CHROM. 6164

Non-aqueous solvent chromatography

II. Separation of benzene derivatives in the anion-exchange and n-butyl alcohol system

Although many successful separations of cations, anions and organic compounds by column chromatography have been reported in the literature, they were generally conducted in an aqueous system and very few efforts have been made to use non-aqueous solvents in chromatography, except in the thin-layer chromatographic (TLC) separation of organic compounds. As pointed out in the previous paper[1], however, one cannot apply the conditions of TLC mechanically to column chromatography, because conditions that are suitable for the former are not always suitable for the latter; neither silica nor alumina can give a reproducible stationary phase, and also the heterogeneous distribution of mixed solvents cannot be neglected.

The object of the present study was to investigate the chromatographic behaviour of aromatic compounds in a system of ion-exchange resin and non-aqueous solvent, to elucidate the mechanism of their adsorption and to establish a new column chromatographic technique for the separation of organic compounds.

Experimental

The distribution coefficients (K_d') of 71 reference compounds were determined by column chromatography using a Hitachi Model 124 ultraviolet spectrophotometer with a flow-cell of 3 mm length, a column of 15 cm × 8 mm diameter filled with an anion-exchange resin (Amberlite CG-400, type I, Cl- or OH-form) and a Milton Roy micro-pump. The distribution coefficients were calculated from

$$K_d' = (V - V_0)/V_c,$$

where V (ml) is the peak position of eluted reference compound, V_0 (ml) is the hold-up of the column, and V_c (ml) is the volume of the resin in the column.

The distribution coefficients measured with the anion-exchange resin (Cl-form) and n-butyl alcohol are summarized in Table I.

The influence of the difference in the type of resin and its ionic form was examined by using the Cl- and the OH-forms of an anion-exchange resin, the Na-form of a cation-exchange resin (Amberlite CG-120, type I), as well as polystyrene gels (Bio-Beads SX-8 and Mitsubishi-Kasei polystyrene gel with 8 % divinylbenzene).

Some typical examples of elution curves are shown in Figs. 1–7, which show the possibility of this method for separating various isomers and compounds.

Results and discussion

The reference compounds were adsorbed on the anion-exchange resins, but not on the polystyrene gels or on the cation-exchange resin (Na-form). Dimethylaniline was adsorbed neither on the cation- nor on the anion-exchange resins. The adsorption on the anion-exchange resin in the OH-form was generally stronger than that of the Cl-form; phenol, in particular, could not be eluted from the OH-form of the resin. As when ethanol was used as the eluant[1], the effect of substituents (R) of benzene of

TABLE I

K_d' VALUES OF SOME BENZENE DERIVATIVES (R–C₆H₄–X)

Values are given in ml/ml; resin: Amberlite CG-400, type I, Cl-form; eluant: n-butyl alcohol.

| X	Position	R							
		$-CH_3$	$-H$	$-Cl$	$-F$	$-CN$	$-NO_2$	$-NH_2$	$-OH$
–CH₃	o-	0.56		0.60	0.59	0.48	0.70	2.06	4.26
	m-	0.55	0.56	0.61	0.60	0.33	0.70	2.43	3.70
	p-	0.51		0.62	0.60	0.54	0.80	2.00	3.39
–H		0.56	0.64	0.77	0.69	1.13	1.40	4.14	4.81
–Cl	o-	0.60		0.70			1.08	2.84	8.26
	m-	0.61	0.77	0.64			1.31	7.68	5.00
	p-	0.62		0.69		1.09	1.22	5.83	5.82
–F	o-	0.59						1.20	5.88
	m-	0.60	0.69					0.72	4.94
	p-	0.60					0.87		4.94
–CN	o-	0.48							
	m-	0.33	1.13						
	p-	0.54		1.09					
–NO₂	o-	0.70		1.08			3.62	5.69	13.37
	m-	0.70	1.40	1.31			2.06	22.51	12.90
	p-	0.80		1.22	0.87			36.16	21.98
–NH₂	o-	2.06		2.84	1.20		5.69	23.16	31.38
	m-	2.43	4.14	7.68	0.72		22.51	29.74	76.97
	p-	2.00		5.83			36.16	5.30	
–OH	o-	4.26		8.26	5.88		13.37	31.38	
	m-	3.70	4.81	5.00	4.94		12.90	76.97	
	p-	3.39		5.82	4.94		21.98		
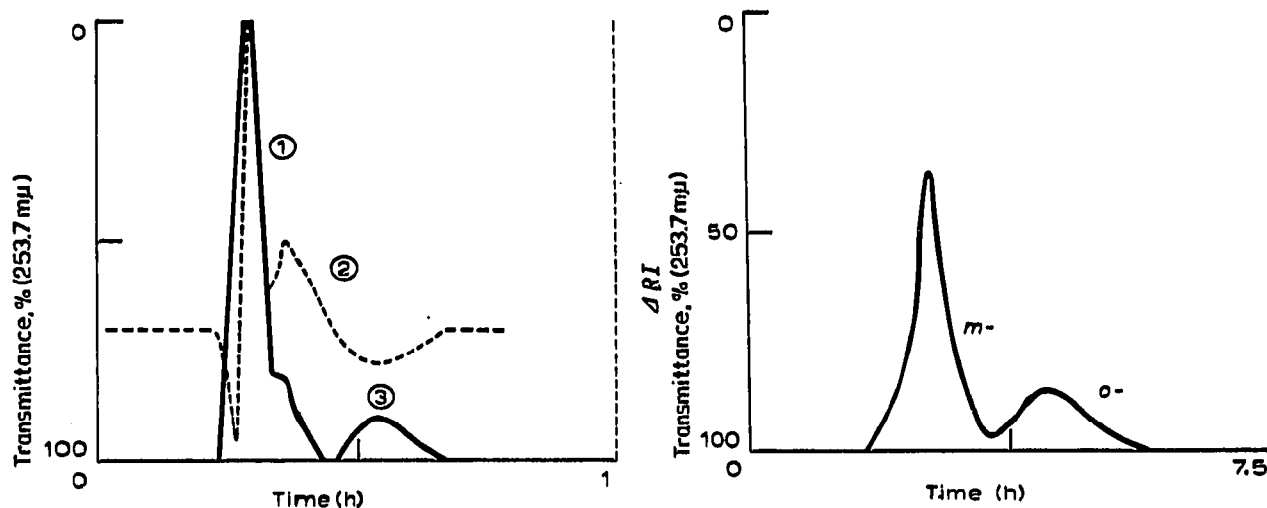
Fig. 1. Elution curves of benzene, naphthalene and anthracene on Amberlite CG-400, type II, Cl-form. Eluant: n-butyl alcohol; flow-rate: 35 ml/h. Column size: 700 mm × 8 mm diameter. 1, benzene; 2, naphthalene; 3, anthracene.

Fig. 2. Elution curve of o- and m-dinitrobenzene on Amberlite CG-400, type II, Cl-form. Eluant: n-butyl alcohol; flow-rate: 35 ml/h. Column size: 700 mm × 8 mm diameter.
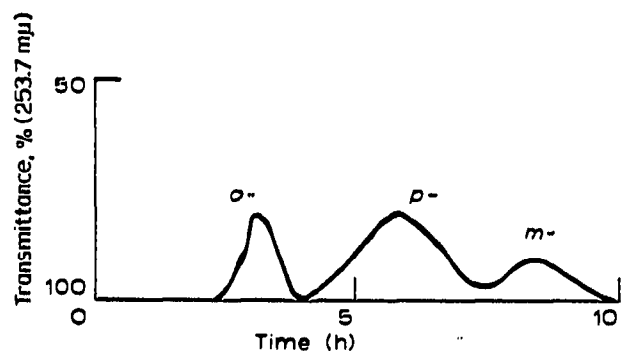
Fig. 3. Elution curve of o-, m- and p-chloroaniline on Amberlite CG-400, type II, Cl-form. Eluant: n-butyl alcohol; flow-rate: 35 ml/h. Column size: 700 mm × 8 mm diameter.
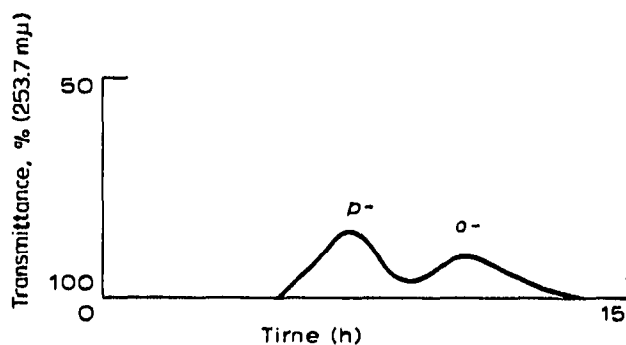
Fig. 4. Elution curve of o- and p-chlorophenol on Amberlite CG-400, type II, Cl-form. Eluant: n-butyl alcohol; flow-rate: 35 ml/h. Column size: 700 mm × 8 mm diameter.

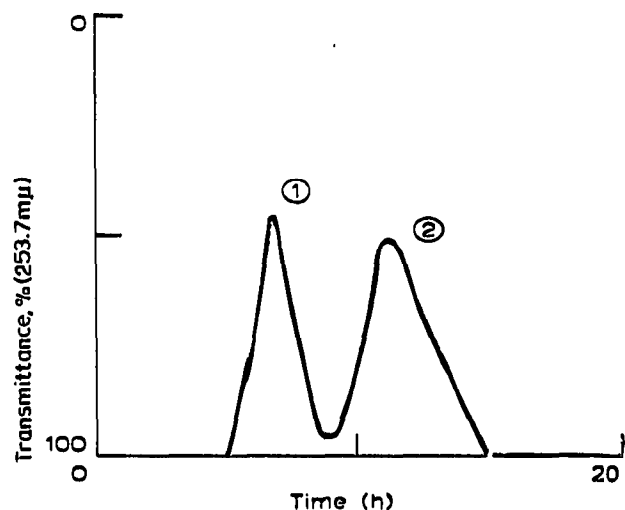Fig. 5. Elution curve of o-nitro-p-chloroaniline and p-nitro-o-chloroaniline on Amberlite CG-400. type II, Cl-form. Eluant: n-butyl alcohol; flow-rate: 35 ml/h. Column size: 700 mm × 8 mm diameter. 1, o-nitro-p-chloroaniline; 2, p-nitro-o-chloroaniline.
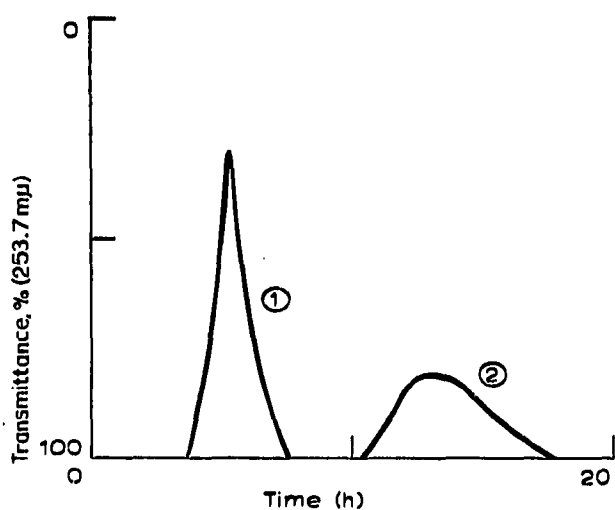
Fig. 6. Elution curve of m-nitro-p-toluidine and o-nitro-p-toluidine on Amberlite CG-400, type II, Cl-form. Eluant: n-butyl alcohol; flow-rate: 35 ml/h. Column size: 700 mm × 8 mm diameter. 1, m-nitro-p-toluidine; 2, o-nitro-p-toluidine.

increasing the K_d' value followed the order $CH_3 \leqslant H \leqslant Cl \leqslant F < CN < NO_2 < NH_2 < OH$.

Except for aniline (R = NH_2) and phenol (R = OH), the strength of adsorption of a benzene derivative on the anion-exchange resin is evidently related to the electronegativity, or the electron-withdrawing ability, of the substituent; the increase of the π-electron density over the benzene ring tends to decrease the strength of adsorption. This is in accord with the finding of GRAHAM and co-workers[2-4], who have reported that the substitution with halogen atoms or nitro groups causes a reduction in R_F values and the substitution with alkyl groups generally increases the R_F values.
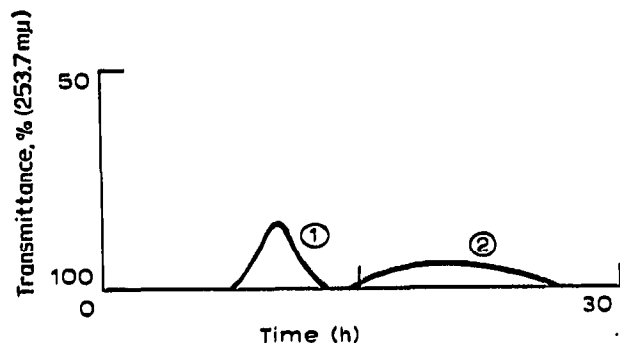
Fig. 7. Elution curve of *p*-nitro-*o*-toluidine and 5-nitro-*o*-toluidine on Amberlite CG-400, type II, Cl-form. Eluant: *n*-butyl alcohol; flow-rate: 35 ml/h. Column size: 700 mm × 8 mm diameter. 1, *p*-Nitro-*o*-toluidine; 2, 5-nitro-*o*-toluidine.

In view of the above experimental results, it seems convenient to classify the substituents into two groups, *viz.*, those of the first kind (CH_3, Cl, F, CN and NO_2) and those of the second kind (NH_2 and OH), because there is a great difference in K_d' values between these two groups.

Similar effects of substituents were observed when a second substituent (X) is introduced to mono-substituted benzenes (C_6H_5–R); when R and X are both of the first kind, the value of K_d' remains small, irrespective of the position of substitution (*o*-, *m*- or *p*-). On the contrary, the values of K_d' of substituted anilines and phenols are influenced considerably by the position of the second substituent.

The great difference in the effect upon K_d' values between the substituent of the first and the second kind may be reasonably explained by assuming that compounds with substituents of the second kind and those with no such substituents are adsorbed by different mechanisms. Probably the latter compounds are adsorbed by the formation of a charge-transfer complex between the benzene ring of the sample and the chloride anion of the resin, as they show little or no adsorption on the polystyrene gels or on the cation-exchange resin in the Na-form, and the decrease of the π-electron density over the benzene ring generally increases the strength of adsorption.

In contrast, compounds with substituents of the second kind, anilines and phenols, are probably adsorbed by the formation of hydrogen bonds between the hydrogen of the substituent and the anion of the resin. The much weaker adsorption of dimethylaniline than that of aniline, which reveals the important effect of hydrogen in increasing the strength of adsorption, provides strong support for this view.

It may be added here that *n*-butyl alcohol is a better solvent than ethyl alcohol for separating isomers by this type of chromatography.

Studies to examine the validity of the above assumptions and to extend the applicability of this new technique are now in progress with other solvents and resins.

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