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RETENTION BEHAVIOUR OF DISUBSTITUTED BENZENE ISOMERS ON ACETYLATED CYCLODEXTRIN STATIONARY PHASES

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SUMMARY

Chemically bonded α- and β-cyclodextrin stationary phases were treated with acetic anhydride. The resulting acetylated stationary phases exhibit selectivity in the separation of disubstituted benzene isomers by liquid chromatography, as do the unmodified, parent cyclodextrin stationary phase. However, the acetylated β-cyclodextrin stationary phase is superior to the unmodified one and can completely separate the *o*-, *m*- and *p*-isomers of toluidine or dinitrobenzene, which cannot be done on the unmodified stationary phase. Acetylation of the α-cyclodextrin stationary phase does not necessarily bring about a similar improvement in the separation.

INTRODUCTION

The chemical modification of cyclodextrins has been investigated in an attempt to improve their complexing and catalytic abilities. Various functional groups have been introduced onto the rim of cyclodextrins[1–4], resulting in changes in the depth of the cyclodextrin cavity, in the hydrogen-bonding ability and various other physical properties, compared with those of the unmodified, parent cyclodextrins. It has been observed that the host–guest interaction in complexes of methylated α-cyclodextrin is quite different from that in those of unmodified α-cyclodextrin, in which the guest molecule (benzaldehyde or *p*-nitrophenol) is positioned upside down[5].

α- and β-cyclodextrins have been immobilized covalently on polyacrylamide[6] and silica[7,8] gels via the spacer arms. The resulting stationary phases yielded efficient, selective separations of various aromatic compounds in liquid chromatography. The retention behaviour of modified cyclodextrin stationary phases is of great interest because selectivity changes in solute retention are expected.

In this work, α- or β-cyclodextrin immobilized on silica was acetylated with acetic anhydride, and the retention behaviours were studied for several disubstituted benzene derivatives and compared with those on the cyclodextrin phases before acetylation.

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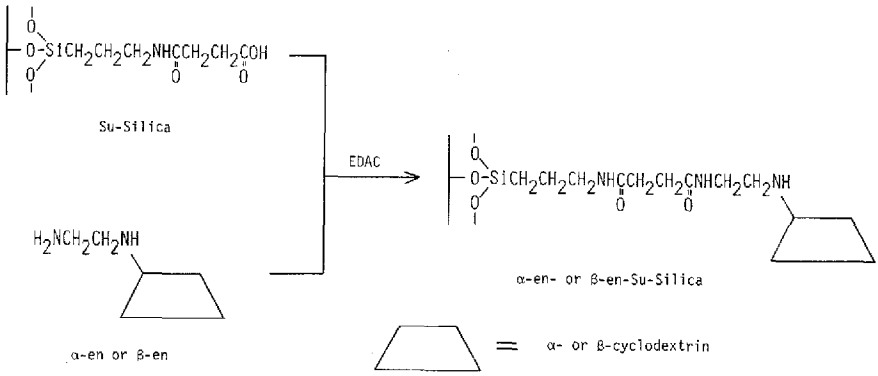


Fig. 1. Immobilization of cyclodextrin on silica gel. EDAC = 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride.

EXPERIMENTAL

The reagents and apparatus used were as described previously[8].

Preparation of acetylated cyclodextrin stationary phases

Ethylenediamine-monosubstituted α- or β-cyclodextrin was immobilized on the carboxylated derivative of silica as described previously[8]. The stationary phase obtained was denoted by α-en-Su-Silica or β-en-Su-Silica, respectively (Fig. 1).

α-en- or β-en-Su-Silica (1.7 g) was suspended in dry pyridine (10 ml). Acetic anhydride (6 ml) was added to this suspension kept at 45°C. After 6 h the acetylated 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 α- or β-cyclodextrin stationary phase thus obtained is denoted by Ac-α-en-Su-Silica or Ac-β-en-Su-Silica, respectively.

Chromatography

The acetylated cyclodextrin stationary phase was packed by a balanced density slurry method into a stainless-steel column (15 cm × 4 mm I.D.). The flow-rate of eluent (water or methanol–water) was 1.0 ml/min. The wavelength used for detection was 254 nm. The concentration of sample solution was 0.2 m*M*, and a volume of 20 μl was injected except where specified.

TABLE I

ANALYTICAL DATA FOR CYCLODEXTRIN STATIONARY PHASES

Phase	*Amount of cyclodextrin immobilized (μmol/g)*	*Elemental analysis (%)*		
		C	*H*	*N*
α-en-Su-Silica	31.5	8.68	1.52	1.15
Ac-α-en-Su-Silica	31.5	10.36	1.71	1.14
β-en-Su-Silica	21.7	8.32	1.63	1.06
Ac-β-en-Su-Silica	21.7	9.59	1.59	1.04

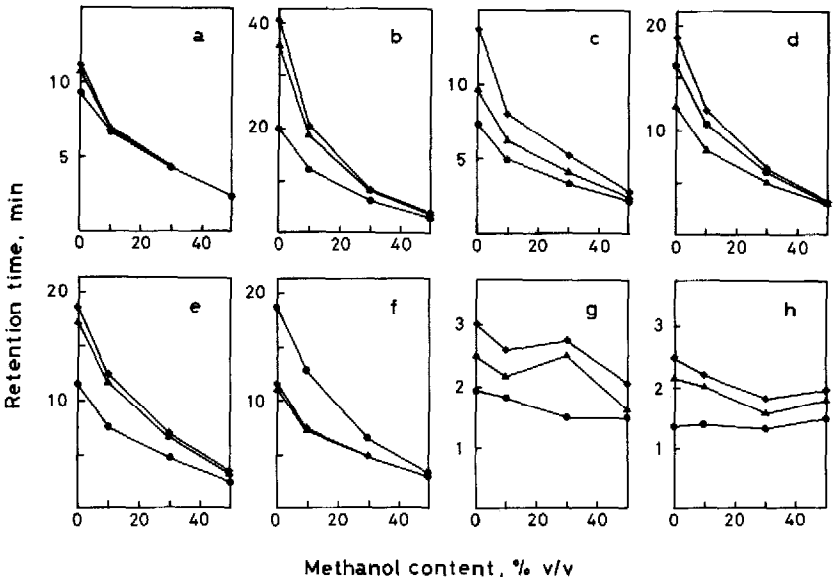


Fig. 2. Effect of methanol content in the eluent on retention times of disubstituted benzene isomers (●, *o*-; ▲, *m*-; and ■, *p*-) on Ac-α-en-Su-Silica. Solutes: *a* = cresol; *b* = iodoaniline; *c* = toluidine; *d* = nitroaniline; *e* = nitrophenol; *f* = dinitrobenzene; *g* = aminobenzoic acid; *h* = nitrobenzoic acid.

RESULTS AND DISCUSSION

The amounts of cyclodextrins immobilized on α-en- and β-en-Su-Silica were evaluated spectrophotometrically by determining D-glucose formed after hydrolysis with H_2SO_4[8]. Table I gives the cyclodextrin capacities of the stationary phases together with the results of elemental analyses before and after acetylation. The increase of the carbon content after acetylation indicates complete modification of the cyclodextrin units in α-en- or β-en-Su-Silica.

Dependence of retention upon eluent composition

The retention times of disubstituted benzene derivatives on both Ac-α-en- and Ac-β-en-Su-Silica were measured by changing the methanol–water ratio in the eluent from 0:100 to 50:50. The results are shown in Figs. 2 and 3, respectively. In the case of aminobenzoic or nitrobenzoic acid, 5 μl instead of 20 μl of a 0.2 m*M* solution were injected because leading effects appeared when the larger sample volume was employed. A decrease in retention with increasing methanol content was found for the disubstituted benzenes studied on Ac-α-en- and Ac-β-en-Su-Silica, except for aminobenzoic and nitrobenzoic acids. For the isomers of the latter benzoic acids the dependence of retention on methanol content was more complicated. On the unmodified phase, α-en-Su-Silica, the elution order of the *o*- and *m*-isomers of cresol, toluidine, nitroaniline or nitrophenol in methanol–water (10:90) ($m < o < p$) was reversed compared with that in water ($o < m < p$)[8]. In the cases of the acetylated cyclodextrin phases, the retention order of the *o*-, *m*- and *p*-isomers of each solute was not dependent upon the methanol content in the eluent.

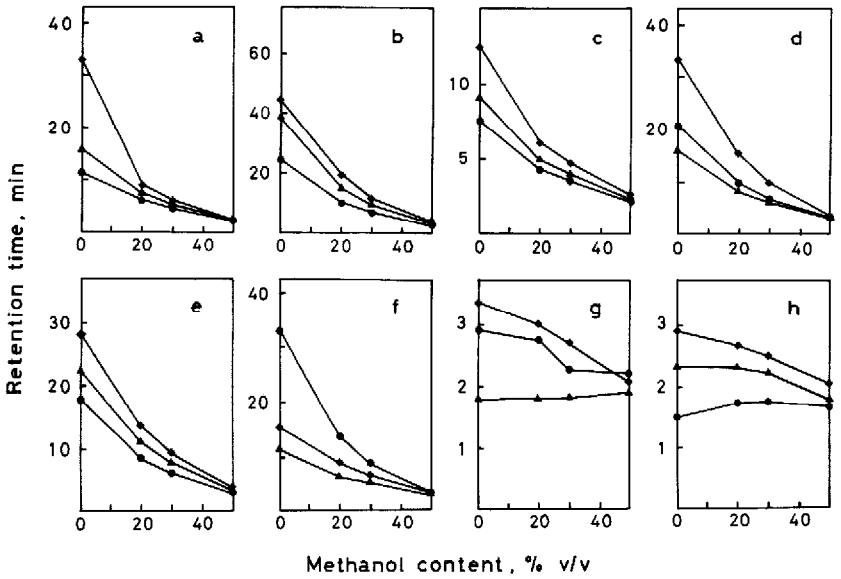


Fig. 3. Effect of methanol content in the eluent on retention times of disubstituted benzene isomers on Ac-β-en-Su-Silica. Solutes as in Fig. 2.

Considering both the separations of the three isomers and the total analysis times, the optimum eluent is pure water for Ac-α-en-Su-Silica. In this case, the isomers can be completely separated, with the exception of the *m*- and *p*-isomers of cresol, dinitrobenzene or nitrobenzoic acid. Similarly, the optimum eluent is methanol–water (20:80) for Ac-β-en-Su-Silica: except for the *o*- and *p*-isomers of aminobenzoic acid and the *m*- and *p*-isomers of nitrobenzoic acid, the isomers can be completely separated. *p*-Iodoaniline gives the longest retention time of 19.13 min. By using pure water as eluent, the three isomers of both aminobenzoic and nitrobenzoic acids can also be separated completely within 4 min on Ac-β-en-Su-Silica, as shown in Fig. 4.

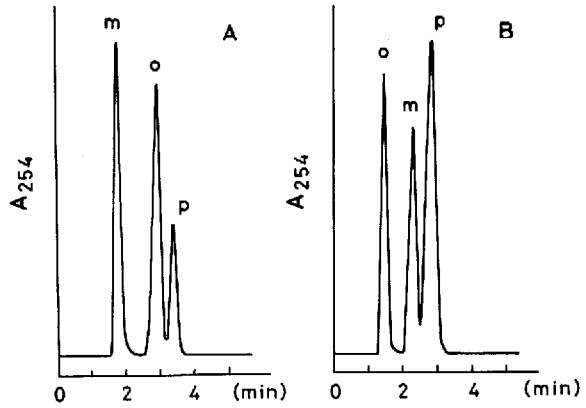


Fig. 4. Liquid chromatograms of aminobenzoic acid isomers (A) and nitrobenzoic acid isomers (B) on Ac-β-en-Su-Silica in water.

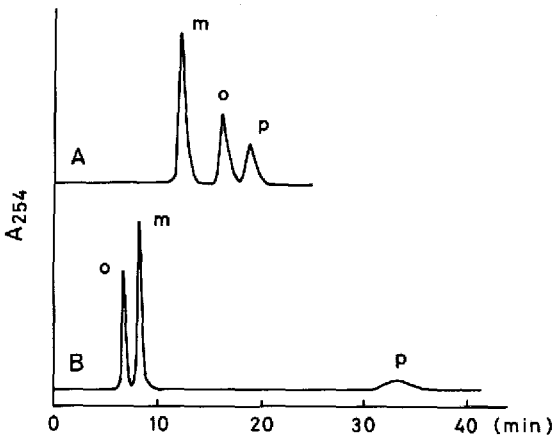


Fig. 5. Liquid chromatograms of nitroaniline isomers on Ac-α-en-Su-Silica (A) and α-en-Su-Silica (B).

Comparison of retention before and after acetylation

Fig. 5 shows typical liquid chromatograms of a mixture of *o*-, *m*- and *p*-isomers of nitroaniline on the α-cyclodextrin stationary phases before and after acetylation (α-en-Su-Silica and Ac-α-en-Su-Silica). A complete separation of the three isomers can be obtained earlier on Ac-α-en-Su-Silica than on α-en-Su-Silica.

Table II gives the retention times of the eight kinds of disubstituted benzene derivatives both on Ac-α-en-Su-Silica and α-en-Su-Silica in water. Both stationary phases must have the same α-cyclodextrin capacity. Therefore, it is reasonable to assume that the difference in the retention time of a solute on the different phases directly reflects the difference in the interaction between unmodified and acetylated α-cyclodextrin with the solute. The *p*-isomers of iodoaniline, nitroaniline and nitro-

TABLE II

RETENTION TIMES ON α-CYCLODEXTRIN STATIONARY PHASES BEFORE AND AFTER ACETYLATION

Eluent: water.

Solute	*Retention time (min)*					
	Ac-α-en-Su-Silica			*α-en-Su-Silica*		
	o-	*m-*	*p-*	*o-*	*m-*	*p-*
Cresol	9.30	10.61	11.09	4.30	5.15	5.76
Iodoaniline	20.05	35.40	40.85	7.35	42.20	115.2
Toluidine	7.39	9.70	13.70	3.19	3.87	4.55
Nitroaniline	16.30	12.25	18.95	6.66	8.17	32.95
Nitrophenol	11.54	16.98	18.50	6.10	10.19	104.3
Dinitrobenzene	18.70	11.25	11.48	4.81	3.96	4.23
Aminobenzoic acid*	1.95	2.51	3.05	–	–	–
Nitrobenzoic acid*	1.35	2.15	2.46	–	–	–

* Not eluted within 60 min on α-en-Su-Silica.

TABLE III

RETENTION TIMES ON β-CYCLODEXTRIN STATIONARY PHASES BEFORE AND AFTER ACETYLATION

Eluent: methanol–water (20:80).

Solute	*Retention time (min)*					
	Ac-β-en-Su-Silica			*β-en-Su-Silica*		
	o-	*m-*	*p-*	*o-*	*m-*	*p-*
Cresol	6.13	7.30	8.39	4.25	5.13	7.22
Iodoaniline	9.85	14.64	19.13	6.44	10.66	20.15
Toluidine	4.31	4.90	6.05	3.05	3.22	4.05
Nitroaniline	9.91	8.40	15.20	5.35	4.50	12.94
Nitrophenol	8.65	11.14	13.49	14.53	7.62	34.70
Dinitrobenzene	13.64	6.48	8.58	5.96	2.95	3.05
Aminobenzoic acid*	2.76	1.80	3.08	–	–	–
Nitrobenzoic acid*	1.75	2.32	2.65	–	–	–

* Not eluted within 60 min on β-en-Su-Silica.

henol and the *m*-isomer of iodoaniline strongly interact with unmodified α-cyclodextrin, which is apparent from the long retention times. However, their retention is considerably reduced by acetylation of the α-cyclodextrin units. This fact indicates a decrease in the complexing ability of acetylated α-cyclodextrin, compared with that of unmodified α-cyclodextrin. On the other hand, the retention times of the other solutes are longer on Ac-α-en-Su-Silica than on α-en-Su-Silica. This may be due to the in-

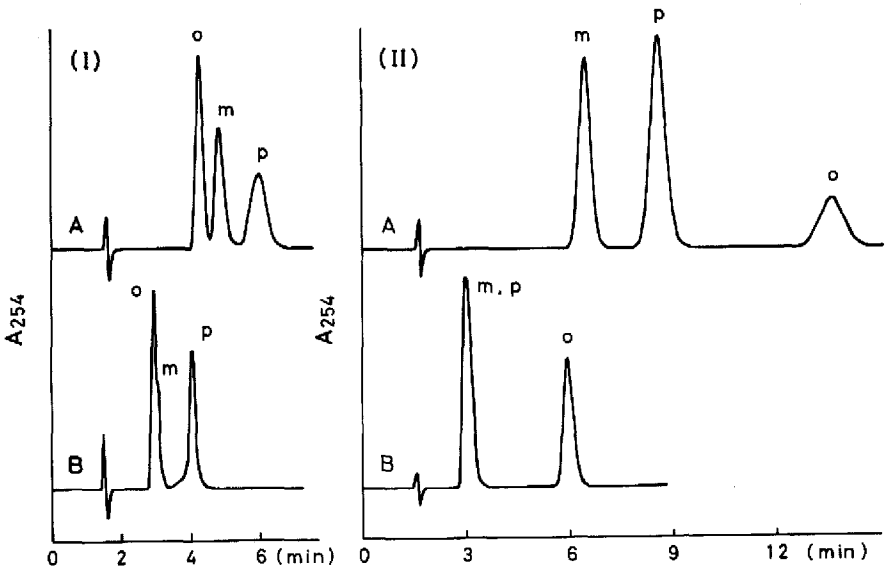


Fig. 6. Liquid chromatograms of toluidine isomers (I) and dinitrobenzene isomers (II) on Ac-β-en-Su-Silica (A) and on β-en-Su-Silica (B).

crease in the hydrophobicity of the phase upon conversion of the hydroxyl groups of α-cyclodextrin into acetoxyl groups. The most remarkable change in retention behaviour after acetylation is that of aminobenzoic and nitrobenzoic acids which can be eluted with pure water within about 3 min; they cannot be eluted within 60 min on α-en-Su-Silica. Although the separation of the toluidine isomers is improved by the acetylation, α-en-Su-Silica is superior to Ac-α-en-Su-Silica in separating the other disubstituted benzene isomers.

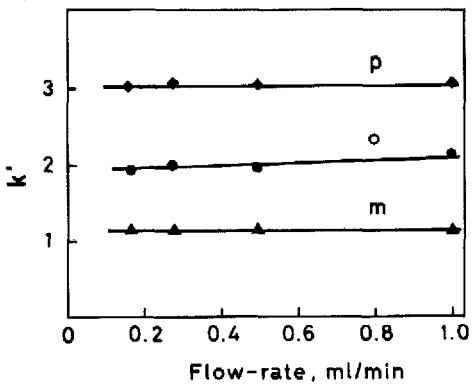
The retention times of the disubstituted benzenes on both Ac-β-en-Su-Silica and β-en-Su-Silica in methanol–water (20:80) are given in Table III. Except for *o*- and *p*-nitrophenol and *p*-iodoaniline, the retention of the solutes tested increases upon acetylation of the β-cyclodextrin units. As mentioned above, the three isomers of all the solutes investigated can be completely separated on Ac-β-en-Su-Silica. The toluidine or dinitrobenzene isomers are not completely separated on β-en-Su-Silica as shown in Fig. 6.

The elution order of the three isomers on Ac-α-en- or Ac-β-en-Su-Silica is the same as that on the corresponding, unmodified cyclodextrin stationary phase with only two exceptions: $m < o < p$ on Ac-α-en-Su-Silica and $o < m < p$ on α-en-Su-Silica for nitroaniline and $o < m < p$ on Ac-β-en-Su-Silica and $m < o < p$ on β-en-Su-Silica for nitrophenol. Considering the elution order of the isomers on octadecylsilyl silica (reversed-phase mode) or carboxylated silica before coupling with cyclodextrins[8], this result strongly suggests that an inclusion process is operative in the cases of the acetylated cyclodextrin stationary phases.

Column efficiency

Fig. 7 shows the effect of the eluent flow-rate on the capacity factors, k', for the nitroaniline isomers on Ac-β-en-Su-Silica. The k' values for each isomer are independent of the flow-rate in the range 0.16–1.0 ml/min; the selectivity is nearly constant.

Fig. 8 shows a plot of the height equivalent to a theoretical plate (HETP) for the nitroaniline isomers on Ac-β-en-Su-Silica *versus* the flow-rate of methanol–water (20:80). The HETP values for the *o*-isomer are larger than those for *m*- and *p*-isomers.



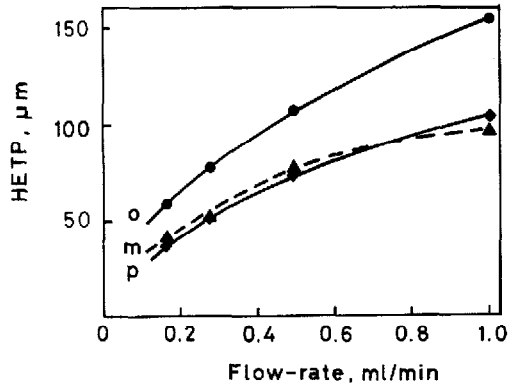


Fig. 7. Effect of eluent flow-rate on capacity factors, k', for nitroaniline isomers on Ac-β-en-Su-Silica.

Fig. 8. Effect of eluent flow-rate on height equivalent to a theoretical plate (HETP) for nitroaniline isomers on Ac-β-en-Su-Silica.

When calculated from the *m*- or *p*-isomer, the HETP value at a flow-rate of 1 ml/min is 100 μm, which corresponds to 1500 theoretical plates, and the HETP value at a flow-rate of 0.16 ml/min is 40 μm. These values are satisfactory for practical purposes.

In conclusion, stationary phases containing α- or β-cyclodextrin chemically bonded to silica have been modified by treatment with acetic anhydride. Compared with the unmodified phase, the acetylated β-cyclodextrin phase gives improved separations of the isomers of disubstituted benzenes.

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