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Note

Separation of some aromatic amino acids by reversed-phase high-performance liquid chromatography using α- or β-cyclodextrin as mobile phase component

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The application of cyclodextrins (CDs) as stationary phase components for chromatographic separation of some aromatic amino acids has given interesting results[1,2]. However, columns containing polymers with incorporated cyclodextrin molecules exhibit low efficiency owing to the complex mechanism of sorption: gel permeation and molecular inclusion.

To avoid gel permeation, we have recently applied α- and/or β-cyclodextrin as mobile phase modifiers for resolution of mandelic acid and some of its derivatives into enantiomers[3–5], and for separation of positional isomers of nitrobenzoic[6] and nitrocinnamic acid[7], using reversed-phase (RP) high-performance liquid chromatography (HPLC). Such systems exhibit greater selectivity, whilst almost fully retaining all the advantageous properties of the original columns.

This paper reports the results of our further studies on the application of these systems for separation of some aromatic amino acids. The model compounds tested included phenylglycine (PHEGLY), phenylalanine (PHEALA), 4-hydroxyphenylglycine (4-OH-PHEGLY), 5-hydroxytryptophan (5-OH-TRP), tyrosine (TYR), histidine (HIS) and 3,4-dihydroxyphenylalanine (DOPA).

It should be mentioned that RP-HPLC has recently emerged as a technique of great importance not only for separation of non-dissociating molecules but also for resolution of ionic solutes with the aid of appropriate modifiers. Attempts at adaptation of RP-HPLC to the separation of free amino acids (without a pre-column derivatization step) have recently been performed using extremely acidic media[8] or upon addition of various modifying agents, *e.g.*, anionic surfactants[9], sodium dodecyl sulphate[10] or copper(II) ions[11].

EXPERIMENTAL

α- and β-CD were supplied by Chinoin (Budapest, Hungary), amino acids by Reanal (Budapest, Hungary). All other materials were of analytical or laboratory reagent grade and were used without further purification.

Chromatographic experiments were performed using an HPLC unit constructed at the Institute of Physical Chemistry, Polish Academy of Sciences (Warsaw, Po-

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land), and was equipped with an UV detector (254 nm). Stainless-steel columns (250 × 4 mm I.D. and 100 × 4 mm I.D.) packed with 10-μm LiChrosorb RP-18 and 10-μm LiChrosorb RP-8 (E. Merck, Darmstadt, F.R.G.), were employed. The mobile phase consisted of an aqueous solution containing various concentrations of α- or β-CD and a suitable phosphate buffer.

RESULTS AND DISCUSSION

The capacity factors, k', of the investigated aromatic amino acids (determined on LiChrosorb RP-18) as a function of the α-CD concentration in the mobile phase solution at pH 3.0 are presented in Fig. 1.

Fig. 2 shows two chromatograms of the same mixture of amino acids, obtained using an RP-18 column and a mobile phase solution (pH 3.0) either containing α-CD (at a concentration marked by an arrow in Fig. 1) or without α-CD.

It is seen that in chromatographic separations of aromatic amino acids, α-CD results in improvement in resolution. Similarly to organic solvents, it reduces the adsorption of these solutes on the RP-18 phase, and thus shortens the analysis time. At the same time, the separation factors, α (for neighbouring peaks), remain unchanged or increase. The latter behaviour is exemplified by the separation of

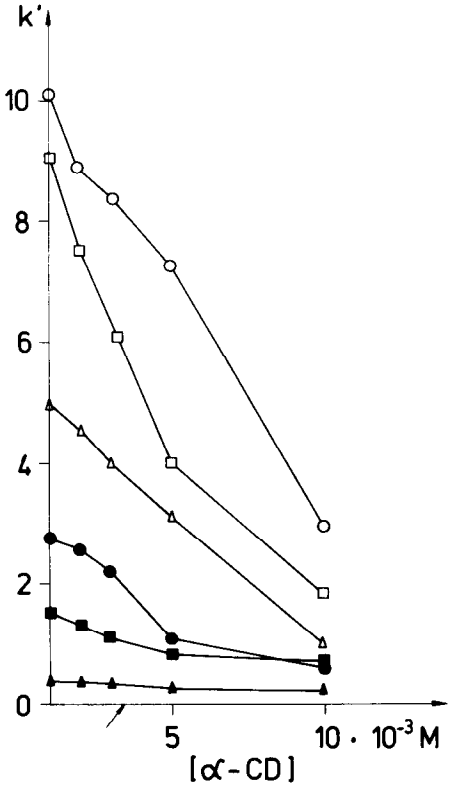

Fig. 1. Plots of capacity factors, k', determined on LiChrosorb RP-18 stationary phase *versus* α-CD concentration in the mobile phase solution at pH 3.0: ○ = PHEALA; □ = 5-OH-TRP; △ = TYR; ● = DOPA; ■ = PHEGLY; ▲ = 4-OH-PHEGLY.

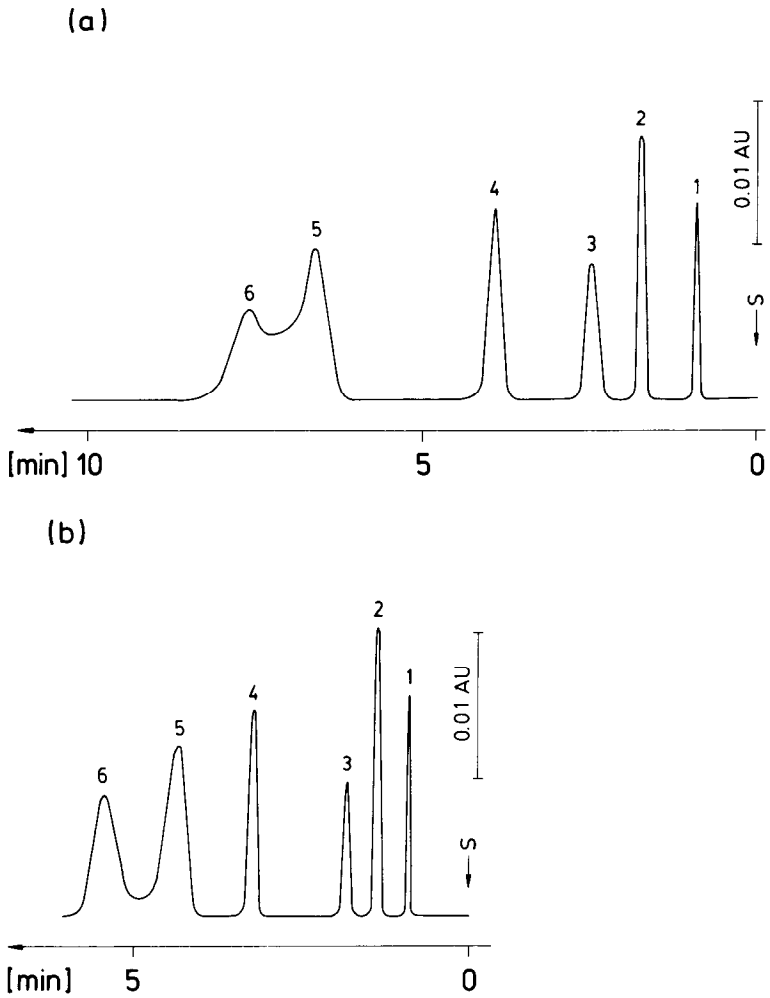

Fig. 2. Chromatograms of a mixture of 10^{-3} *M* 4-OH-PHEGLY (1), PHEGLY (2), DOPA (3), TYR (4), 5-OH-TRP (5) and PHEALA (6) on LiChrosorb RP-18 (100 × 4 mm I.D.) with an aqueous mobile phase solution at pH 3.0, without α-CD (a) and containing $3 \cdot 10^{-3}$ *M* α-CD (b).

PHEALA and 5-OH-TRP for which α rises from 1.11 (without α-CD) to 1.36 ($3 \cdot 10^{-3}$ *M* α-CD). This effect is much more pronounced in the case of β-CD, as seen in Fig. 3. On the RP-8 phase at pH 5.0, $\alpha_{\text{PHEALA/5-OH-TRP}}$ increases from 1.2 (without β-CD) to 2.1 ($2 \cdot 10^{-3}$ *M* β-CD).

Evaluation of the number of theoretical plates of the columns shows that in the case of amino acids the introduction of α- or β-CD results in increased column efficiency (*ca.* 50%). This behaviour is contrary to that observed for nitrobenzoic, nitrocinnamic and mandelic acid derivatives, where there is a small decrease in efficiency[5–7].

To summarize, α- or β-CD may be used to modify the separation of aromatic amino acids on RP columns, and hence for optimization of such procedures. Un-

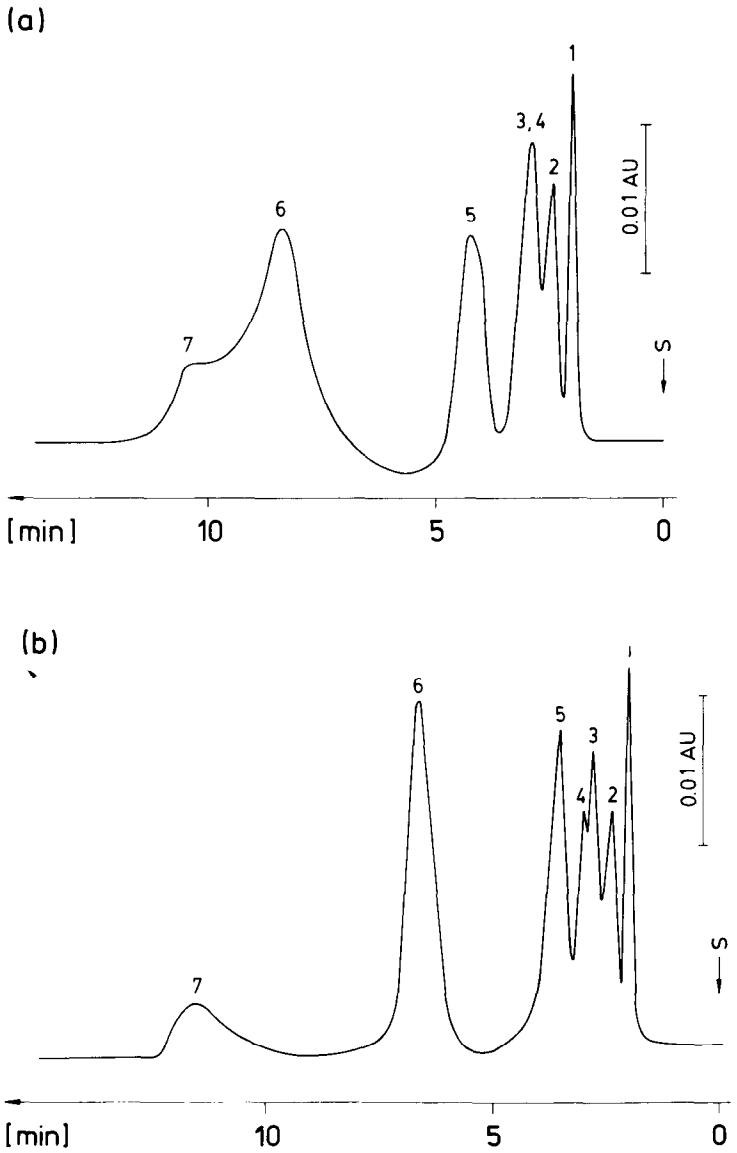

Fig. 3. Chromatograms of a mixture of 10^{-3} *M* 4-OH-PHEGLY (1), HIS (2), DOPA (3), PHEGLY (4), TYR (5), 5-OH-TRP (6) and PHEALA (7) on LiChrosorb RP-8 (250 × 4 mm I.D.) with an aqueous mobile phase solution at pH 5.0, without β-CD (a) and containing $2 \cdot 10^{-3}$*M* β-CD (b).

fortunately, the model of equilibria and the equations deduced earlier[6,7] for optimization of the separation conditions (pH, [CD]) for nitrobenzoic and nitrocinnamic acids on RP columns are not valid in the case of the present aromatic amino acids; the ionogenic nature of the latter is more complex. In this case, the optimization procedure still remains at the "trial and error" stage.

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