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AUTOMATIC LIQUID CHROMATOGRAPHY OF ORGANIC COMPOUNDS

II. ADSORPTION CHROMATOGRAPHY OF BENZENE DERIVATIVES ON STYRENE-BASED CATION-EXCHANGE RESIN*

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SUMMARY

The adsorption chromatography of benzene derivatives on styrene-based cation-exchange resin has been investigated. Adsorption isotherms of benzene derivatives are discussed and the possibility of separation and quantitative treatment is considered for some cases of non-linear isothermal adsorption.

The distribution coefficient and the height equivalent to a theoretical plate, important for separation and quantitative analysis, have been calculated for thirty-eight derivatives of benzene.

INTRODUCTION

DAVIES AND THOMAS² and SAMUELSON³ reported on the adsorption of carboxylic acids on sulfonic acid-type ion-exchange resin. Salting-out chromatography of aromatic compounds has been reported by SARGENT AND RIEMAN⁴ and FUNASAKA *et al.*^{5,6} The adsorption of aromatic compounds on the skeleton of ion-exchange resins has been considerably investigated⁷⁻¹⁰.

Although some relationships between isotherms and liquid chromatograms have been discussed, there has been little experimental study¹¹. The experimental design and column standardization are very difficult, and the experimental conditions used must therefore be carefully controlled. Thus the diameters of resin particles should be controlled, the resin should be packed into the column as homogeneously as possible, the extra column volume and the sample injection volume should be decreased, and the column temperature and the flow rate should be maintained constant. Under these controlled conditions, an isothermal curve can be predicted from the variation of the distribution coefficients (K_D) with change of solute concentration.

In this paper, K_D and height equivalent to a theoretical plate (HETP) of thirty-eight derivatives of benzene were obtained, and the variation of retention volumes (V_R) with the change of concentration of several compounds was studied. The relation between the type of adsorption isotherm and the separative effect of admixed solutes in liquid chromatography was demonstrated for phenol derivatives.

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EXPERIMENTAL

Samples

Samples used as solutes were recrystallized or redistilled and their purity was determined by means of the melting point, organic microanalysis and IR spectroscopy. Some of them were kindly supplied by WAKO Pure Chemicals Co., Ltd. The samples were weighed with an accuracy of 10 μg and dissolved in deionized and redistilled water to give solutions of various concentrations. The injection volume of each sample solution was 100 μl .

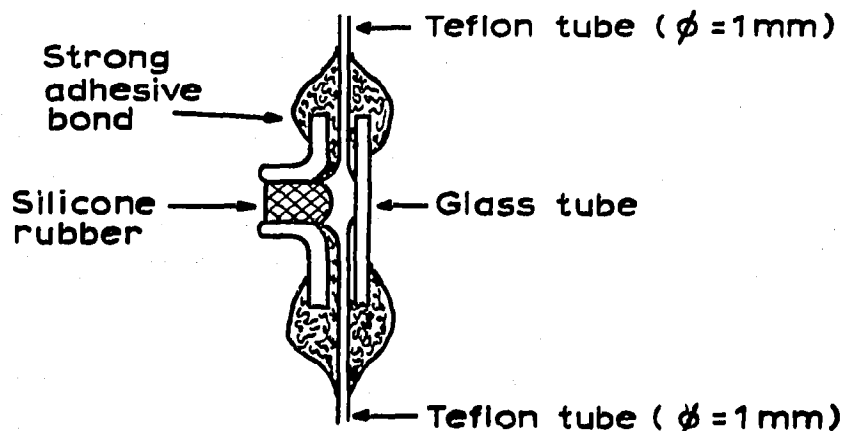


Fig. 1. Special sample injector.

Equipment

A UV-detecting, automatic recording liquid chromatograph was used, fitted with a reverse phase duplex plunger pump, JLC-P₂ (JEOL Co., Ltd.); a constant-temperature water circulator connected to the column jacket, TAIYO Thermo Unit C-550 (TAIYO Scientific Fabrication Co., Ltd.); a specially designed sample injector depicted in Fig. 1; a UV detector, JLC-B₁ UV Detector (JEOL Co., Ltd.), which had four light interference filters (transmittances were about 40% at 260, 273, 280 and 287 nm) and a quartz flow cell (lightpath 3 mm); and a 6-pen electronic recorder (OHKURA Electric Co., Ltd.). The connector between the column and the flow cell was a 38-cm Teflon tube of 1 mm I.D. that was as short as possible.

The inner diameter of the glass column used was 0.8 cm. The column length was controlled by a variable column plunger. The outlet pressure was controlled by a valve.

Preparation of stationary phase

Dowex 50W X2, 4, 8 and 12, hydrogen form, (each 200–400 mesh), Aminex X12 (21–29 μm), Amberlite CG-120 (X8.5) (400–600 mesh) and Bio-Rad AG 50W X8 (minus 400 mesh) were passed through a sieve to obtain a particle size of minus 400 mesh, and the particle size was further adjusted to be uniform by the sedimentation method described previously¹.

The sodium form of the ion-exchange resin was formed with a large excess of 2 N sodium hydroxide solution and washed with water in a long column. The diameters of the particles of both types of resin were tested by microscopy and are tabulated in Table I.

TABLE I

CROSS-LINKING, PARTICLE DIAMETERS AND WEIGHTS IN COLUMNS OF ION-EXCHANGE RESINS USED IN EXPERIMENTS

	Commercially available resin Dowex 50W				Aminex	Amberlite CG-120	Bio-Rad AG 50W
Cross-linking	2	4	8	12	12	(8.5)	8
Mean diameter of particles (μm) (as H^+ type)	80	65	60	60	25	60	28.1
Resin weight (g) (column: 18.5×0.8 cm)							
H^+ form	1.544	2.350	3.670	4.387	4.144	3.234	3.735
Na^+ form	1.716	2.816	4.241	4.965	4.747	3.990	4.301

Ion-exchange resin was packed into a column homogeneously by means of a supporting tube which had the same diameter as the column connected to the top of the column, and the supporting tube was then detached. By using such a supporting tube, the column length was proportional to the weight of the packed resin (Fig. 2).

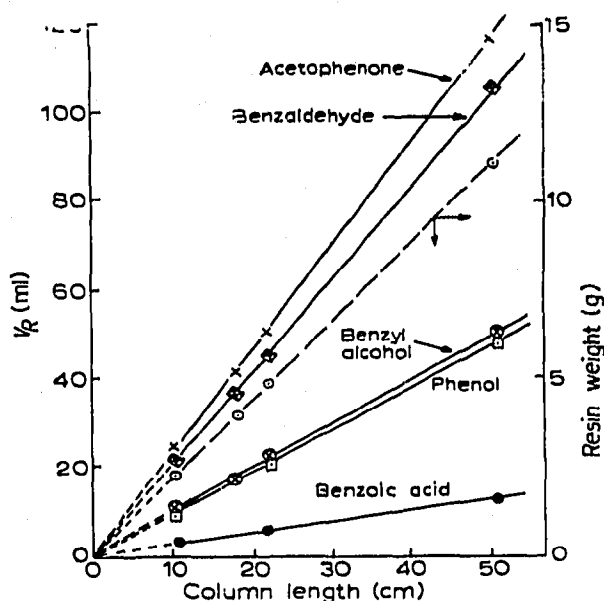


Fig. 2. Correlation of the column length with weight of packed resin and V_R value of some benzene derivatives. Amberlite CG-120, Na^+ form. Mean diameter of particles, $60 \mu\text{m}$. Column diameter, 0.8 cm. Eluent: water. Temperature, 40° . Flow rate, 0.45 ml/min.

The variation of V_R values with the change in the degree of cross-linking is shown in Fig. 3A and B. This indicates that the effect of cross-linking on V_R values is an important problem which should be investigated, but in this paper a degree of cross-linking of 8% was selected.

Measuring conditions

Deionized and redistilled water was run as eluent at rates of 0.45 and 0.36 ml/min. The column temperature was controlled at 30 , 40 , 50 , 52.5 , 60 and 70° . The wave-

length of the light interference filter used was selected as near as possible to the absorption maximum of the solute.

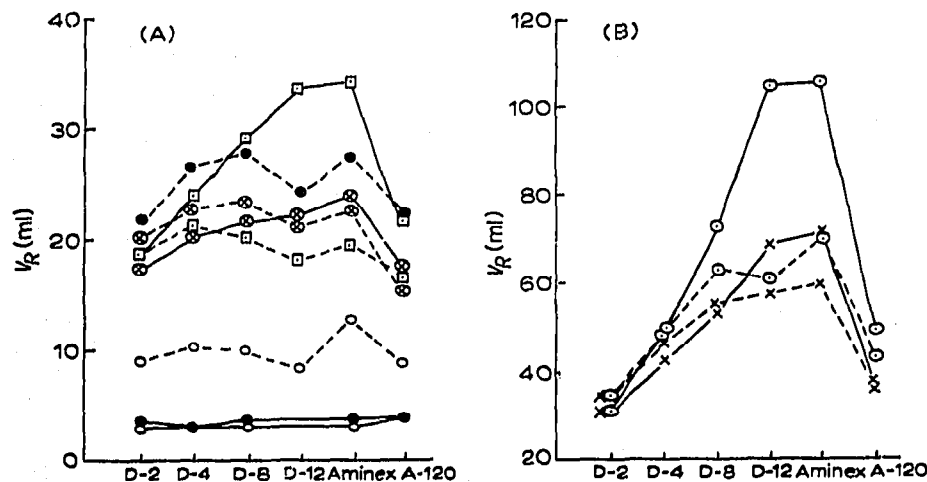


Fig. 3. Variation of V_R values of some benzene derivatives with change of cross-linking, of ion-exchange resins. Column, 18.5×0.8 cm. Eluent, water. Flow rate, 0.45 ml/min. Temperature, 52.5° . Symbols of resins: D-2, Dowex 50W X₂; D-4, Dowex 50W X₄; D-8, Dowex 50W X₈; D-12, Dowex 50W X₁₂; Aminex, Aminex X₁₂; A-120, Amberlite CG-120. (—), Na⁺ type; (---), H⁺ type; □, phenol, ⊗, benzylalcohol; ○, *p*-nitrobenzoic acid, ●, benzoic acid; ×, acetophenone; ⊙, nitrobenzene.

Calibration curve

A linear relation between peak area and solute concentration was obtained. Some solutes gave a non-linear adsorption isotherm, and the calibration curves were almost linear or very slightly curved (Fig. 4A and B).

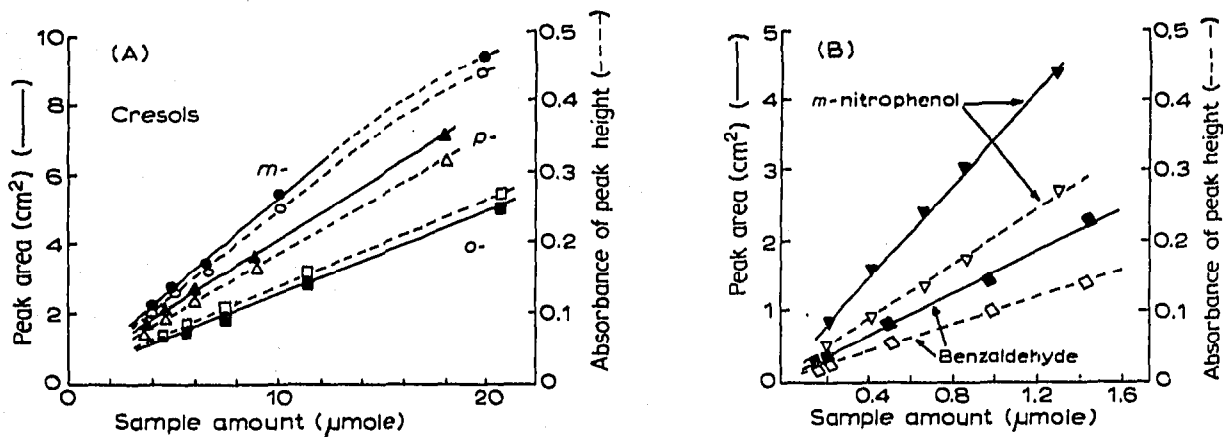


Fig. 4. Calibration curves of benzene derivatives. Bio-Rad AG 50W X₈, Na⁺ form. (A): *o*-, *m*- and *p*-cresols. (B): *m*-nitrophenol and benzaldehyde.

RESULTS AND DISCUSSION

Usually aqueous solvent systems are used in ion-exchange chromatography. Such systems were used in this work since it is preferable to use an aqueous system

for the investigation of the effect of physical adsorption mechanisms on strong ion-exchange resins.

Linear relations were obtained between the column length and the weight of packed resin and V_R value (Fig. 2). Also, $\log K_D$ was proportional to the reciprocal of absolute temperature in the range 30–70°C (as shown in Fig. 5). These facts showed that the adsorption mechanism should be nearly constant under these experimental conditions. V_R values and peak widths for benzene derivatives were determined from their chromatograms at the concentrations given in Tables II and III, and their K_D values and HETP values were calculated from them. Relative standard deviations of V_R values and widths were less than 2.5 % and less than 5 %, respectively ($n \geq 5$).

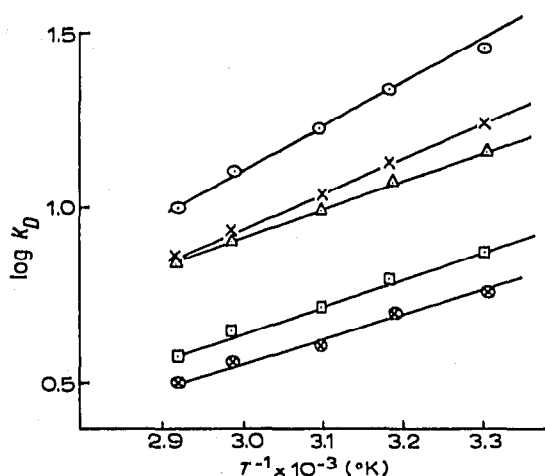


Fig. 5. Plot of $\log K_D$ of some benzene derivatives on Bio-Rad AG 50W XS, Na⁺ form, against the reciprocal of absolute temperature of the column. ⊙, benzyl alcohol; □, phenol; △, *p*-cresol; ×, acetophenone; ⊙, nitrobenzene.

The K_D values on Bio-Rad AG were less than on Dowex and the HETP values on Bio-Rad AG were 20–50 % of those on Dowex. Particle diameters, column temperatures and flow rates are given in Tables II and III.

As benzenesulfonic acid and *p*-toluenesulfonic acid were not adsorbed on the hydrogen form of strong ion-exchange resins, the volume of the mobile phase in the column was made the same as their V_R value. *o*- and *p*-nitrobenzoic acids were sparingly adsorbed and other substituted benzoic acids were adsorbed on the hydrogen form of the resins.

On the other hand, benzenesulfonic acid, *p*-toluenesulfonic acid and benzoic acids⁸ were exchanged with sodium ions on the sodium form of strong ion-exchange resins. Accordingly, the sodium salts of these compounds were used as solutes, and the volume of the mobile phase in the column was made the same as the V_R value of sodium benzenesulfonate. Sodium benzoic acids were sparingly adsorbed on the sodium form of the resins.

Nitrophenols were partially exchanged with the sodium form of the resins, as demonstrated by UV spectroscopy. The other solutes in Tables II and III were adsorbed similarly on the hydrogen and sodium forms of the resins.

The simplest mechanism of adsorption is the case in which the adsorption iso-

TABLE II

K_D VALUES AND HETP VALUES OF BENZENE DERIVATIVES ON H^+ FORM BIO-RAD AG 50W AND DOWEX 50W IN LIQUID CHROMATOGRAPHY

UV filters: **, 260 nm; ***, 273 nm; ****, 280 nm. Column: 18.5 × 0.8 cm. A = Bio-Rad AG 50W X8, H^+ form (particle diameter 28 μ m). Flow rate, 0.36 ml/min; temperature, 60°. B = Dowex 50W X8, H^+ form (particle diameter 60 μ m). Flow rate, 0.45 ml/min; temperature, 52.5°.

$X \cdot C_6H_4 \cdot Y$		Position of Y	UV filter	A			B		
X	Y			Sample amount (μ mole)	K_D (ml/g)	HETP (mm)	Sample amount (μ mole)	K_D (ml/g)	HETP (mm)
H	SO ₃ H		**	0.745		0.635			
	COOH		***	2.37	4.97	0.238	2.37	6.38	
	OH		**	2.46	3.63	0.476	44.1	4.35	
	NO ₂		**	1.66	11.8	0.156	1.66	14.9	
	CH ₂ OH		**	35.2	4.51	0.141	35.2	4.92	
	COCH ₃		****	4.49	9.99	0.314	4.49	12.8	
	CHO		****	2.38	8.56	0.181	3.77	0.734	
COOH	NO ₂	2	****	0.544	0.680	0.378	4.10	1.17	
		3	****	0.807	3.50	5.65	1.86	4.84	
		4	****	1.25	1.08	2.61	2.51	1.50	
	OH	2	****	0.725	1.01	3.68			
		3	****		2.67	(0.743)			
		4	****	3.62	3.35	(0.455)	3.62	4.26	
	Cl	2	**	12.8	2.77	(2.57)			
		3	**	16.0	5.63	2.29			
	CH ₃	3	****		(7.44)	(1.50)			
CH ₃	SO ₃ H	4	**	2.00		0.782	4.00	1.41	
		2	****	0.216	20.7	0.726			
	NO ₂	3	****	0.363	24.9	0.165	0.363	31.1	
		4	****	0.322	23.1	0.134		0.472	
OH	CH ₃	2	**	1.70	5.90	0.201	22.7	6.79	
		3	**	1.48	6.09	0.204	20.3	7.46	
		4	**	1.79	6.68	0.145	17.9	7.97	
		2	***	1.67	11.1	0.210			
	iso-C ₃ H ₇	3	***	1.69	12.3	0.182			
		4	***	1.20	13.6	0.220			
		2	****	0.511	2.19	0.235		2.71	
	OH	3	****	0.737	1.95	0.270		2.60	
		4	****	0.602	1.79	0.295	72.8	2.44	
		2	**	1.50	9.59	0.0997	1.50	11.8	
	NO ₂	3	**	1.93	8.67	0.156	15.4	11.1	
		4	**	1.71	8.71	0.269	9.49	12.4	
		2	****	4.88	8.23	0.303	19.6	11.4	
	Cl	3	****	5.04	10.1	0.305	17.9	12.9	
		4	****	4.48	10.3	0.202	17.9	13.3	
		2	****	0.647	11.8	0.159		0.866	
	Br	3	****	0.601	15.9	0.300			
		4	****	0.688	15.7	0.184			

therm is linear and ideal. Cases of linear ideal isotherms in adsorption phenomena are few and there are many examples of non-linear and non-ideal isotherms.

Chromatograms obtained in this experiment showed leading, tailing or Gaussian curves (some examples are shown in Fig. 6A, B and C). In the case of non-ideal isotherms, it is impossible to obtain the isotherm directly from its chromatogram. In such cases, the variation of the V_R value with change of solute concentration was

measured and its influence examined on separation and quantification. A range of solute concentration in which there was little or no change in the K_D value was found (Fig. 7A and B). In such a concentration range, the design of a separation and quantitative analysis method in non-linear isothermal adsorption chromatography should be practical in some cases. An example of the separation of *o*-, *m*- and *p*-nitrophenol is shown in Fig. 6A. Chromatograms of *o*- and *p*-nitrophenol showed leading, corresponding to an isotherm convex to the horizontal axis, but *m*-nitrophenol showed tailing corresponding to a concave isotherm. The resolution of these chromatograms

TABLE III

K_D VALUES AND HETP VALUES OF BENZENE DERIVATIVES ON THE Na FORM OF BIO-RAD AG 50W AND DOWEX 50W IN LIQUID CHROMATOGRAPHY

UV filters: **, 260 nm; ***, 273 nm; ****, 280 nm; *****, 287 nm. Column: 18.5 × 0.8 cm; A = Bio-Rad AG 50W X8, Na⁺ form (particle diameter 28 μm). Flow rate, 0.36 ml/min; Temperature 60°. B = Dowex 50W X8, Na⁺ form (particle diameter 60 μm). Flow rate, 0.45 ml/min; Temperature, 52.5°.

$X \cdot C_6H_4 \cdot Y$		Position of Y	UV filter	A			B		
X	Y			Sample amount (μmole)	K_D (ml/g)	HETP (mm)	Sample amount (μmole)	K_D (ml/g)	HETP (mm)
H	SO ₃ Na COONa OH		**	1.00		9.42			
			****	2.55	0.688	1.94	2.37	0.561	2.25
			***	2.22	4.98	0.319	44.1	6.46	1.39
	NO ₂ CH ₂ OH COCH ₃ CHO ^a		*****	1.66	12.7	0.307	1.66	17.7	1.21
			**	35.2	3.88	0.377	35.2	4.80	1.43
			****	4.49	8.63	0.717	4.49	12.9	1.68
			****	2.45	7.79	0.406	3.77	11.2	1.33
COONa	CH ₃	2	****	2.71	0.660	4.28			
		3	****	2.57	1.16	4.28			
		4	****	2.50	1.27	4.96			
	OH NO ₂	2	****	0.478	0.0802	1.60			
		3	****	0.0270	0.137	1.60	1.86	0.104	1.57
		4	****	0.145	0.0930	1.28	0.251	0.0280	1.54
CH ₃	SO ₃ Na NO ₂ ^a	4	**				4.00		
		2	****	0.475	20.8	0.402			
		3	*****	0.363	26.0	0.465			
		4	*****	0.322	23.5	0.487			
OH	CH ₃	2	***	4.54	7.99	0.441	22.7	9.33	2.73
		3	***	5.05	8.16	0.561	20.3	10.2	2.07
		4	***	4.50	8.38	0.439	17.9	11.1	1.95
	OH	2	***	1.12	4.42	0.222		3.54	
		3	***	0.976	3.78	0.250		3.49	
		4	***	1.20	3.40	0.329	72.8	4.25	
	NO ₂ ^a	2	***	0.558	6.74	0.445	1.50	8.54	1.58
		3	***	0.827	13.9	0.362	15.4	20.3	1.18
		4	***	0.863	11.1	0.539	9.49	12.4	1.51
	Cl ^a	2	*****	4.88	11.7	0.520	19.5	7.02	
		3	****	5.93	17.2	0.486	17.9	14.3	
		4	****	4.48	16.8	0.588	17.9	14.9	
	Br ^a	2	****	2.37	17.1	0.506			
		3	****	2.57	24.0	0.716			
		4	****	2.06	25.5	0.571			

^a Column 6.7 × 0.8 cm.

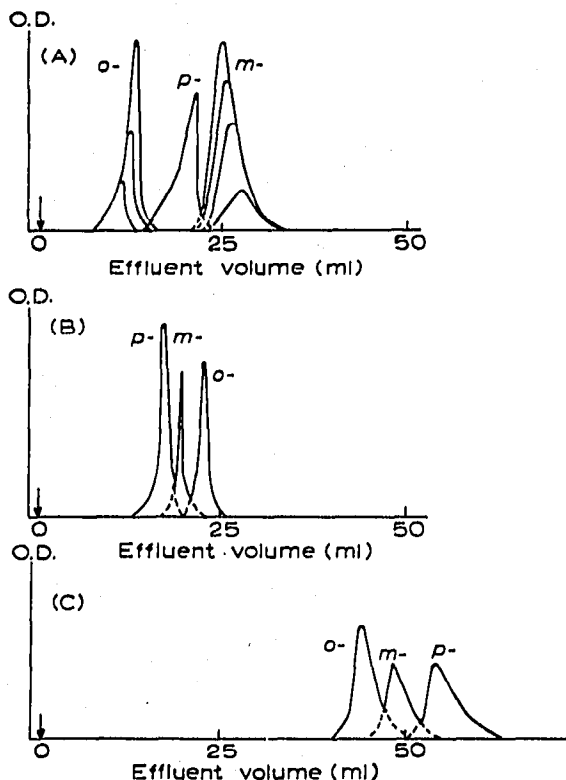


Fig. 6. Liquid chromatograms of phenols. Bio-Rad AG 50W X8. Diameter of particles, $28.1 \mu\text{m}$ (in H^+ form). Column diameter, 0.8 cm. Eluent, water. Flow rate, 0.36 ml/min. Temperature, 60° . (A) Nitrophenols: *ortho*, 0.56, 0.28 and 0.16 μmole ; *meta*, 0.83, 0.65, 0.42 and 0.21 μmole ; *para*, 0.18 μmole . Column length, 6.7 cm, Na^+ form. (B) Hydroxyphenols, *ortho*, 1.1 μmole ; *meta* 0.98 μmole ; *para* 1.2 μmole . Column length, 18.5 cm, Na^+ form. (C) Isopropylphenols, *ortho*, 1.67 μmole ; *meta*, 1.69 μmole ; *para*, 1.20 μmole . Column length: 18.5 cm, H^+ form.

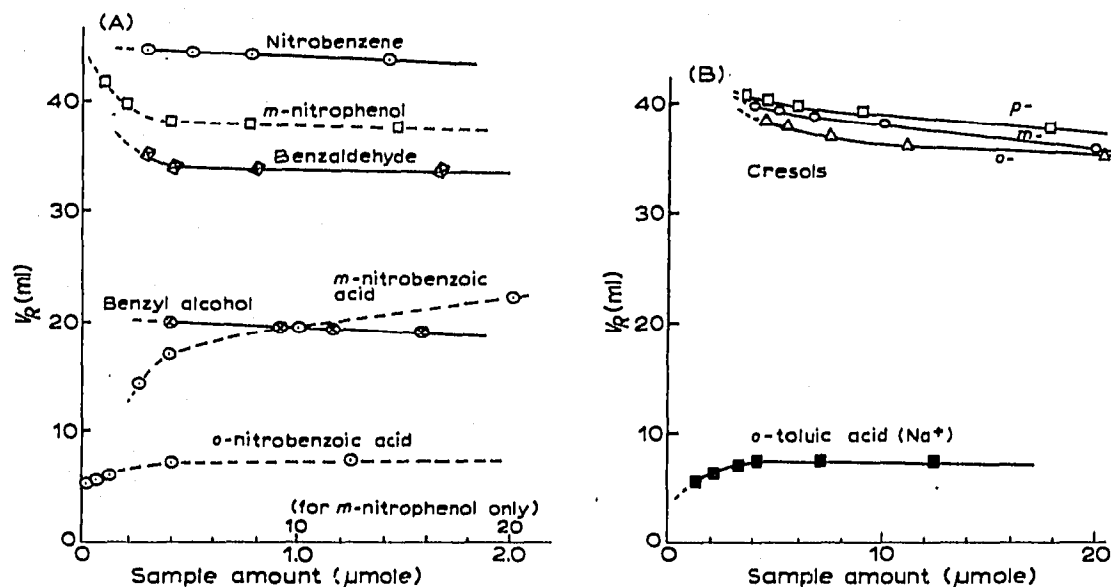


Fig. 7. Variation of V_R values of some benzene derivatives with change of solute concentration. (A) Dowex 50W X4. Mean diameter of particles, $65 \mu\text{m}$. Column, 18.5×0.8 cm. Eluent, water. Flow rate, 0.45 ml/min. Temperature, 52.5° . (—), Na^+ form; (---), H^+ form. (B) Bio-Rad AG 50W X8. Mean diameter of particles; $28.1 \mu\text{m}$ (in H^+ form). Flow rate, 0.36 ml/min. Temperature, 60° . (—), Na^+ form.

was therefore increased by dilution of the solutes, but decreased by an increase in concentration.

As another example, the separation of *o*-, *m*- and *p*-dihydroxybenzene shows an almost linear adsorption isotherm (Fig. 6B). Chromatograms of *o*-, *m*- and *p*-isopropylphenol showed tailing and their complete separation was not achieved with the 18.5-cm column length (Fig. 6C). However, the separation of *o*- and *p*-isopropylphenol was complete.

The relation between the chemical structure of solutes and their K_D values will be discussed in a future paper.

CONCLUSIONS

The adsorption of benzene derivatives on the hydrogen and sodium forms of styrene-based strong cation-exchange resins was examined in aqueous solutions.

Hitherto, the character of non-ionic adsorption of aromatic compounds on strong cation-exchange resins was not certain. The present results explain some aspects of non-ionic adsorption. Thus there are some cases of linear adsorption isotherms and some cases of non-linear adsorption isotherms for which the variation of V_R value with solute concentration is not large. In these cases, a linear relation between peak heights or peak areas and solute concentration was obtained and quantitative analyses were possible.

Based on these results, some examples of possible separations were given, and K_D and HETP values, important data for the practical design of quantitative and qualitative analysis of benzene derivatives by liquid chromatography, were obtained for thirty-eight compounds.

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