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NON-AQUEOUS SOLVENT CHROMATOGRAPHY

IV. EFFECTS OF SOLVENTS AND ION-EXCHANGE RESINS ON ADSORPTION MECHANISMS AND THEIR APPLICATION IN HIGH-SPEED LIQUID CHROMATOGRAPHY

WATARU FUNASAKA, TOSHIHIKO HANAI, TSUYOSHI MATSUMOTO, KAZUMI FUJIMURA and TEIICHI ANDO

Department of Industrial Chemistry, Faculty of Engineering, Kyoto University, Sakyo-ku, Kyoto (Japan)

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SUMMARY

In order to elucidate the adsorption mechanisms of aromatic compounds on an anion-exchange resin, the effects of solvents and ion-exchange resins were studied. The results are presented as "chromatographic spectra" which suggest that the adsorption mechanisms are hydrogen bond or π-bond attraction between an aromatic compound and a counter ion of the anion-exchange resin. The most important factor in the adsorption is the ability of the solvent to form a hydrogen bond, while Van der Waals' forces have little influence on K_d (distribution coefficient) values. Some isomers of benzene and naphthalene derivatives have been separated by high-speed liquid chromatography.

INTRODUCTION

In previous papers[1,2], we described the new chromatographic technique of non-aqueous solvent chromatography with an anion-exchange resin and alcohol, and discussed how the K_d'* values varied with molecular structure. This paper describes the extension of this work to a study of the effects of solvents and ion-exchange resins. Lee *et al.*[3] used a ferric chloride organic solution as mobile phase for separating some aromatic organic acids with Dowex 1-X8 (Cl^-) and Gilmer and Pietrzyk[4] anticipated that interactions between ion-exchange resins and electrolytes or non-electrolytes would be affected by non-aqueous solvents. Streuli[5] reported that the adsorption of aromatic compounds on Sephadex LH-20 occurred through π- or hydrogen bond mechanisms, with the dominant mechanism determined by the solvent employed. They tried to introduce resonance energies for calculating K_d values. Waksmundzki and Różyło[6] chose five typical solvents for investigating the adsorption mechanism on silica gel. To demonstrate experimentally hydrogen bond formation between a sample and a counter ion of the anion-exchange resin, six solvents of different types were chosen according to the classification of Ewell

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* K_d' and K_d are distribution coefficients with V_c= volume of wet column resin and V_c= weight of dry column resin, respectively (*cf.* Experimental).

et al.[7] and Pimentel and McClellan[8]. Also, for investigating the effect of ion-exchange resins on K_d values, different ion-exchange resins were used.

The separation of some benzene and naphthalene derivatives has also been examined by high-speed liquid chromatography[9] by the use of Zipax SAX as adsorbent.

EXPERIMENTAL

Various resin adsorbents were used to investigate the effect of the ion forms on K_d values: SA-X^-, strong anion-exchange resin, Amberlite CG-400, type I, X^- (X=OH, Cl, F or I); WA, weak anion-exchange resin, Amberlite CG-45, type I, Cl^-; Py, weak anion-exchange resin (pyridinium), Bio-Rex 9, 100–200 mesh, Cl^-; PG, polystyrene gel, Bio-Beads SX-8, 200–400 mesh; WC, weak cation-exchange resin, Amberlite CG-50, type I, H^+; SC, strong cation-exchange resin, Amberlite CG-120, type I, Na^+; SAX, strong anion-exchange material for high-speed liquid chromatography, Zipax SAX, Cl^-, DuPont.

TABLE I

SOLVENTS USED AS ELUENTS[7,8]

Class	*Effect*	*Solvent*
AB	Hydrogen bond breaking and forming	Ethanol, *n*-butanol
A	Hydrogen bond forming	Chloroform, dichloromethane
N	Hydrogen bond effect absent	Carbon tetrachloride, *n*-hexane

To take into account solvent effects on adsorption, six typical solvents were chosen from three different classes in the classification of Ewell *et al.*[7] and Pimentel and McClellan[8] (Table I). The solutes studied differed in molecular structure, so that their contribution to the chromatographic phenomena could be realistically estimated.

The distribution coefficients (K_d) were determined by column chromatography using a Hitachi Model 124 ultraviolet spectrometer monitor with a flow cell of 3 mm path length. The column (15 cm length × 8 mm I.D.) was filled with resin and fitted with 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 compounds, V_0 (ml) is the hold-up of the column and V_c is the weight of the dry resin in the column.

The separation of some benzene and naphthalene derivatives was also carried out, with a 120 cm length×2.1 mm I.D. stainless-steel column charged with Zipax SAX and fitted with a Kyowa Seimitsu SD-1 micro pump. A comparison of the classical and the new column techniques was also made.

RESULTS AND DISCUSSION

The experimental results are presented as chromatographic spectra, the K_d values being plotted against the six solvents arranged on the abscissa according to the classification of Ewell *et al.*[7] and Pimentel and McClellan[8]. Such spectra

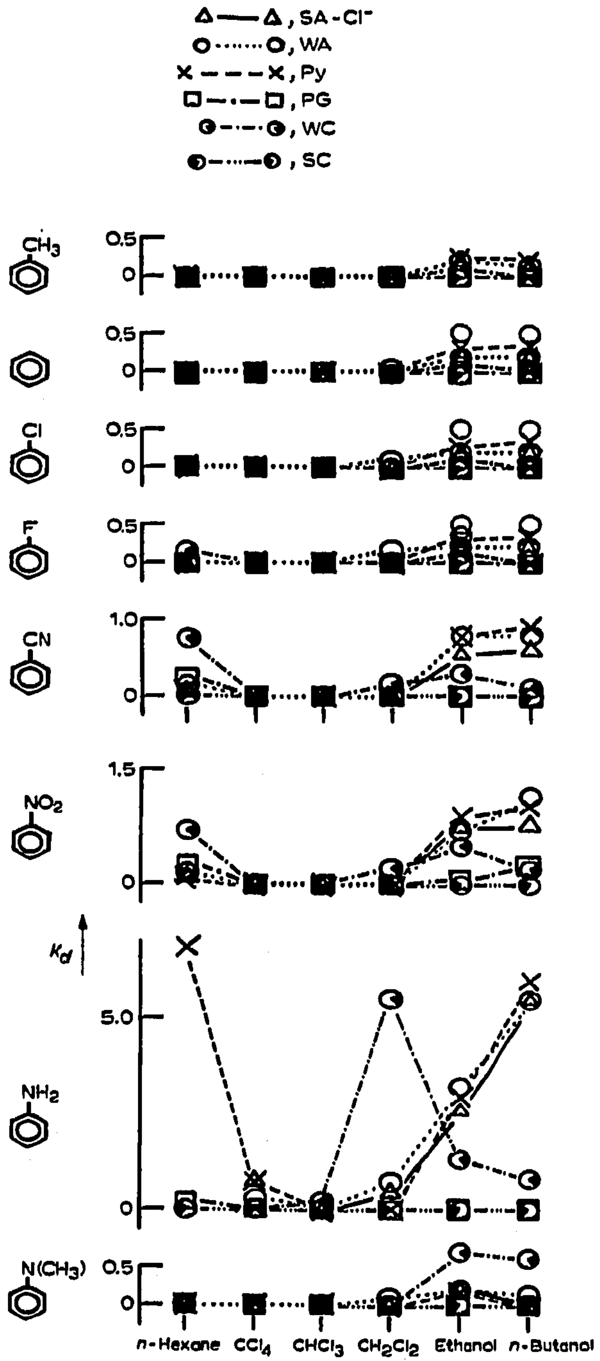


Fig. 1. Chromatographic spectra of benzene derivatives using various solvents arranged according to the classification of Ewell *et al.*[7] and Pimentel and McClellan[8] and several resins.

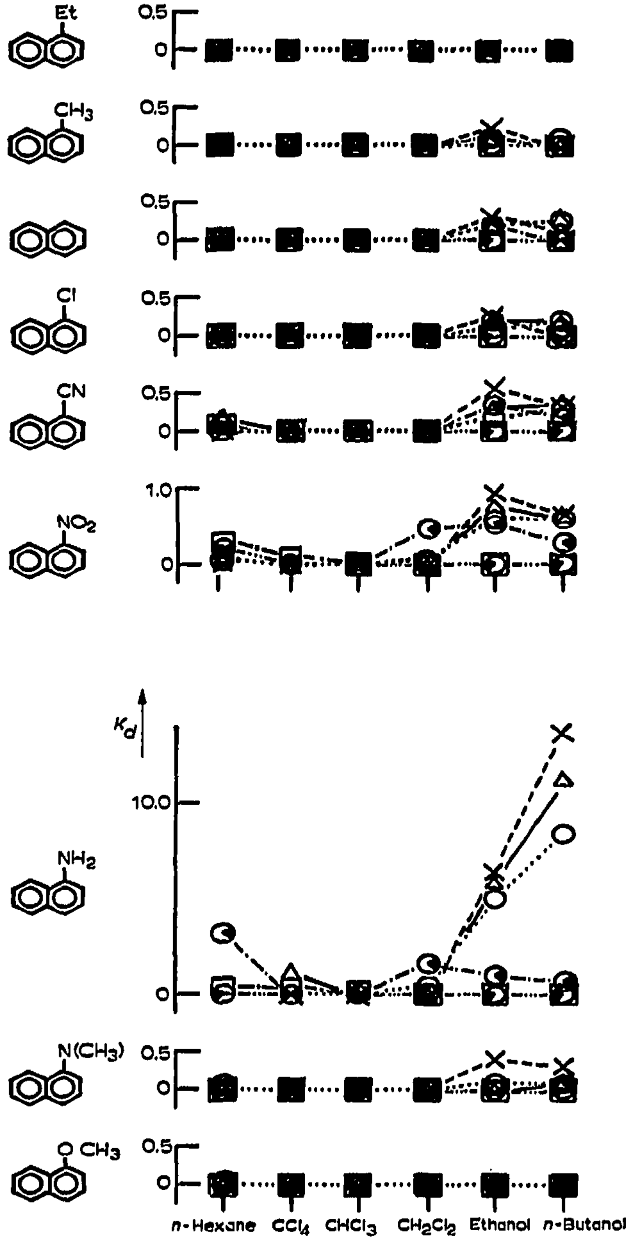
for various resins are given in Figs. 1–3, the spectra of benzene derivatives being shown in Fig. 1.

As described in previous papers[1,2], benzene with an amino or a hydroxyl group was adsorbed very strongly on to an anion-exchange resin in alcohol and the adsorption mechanism suggested was based on hydrogen bond formation. When an amino or a hydroxy group forms a hydrogen bond with a counter ion on the anion-exchange resin, the solvent (Class AB) affects the nature of the hydrogen bond. Thus, since ethanol or *n*-butanol (Class AB) can form a hydrogen bond with a counter ion of the anion-exchange resin (this is apart from hydrogen bond formation with the solute), aromatic compounds with an amino or a hydroxy group are less readily adsorbed on the anion-exchange resin. The reason why the K_d values in ethanol are smaller than those in *n*-butanol is related to the lesser +I effect of the alkyl groups resulting in ethanol forming a stronger hydrogen bond than does *n*-butanol. Chloroform or dichloromethane (Class A) forms a hydrogen bond with a counter ion of the anion-exchange resin, so that compounds cannot be easily adsorbed and K_d values are very small. *n*-Hexane (Class N) cannot form a hydrogen bond, and in this solvent samples having an amino or a hydroxy group are adsorbed on the anion-exchange resin and cannot easily be desorbed. When carbon tetrachloride (Class N) is used as solvent, a feeble π-complex formed between carbon tetrachloride and a counter ion of the anion-exchange resin would obstruct the adsorption of the samples. The difference between *n*-hexane and carbon tetrachloride would depend upon proton-acceptor solubility and solvent strength[10]. On the other hand, since tetrabutylammonium bromide forms a π-complex with nitrobenzene[11], the formation of a π-complex between a counter ion of the anion-exchange resin and a benzene ring is also reasonable. Thus the ease of π-complex formation should depend upon the electronegativities of the substituents. In fact, the increasing K_d' values of benzene derivatives followed the order $NO_2 > CN >$

TABLE II

K_d' VALUES OF SOME BENZENE AND NAPHTHALENE DERIVATIVES
Values are given as ml/ml; resin: Amberlite CG-400, type I, Cl− and OH−.

Group	*Solvent*								
	Ethanol						*n-Butanol*		
	Cl−			*OH−*			*Cl−*		
	Benzene	*α-Naphthalene*	*β-Naphthalene*	*Benzene*	*α-Naphthalene*	*β-Naphthalene*	*Benzene*	*α-Naphthalene*	*β-Naphthalene*
$-C_2H_5$		0.26	0.28		0.50	0.53		0.59	0.60
$-CH_3$	0.25	0.35	0.32	0.41	0.44	0.45	0.56	0.73	0.74
$-H$	0.27	0.39		0.44	0.57		0.64	0.82	
$-Cl$	0.27	0.37	0.40	0.44	0.55	0.59	0.77	0.77	0.84
$-F$	0.31		0.35	0.41	–	0.53	0.70		0.86
$-CN$	0.35	0.46	0.46	0.50	0.66	0.64	1.09	1.15	1.32
$-NO_2$	0.45	0.65		0.61	0.78		1.35	1.39	
$-NH_2$	1.08	1.98	2.33	1.12	2.12	2.22	4.14	8.85	
$-OH$	1.92	3.13	2.83				4.81	8.66	8.42



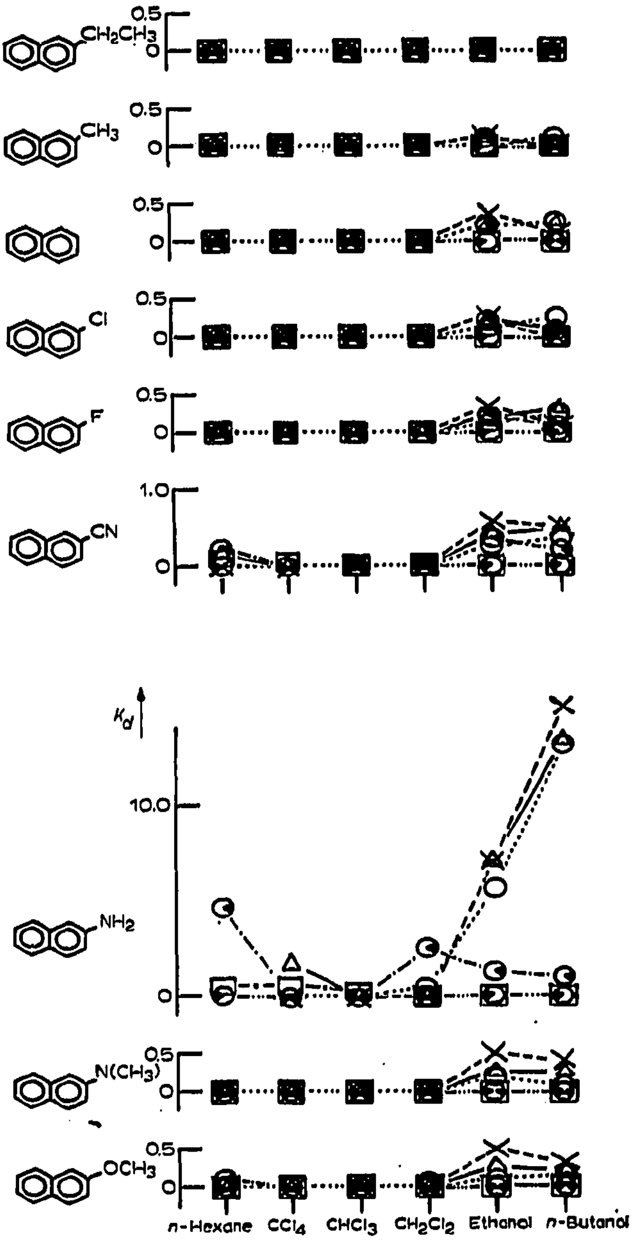
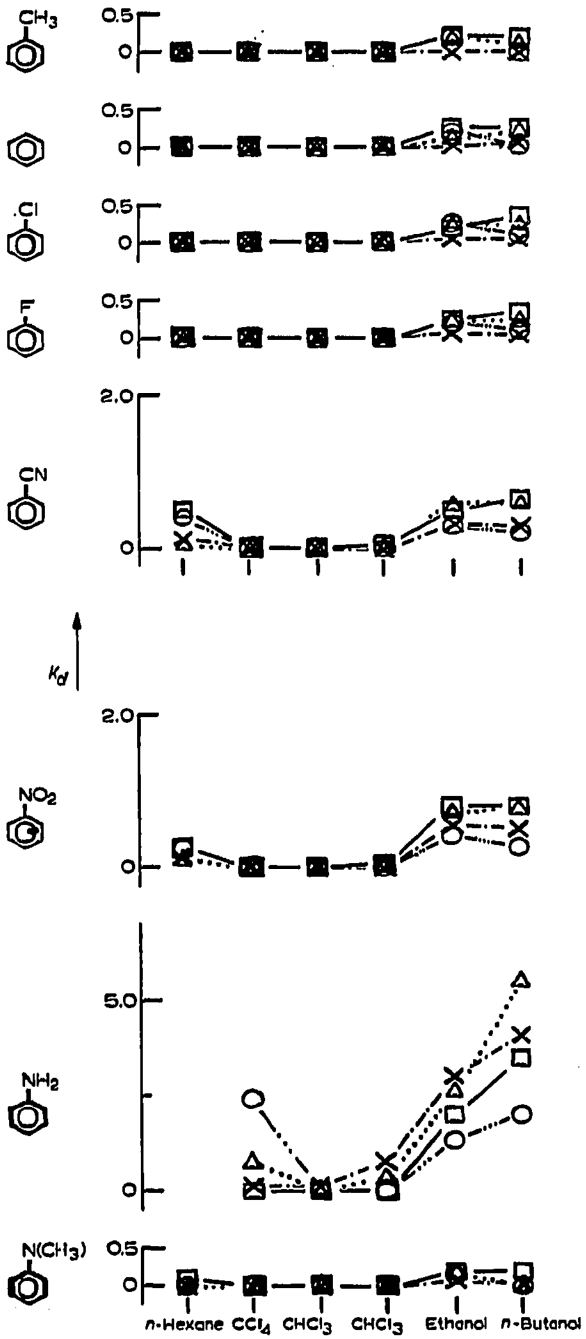


Fig. 2. Chromatographic spectra of naphthalene derivatives using various solvents arranged according to the classification of Ewell *et al.*[7] and Pimentel and McClellan[8] and several resins. Symbols as in Fig. 1.

Fig. 3. Chromatographic spectra of naphthalene derivatives using various solvents arranged according to the classification of Ewell *et al.*[7] and Pimentel and McClellan[8] and several resins. Symbols as in Fig. 1.



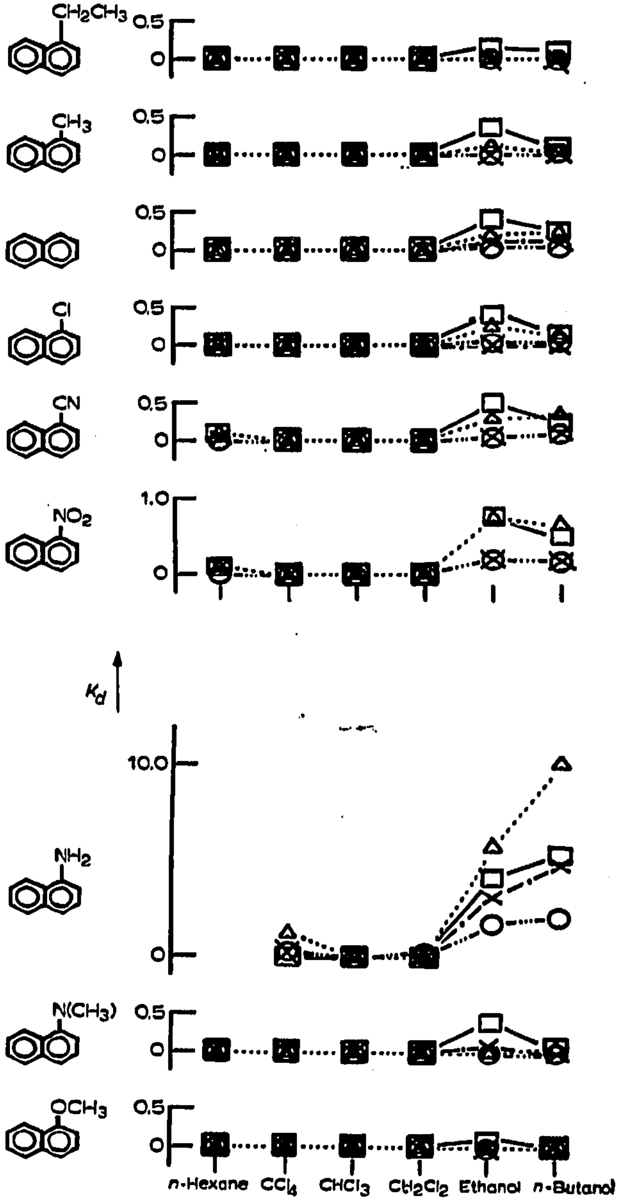


Fig. 4. Chromatographic spectra of benzene derivatives using various solvents arranged according to the classification of Ewell *et al.*[7] and Pimentel and McClellan[8] and SA-X⁻.
□—□, X= OH; △····△, X= Cl; ○-···-○, X= F; ×-·-×, X= I.

Fig. 5. Chromatographic spectra of naphthalene derivatives using various solvents arranged according to the classification of Ewell *et al.*[7] and Pimentel and McClellan[8] and SA-X⁻. Symbols as in Fig. 4.

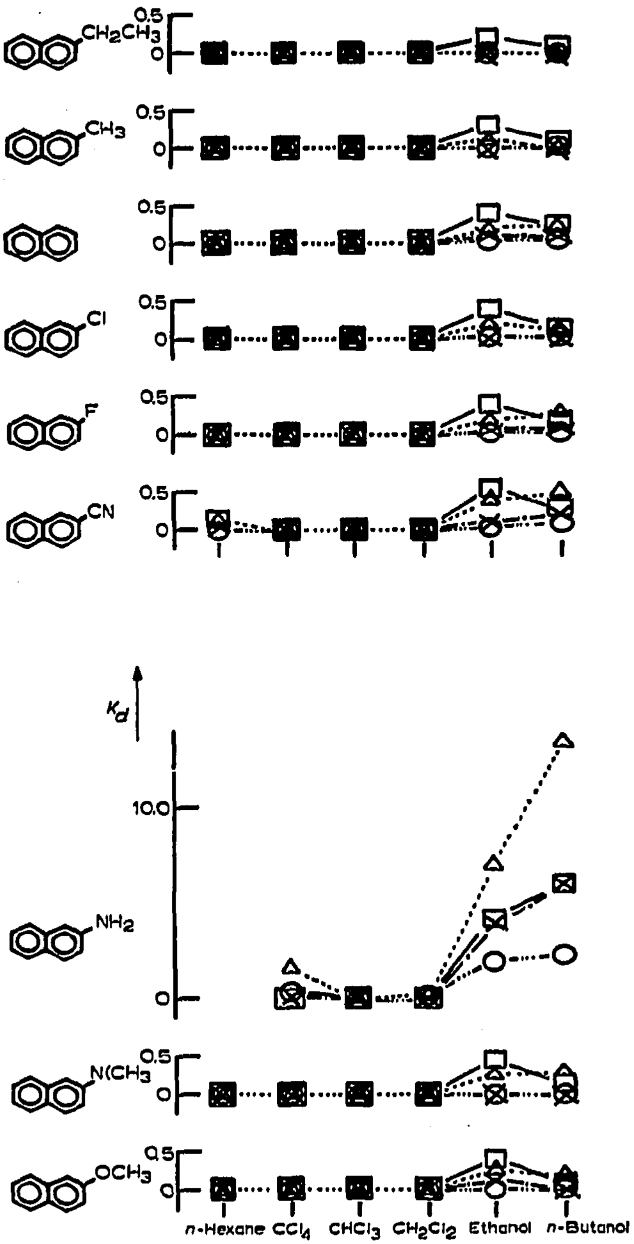


Fig. 6. Chromatographic spectra of naphthalene derivatives using various solvents arranged according to the classification of Ewell *et al.*[7] and Pimentel and McClellan[8] and SA-X⁻. Symbols as in Fig. 4.

$Cl \geqslant F \geqslant H \geqslant CH_3$ and the K_d' values are presented in Table II. This result was the same as the adsorption of aromatic compounds on Sephadex LH-20 (ref. 5).

Poor adsorption on polystyrene gel or a strong cation-exchange resin would depend upon a feeble π-complex between π-electrons of the gel and an aromatic

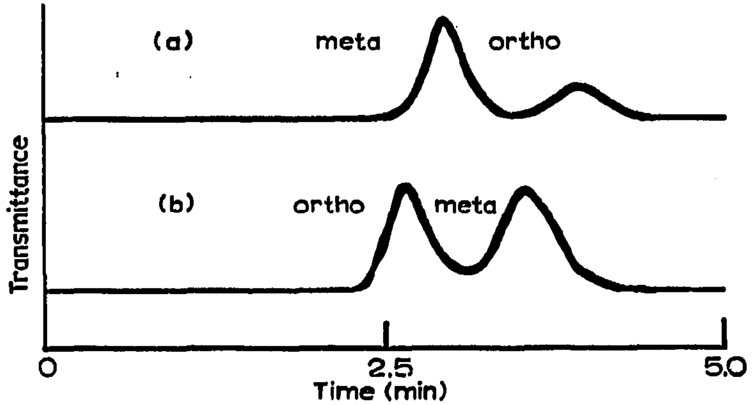
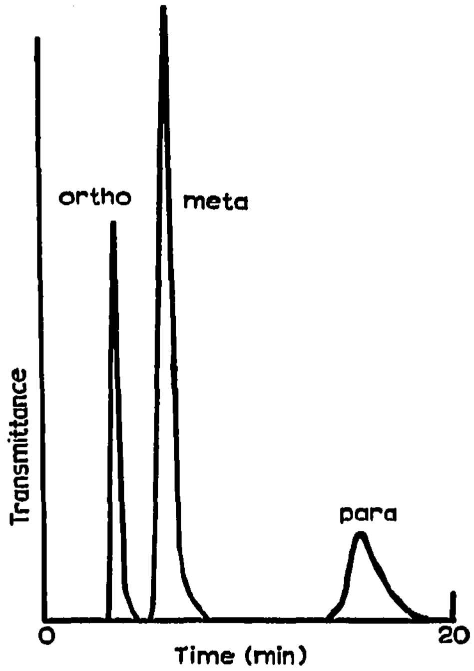


Fig. 7. Elution curves of *o*- and *m*-dinitrobenzenes (a) and *o*- and *m*-chloroanilines (b) on SAX. Eluent, *n*-butanol–*n*-hexane (1:9); column, 1.2 m × 2.1 mm I.D. stainless steel; flow-rate, 39.7 ml/h (60 kg/cm^2).



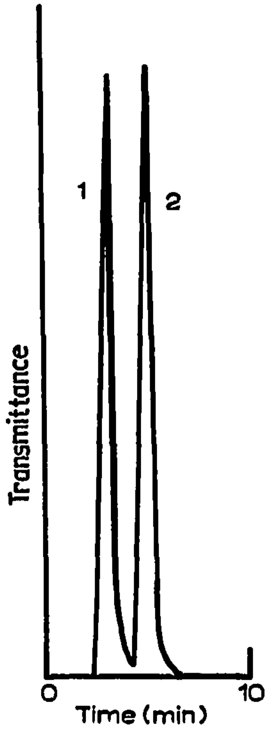


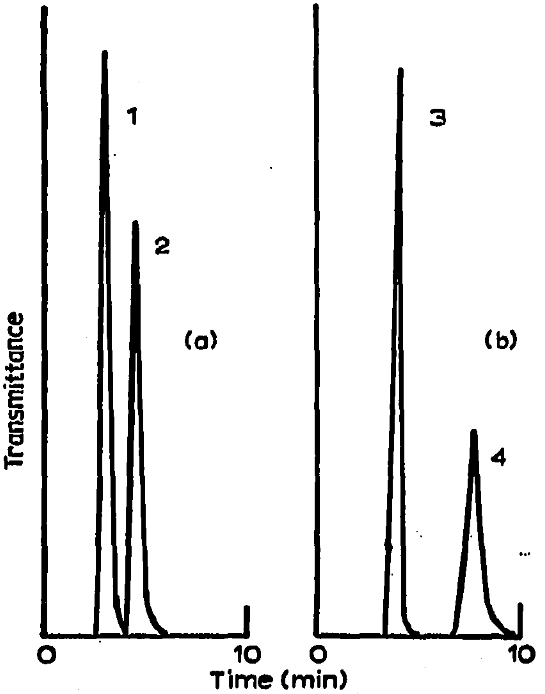
Fig. 8. Elution curve of *o*-, *m*- and *p*-nitroanilines on SAX. Eluent, *n*-butanol–*n*-hexane (1:9); column, 1.2 m × 2.1 mm I.D. stainless steel; flow-rate, 45.5 ml/h (65 kg/cm^2).

Fig. 9. Elution curve of *o*-nitro-*p*-chloroaniline (1) and *p*-nitro-*o*-chloroaniline (2) on SAX. Eluent, *n*-butanol–*n*-hexane (1:9); column, 1.2 m × 2.1 mm I.D. stainless steel; flow-rate, 45.5 ml/h (65 kg/cm^2).

ring of a sample molecule, so that alcohol compounds with a hydrophobic group are adsorbed more feebly than those with hydrophilic substituents.

To confirm these trends, naphthalene derivatives were also used as solutes (Figs. 2 and 3). These results show that the adsorption mechanism is a hydrogen bond or a π-bond. The increase of K_d' values followed the order of $NH_2 \gg NO_2 > CN > Cl \geqslant F \geqslant H \geqslant CH_3 \geqslant C_2H_5$ (Table II). Aminonaphthalene was adsorbed very strongly on anion-exchange resin and naphthol could not be desorbed, but the K_d values of N,N-dimethylnaphthylamine and methoxynaphthalene were very small. These results confirm the formation of a hydrogen bond between a counter ion of the anion-exchange resin and an amino or hydroxyl group of the aromatic compound. Generally, the K_d values of β-derivatives of naphthalene were somewhat larger than those of α-derivatives (Fig. 3). This is probably due to differences in the resonance energies.

Adsorption on weak cation-exchange resins (WC) would depend upon a π-electron of the carbonium group of the resin in dichloromethane. However, dichloromethane is not a stable liquid at room temperature, so this chromatographic system is not a very practical one for separation chromatography. The differences in sorption between strong anion-exchange resin (SA-Cl$^-$) and weak anion-exchange



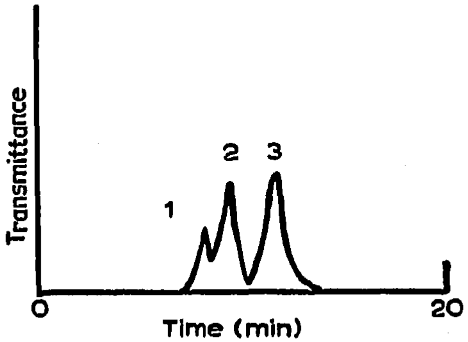


Fig. 10. Elution curves of (a) *m*-nitro-*p*-toluidine (1) and *o*-nitro-*p*-toluidine (2) and (b) *p*-nitro-*o*-toluidine (3) and 5-nitro-*o*-toluidine (4) on SAX. Eluent, *n*-butanol–*n*-hexane (1:9); column, 1.2 m × 2.1 mm I.D. stainless steel; flow-rate, 45.5 ml/h (65 kg/cm^2).

Fig. 11. Elution curve of 3-nitro-*p*-anisidine (1), 4-nitro-*o*-anisidine (2) and 5-nitro-*o*-anisidine (3) on SAX. Eluent, *n*-butanol–*n*-hexane (1:9); column, 1.2 m × 2.1 mm I.D. stainless steel; flow-rate, 19.1 ml/h.

resins (WA and Py) are generally low, except in alcohols. Strong anion-exchange resin is a stable adsorbent, so that an effective system appears to be that of strong anion-exchange resin and alcohol (Figs. 1–3).

To investigate the influence of ionic forms of the anion-exchange resin, Amberlite CG-400, I^-, OH^-, Cl^- and F^- forms were used as adsorbents and the chromatographic spectra are presented in Figs. 4–6. These results suggest that the Van der Waals' radius and the electronegativities of the counter ions of the anion-exchange resin did not regularly affect the adsorption. Thus, if the former was the predominant factor, K_d values would be maximal with SA-I^- and if the latter was predominant, K_d values would be maximal with SA-F^-; the spectra observed do not fulfil these expectations, so that both effects are apparently present.

Actual separation results for isomers of benzene and naphthalene derivatives by high-speed liquid chromatography are presented in Figs. 7–13.

In Fig. 7, the reason for the retention time of *o*-dinitrobenzene being longer than that of *m*-dinitrobenzene depends upon the difference in resonance energies between the *o*- and *m*-isomers, that is, it is the difference in polarizabilities or dipole moments. On the other hand, *o*-chloroaniline was eluted more quickly than the *m*-isomer because of the *ortho* effect on the formation of a hydrogen bond between the amino group and the counter ion of the anion-exchange resin. The order of retention times of nitroanilines is *o*-<*m*-<*p*-isomers (Fig. 8), suggesting that a

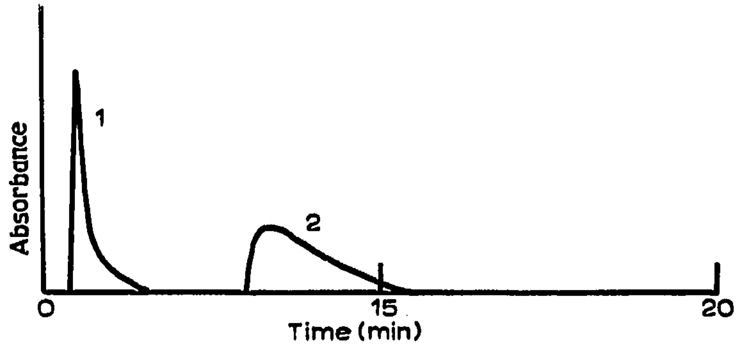


Fig. 12. Elution curve of (1) 1,5- and (2) 1,8-dinitronaphthalene on SAX. Eluent, *n*-butanol–*n*-hexane (1:99); column, 1.2 m×2.1 mm I.D. stainless steel; flow-rate, 78 ml/h (45 kg/cm²).

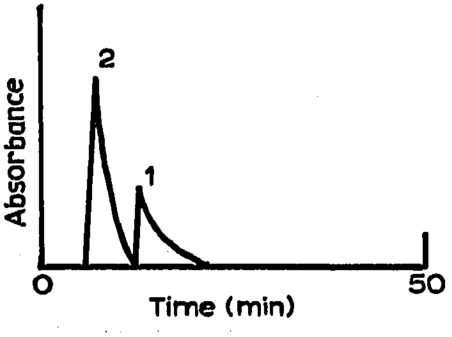


Fig. 13. Elution curve of (1) 1,5- and (2) 1,8-diaminonaphthalene on SAX. Eluent, *n*-butanol–*n*-hexane (1:4); column, 1.2 m×2.1 mm I.D. stainless steel; flow-rate, 35 ml/h (21 kg/cm²).

nitro group which has a high electronegativity influences the hydrogen bond between an amino group and a counter ion of the anion-exchange resin. This steric effect would depend upon resonance energies, but not on delocalization energies[12]. In Fig. 9, the separation of *o*-nitro-*p*-chloroaniline from *p*-nitro-*o*-chloroaniline depends upon the differences in electronegativity between chloro and nitro groups of the *o*-position, *i.e.*, it depends upon the difference in the *ortho* effect. In Fig. 10, the order of retention times of nitrotoluidines is (1)<(3)<(2)<(4), which also depends upon *ortho* and steric effects on hydrogen bonding. The elution curve of nitroanisidines also suggests an *ortho* and steric effect in Fig. 11. In Fig. 12 differences of retention time of dinitronaphthalenes can be explained by resonance energies influencing a π-complex. The differences in retention times of diaminonaphthalenes in Fig. 13 is related to steric hindrance.

These results suggest that the modern high-speed liquid chromatographic technique is very useful for good separations. The separation time is shorter than that of classical column chromatography[1,2], but the poor adsorptive ability of some adsorbents under the conditions required is a limitation.

CONCLUSION

The chromatographic system using a strong anion-exchange resin as adsorbent and non-aqueous solvents as eluents is useful for separating organic isomers, and has a better reproducibility than in the case of inorganic adsorbents, which has a poor reproducibility.

The possible adsorption mechanisms include the formation of a π-complex or of a hydrogen bond between a counter ion of the anion-exchange resin and a molecule of the sample. Resonance energies can also play a part.

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