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UNMODIFIED AND ACYLATED CYCLODEXTRIN STATIONARY PHASES FOR LIQUID CHROMATOGRAPHIC SEPARATION OF AROMATIC COMPOUNDS

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SUMMARY

α- or β-cyclodextrin was immobilized on 3-μm silica particles. The resulting stationary phases are much more efficient than the corresponding stationary phases obtained from 10-μm silica particles in the separation of aromatic compounds. The former stationary phases can separate the *o*-, *m*- and *p*-isomers of toluidine or dinitrobenzene, whereas the latter cannot. These unmodified cyclodextrin stationary phases were acetylated, propionylated or benzoylated, and the retention behaviour of some aromatic compounds was studied. Acylation of the β-cyclodextrin stationary phase in particular improves the peak shapes and effects a selectivity change in the separation.

INTRODUCTION

Cyclodextrins and their chemically modified derivatives have been the subject of numerous investigations and been used for various purposes. It is well known that the chemical modification brings about changes in the depth of the cyclodextrin cavity, in the hydrogen-bonding ability and in various other physical properties. A review has been published on chemically modified cyclodextrins[1].

We recently prepared unmodified and acetylated cyclodextrin stationary phases and reported the specific separations of some aromatic compounds by liquid chromatography[2,3]. In these instances using 10-μm irregularly shaped silica particles, *o*- and *m*-toluidine and *m*- and *p*-dinitrobenzene could not be separated on either the α- or β-cyclodextrin stationary phase. The peak resolutions, however, increased as the amount of cyclodextrin immobilized on silica gel increased. The maximum

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amounts of α- and β-cyclodextrin immobilized were 31.5 and 49.2 μmol/g, respectively.

In order to increase the cyclodextrin capacities further, 3-μm spherical silica particles were used instead of the 10-μm particles mentioned above. It was found that the 3-μm cyclodextrin columns brought about a large improvement in the separation. In this work, α- or β-cyclodextrin was immobilized on 3-μm spherical silica particles and the resulting cyclodextrin stationary phases were acetylated with acetic anhydride. Further, the propionylated or benzoylated cyclodextrin stationary phases were prepared, and retention behaviour on these stationary phases was studied systematically for several aromatic compounds, especially disubstituted benzene derivatives.

EXPERIMENTAL

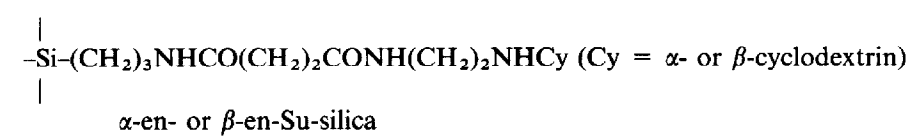
Materials and chromatographic conditions

Silica gel (Develosil 60-3, particle size 3 μm) was purchased from Nomura Kagaku (Nagoya, Japan); all other reagents were as described previously[2].

A Waters ALC/GPC 244 liquid chromatograph equipped with a U6K injector and a 440 absorbance detector (254 nm) was used. Each stationary phase was packed into a stainless-steel column (10 × 0.4 cm I.D.). The flow-rate of the eluent (water, 0.15 *M* phosphate buffer, methanol–water or methanol–0.15 *M* phosphate buffer) was 0.7 ml/min. The concentration of sample solutes was 1.0 m*M* and a volume of 4 μl was injected.

Preparation of cyclodextrin stationary phases

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



α-en- or β-en-Su-silica

The α-en- or β-en-Su-silica (1.7 g) was suspended in dry pyridine (30 ml). Acetic (18 ml), propionic (18 ml) or benzoic anhydride (10 g) was added to this suspension, kept at 45°C. After 6 h the acylated cyclodextrin phase was filtered off, thoroughly washed successively with methanol, water and methanol and dried *in vacuo* at 80°C for 12 h. The acetylated, propionylated or benzoylated cyclodextrin stationary phases are denoted by adding Ac-, Pr- or Bz-, respectively, to the front of the names for the unmodified stationary phases.

RESULTS AND DISCUSSION

Cyclodextrin content in the stationary phases

Table I gives the cyclodextrin capacities of the unmodified cyclodextrin stationary phases. The amount of β-cyclodextrin coupled to the 10-μm irregular or

TABLE I

CYCLODEXTRIN CONTENTS IN UNMODIFIED CYCLODEXTRIN STATIONARY PHASES

Stationary phase	*Cyclodextrin content (μmol/g)**
α-en-Su-silica (3 μm, spherical)	92.5
β-en-Su-silica (3 μm, spherical)	104.6
β-en-Su-silica (10 μm, spherical)	47.9
β-en-Su-silica (10 μm, irregular)	58.3

* Determined spectrophotometrically[2].

spherical silica particles was at most about 60 μmol/g. However, the cyclodextrin capacity was approximately doubled on using 3-μm silica particles. The maximum capacity of α-cyclodextrin was 31.5 μmol/g for the 10-μm irregular silica particles. About a 3-fold amount of α-cyclodextrin can be introduced on the 3-μm spherical silica particles. This increase in the capacities is expected to strengthen the specificity of the liquid chromatographic separations on cyclodextrin stationary phases.

Unmodified cyclodextrin stationary phases

The capacity factors of six disubstituted benzene derivatives on the 3-μm columns of α-en- and β-en-Su-silica were measured by changing the methanol:water ratio in the eluent from 0:100 to 40:60. Fig. 1 shows the results obtained on α-en-Su-silica. A decrease in retention with increasing methanol content is found except

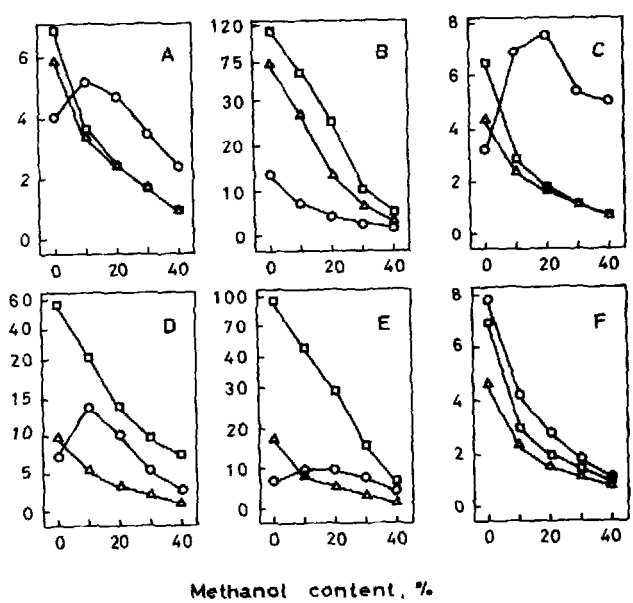


Fig. 1. Effect of methanol content in the eluent on capacity factors of disubstituted benzene isomers (○, *o*-; △, *m*-; □, *p*-) on 3-μm α-en-Su-silica. Solutes: A = cresol; B = iodoaniline; C = toluidine; D = nitroaniline; E = nitrophenol; F = dinitrobenzene.

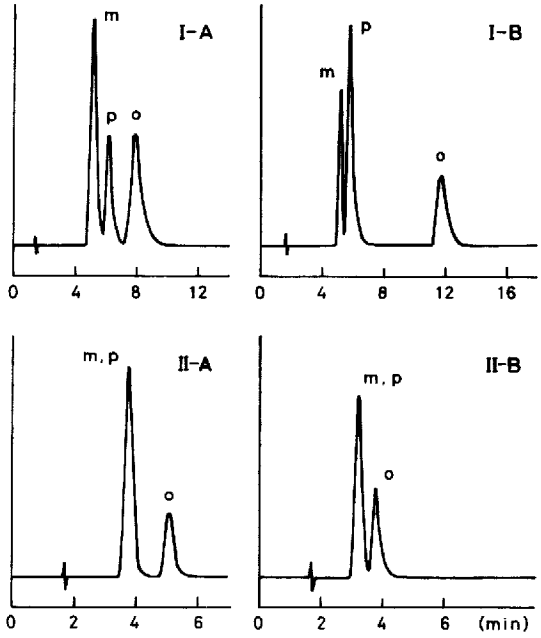


Fig. 2. Liquid chromatograms of (A) dinitrobenzene isomers and (B) toluidine isomers on (I) 3-μm and (II) 10-μm α-en-Su-silica in methanol–water (10:90).

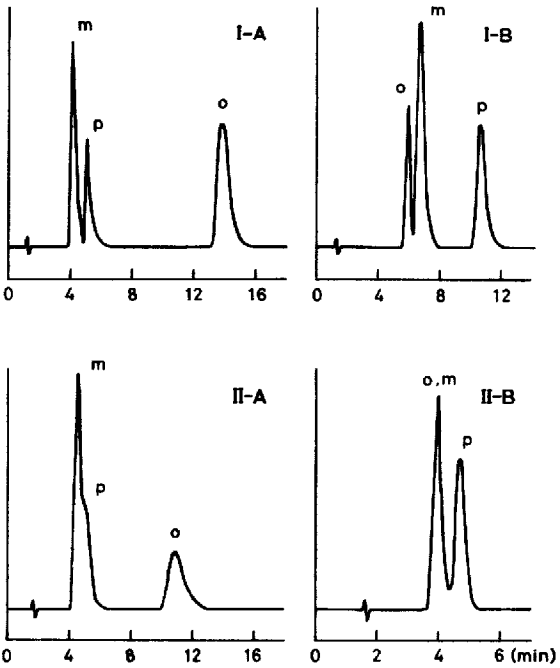


Fig. 3. Liquid chromatograms of (A) dinitrobenzene isomers and (B) toluidine isomers on (I) 3-μm and (II) 10-μm β-en-Su-silica in methanol–water (20:80).

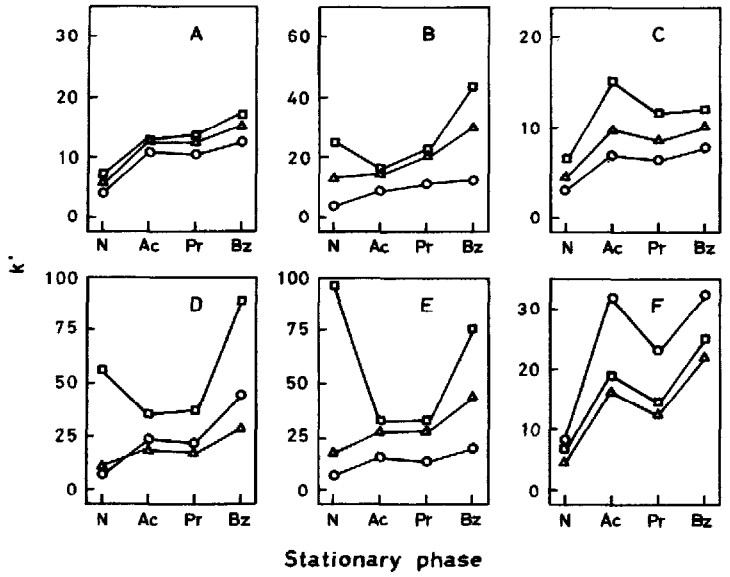


Fig. 4. Capacity factors of disubstituted benzene isomers (○, *o*-; △, *m*-; □, *p*-) on α-cyclodextrin stationary phases. Eluent: water except for B [methanol–water (20:80)]. Stationary phases: N = unmodified; Ac = acetylated; Pr = propionylated; Bz = benzoylated. Solutes as in Fig. 1.

for the *o*-isomers of cresol, toluidine, nitroaniline and nitrophenol, which exhibit abnormal retention behaviour. A convincing explanation of this abnormality has not been given. The interaction of methanol with the α-cyclodextrin units supposedly plays an important role. Further work is needed and is now in progress. On β-en-

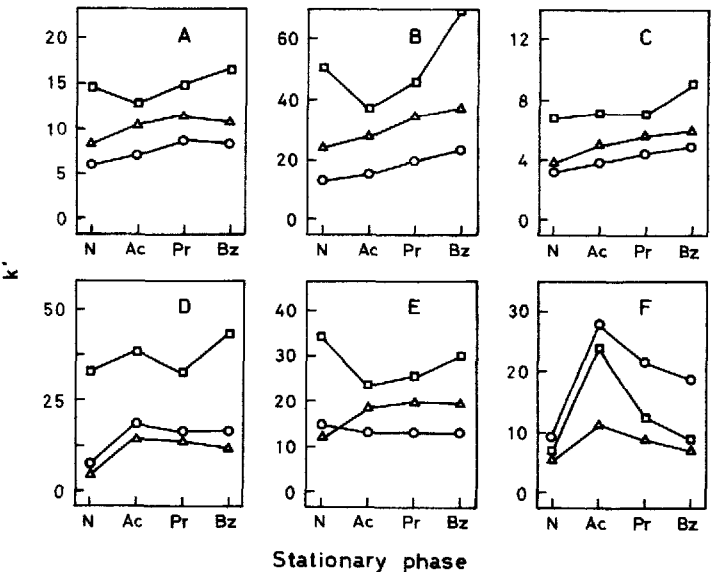


Fig. 5. Capacity factors of disubstituted benzene isomers on β-cyclodextrin stationary phases in methanol–water (20:80). Others as in Fig. 4.

Su-silica, such an abnormality was not observed and the retentions of all the solutes decreased as the methanol content increased.

The three isomers of toluidine or dinitrobenzene cannot be separated on the 10-μm columns of either α-en- or β-en-Su-silica (15 cm long). On the other hand, they can be separated on the two 3-μm columns (10 cm long), as shown in Figs. 2 and 3. The *m*- and *p*-isomers of cresol or toluidine cannot be separated on 3-μm α-en-Su-silica when eluents containing more than 20% of methanol are used (Fig. 1). On 3-μm β-en-Su-silica, separation of the three isomers can be achieved for each solute in the methanol–water eluents (0:100 to 40:60). It is found that the 3-μm columns are superior to the 10-μm columns for separating disubstituted benzene isomers.

Acylated cyclodextrin stationary phases

The retention behaviour of acylated cyclodextrin stationary phases is of great interest because selectivity changes in the solute retention are expected. Therefore, we acetylated, propionylated and benzoylated α- and β-cyclodextrins with the corresponding acid anhydride on the 3-μm silica. The retention behaviours of the solutes on the cyclodextrin stationary phases before and after acylation were compared. The results for the α-cyclodextrin stationary phases in water and the β-cyclodextrin stationary phases in methanol–water (20:80) are shown in Figs. 4 and 5, respectively. As shown in Fig. 1, the retention of the *o*-isomers of cresol, toluidine, nitroaniline and nitrophenol on α-en-Su-silica is increased by the addition of methanol to water as the eluent. Such a phenomenon was not observed on the acylated α- and β-cyclodextrin stationary phases, and the retention of each solute decreased with increasing methanol content in the eluent.

The elution order of *o*-, *m*- and *p*-nitroaniline was changed from *o*- < *m*- < *p*- on α-en-Su-silica to *m*- < *o*- < *p*- on Ac-, Pr- and Bz-α-en-Su-silica. A similar change in the elution order was observed for nitrophenol on the β-cyclodextrin sta-

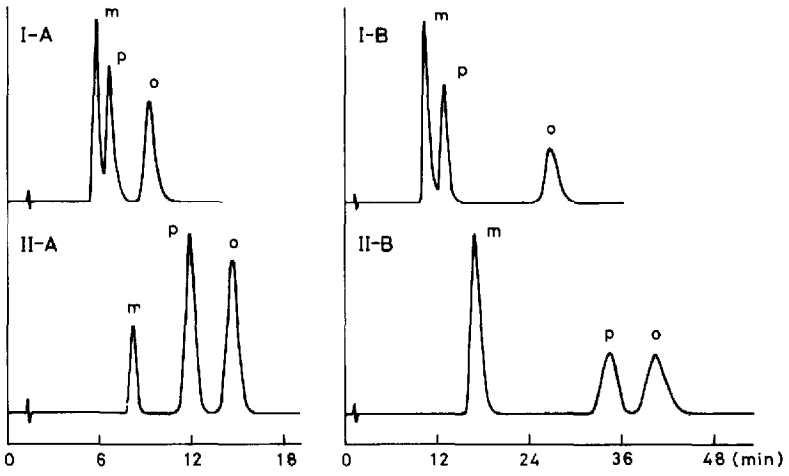


Fig. 6. Liquid chromatograms of dinitrobenzene isomers on (I) Bz-β-en-Su-silica and (II) Ac-β-en-Su-silica in methanol–water, (A) 40:60 and (B) 20:80.

tionary phases: *m*- < *o*- < *p*- on β-en-Su-silica to *o*- < *m*- < *p*- on acylated β-en-Su-silica. Most of the solutes are retained more strongly on the acylated (especially benzoylated) cyclodextrin stationary phases than on the unmodified stationary phases. *p*-Nitrophenol is a typical exception and is retained most strongly on the unmodified cyclodextrin stationary phases. This may imply that there is an interaction between the hydroxy groups of the cyclodextrin units and *p*-nitrophenol.

As can be seen from Fig. 4, acetylation or propionylation of α-en-Su-silica does not necessarily improve the separation of the isomers (*e.g.*, cresol and iodoaniline). On the other hand, acylation of β-en-Su-silica improves the resolution of the *o*- and *m*-isomers for toluidine, nitroaniline and nitrophenol and the *m*- and *p*-isomers for dinitrobenzene (Fig. 5). Moreover, most of the solutes are retained on the β-cyclodextrin more strongly than on the α-cyclodextrin stationary phases. These results enable us to use a short column and an eluent containing more methanol with the β-cyclodextrin stationary phases. This is advantageous because the higher the methanol content in the eluent, the sharper are the peaks. Fig. 6 shows typical liquid chromatograms of the dinitrobenzene isomers on both Bz- and Ac-β-en-Su-silica by using 40:60 or 20:80 methanol–water. On the latter column, a complete baseline separation of the three isomers can be obtained. The number of theoretical plates for the *o*-isomer (t_R = 14.50 min, Fig. 6, IIA) and the *m*-isomer (t_R = 16.83 min, Fig. 6, IIB) on Ac-β-en-Su-silica, for instance, are 2400 and 1500, respectively. The peak shapes on the acylated cyclodextrin stationary phases are more symmetrical than those on the unmodified phases.

Separation of other compounds

The isomers of aminobenzoic, chlorobenzoic, methylbenzoic and nitrobenzoic acid can be completely separated on β-en-Su-silica. In this instance, methanol–0.15

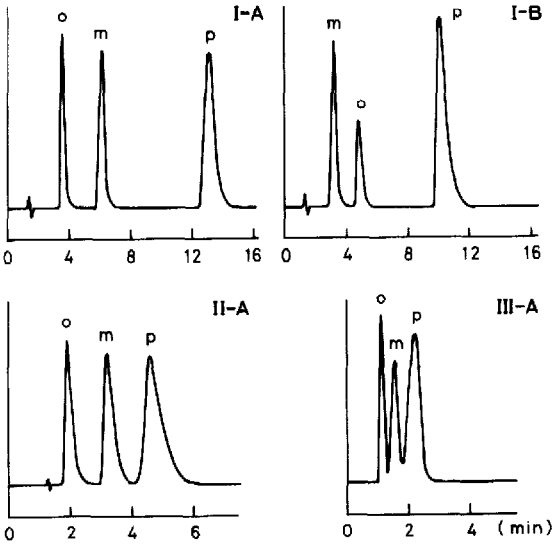


Fig. 7. Liquid chromatograms of (A) nitrobenzoic acid isomers and (B) aminobenzoic acid isomers (I) on β-en-Su-silica in methanol–phosphate buffer of pH 6.5 (20:80), (II) on Bz-β-en-Su-silica in methanol–water (20:80) and (III) on Ac-β-en-Su-silica in water.

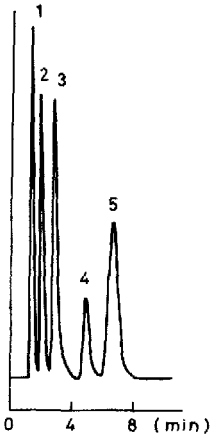


Fig. 8. Separation of amino acids on β-en-Su-silica in phosphate buffer of pH 6.8. Peaks: 1 = cystine; 2 = phenylglycine; 3 = β-(3,4-dihydroxyphenyl)alanine; 4 = phenylalanine; 5 = tyrosine.

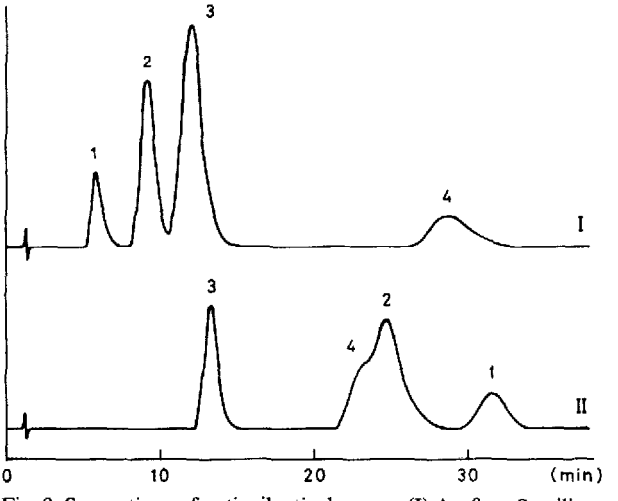


Fig. 9. Separations of antiepileptic drugs on (I) Ac-β-en-Su-silica and (II) β-en-Su-silica in methanol–water (20:80). Peaks: 1 = primidone; 2 = phenobarbital; 3 = carbamazepine; 4 = phenytoin.

M phosphate buffer of pH 6.5 (20:80) was used as the eluent instead of methanol–water, because the acids could not be eluted with the latter eluent. Fig. 7 shows the chromatograms of nitrobenzoic (IA) and aminobenzoic acid (IB) on β-en-Su-silica. The three isomers of chlorobenzoic and methylbenzoic acids were eluted in the order *o-* < *m-* < *p-* with retention times of 6.56, 13.92 and 26.08 min (chlorobenzoic acid) and 6.88, 14.40 and 27.14 min (methylbenzoic acid). The acid isomers on the modified β-cyclodextrin stationary phases can be eluted with water or methanol–water. This is a remarkable change in retention behaviour after acylation. Fig. 7 also shows typical liquid chromatograms of nitrobenzoic acid on Bz-β-en-Su-silica (IIA) and Ac-β-en-Su-silica (IIIA).

Phenylglycine, β-(3,4-dihydroxyphenyl)alanine, phenylalanine and tyrosine,

which have similar structures, can be separated within 8 min on 3-μm β-en-Su-silica (10 cm long), as shown in Fig. 8. On the other hand, it took about 18 min to separate these amino acids completely on 10-μm β-en-Su-silica (30 cm long)[2].

Fig. 9 shows a remarkable change in selectivity of the solute retention after acetylation: primidone is retained least on Ac-β-en-Su-silica and most on β-en-Su-silica. Phenobarbital and phenytoin could not be separated on the latter unmodified stationary phase.

It has been reported that the pairs *m*–*p*-cresol, benzoic acid–2,6-dimethylphenol, 4-hydroxy-3-methoxybenzoic acid–4-hydroxybenzaldehyde and (4-hydroxy-3-methoxyphenyl) acetic acid–3,4-dihydroxycinnamic acid could not be resolved using reversed-phase liquid chromatography with gradient elution[4]. The two solutes of each pair can be easily separated on the β-cyclodextrin stationary phases.

In conclusion, α- and β-cyclodextrin stationary phases obtained from 3-μm silica particles provide efficient, selective separations of aromatic compounds. Acylation of the β-cyclodextrin stationary phase in particular effects a selectivity change and an improvement in the separations and peak shapes of the solutes.

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