

CHROM. 12,788

RETENTION BEHAVIOUR OF *o*-, *m*- AND *p*-ISOMERS OF BENZENE DERIVATIVES ON A SILICA GEL HYDROXYLATED SURFACE IN LIQUID CHROMATOGRAPHY

A. V. KISELEV*

Laboratory of Surface Chemistry, Institute of Physical Chemistry, U.S.S.R. Academy of Sciences, Leninsky Prospect 31, 117071 Moscow (U.S.S.R.)

and

A. A. ARATSKOVA, T. N. GVOZDOVITCH and Ya. I. YASHIN

Experimental Design Bureau of Automatics, Selskokhozaystvennaya 20, 129226 Moscow (U.S.S.R.)

(First received December 18th, 1979; revised manuscript received February 27th, 1980)

SUMMARY

The retention order of *o*-, *m*- and *p*-isomers of polar substituted benzenes has been investigated on columns packed with silica gel with a hydroxylated surface, the eluent being ϵ -hexane with different polar additions. The elution order is determined by the nature and position of the substituent groups, their influence on the electron density distribution in the benzene ring, the possibility of the formation of intra-molecular and inter-molecular hydrogen bonds and the orientation of adsorbate molecules relative to the adsorbent surface.

INTRODUCTION

In gas-adsorption chromatography (GAC) on polar adsorbents the additional non-specific inter-molecular interaction of the hydrocarbon parts of molecule with the adsorbent considerably diminishes the difference in total adsorption energy of *o*-, *m*- and *p*-isomers of aromatic compounds. Therefore, the selectivity of such well known polar adsorbents as porous silica with a hydroxylated surface towards these isomers in GAC is usually lower than in liquid-adsorption chromatography (LAC). Adequate separation of these isomers in GAC is obtained only upon a further increase in the adsorbent specificity, for example when using such ion adsorbents as BaSO₄ (ref. 1). When using non-polar or weakly polar eluents, LAC provides the separation of *o*-, *m*- and *p*-isomers of aromatic compounds even on such a weakly specific adsorbent as hydroxylated silica. The higher selectivity towards these isomers in LAC compared with GAC is caused by the fact that LAC separation is much more influenced by differences in specific inter-molecular interactions between polar groups of the molecule and polar groups or ions of the adsorbent²⁻⁴. The non-specific inter-molecular interaction of an adsorbent with methyl and methylene groups of benzene derivatives is not as important as in GAC, because the molecules of the

eluent (saturated hydrocarbons) also contain these groups. However, inter-molecular interactions of methyl and methylene groups of benzene derivatives with similar groups of eluent molecules are of considerable importance. It has been shown⁵ that with mono-*n*-alkylbenzenes, beginning with ethylbenzene, the increase in the length of the *n*-alkyl substituent leads to a decrease in the retention on the hydroxylated surface of silica gel owing to the inter-molecular interaction with the eluent (*n*-hexane).

The LAC separation of isomers of substituted benzenes with polar groups in *o*-, *m*- and *p*-positions on silica gel, eluted with saturated hydrocarbons containing some polar additions, is ensured by the different abilities of the polar substituents to form hydrogen bonds with silanol surface groups, the electron density distribution in the benzene nucleus of these molecules and their orientation relative to the adsorbent surface.

LAC has been used successfully for the separation of *o*-, *m*- and *p*-isomers of alkylphenols and chlorophenols^{6,7}, methyl- and chloroanilines^{8,9} and other isomers^{10,11} the aim being to maximize adsorbent selectivity; the adsorbent was impregnated with cadmium and silver salts.

In this work we studied the regularities of the order of retention of benzene derivatives with different substituents in the *o*-, *m*- and *p*-positions on the hydroxylated surface of silica gel when eluting with weakly polar eluents.

EXPERIMENTAL

A TSVET-304 liquid chromatograph equipped with a UV detector (254 nm) was used at room temperature for the measurements. Stainless-steel columns (length 20 cm, I.D. 4 mm) were packed with hydroxylated silica gel C-3 (with a specific surface area of 260 m²/g and a particle size of 10–15 μm) by the slurry packing method from chloroform. *n*-Hexane–chloroform–isopropanol was used as the eluent. The retention of *o*-, *m*- and *p*-isomers of benzene derivatives containing CH₃, *tert*-C₄H₉, Cl, Br, OH, COOCH₃, NH₂ and NO₂ substituents was investigated.

RESULTS AND DISCUSSION

At a small surface coverage adsorption from non-polar or weakly polar eluents on silica gel with a hydroxylated surface, and the corresponding retention volumes, are determined mainly by specific intermolecular interactions and, if it is possible, by hydrogen bonding between polar groups of the adsorbed molecules and surface silanol groups^{4,12}. When separating alkyl-substituted *o*-, *m*- and *p*-isomers of aromatic hydrocarbons (+R substituents) the order of elution of these isomers is influenced by the change in the electron density distribution in the benzene ring⁴.

In the separation of *o*-, *m*- and *p*-isomers with polar +R and –R substituents three basic cases may be observed. In the first case, both polar substituents are similar type, in the *o*-position, they do not form strong intra-molecular bonds, or intra-molecular hydrogen bonds in particular (the polar substituents may be different, but it is important that they do not exhibit strong intra-molecular interactions in the *o*-position). In this case the dipole moment increases in the order $p < m < o$. The same order would be expected in the retention of these isomers.

In the second case, both substituents are also polar, identical or not identical and in the *o*-position strong, intra-molecular interactions come into play. Therefore,

the *o*-isomers of such compounds should be retained less than the *m*- and *p*-isomers. Their dipole moments depend on the electron-donating or electron-accepting character of the substituent groups which influence the electron density in the benzene ring. For most of the investigated compounds falling into this group, the dipole moments follow the order $o < m < p$. However, in addition to the electron density distribution, the orientation of the molecule relative to a particular surface and also the influence of the eluent must be considered.

In the third case, one substituent is polar and the other is non-polar (alkyl group). The contribution of the non-polar substituent itself to the retention volume in LC is negligible, but a +R substituent increases the electron density in the benzene ring. However, this effect is smaller than the influence of strong inter-molecular interactions between the substituent polar group of the molecule and surface silanol groups. Therefore, an increase in retention may be expected in the sequence $o < m < p$, *i.e.*, in the order of decrease in the shielding of polar group with alkyl substituent.

Retention volumes of isomers of 16 benzene derivatives were measured in order to establish the retention regularities of such *o*-, *m*- and *p*-isomers on silica gel with a hydroxylated surface (Table I). An increase in the capacity ratio, K_e , for silica gel columns with increase in dipole moment is observed for most of the benzene derivatives investigated. With dioxybenzenes and toluidines a decrease in K_e is observed with the increase in dipole moment, corresponding to the elution order $p < m < o$. It should be noted, however, that the molecular dipole moment does not represent the total capacity of the molecule to engage in specific interactions with the adsorbent.

When the substituent polar groups do not form intra-molecular bonds, the isomers are generally retained in the order $p < m < o$. This order corresponds to the increase in the total dipole moment of the molecule and to the probability that both polar groups interact with the hydroxyl groups of the silica gel surface.

Fig. 1 shows the chromatograms of *o*-, *m*- and *p*-isomers of dimethyl phthalate, methyl nitrobenzoate and xylene. All of these isomers are retained in the order $p < m < o$ because in this instance no strong intra-molecular bonds are formed between the substituent groups. Therefore, *o*-isomers are the most retained as their dipole moments are the highest and they can take the most advantageous position relative to the hydroxyl groups on the silica gel surface.

Fig. 2 shows the chromatograms of nitroaniline, nitrophenol, bromophenol and dihydroxybenzene isomers. The order of retention of these compounds is $p < m < o$, which it is opposite to that of the isomers of the first group. In this instance *o*-isomers are the least retained as the inter-molecular interaction of their polar groups with the silanol groups of the adsorbent surface are weakened by intra-molecular hydrogen bonds between closely positioned substituent groups ($\text{NH}_2 \dots \text{NO}_2$; $\text{OH} \dots \text{NO}_2$; $\text{OH} \dots \text{Br}$; $\text{OH} \dots \text{OH}$).

Fig. 3 shows chromatograms of isomers of *tert*-butylphenol, nitrotoluene and cresol. The substituent groups in the molecules of these compounds do not form strong intra-molecular bonds. However, the retention order $o < m < p$ in this instance is the same as for molecules in which both substituent groups are polar. This retention order is explained by the fact that in these instances the polar group is shielded by methyl or *tert*-butyl groups, the effect being most notable when two *tert*-butyl groups are in the *o*-position relative to the hydroxyl group.

TABLE I
 VALUES OF CAPACITY RATIO, K_a , AND SELECTIVITY COEFFICIENT, α , OF BENZENE DERIVATIVES ON SILICA GEL WITH A
 HYDROXYLATED SURFACE
 Eluents: (1) *n*-hexane; (2) *n*-hexane-chloroform (90:10); (3) *n*-hexane-chloroform-isopropanol (78:20:2); (4) *n*-hexane-chloroform-isopropanol (75:20:5);
 Dipole moments¹³ (μ) are also given.

Compound	μ (D)			Eluent			α								
	<i>o</i> -	<i>m</i> -	<i>p</i> -	<i>o</i> -	<i>m</i> -	<i>p</i> -	<i>o</i> -	<i>m</i> -	<i>p</i> -	<i>m/p</i>	<i>o/m</i>	<i>o/p</i>	<i>m/o</i>	<i>p/m</i>	<i>p/o</i>
Bromonitrobenzenes	4.0		2.45	1			9.3			6.7		1.4			
Dimethyl phthalates	2.8	2.46	2.2	2	2.46		3.3	1.9	1.3	1.8	1.5	2.6			
Methyl nitrobenzoylates				2			3.6	2.5	2.1	1.2	1.5	1.8			
Dinitrobenzenes	6.05	3.81	0.32	3	3.81		—	0.7	0.3	2.3					
Xylenes	0.58	0.37	0.12	1	0.37		2.25	2.09	1.92	1.09	1.08	1.17			
Bromophenols	1.36	—	2.8	3	—		1.1	5.1	7.5				4.7	1.3	5.9
Chlorophenols	1.43	2.17	2.68	3	2.17		0.95	—	7.5				—	—	6.8
Nitrophenols	3.11	3.9	5.05	4	3.9		0.3	0.9	1.4				2.7	1.5	4.1
Nitroanilines	4.06	4.91	6.32	4	4.91		0.95	2.7	5.4				3.0	1.9	5.7
Dihydroxybenzenes	2.58	1.53	0	4	1.53		2.5	3.3	4.0				1.3	1.24	1.64
Chloroanilines	1.84	2.91	3.0	3	2.91		0.6	—	3.4				—	—	5.9
Aminophenols				4			4.6	13.5	15.1				3.6	1.2	4.4
Nitrotoluenes	3.66	4.14	4.42	1	4.14		7.5	8.9	11.1				1.2	1.3	1.5
<i>tert</i> -Butylphenols	—	—	1.65	4	—		1.7	—	7.1				—	—	4.0
Cresols	1.44	1.6	1.64	3	1.6		0.3	1.8	1.9				5.9	1.07	6.3
Toluidines	1.58	1.44	1.31	3	1.44		1.4	1.8	2.05				1.3	1.14	1.5

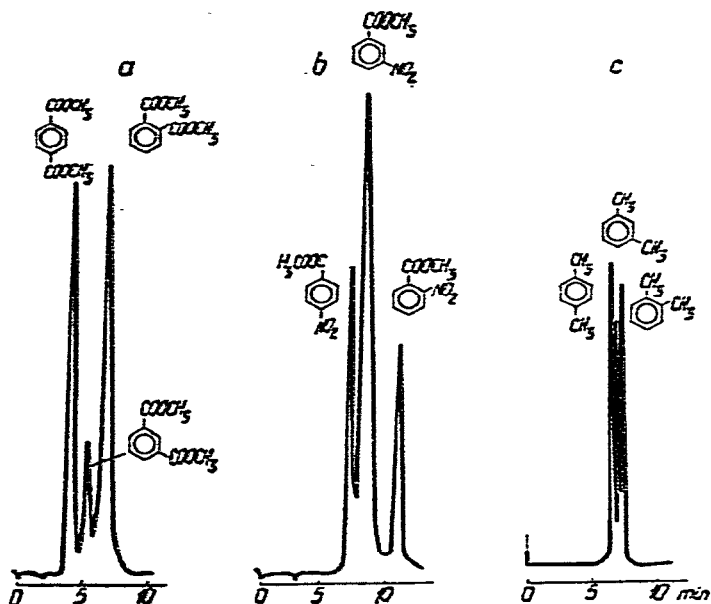


Fig. 1. Chromatogram of isomers (elution order $p < m < o$) on silica gel with a hydroxylated surface. (a) and (b) column 20 cm \times 4 mm I.D., silica gel C-3, particle size 10–15 μm , eluent *n*-hexane–chloroform (90:10), flow-rate 1.2 cm^3/min ; (c) column 30 cm \times 4 mm I.D., silica gel KCC-4, particle size 5–8 μm , eluent *n*-hexane, flow-rate 3.7 cm^3/min [data in (c) from ref. 5].

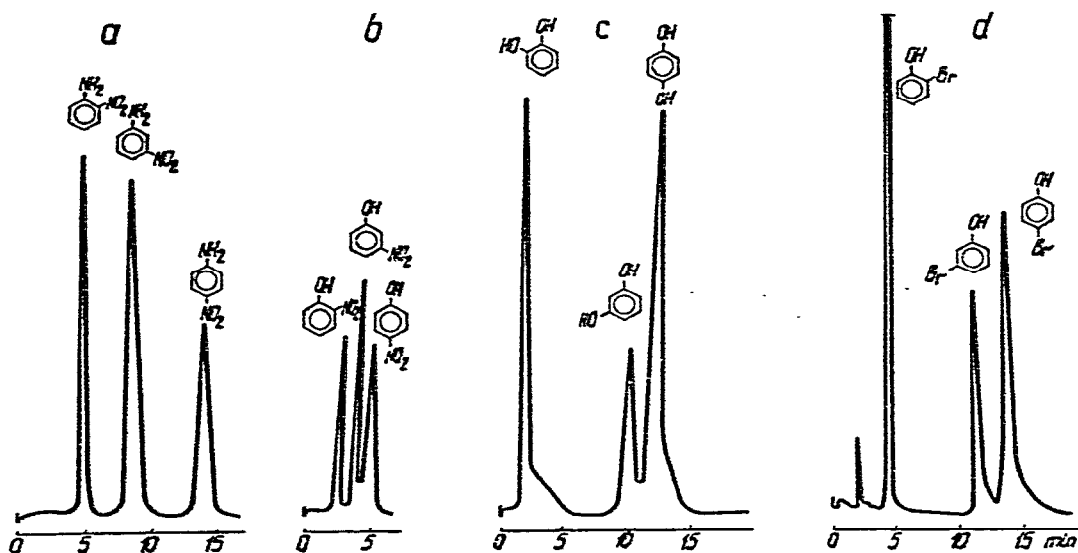


Fig. 2. Chromatogram of isomers (elution order $o < m < p$) on silica gel C-3 with a hydroxylated surface, particle size 10–15 μm , column 20 cm \times 4 mm I.D. (a), (b) and (c) eluent *n*-hexane–chloroform–isopropanol (75:20:5), flow-rate 1.2 cm^3/min ; (d) eluent *n*-hexane–chloroform–isopropanol (78:20:2), flow-rate 1.5 cm^3/min .

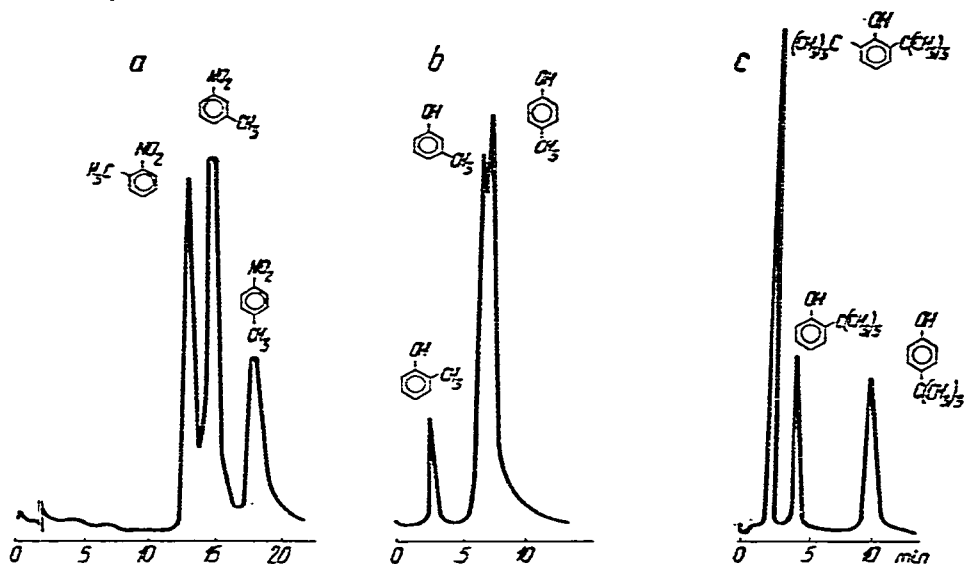


Fig. 3. Chromatogram of isomers (elution order $o < m < p$) on silica gel C-3 with a hydroxylated surface, particle size 10–15 μm , column 20 cm \times 4 mm I.D. (a) Eluent *n*-hexane, flow-rate 2 cm³/min; (b) eluent *n*-hexane–chloroform–isopropanol (78:20:2), flow-rate 1 cm³/min; (c) eluent *n*-hexane–chloroform–isopropanol (75:20:5), flow-rate 2 cm³/min.

Hence it has been shown that the order of retention of *o*-, *m*- and *p*-isomers with different functional groups is influenced by several factors such as the nature of the substituents, their probable intra-molecular interaction in the *o*-position, their influence on the electron density distribution in the benzene ring (+R and –R substituents) and steric hindrance (caused by non-polar substituents in the *o*-position) to the formation of strong specific inter-molecular interactions between a polar substituent of the molecule and the silanol group of the adsorbent surface. The regularities obtained for the order of retention of *o*-, *m*- and *p*-isomers help in predicting the retention order for isomers of many similar compounds, taking into account the character of the substituent groups, their mutual influence (possibility of intra-molecular bond formation) and their specific inter-molecular interaction with the polar adsorbent with a favourable orientation.

REFERENCES

- 1 L. D. Belyakova, A. V. Kiselev and G. A. Soloyan, *Chromatographia*, 3 (1970) 254.
- 2 L. R. Snyder, *Principles of Adsorption Chromatography*, Marcel Dekker, New York, 1968.
- 3 J. J. Kirkland, *Modern Practice of Liquid Chromatography*, Wiley-Interscience, New York, 1971.
- 4 A. V. Kiselev and Ya. I. Yashin, *Adsorbtsionnaya Gazovaya i Zhidkostnaya Khromatografiya (Gas and Liquid Adsorption Chromatography)*, Khimiya, Moscow, 1979.
- 5 A. N. Ageyev, A. V. Kiselev and Ya. I. Yashin, *Dokl. Akad. Nauk SSSR*, 249 (1979) 377.
- 6 S. Husain, P. Kunzelmann and H. Schildknecht, *J. Chromatogr.*, 137 (1977) 53.
- 7 S. Husain and M. Kifayatulla, *J. Chromatogr.*, 168 (1979) 517.
- 8 D. Kunzru and R. W. Frei, *J. Chromatogr. Sci.*, 12 (1974) 191.
- 9 C. R. Vogt, T. R. Ryan and J. S. Baxter, *J. Chromatogr.*, 136 (1977) 221.
- 10 M. Popl, V. Dolanský and J. Mostecký, *J. Chromatogr.*, 117 (1976) 117.
- 11 R. Aigner and H. Spitz, *Anal. Chem.*, 48 (1976) 381.
- 12 W. Golkiewicz, *Wiad. Chem.*, 29 (1975) 645.
- 13 O. V. Osipov and V. I. Minkin, *Spravochnik po Dipolnym Momentam (Reference Book on Dipole Moments)*, Vysshaya Shkola, Moscow, 1965.