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HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC SEPARATION OF AROMATIC CARBOXYLIC ACIDS WITH β-CYCLODEXTRIN-BONDED STATIONARY PHASES

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SUMMARY

Three β-cyclodextrin-bonded stationary phases, having amino-type spacers of different length, were prepared and their ability to separate isomers of aromatic carboxylic acids based on inclusion complex formation was examined. Two retention mechanisms, inclusion complex formation and anion exchange, were found to determine the elution order of the isomers. An increase in the length of the spacer of bonded cyclodextrin moieties on a silica surface increases the retention and the selectivity by inclusion complex formation.

INTRODUCTION

An advantage of applying a retention mechanism based on the formation of inclusion complexes, *i.e.*, host–guest compounds, with cyclodextrins (CDs) to chromatography over the application of conventional mechanisms such as partition, adsorption or size exclusion is that this technique can offer a high selectivity for the separation of structural, geometric and, in some instances, optical isomers. This comes from the ability of CD molecules to recognize not only the hydrophobicity but also the suitability of the size and/or the shape of a guest compound for the formation of inclusion complexes.

In general, two methods have been advanced; one involves the use of CDs as an additive to an aqueous mobile phase in liquid chromatography and the other employs CDs as a stationary phase in either liquid or gas chromatography; CDs are bonded to or coated on appropriate supports. The former method has been applied to ion-exchange and reversed-phase high-performance column chromatography, and also to thin-layer chromatography, with conventional stationary phases. It has been shown that the retention value of solutes depends largely on the concentration of CD in aqueous–organic or aqueous mobile phases, although other factors such as pH, ionic strength and the type or content of organic modifier in the mobile phase also have an influence[1–13,21]. In spite of many studies having been made, problems are

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often still encountered with this additive method, *e.g.*, contamination of solutes in the effluent with CDs or the loss of CDs, which is expensive. The most serious problem in column chromatography is partial plugging of the mobile phase delivery line in the injector or at the column inlet frit owing to the formation of less soluble inclusion complexes, which in turn increases the column inlet pressure or changes the flow-rate of the mobile phase. In contrast, such problems do not arise in the latter approach, although some difficulty still exists in preparing high-performance CD-bonded stationary phases[14–17].

In previous work[18], an attempt was made to bind CD molecules on a silica surface by treating an amino-bonded silica gel with monotosylated CD, and the retention behaviour of some aromatic compounds on this CD-bonded phase was studied.

In this study, to clarify the effect of the length of the spacer on the retention of aromatic carboxylic acids based on inclusion complex formation, three types of CD-bonded silica stationary phases having amino-type spacers of different length were prepared and examined for their separation ability. Another β-CD-bonded phase (carbamate type) having no amino-type spacer was also prepared for comparison.

EXPERIMENTAL

Apparatus and procedure

The liquid chromatograph consisted of a Waters Model 6000A solvent delivery pump (Waters Assoc., Milford, MA, U.S.A.), a Rheodyne 7120 sample injector with a 20-μl loop (Rheodyne, Berkeley, CA, U.S.A.) and a JASCO Uvidec-100-II variable-wavelength UV detector (JASCO, Tokyo, Japan).

Columns (20 cm × 4 mm I.D.) were packed by the balanced viscosity slurry method, using a Chemco Model 124 slurry packing apparatus (Chemco Scientific, Osaka, Japan) at *ca.* 450 kg/cm^2. The slurry solvent was methanol–2-propanol–carbon tetrachloride–cyclohexanol–toluene (5:5:18:10.8:1.2 by volume) and the pumping solvent was methanol. Sample solutions of aromatic carboxylic acids were prepared by dissolving them in methanol to give a concentration of 1 mg/l and 0.5-μl volumes were usually injected. The flow-rate was 0.7 ml/min, and the detector was operated at 230 nm. The mobile phase was 0.002 *M* potassium dihydrogen phosphate–methanol (1:1), the pH of which was adjusted with 85% phosphoric acid. It was filtered through a membrane filter of 0.7 μm pore size and degassed by ultrasonic vibration under vacuum prior to use. All retention data were obtained under ambient conditions. The column dead volume was determined using potassium nitrite solution.

Reagents and materials

Aromatic carboxylic acids were purchased from various suppliers and were of the highest quality available. Guaranteed-reagent grade β-cyclodextrin and LC-grade methanol were obtained from Nakarai Chemicals (Kyoto, Japan). All silylating agents were purchased from Petrarch Systems (Bristol, PA, U.S.A.) and were purified by fractional distillation in a nitrogen atmosphere under vacuum to exclude any dimeric or polymeric siloxanes before use. Cosmosil 5SL (Nakarai Chemicals), totally porous spherical silica gel with a mean particle diameter of 5 μm, a mean pore size of 11

Å and a specific surface area of 330 m^2/g, was used for the preparation of cyclodextrin-bonded phases. Deionized water, obtained by ion exchange, was further purified with a Milli-Q Water Purification System (Millipore, Bedford, MA, U.S.A.).

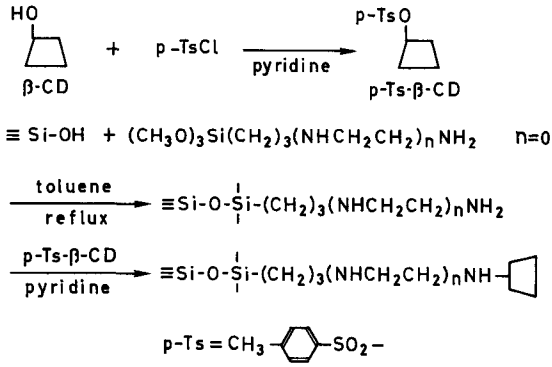
Preparation of β-CD bonded phase

Three types of β-CD bonded stationary phases were prepared by treating amino-bonded silicas with monotosylated β-CD.

Triamine (N3) type. Ten grams of silica gel were dried *in vacuo* (about 5 Torr) at 120°C for 6 h using a vacuum drying apparatus. After cooling, it was transferred into a round-bottomed flask containing 100 ml of dry toluene with gentle shaking and then 25 g (0.094 mol) of 3-[2-(2-aminoethylaminoethylamino)propyl]-trimethoxysilane were added. The reaction apparatus was purged with dry nitrogen and the mixture was stirred under reflux for 20 h. The reaction mixture was filtered through a membrane filter and washed successively with toluene, acetone and methanol. The N3-bonded silica gel thus obtained was vacuum-dried at 120°C for 6 h and characterized by carbon and nitrogen elemental analyses. Tosylation of primary hydroxy groups of β-CD was performed according to the literature method[19].

In the bonding procedure, 7 g of monotosylated β-CD, which had been dried at 50°C *in vacuo* for 5 h, were introduced into a reaction flask containing 150 ml of dry pyridine and the resulting solution was treated with 3 g of vacuum-dried N3-bonded silica gel at 50°C for 2 days under a dry nitrogen atmosphere with stirring. The product was filtered, washed with pyridine, acetone and methanol, then dried at 50°C *in vacuo* for 5 h.

Diamine (N2) and monoamine (N1) types. For the modification of silica gel, 3-[(2-aminoethyl)amino]propyltrimethoxysilane and 3-aminopropyltrimethoxysilane were used to prepare N2- and N1-type stationary phases, respectively. The silylating reaction and bonding reaction were carried out in the same way as for the N3-type bonded phase. Synthetic schemes are shown in Scheme 1.



Scheme 1.

Details of the preparation of carbamate-type β-CD-bonded phase will be reported in a separate paper[20].

RESULTS AND DISCUSSION

Characterization of bonded phases

The amounts of bonded β-CD molecules in the three types of stationary phase were calculated from their carbon and nitrogen contents and the specific surface area of the starting silica gel. Table I shows the characteristics of the six bonded phases used in this study.

TABLE I

CHARACTERIZATION OF BONDED PHASES

Designation	*Elemental analysis*		*Bonded amino group (mol/g × 10^{-6})*	*Bonded β-CD molecule (mol/g × 10^{-6})*
	C (%)	*N (%)*		
Monoamine (N1)	3.10	–	861	–
Monoamine-β-CD (N1-CD)	5.00	0.68	486	64.5
Diamine (N2)	4.60	–	767	–
Diamine-β-CD (N2-CD)	5.40	1.29	461	52.3
Triamine (N3)	8.43	–	910	–
Triamine-β-CD (N3-CD)	8.27	2.78	662	53.8

The amounts of amino groups on these stationary phases decreased to 56% for N1, 60% for N2 and 73% for N3 after bonding with β-CD. The extents of immobilization of β-CD molecules to the spacer of N1, N2 and N3 types were 13.3, 11.3 and 8.1%, respectively. It should be noted that, as shown in Table I, the amounts of β-CD molecules on these three stationary phases were nearly the same, irrespective of the difference of the chain length of the spacer.

Effect of pH on retention on N3-CD phase

In this study, methanol–water (1:1) was selected as the mobile phase because, as discussed earlier, β-CD forms inclusion complexes more easily in aqueous methanol than in the other aqueous–organic solvents usually employed in reversed-phase chromatography[14,15].

In liquid chromatography based on inclusion complex formation with CDs, the retention behaviour of ionizable compounds is influenced significantly by the pH of the mobile phase. If a CD-bonded stationary phase is employed, it is to be expected that charged species will be eluted rapidly, whereas neutral molecules will be strongly retained. Table II shows the pH dependence of capacity factors (k') of aromatic carboxylic acids obtained on an N3-CD column. The k' values of all the compounds increased significantly with increase in the pH from 2.8 to 4.0, in contrast to the above generalization and to results reported in the literature[7,10,15]. Table II also shows that the elution order of the isomers in each series was not changed by changes in pH, except for aminobenzoic acid. It should be noted that the observed orders do not necessarily agree with the order *ortho* < *meta* < *para* reported in the literature[15] or generally expected from the relative stability of their CD inclusion complexes.

This anomalous retention behaviour of aromatic carboxylic acids on the N3-

TABLE II

EFFECT OF pH ON CAPACITY FACTORS OF AROMATIC CARBOXYLIC ACIDS ON TRIAMINE-β-CD BONDED PHASE

Column, 20 cm × 4 mm I.D.; mobile phase, 0.002 *M* phosphate buffer–methanol (1:1); flow-rate, 0.7 ml/min.

Substance	*pH*						
	2.8	*3.0*	*3.2*	*3.4*	*3.6*	*3.8*	*4.0*
o-Aminobenzoic acid	1.80	1.92	2.07	2.28	2.62	3.20	4.02
m-Aminobenzoic acid	1.00	1.20	1.50	1.95	2.68	3.70	5.15
p-Aminobenzoic acid	2.45	2.60	2.86	3.25	3.80	4.61	6.01
o-Hydroxybenzoic acid	–*	–	–	–	–	–	–
m-Hydroxybenzoic acid	2.60	2.75	3.10	3.81	4.80	5.87	6.95
p-Hydroxybenzoic acid	2.67	2.80	3.13	3.72	4.39	5.22	6.15
o-Nitrobenzoic acid	–	–	–	–	–	–	–
m-Nitrobenzoic acid	3.14	4.16	5.58	7.70	11.03	15.39	19.95
p-Nitrobenzoic acid	3.55	4.75	6.40	9.02	13.04	18.00	23.66
o-Chlorobenzoic acid	5.70	7.55	–	–	–	–	–
m-Chlorobenzoic acid	3.03	3.58	4.46	5.98	8.50	11.93	15.36
p-Chlorobenzoic acid	2.81	3.30	4.19	5.57	7.78	10.57	13.52
o-Toluic acid	1.70	1.85	2.13	2.55	3.15	4.01	5.20
m-Toluic acid	2.12	2.29	2.55	3.04	3.82	5.02	6.56
p-Toluic acid	2.35	2.75	3.36	4.20	5.42	7.03	8.80
1-Naphthoic acid	2.20	2.70	3.42	4.38	5.83	8.17	11.70
2-Naphthoic acid	2.78	3.30	3.98	5.10	6.72	9.20	13.03

* Not eluted.

CD phase may be attributed to the chemical nature of the spacer, *i.e.*, three secondary amino groups in the spacer and unreacted N-propyldiethylenetriamine moieties on the silica surface behave as anion exchangers. In order to confirm the contribution of anion-exchange sorption of the carboxylic acids on the N3-CD phase, the k' values on non-β-CD-bonded phase (N3) were measured as a function of the pH of the mobile phase. The results are given in Table III, together with the pK_a values of the samples. Table III reveals that, in anion-exchange sorption, the k' values of the carboxylic acids increase with increase in the pH of the mobile phase, reflecting an increase in ionized species in the solution. This tendency is the same as that observed on the N3-CD phase, although the k' values obtained on the N3 phase for each compound, especially for *para* isomers, are lower at higher pH.

Independent of the pH of the mobile phase, the elution orders of the isomers of benzoic acids and of naphthoic acid were *para* < *meta* < *ortho* and 2- < 1-, respectively, which are in agreement with the decreasing order of their pK_a values.

With aminobenzoic acids, the elution order *meta* < *para* < *ortho* observed at lower pH also agrees with the decreasing order of their pK_a values, although it changed to *para* < *ortho* < *meta* at higher pH owing to their amphoteric nature.

Similar retention behaviour of these carboxylic acids was also observed on both N2 and N1 phases, in spite of the differences in the number of amino groups and the chain lengths of the bonded moieties. These findings strongly suggest that

TABLE III

EFFECT OF pH ON CAPACITY FACTORS OF AROMATIC CARBOXYLIC ACIDS ON TRIAMINE PHASE

Column, 20 cm × 4 mm I.D.; mobile phase, 0.002 *M* phosphate buffer–methanol (1:1); flow-rate, 0.7 ml/min.

Substance	*pH*							*pK$_a$ (25°C)*
	3.0	*3.2*	*3.4*	*3.6*	*3.8*	*4.0*	*4.4*	
o-Aminobenzoic acid	1.85	1.90	2.05	2.30	2.57	3.05	4.50	2.018, 4.946
m-Aminobenzoic acid	1.32	1.50	1.80	2.30	2.92	3.90	7.20	3.124, 4.744
p-Aminobenzoic acid	1.80	1.90	1.95	2.05	2.23	2.55	3.70	2.413, 4.853
o-Hydroxybenzoic acid	6.55	8.63	11.50	16.20	–*	–	–	2.996
m-Hydroxybenzoic acid	1.98	2.26	2.53	2.96	3.70	4.91	9.25	4.080
p-Hydroxybenzoic acid	1.70	1.84	2.01	2.20	2.55	3.10	5.02	4.582
o-Nitrobenzoic acid	14.72	18.92	–	–	–	–	–	2.170
m-Nitrobenzoic acid	2.71	3.90	5.22	7.11	9.76	12.84	19.58	3.450
p-Nitrobenzoic acid	2.70	3.89	5.20	7.10	9.51	12.37	19.11	3.442
o-Chlorobenzoic acid	4.81	6.23	8.37	11.70	15.97	20.93	–	2.943
m-Chlorobenzoic acid	2.27	2.61	3.00	3.83	5.10	7.18	13.26	3.824
p-Chlorobenzoic acid	2.01	2.20	2.62	3.30	4.15	5.52	10.34	3.986
o-Toluic acid	1.85	2.05	2.35	2.71	3.28	4.15	7.28	3.91
m-Toluic acid	1.85	2.00	2.20	2.47	2.90	3.60	6.20	4.27
p-Toluic acid	1.73	1.85	2.05	2.35	2.72	3.40	5.40	4.36
1-Naphthoic acid	2.30	2.75	3.39	4.24	5.79	8.00	15.25	3.695
2-Naphthoic acid	1.82	2.08	2.42	2.94	3.70	4.72	9.00	4.161

* Not eluted.

the contribution of the anion-exchange sorption mechanism is not negligible for the retention of aromatic carboxylic acids on the N3-CD. However, inclusion complex formation is still responsible for determining the retention of the carboxylic acids on β-CD-bonded phases; the k' values of *para* or *meta* isomers obtained on a β-CD-bonded phase are nearly 1.5–2.6 or 1.3–2.1 times larger than those obtained on the corresponding non-CD-bonded phase, independent of the pH of the mobile phase. Further, the elution orders of the isomers of toluic, aminobenzoic and naphthoic acids on the N3-CD phase are nearly invariably the reverse of those observed on the N3 phase, implying that the elution orders of these substances follow the increasing order of the stability of their inclusion complexes. The elution order of nitrobenzoic acid isomers is also changed from *para* < *meta* < *ortho* on the N3 phase to *meta* < *para* < *ortho* on the N3-CD phase. Although the latter is still different from the order *ortho* < *meta* < *para*, which is accepted as the increasing order of the stability of their β-CD inclusion complexes[15], this inconsistency can be explained by assuming that the *o*-nitrobenzoate ion interacts with amino groups in the spacer to nearly the same extent as with bonded β-CD molecules, without decreasing its retention value compared with *para* or *meta* isomers. The same rationalization may also be applied to the elution order of the isomers of hydroxy- and chlorobenzoic acids.

Hence it is very probable that substances that have a greater capability to form inclusion complexes are retained mainly by β-CD molecules, whereas the others are retained by both inclusion complex formation and anion-exchange sorption mechanisms.

TABLE IV

EFFECT OF pH ON CAPACITY FACTORS OF AROMATIC CARBOXYLIC ACIDS ON DI-AMINE-β-CD BONDED PHASE

Column, 20 cm × 4 mm I.D.; mobile phase, 0.002 *M* phosphate buffer–methanol (1:1); flow-rate, 0.7 ml/min.

Substance	*pH*						
	2.8	*3.0*	*3.2*	*3.4*	*3.6*	*3.8*	*4.0*
o-Aminobenzoic acid	1.60	1.88	2.21	2.60	3.04	3.53	4.08
m-Aminobenzoic acid	1.30	1.60	1.98	2.49	3.13	3.90	4.76
p-Aminobenzoic acid	2.15	2.36	2.64	2.96	3.38	3.86	4.44
o-Hydroxybenzoic acid	9.62	13.53	–*	–	–	–	–
m-Hydroxybenzoic acid	2.30	2.59	3.02	3.62	4.43	5.45	6.68
p-Hydroxybenzoic acid	2.12	2.25	2.50	2.88	3.37	4.00	4.74
o-Nitrobenzoic acid	11.80	13.75	16.45	20.20	–	–	–
m-Nitrobenzoic acid	3.01	3.55	5.08	7.70	10.98	14.75	18.98
p-Nitrobenzoic acid	3.10	3.82	5.44	8.18	11.55	15.40	19.80
o-Chlorobenzoic acid	4.00	5.35	9.10	13.60	18.01	–	–
m-Chlorobenzoic acid	2.50	3.03	4.02	5.50	7.50	9.87	12.48
p-Chlorobenzoic acid	2.30	2.65	3.35	4.43	5.85	7.50	9.30
o-Toluic acid	2.04	2.17	2.48	2.94	3.53	4.25	5.03
m-Toluic acid	2.05	2.26	2.66	3.16	3.79	4.59	5.46
p-Toluic acid	2.88	2.57	3.05	3.57	4.42	5.40	6.52
1-Naphthoic acid	2.50	2.80	3.45	4.50	5.90	7.82	10.40
2-Naphthoic acid	2.45	2.70	3.25	4.20	5.55	7.40	9.86

* Not eluted.

TABLE V

EFFECT OF pH ON CAPACITY FACTORS OF AROMATIC CARBOXYLIC ACIDS ON MONO-AMINE-β-CD BONDED PHASE

Column, 20 cm × 4 mm I.D.; mobile phase, 0.002 *M* phosphate buffer–methanol (1:1); flow-rate, 0.7 ml/min.

Substance	*pH*						
	2.8	*3.0*	*3.2*	*3.4*	*3.6*	*3.8*	*4.0*
o-Aminobenzoic acid	1.02	1.18	1.34	1.54	1.77	2.07	2.40
m-Aminobenzoic acid	0.90	1.03	1.22	1.58	2.09	2.64	3.19
p-Aminobenzoic acid	1.08	1.22	1.38	1.58	1.83	2.10	2.44
o-Hydroxybenzoic acid	7.85	11.64	–*	–	–	–	–
m-Hydroxybenzoic acid	1.20	1.44	1.80	2.28	2.88	3.67	4.58
p-Hydroxybenzoic acid	1.19	1.31	1.49	1.73	2.01	2.45	3.00
o-Nitrobenzoic acid	10.28	–	–	–	–	–	–
m-Nitrobenzoic acid	2.61	3.43	4.76	6.83	9.50	12.16	14.91
p-Nitrobenzoic acid	2.40	3.30	4.72	6.75	9.20	11.75	14.22
o-Chlorobenzoic acid	3.05	5.02	7.22	10.30	–	–	–
m-Chlorobenzoic acid	1.64	2.33	3.19	4.21	5.57	7.52	10.50
p-Chlorobenzoic acid	1.46	1.80	2.33	3.06	4.02	5.50	7.83
o-Toluic acid	1.21	1.38	1.62	1.93	2.34	2.90	3.69
m-Toluic acid	1.21	1.42	1.69	2.04	2.50	3.14	4.12
p-Toluic acid	1.22	1.48	1.81	2.20	2.69	3.34	4.40
1-Naphthoic acid	1.70	2.25	2.92	3.79	4.97	6.69	9.45
2-Naphthoic acid	1.58	2.00	2.56	3.30	4.31	5.75	8.00

* Not eluted.

Effect of chain length of spacer on retention and resolution

To elucidate the effect of the chain length of the spacer on retention by inclusion complex formation, k' values were measured as a function of the pH of the mobile phase using N2-CD and N1-CD phases. Comparison of the data in Tables IV and V with those in Table II shows that, in general, the longer the spacer, the larger are the k' values of each substance, although the amount of bonded β-CD molecules is, as listed in Table I, not necessarily larger on the phases having a longer spacer.

The ratio of the capacity factors of *para* to *meta* or of *para* to *ortho* isomers is a useful measure for evaluating the effect of spacer length in stationary phases containing different amounts of bonded β-CD moieties, because these values reflect the ease of separation based on inclusion complex formation. The values obtained at pH 3.8 and 3.0 are given in Table VI, together with the values obtained with non-CD-bonded phases. It is found that the above-defined values increase with increase in the spacer length of β-CD-bonded stationary phases, whereas these values on non-CD-bonded phases remain nearly the same, irrespective of the difference in the spacer length. This fact strongly suggests that on a stationary phase with a longer and more flexible spacer, the steric hindrance for complexation in the vicinity of the bulk silica surface would diminish and the effective interaction between the bonded CD molecule and the guest compound would increase. The validity of this explanation is also supported by Fig. 1, where the change in k' values caused by the introduction of CD molecules on to N1, N2 and N3 stationary phases is illustrated.

TABLE VI

EFFECT OF CHAIN LENGTH OF THE SPACER ON SELECTIVITY

Column, 20 cm × 4 mm I.D.; mobile phase, 0.002 *M* phosphate buffer–methanol (1:1), pH 3.8 (except values in parentheses, which are at pH 3.0); flow-rate, 0.7 ml/min.

Substance	*Ratio of k' values*	*β-CD bonded*			*Non-bonded*		
		Monoamine	*Diamine*	*Triamine*	*Monoamine*	*Diamine*	*Triamine*
Aminobenzoic acid	*p-/m-*	0.80 (1.18)	0.99 (1.48)	1.25 (2.17)	0.69	0.67	0.76
	p-/o-	1.01 (1.03)	1.09 (1.26)	1.44 (1.35)	0.87	0.80	0.87
Hydroxybenzoic acid	*p-/m-*	0.67 (0.91)	0.73 (0.87)	0.89 (1.02)	0.72	0.62	0.69
Nitrobenzoic acid	*p-/m-*	0.97 (0.96)	1.04 (1.08)	1.17 (1.14)	1.01	1.00	0.97
Chlorobenzoic acid	*p-/m-*	0.73 (0.89)	0.76 (0.87)	0.89 (0.92)	0.79	0.76	0.81
Toluic acid	*p-/m-*	1.06 (1.04)	1.18 (1.14)	1.40 (1.20)	0.93	0.88	0.94
	p-/o-	1.15 (1.07)	1.27 (1.18)	1.75 (1.49)	0.80	0.78	0.83
Naphthoic acid	2-/1-	0.86 (0.89)	0.95 (0.96)	1.13 (1.22)	0.63	0.63	0.64

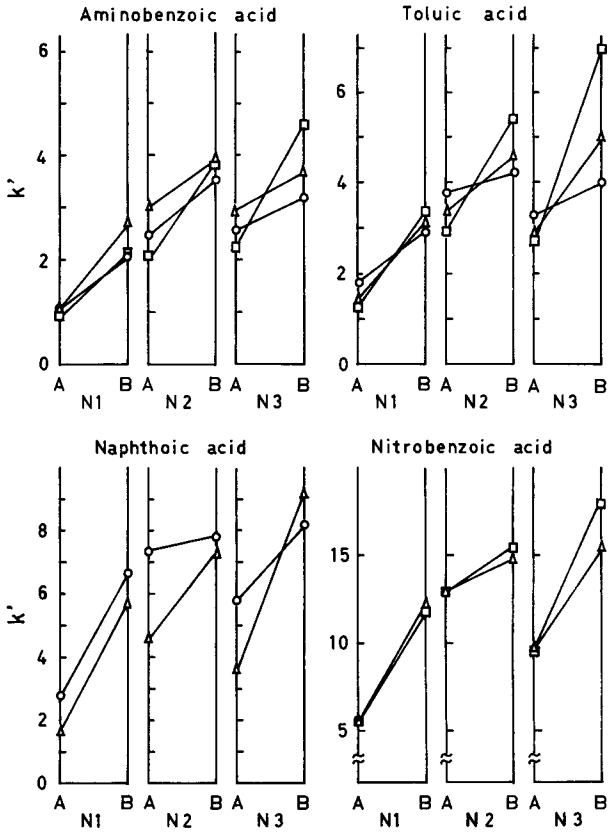


Fig. 1. Retention patterns on (B) β-CD-bonded and (A) non-bonded N1, N2 and N3 phases. For benzoic acids: ○, *o*-isomer; △, *m*-isomer; □, *p*-isomer. For naphthoic acids: ○, 1-naphthoic acid; △, 2-naphthoic acid. Columns, 20 cm × 4 mm I.D.; mobile phase, 0.002 *M* phosphate buffer–methanol (1:1), pH 3.8.

Separation of isomers

The chromatographic separation of the isomers of some aromatic carboxylic acids using the N3-CD bonded phase was attempted. Figs. 2 and 3 illustrate chromatograms of toluic and aminobenzoic acid isomers. As was expected, nearly baseline separations of three isomers were achieved in both series; 1- and 2-naphthoic acids could also be separated under the same conditions as in Fig. 2, but separations of hydroxy- and chlorobenzoic acid isomers were unsuccessful. It should be noted, however, that the isomers of hydroxy- and chlorobenzoic acids can be separated successfully by the anion-exchange mechanism on an N3 column, although the anion-exchange separation of aminobenzoic or toluic acids failed. In view of the fact that the separations of the isomers achieved by inclusion complex formation on the N3-CD column are unsuccessful by the anion-exchange mechanism on the N3 column, and *vice versa*, the separations obtained on N2-CD and N1-CD columns are considered to be partly based on an anion-exchange mechanism. More effective separations based only on inclusion complex formation have been achieved, as shown in Fig. 4, by using a carbamate-type β-CD-bonded stationary phase. An application of this stationary phase to the separation of some enantiomers will be reported elsewhere[20].

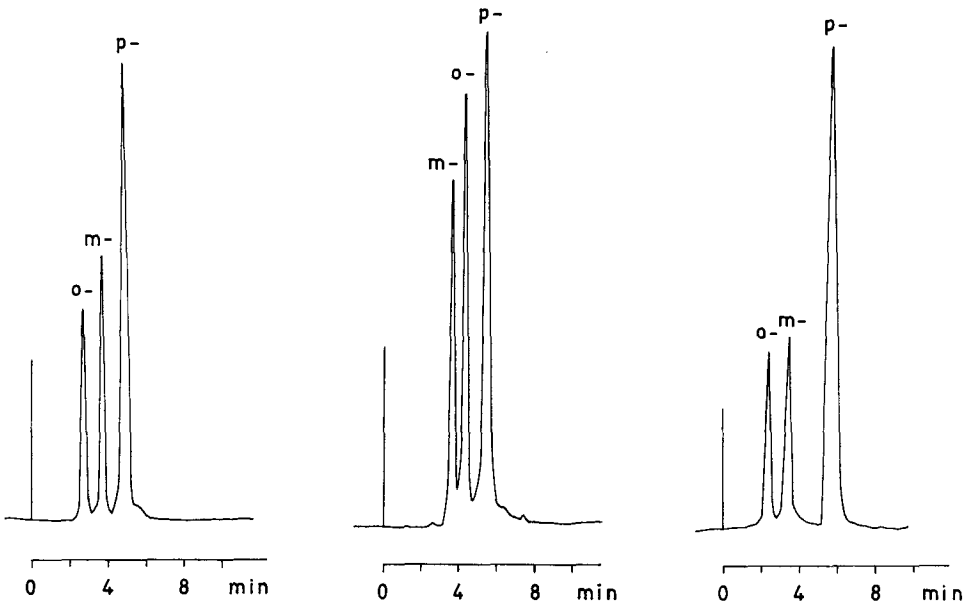


Fig. 2. Separation of toluic acid isomers. Column, N3-CD, 20 cm × 4 mm I.D.; mobile phase, 0.002 *M* phosphate buffer–methanol (1:1), pH 3.0; flow-rate, 0.7 ml/min.

Fig. 3. Separation of aminobenzoic acid isomers. Chromatographic conditions as in Fig. 2.

Fig. 4. Separation of chlorobenzoic acid isomers. Column, carbamate-type β-CD-bonded phase, 20 cm × 4 mm I.D.; mobile phase, 40% aqueous methanol; flow-rate, 0.7 ml/min.

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