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CARBAMOYLATED CYCLODEXTRIN-BONDED STATIONARY PHASES FOR LIQUID CHROMATOGRAPHY

MINORU TANAKA*, HIROKAZU IKEDA and TOSHIYUKI SHONO
Department of Applied Chemistry, Faculty of Engineering, Osaka University, Yamada-oka, Suita, Osaka 565 (Japan)
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SUMMARY

Ethylenediamine-monosubstituted α- and β-cyclodextrins were coupled to succinamidopropyl silica. These unmodified cyclodextrin stationary phases were treated with methyl, ethyl or phenyl isocyanate in pyridine. The cyclodextrin stationary phases exhibited remarkable selectivity changes in solute retention due to the carbamoylation. In particular, there was a great decrease in the retention of aminobenzoic and nitrobenzoic acids, and a remarkable improvement in the separation of the *ortho*, *meta* and *para* isomers of toluidine or dinitrobenzene. It is reasonable that the unreacted carboxyl groups on the unmodified cyclodextrin stationary phases react with the isocyanates as do the cyclodextrin moieties. Evidence in support of this is provided by a model reaction of 4-phenyl-*n*-butyric acid with methyl isocyanate.

INTRODUCTION

Chemical modification of cyclodextrins (CDs) with various functional groups has extensively been investigated in an attempt to improve the complexing and catalytic abilities of CDs. Various functional groups have been introduced on to their rims[1]. It is well known that such chemical modification of CDs brings about changes in the shape and size of their cavities, in hydrogen-bonding ability and in other physical properties.

In previous papers[2–5], we reported the specific selectivity in liquid chromatographic separations of some aromatic compounds on unmodified, acylated and methylated CD stationary phases. Both acylation and methylation resulted in remarkable changes in solute retention, as expected. Recently, the phenylcarbamoylation of saccharides, *i.e.*, complex formation of phenylcarbamoylated β-CD with organic salts in organic solvents[6], and chiral discrimination of phenylcarbamoylated, linear polysaccharides[7] have been reported. Therefore, the retention behaviour of carbamoylated CD stationary phases is of greast interest.

In this work, unmodified CD stationary phases, obtained by reaction of ethylenediamine-monosubstituted CDs with succinamidopropyl silica (Su-silica), were chemically modified with methyl, ethyl or phenyl isocyanate in pyridine. The reten-

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tion behaviour of some aromatic compounds on these stationary phases was studied and compared with that on the unmodified CD stationary phases.

EXPERIMENTAL

Materials and chromatography

Silica gel (Super micro bead B-5, mean particle diameter 5.9 μm and specific surface area 411 m^2/g) and α- and β-CDs were gifts from Fuji-Davison (Kasugai, Japan) and Wako (Osaka, Japan), respectively. All other reagents of analytical reagent grade were obtained from Wako or Tokyo Kasei (Tokyo, Japan).

All chromatographic studies were carried out with an LC-6A pump (Shimadzu, Kyoto, Japan), a special damper (Gasukuro Kogyo, Tokyo, Japan), a KHP-UI-130A injector (Kyowa Seimitsu, Tokyo, Japan) and a JASCO 875 variable-wavelength UV detector (Japan Spectroscopic, Tokyo, Japan).

Each stationary phase was packed into a stainless-steel column (10 cm × 0.4 cm I.D.) by a balanced density slurry method. The flow-rate of the eluent (methanol–water or –phosphate buffer) was 1.0 ml/min. The concentration of the sample solutes was 0.2 m*M*, and a volume of less than 20 μl was injected.

Preparation of CD stationary phases

Ethylenediamine-monosubstituted α- or β-CD (α-en or β-en) was coupled to Su-silica; the stationary phase obtained is denoted by α-en-Su-silica or β-en-Su-silica, respectively[2]:

$$\begin{array}{l} \quad | \\ -\mathrm{Si(CH_2)_3NHCO(CH_2)_2CONH(CH_2)_2NHCD} \\ \quad | \end{array} \quad (\mathrm{CD} = \alpha\text{- or } \beta\text{-CD})$$

The amounts of α-en and β-en immobilized were evaluated by elemental analysis: 42.2 μmol/g for α-en-Su-silica and 40.8 μmol/g for β-en-Su-silica.

After endcapping of the silanols with 1,1,1,3,3,3-hexamethyldisilazane in hexane, α-en- or β-en-Su-silica (2.5 g) was suspended in dry pyridine (80 ml), and then methyl, ethyl or phenyl isocyanate (2.5 g) was added. After stirring for 48 h at 60°C (for methyl or ethyl isocyanate) or for 10 h at 70°C (for phenyl isocyanate), the carbamoylated CD stationary phase was filtered off, thoroughly washed successively with hexane and methanol and dried *in vacuo*. The methyl-, ethyl- or phenylcarbamoylated CD stationary phases are denoted by prefixing the names of the parent stationary phases by MeC-, EtC- or PhC-, respectively.

RESULTS AND DISCUSSION

Effect of methanol content of the eluent on retention

The retention of eight disubstituted benzene isomers having various functional groups (cresol, iodoaniline, toluidine, nitroaniline, nitrophenol, dinitrobenzene, aminobenzoic acid or nitrobenzoic acid) was measured on all the six carbamoylated CD stationary phases by changing the methanol–water ratio in the eluent from 10:90 to 40:60. A decrease in retention with increasing methanol content was found, except

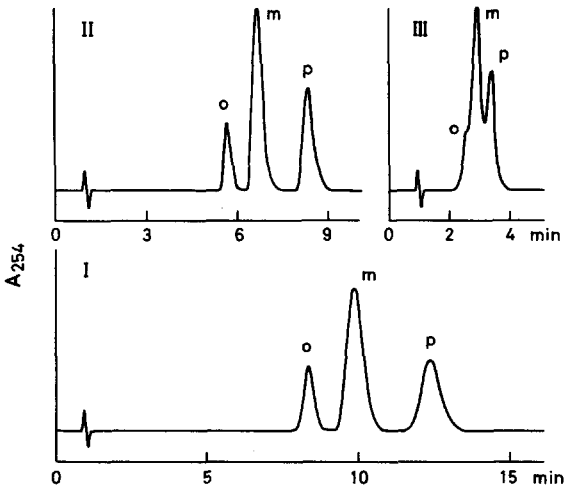

Fig. 1. Liquid chromatograms of cresol isomers on MeC-β-en-Su-silica. Eluents: methanol–water (10:90) (I), (20:80) (II) and (40:60) (III). o, m and p denote *ortho, meta* and *para* isomers, respectively.

for a few cases of aminobenzoic and nitrobenzoic acids. Considering both the separation of the *ortho, meta* and *para* isomers and the total analysis times, the optimum ratio of methanol:water is 20:80. Fig. 1 shows typical liquid chromatograms of a mixture of *o*-, m- and *p*-cresol on MeC-β-en-Su-silica. The complete baseline separation of the three isomers was achieved at the ratio of 10:90 and 20:80. The retention of the solutes tested in this study on the ethylcarbamoylated CD stationary phases was quite similar to that on the corresponding methylcarbamoylated derivatives. Therefore, the methyl- and phenylcarbamoylated CD stationary phases are described in the subsequent discussion.

Effects of eluent pH and ionic strength on retention

Methanol–1/15 *M* phosphate buffer (20:80) at pH 4.5, 5.6 or 6.7 was used as the eluent in order to investigate the effect of pH on the retention. Fig. 2 shows the results obtained on PhC-β-en-Su-silica. Each solute exhibited similar retention behaviour on the other carbamoylated CD stationary phases. The retention of cresol, iodoaniline, nitroaniline and dinitrobenzene was only slightly affected, whereas that of toluidine, nitrophenol and aminobenzoic and nitrobenzoic acids was affected significantly. The retention of *o*- and *p*-nitrophenol and the benzoic acids decreased, while that of toluidine increased, with increasing pH. This is reasonably explained by the well known fact that the inclusion of charged groups into the CD cavity is not favoured. As the pH of the eluent increases, the amounts of the anionic forms of *o*- and *p*-nitrophenol and aminobenzoic and nitrobenzoic acids increase significantly. On the other hand, *m*-nitrophenol has a larger pK_a value, and the amount of its anionic form does not increase significantly by changing the pH from 4.5 to 6.7 (only about 2.4% increase). The amounts of the cationic forms of the toluidine isomers decrease as the pH is changed from 4.5 to 6.7.

On the unmodified CD stationary phases[2,5] the retention of nitrophenol increased with increasing eluent pH. This is the opposite of the above-mentioned re-

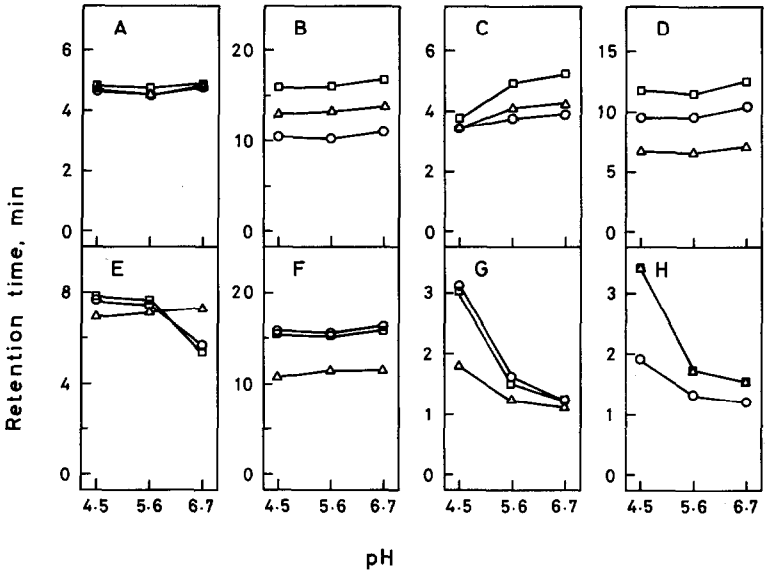

Fig. 2. Effect of eluent pH on the retention times of disubstituted benzene isomers, *ortho* (○), *meta* (△) and *para* (□), on PhC-β-en-Su-silica in methanol–1/15 *M* phosphate buffer (20:80). Solutes: (A) cresol; (B) iodoaniline; (C) toluidine; (D) nitroaniline; (E) nitrophenol; (F) dinitrobenzene; (G) aminobenzoic acid; (H) nitrobenzoic acid.

tention of nitrophenol on the carbamoylated CD stationary phases. Eftink and Harrison[8] reported the preferential binding of the anionic form of *p*-nitrophenol to unmodified CDs, compared with the neutral form of *p*-nitrophenol. Therefore, the retention behaviour of nitrophenol on the unmodified CD stationary phases is not

TABLE I

EFFECT OF IONIC STRENGTH OF THE ELUENT ON RETENTION TIMES (min) ON MeC-β-en-Su-silica

Eluents: methanol–phosphate buffer or sodium chloride solution (20:80) for toluidine and nitrophenol and (0:100) for aminobenzoic acid.

Solute		*Phosphate buffer*			*Sodium chloride solution*		
		1/45 M	*1/30 M*	*1/15 M*	*1/45 M*	*1/30 M*	*1/15 M*
Toluidine	*o-*	3.40	3.50	3.50	3.65	3.60	3.50
	m-	3.80	3.85	3.90	4.20	4.05	3.95
	p-	4.90	4.95	5.00	5.50	5.20	4.80
Nitrophenol	*o-*	5.90	6.05	6.20	7.90	7.90	7.95
	m-	8.70	9.10	9.10	9.50	9.60	9.65
	p-	9.50	10.20	10.55	13.00	13.25	13.40
Aminobenzoic acid	*o-*	1.50	1.65	1.80	5.70	5.10	4.80
	m-	1.15	1.25	1.35	2.35	2.20	2.10
	p-	1.50	1.55	1.65	8.40	7.20	6.70

unreasonable. These opposite retention behaviours of nitrophenol before and after the carbamoylation may be ascribed to differences in its orientation in the cavities of unmodified and carbamoylated CDs, and is of great interest.

It is important to test the effect of the ionic strength of the phosphate buffer on retention, because the ionic strength increases with increasing pH. Over the concentration range 1/45–1/15 *M* at a constant pH of 6.5, the retention times were measured for toluidine, nitrophenol and aminobenzoic and nitrobenzoic acids. Table I gives the results for the first three solutes on MeC-β-en-Su-silica eluted with the phosphate buffer at pH 6.5 together with those with the sodium chloride solution. We had expected their retention to increase with decreasing ionic strength of the phosphate buffer in the same manner as the retention on the unmodified CD stationary phases. In contrast, the solute retention increased with increasing ionic strength. This seems to suggest a salting-out and/or promotive ion-pairing effect. If this inference is reasonable, a similar increase in the retention is expected for an eluent containing sodium chloride. With the latter, the retention of nitrophenol increased similarly, but those of toluidine and aminobenzoic acid (as well as nitrobenzoic acid) decreased. Therefore, the retention behaviours of the solutes listed in Table I cannot be explained by only a salting-out and/or ion-pairing effect. Further work is needed to explain the results.

Comparison of retention before and after carbamoylation

The comparison of the retention behaviours of the solutes on the CD stationary phases before and after carbamoylation is of great interest, because selectivity changes in the solute retention are expected. The retention times of eight disubstituted

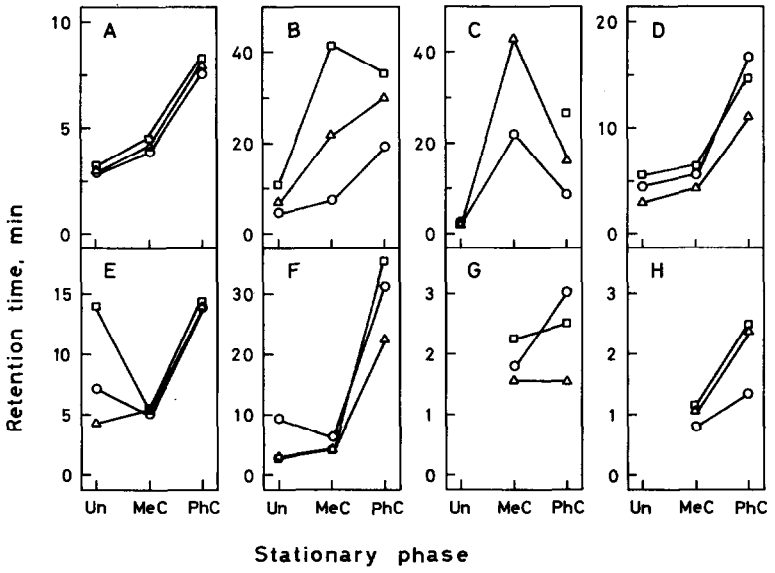

Fig. 3. Retention times of disubstituted benzene isomers on α-en-Su-silica (Un), MeC-α-en-Su-silica (MeC) and PhC-α-en-Su-silica (PhC) in methanol–water (20:80). Solutes as in Fig. 2. *p*-Toluidine and aminobenzoic and nitrobenzoic acids were not eluted from MeC and Un, respectively, within 60 min.

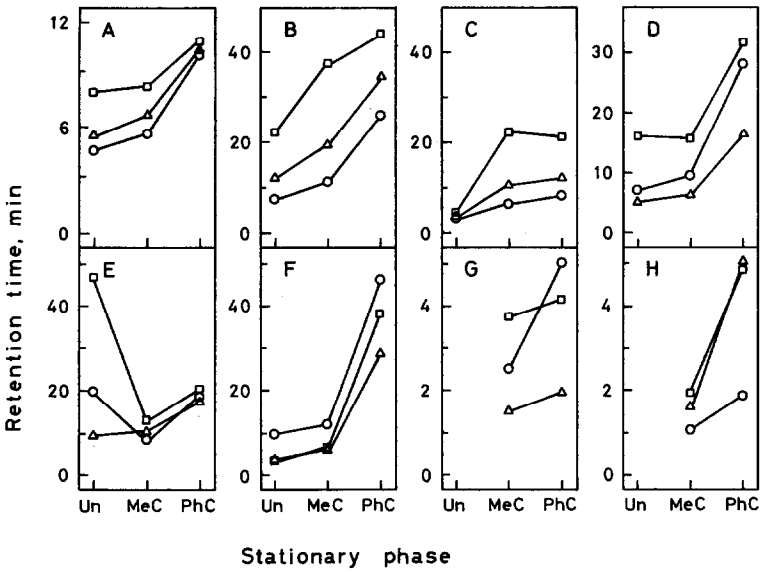

Fig. 4. Retention times of disubstituted benzene isomers on β-en-Su-silica (Un), MeC-β-en-Su-silica (MeC) and PhC-β-en-Su-silica (PhC). Other details as in Fig. 3. Aminobenzoic and nitrobenzoic acids were not eluted from Un within 60 min.

benzene isomers, both on the α- and β-CD stationary phases, are shown in Figs. 3 and 4, respectively. The carbamoylation of the unmodified CD stationary phases brought about an increase in the solute retention except for *o*- and *p*-nitrophenol and aminobenzoic and nitrobenzoic acids. The benzoic acids, which could not be eluted from the unmodified CD stationary phases, exhibited a remarkable decrease in retention after the carbamoylation. This selectivity change is reasonably interpreted as follows. These benzoic acid isomers are almost predominantly in their anionic forms at pH near 7. The negatively charged solutes strongly interact with the secondary amino group in the spacer arm linking the CD units to silica gel[9]. In the treatment of the unmodified CD stationary phases with the isocyanates, it is possible to carbamoylate both the hydroxyl groups in the CD units and the secondary amino group in the spacer arm. By carbamoylation of the amino group, the strong, ionic interaction mentioned above is removed or greatly reduced.

A complete separation of the *ortho, meta* and *para* isomers of toluidine could be obtained after methyl- or phenylcarbamoylation, even when methanol–water (40:60) was used as the eluent. However, the three isomers could not be separated on α-en- or β-en-Su-silica. Fig. 5 exemplifies the improvement in the separation on MeC- or PhC-β-en-Su-silica. The same is also true for the α-CD stationary phases.

On the whole, the retention of the solutes was stronger on the phenylcarbamoylated CD stationary phases than on the methylcarbamoylated ones, with the exception of the toluidine isomers as in Fig. 3. Especially, the three isomers of dinitrobenzene were retained more strongly and could be completely separated only on the phenylcarbamoylated CD stationary phases. These results suggest the importance of the attractive interaction between the solutes and the phenyl moieties in the stationary phases.

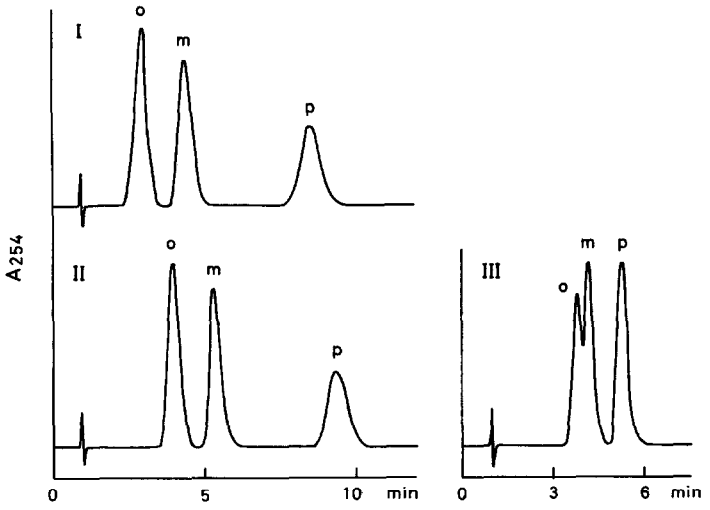

Fig. 5. Liquid chromatograms of toluidine isomers on MeC-β-en-Su-silica in methanol–water (40:60) (I), on PhC-β-en-Su-silica in methanol–water (40:60) (II) and on β-en-Su-silica in methanol–water (10:90) (III).

Table II shows the remarkable change in selectivity after methyl- or phenylcarbamoylation. A difference in the retention order of the antiepileptic drugs on the unmodified and carbamoylated CD stationary phases is apparent. The drugs could be separated completely on the phenylcarbamoylated CD stationary phases.

Generally speaking, it is found from a comparison of Figs. 3 and 4 that the carbamoylation of the β-CD units results in better resolution of the solutes than does the carbamoylation of the α-CD units.

Effect of unreacted terminal groups on retention

The amount of carboxyl groups on Su-silica is about 1 mmol/g, and the proportion of the carboxyl groups coupled to ethylenediamine-monosubstituted CD is only about 1/20. The unreacted terminal groups, therefore, may affect the retention

TABLE II

RETENTION TIMES (min) OF ANTIEPILEPTIC DRUGS ON CD STATIONARY PHASES

Eluent: methanol–water (20:80).

Stationary phase	*Primidone*	*Phenobarbital*	*Carbamazepine*	*Phenytoin*
α-en-Su-silica	2.15	3.25	8.15	7.50
MeC-α-en-Su-silica	2.70	4.20	14.40	14.50
PhC-α-en-Su-silica*	3.10	4.80	9.65	11.80
β-en-Su-silica	12.25	19.10	13.25	19.20
MeC-β-en-Su-silica	9.60	10.00	18.60	23.60
PhC-β-en-Su-silica*	3.55	5.10	10.95	12.65

* Eluted with methanol–water (40:60).

TABLE III

RETENTION TIMES (min) OF DISUBSTITUTED BENZENE ISOMERS ON Su-silica, MeC-Su-silica AND PhC-Su-silica

Eluent: methanol–water (20:80).

Solute	*Su-silica**			*MeC-Su-silica*			*PhC-Su-silica*		
	o-	*m-*	*p-*	*o-*	*m-*	*p-*	*o-*	*m-*	*p-*
Cresol	1.80	1.90	1.90	4.65	5.00	5.10	5.15	5.40	5.70
Iodoaniline	2.45	2.55	2.55	7.55	8.50	8.55	10.35	15.30	18.00
Toluidine	1.90	2.20	2.80	5.00	7.85	13.05	20.75	40.50	–**
Nitroaniline	2.30	1.80	2.05	6.70	4.05	4.90	8.15	5.50	5.90
Nitrophenol	1.95	1.95	2.00	6.30	5.65	5.25	7.60	6.40	5.95
Dinitrobenzene	2.40	1.75	1.65	7.95	4.65	4.30	11.45	7.80	7.15
Aminobenzoic acid	1.10	1.45	1.50	2.00	1.15	1.60	2.10	1.40	1.80
Nitrobenzoic acid	1.60	1.70	1.50	0.85	1.10	1.00	0.80	1.05	1.00

* Data from ref. 9.

** Not eluted within 60 min.

of solutes. The retention times of the eight solutes were measured on the stationary phases MeC- and PhC-Su-silica, prepared by reaction of Su-silica with methyl isocyanates in pyridine. Table III gives the results together with the retention times on Su-silica. The solutes were eluted rapidly and did not interact significantly with the unreacted carboxyl groups on Su-silica. Also, the solutes other than aminobenzoic and nitrobenzoic acids were retained more strongly on MeC-Su-silica and much more on PhC-Su-silica than on Su-silica. On an octadecylsilyl stationary phase the retention of the solutes was similar to that on MeC- or PhC-Su-silica: the benzoic acids were eluted quite rapidly, while the other solutes were retained much more strongly. This fact suggests a considerable change in the surface after reacting Su-silica with the isocyanates (increase in hydrophobicity).

In order to explain this change, 4-phenyl-*n*-butyric acid (1 g) was treated with methyl isocyanate (4.5 g) in pyridine (50 ml) for 48 h at room temperature. The compound isolated gave a molecular ion peak at $m/e = 177$ in its mass spectrum corresponding to a decarboxylation product from the two reactants, presumed to be N-methyl-4-phenyl-*n*-butyramide. This model reaction suggests that the carboxyl groups on Su-silica are replaced with N-methylamido groups after the treatment. Consequently, a similar replacement is possible for the unreacted carboxyl groups on the unmodified CD stationary phases after the carbamoylation. It seems most likely that this replacement partially contributed to the above-mentioned selectivity changes in the solute retention before and after the carbamoylation of the unmodified CD stationary phases. Needless to say, there is a positive contribution of the CD moieties to the retention, considering the differences in the elution orders of the solutes between the CD stationary phases and those without CD units (see Figs. 3 and 4 and Table III).

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