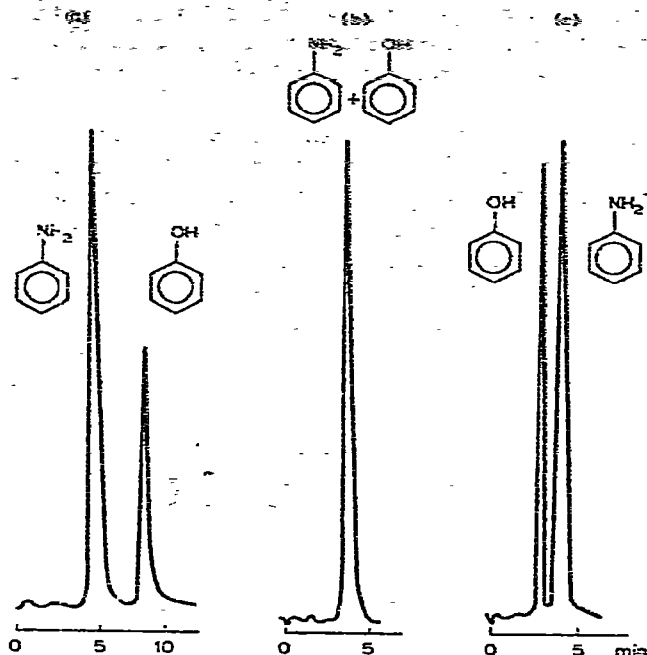
Fig. 5. Separation of phenol and aniline on a column (10 × 0.4 cm I.D.) of C-3 silica gel, particle size 10–15 μm. Flow-rate, 1.5 cm³/min; temperature, 25°C; eluent, hexane–isopropanol. Concentration of isopropanol in hexane: (a) 1%; (b) 3%; (c) 5%.

surface into the mobile phase. Thus in reversed-phase chromatography the retention is determined by non-polar non-specific substance–adsorbent interactions on the one hand and by polar specific substance–eluent interactions on the other, the latter decreasing the retention.

This variant is characterized by the following features:

(a) strongly polar substances are retained weakly, being eluted earlier than non-polar substances; strongly polar substances in the normal-phase variant are retained strongly and stepwise or gradient elution is required for analysis, whereas in the present variant strongly polar substances elute rapidly;

(b) substance–eluent interactions show up most clearly; variation of the nature of the eluent may sometimes change the retention volumes and the selectivity of separation to a greater extent than variation of the nature of the adsorbent;

(c) columns in reversed-phase chromatography have a long service life; under proper operating conditions they may be used as long as required.

The retention regularities are often correlated with the solubilities of the substances in the mobile phase. However, the solubility is determined by substance–eluent intermolecular interactions. In the given instance selectivity is determined by methyl group adsorption. It has been shown[19] that the selectivity of carbon adsorbents is higher.

In reversed-phase chromatography two variants (4 and 5, below) from those listed in Table 1 may be realized.
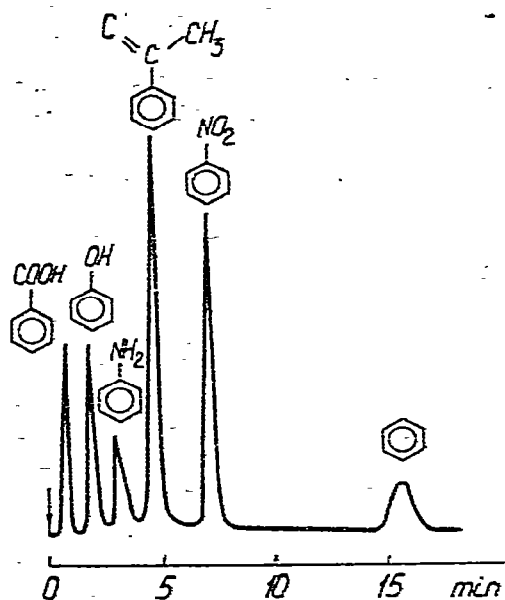
Fig. 6. Chromatogram of benzene derivatives on a 10 × 0.6 cm I.D. column of KCK-2 silica gel (C_{16} bonded phase); temperature, 50°C; eluent, water–isopropanol (3:2).

Variant 4

When using non-polar adsorbents and strongly polar eluents, the separation of non-polar and weakly polar substances is mainly carried out due to non-specific interactions substance–adsorbent. This is used for the separation of homologues by LC. Numerous examples may be found in the literature[20-22].

Variant 5

Retention of substances due to non-specific substance–adsorbent and specific substance–eluent interactions takes place in the system non-polar adsorbent–strongly polar eluent–polar separated substance.

For the chromatography of compounds having polar functional groups (on a non-polar adsorbent from a polar eluent), the intermolecular interaction of these compounds with the eluent is important. However, this intermolecular interaction is of a specific character and it is determined mostly by the formation of hydrogen bonds between π-bonds or polar functional groups of polar compounds and polar groups of the eluent[23]. The contribution of polar compound–eluent intermolecular interactions to the total intermolecular interaction of the given compound with the adsorbent and the eluent may be considerable. It may be increased if substances capable of stronger specific intermolecular interactions are added to the polar eluent[24].

The influence of intermolecular interactions between aromatic hydrocarbons and a polar eluent on retention volumes and separation selectivity on silanized silica gel can be easily established using derivatives differing in the nature of their polar functional groups. The influence of intermolecular interactions of these derivatives with a polar eluent may be estimated relative to benzene and toluene and their deriva-

tives having polar functional groups in *para*-positions. Fig. 6 shows the chromatogram of benzene derivatives.

The interaction of polar functional groups of benzene derivatives with the polar groups of the eluent decreases their retention volume and they elute earlier than benzene.

Increments in the free energy of adsorption per polar functional group are given in Table 2.

Intramolecular interactions (intramolecular hydrogen bonding in particular) have a strong influence on substance retention and separation selectivity. Intramolecular hydrogen bonding weakens specific substance–eluent interactions. In mono-, di- and trihydroxy benzenes the intramolecular hydrogen bond plays a particularly important role (Table 2).

Trihydroxybenzene isomers (phloroglucinol, pyrogallol) differ considerably in their retention times, because in the pyrogallol molecule the hydroxyl groups, being in *ortho*-positions relative to each other, may form an intramolecular hydrogen bond, so that the specific intermolecular interaction with the eluent is weakened and nonspecific interaction with the adsorbent is increased. In the phloroglucinol molecule all hydroxyl groups are in *meta*-positions relative to each other, so that they are free and cannot enter the intramolecular hydrogen bond. They therefore form a strong intermolecular hydrogen bond with the hydroxyl groups in the eluent molecule (water and alcohol). In this instance the retention order of hydroxybenzenes is the reverse of that in variant 1.

TABLE 2

RELATIVE VARIATIONS OF FREE ENERGY OF ADSORPTION, $\Delta(\Delta F)$, ON SILANIZED SILICA GEL WHEN ELUTING WITH WATER–ISOPROPANOL (4:1) AT 50°C

Flow-rate: 1 cm³/min (relative to benzene and phenol)

Compound	Functional group	$\Delta(\Delta F)$ (J/mole)
Benzene derivatives		
Aniline	NH_2	2780
Phenol	OH	2390
Benzyl alcohol	CH_2OH	1600
Anisole	OCH_3	1540
Acetophenone	$COCH_3$	1340
Benzonitrile	CN	907
Nitrobenzene	NO_2	500
Fluorobenzene	F	−437
Toluene	CH_3	−1480
Chlorobenzene	Cl	−1730
Bromobenzene	Br	−1810
Iodobenzene	I	2490
Phenol derivatives		
Phloroglucinol	OH (2)	5050
Hydroquinone	OH	3060
Resorcinol	OH	2360
Pyrocatechol	OH	1640
Pyrogallol	OH (2)	−330

Finally, it should be noted that conditions may be created such that interactions with the adsorbent do not take place. In this instance the retention of a substance in the column will be determined by the void volume (dead volume) accessible to the molecules (gel permeation chromatography[25]).

In conclusion, modern column liquid chromatography with high column efficiency possesses wide possibilities for the variation of the selectivity of separation; it makes the method exclusively applicable to the separation and analysis of multi-component mixtures including high-molecular-weight and unstable compounds.

3. SUMMARY

Classification of liquid adsorption chromatography variants according to the main types of intermolecular interactions responsible for retention is discussed. Five variants are specified in which retention is determined by combinations of specific and non-specific intermolecular interactions of the types substance–adsorbent, eluent–adsorbent and substance–eluent. The classifications are supported by examples.

REFERENCES

1 Z. Deyl, K. Macek and J. Janák (Editors), *Liquid Column Chromatography —A Survey of Modern Techniques and Applications*, Elsevier, Amsterdam, 1975.
2 P. A. Bristow, *Liquid Chromatography in Practice*, hetp, Wilmslow, 1977.
3 A. Pryde and M. T. Gilbert, *Application of High Performance Liquid Chromatography*, Chapman and Hall, London, 1979.
4 H. Engelhardt, *High Performance Liquid Chromatography —Chemical Laboratory Practice*, Springer, Berlin, 1979.
5 L. R. Snyder and J. J. Kirkland, *Introduction to Modern Liquid Chromatography*, Wiley, New York, 2nd ed., 1979.
6 A. V. Kiselev and Ya. I. Yashin, *Adsorbtionnaja Gazovaja i Zidkostnaja Khromatografja (Gas and Liquid Adsorption Chromatography)*, Khimiya, Moscow, 1979.
7. G. Guiochon, *J. Chromatogr.*, 185 (1979) 3.
8 R. P. W. Scott and P. Kucera, *J. Chromatogr.*, 169 (1979) 51.
9 A. V. Kiselev and Ya. I. Yashin, *Gas-Adsorption Chromatography*, Plenum Press, New York, 1969.
10 A. V. Kiselev, V. V. Tarasova and Ya. I. Yashin, *Chromatographia*, 13 (1980) 610.
11 A. V. Kiselev, A. A. Aratskova, T. N. Gvosdovitch and Ya. I. Yashin, *J. Chromatogr.*, 195 (1980) 205.
12 C. Liteanu and S. Gocan, *Gradient Liquid Chromatography*, Ellis Horwood, Chichester, 1974.
13 L. G. Galya and J. C. Suatoni, *J. Liquid Chromatogr.*, 3 (1980) 229.
14 R. P. W. Scott and S. Traiman, *J. Chromatogr.*, 196 (1980) 193.
15 A. N. Ageev, A. V. Kiselev and Ya. I. Yashin, *Dokl. Akad. Nauk USSR*, 249 (1980) 377.
16 A. N. Ageev, A. V. Kiselev and Ya. I. Yashin, *Chromatographia*, 13 (1980) 669.
17 R. P. W. Scott and P. Kucera, *J. Chromatogr.*, 149 (1978) 93.
18 R. P. W. Scott and P. Kucera, *J. Chromatogr.*, 171 (1979) 37.
19 H. Colin and G. Guiochon, *J. Chromatogr.*, 141 (1977) 289.
20 N. K. Bebris, R. G. Vorobieva, A. V. Kiselev, Yu. S. Nikitin, L. V. Tarasova, I. I. Frolov and Ya. I. Yashin, *J. Chromatogr.*, 117 (1976) 257.
21 N. K. Bebris, A. V. Kiselev, Yu. S. Nikitin, I. I. Frolov, L. V. Tarasova and Ya. I. Yashin, *Chromatographia*, 11 (1978) 206.
22 H. Colin and G. Guiochon, *J. Chromatogr.*, 141 (1977) 289.
23 B. Vonach and G. Schomburg, *J. Chromatogr.*, 149 (1978) 417.