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Note

Host–guest complexation in capillary isotachophoresis

I. α-, β- and γ-cyclodextrins as complexing agents for the resolution of substituted benzoic acid isomers*

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Isotachophoresis is a useful electrophoretic technique for the separation of inorganic and organic ions. Separation of ions is achieved when their effective mobilities are sufficiently different owing to both their intrinsic charge and their equilibria in solution.

Several approaches have been applied to modify the effective mobilities of ions in order to improve their separations, *e.g.*, by using solvents with different polarities, by changing the pH of the leading solution and by adding to the leading electrolyte charged and/or uncharged compounds that are able to form complexes with the sample.

In capillary isotachophoresis, complex-forming equilibria have been studied[1]. The use of uncharged complexing agents such as crown ethers and cyclodextrins is well known in chromatography for improving the selectivity, especially when molecules with similar chemical properties have to be separated, *e.g.*, optical, geometrical and structural isomers[2–5].

Crown ethers and cyclodextrins were employed in capillary isotachophoresis by Tazaki *et al.*[6] to separate cations and anions, respectively, and more recently cyclodextrins have been used as complex forming agents for the separation of antibiotics[7] and aromatic compounds, including substituted benzoic acids[8,9].

The aim of this work was to study the effect of adding cyclodextrins to the leading electrolyte on the mobilities of structural isomers of benzoic acid derivatives by measuring the step heights.

The influence of the cyclodextrin cavity, the molecular shape of the sample anion and the solvent was studied. The resolution of *ortho*, *meta* and *para* isomers of benzoic acid derivatives by host–guest complexation is reported.

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EXPERIMENTAL

Apparatus

Isotachophoresis was performed with an LKB (Bromma, Sweden) Tachophor 2127 instrument equipped with a conductivity detector in a polytetrafluoroethylene (PTFE) capillary tube (23 cm × 0.5 mm I.D.). TPX electrolyte reservoirs for non-aqueous solvents were used. Isotachopherograms were recorded with a LKB 2210 line recorder at a chart speed of 50 mm/min. The current used was 150 μA, changed to 50 μA during the detection. The analysis time was less than 14 min.

Detector conductivity cell

The laboratory-made detector conductivity cell was similar to that described by Everaerts *et al.*[10]. The resin block was made with Araldite 502 polymerized with 1.5% (w/w) of 2,4,6-tris(dimethylaminomethyl)phenol (DMP) at 70°C for 7 h. The low percentage of activator allowed an extremely homogeneous resin to be obtained. The cell was of 0.5 mm I.D. and was threaded for connection to a PTFE capillary with a commercial LKB fitting. The conductivity detector was connected to the cell by insulated cables (LKB). Platinum (0.020 mm thick) was used for sensing electrodes. Cyanolite served to glue the platinum and Araldite.

Chemicals

Doubly distilled water was used to prepare the solutions. Poly(vinyl alcohol) 28/20 (PVA) and morpholinoethanesulphonic acid (MES) were purchased from Serva (Heidelberg, F.R.G.) and 30% Suprapur hydrochloric acid, creatinine and ethanol were obtained from Merck (Darmstadt, F.R.G.). Tris(hydroxymethylamino)methane (Tris), 2-, 3- and 4-chlorobenzoic acid, 2-, 3- and 4-bromobenzoic acid, 2-, 3- and 4-fluorobenzoic acid, 2-, 3- and 4-aminobenzoic acid and α- and γ-cyclodextrins (α- and γ-CD) were purchased from Fluka (Bonaduz, Switzerland), β-cyclodextrin (β-CD) from Sigma (St. Louis, MO, U.S.A.), 3-nitrobenzoic acid and benzoic acid from Carlo Erba (Milan, Italy) and Araldite 502 and DMP from EMS (Fort Washington, PA, U.S.A.).

All chemicals were of analytical-reagent grade and used as received, except for PVA, which was purified by passing it through a mixed-bed ion exchanger. Halogenobenzoic acid derivatives were dissolved in water–methanol (1:1, v/v) and nitro-, hydroxy- and aminobenzoic acid derivatives in water–ethanol (9:1, v/v) to give 2 m*M* solutions.

Electrolytes

In system A (aqueous solution), mixtures of 10 m*M* hydrochloric acid containing 0.1% (w/v) of PVA adjusted to pH 5.1 with creatinine and the appropriate amount of α-, β- and γ-cyclodextrins, and 10 m*M* MES adjusted to pH 6.0 with Tris, were used as the leading and terminating electrolyte, respectively.

In system B [water–methanol (9:1, 8:2 and 7:3) and water–ethanol (8:2) solutions], the leading electrolyte contained 10 m*M* hydrochloric acid, 0.1% (w/v) PVA and appropriate amount of cyclodextrins adjusted to an apparent pH of 5.1 with creatinine, and the terminating electrolyte was 10 m*M* MES adjusted to an apparent pH of 6.0 with Tris.

Cyclodextrins were dissolved daily in the leading electrolyte.

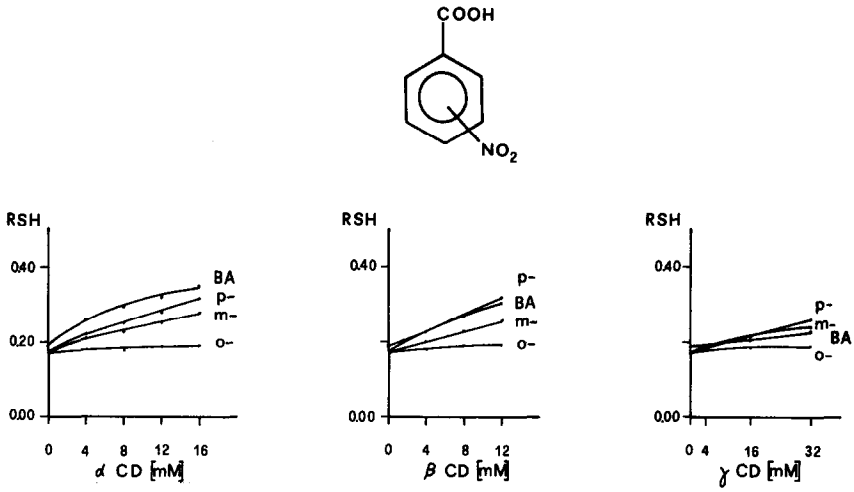

Fig. 1. Effect of α-, β- and γ-CD concentration on the RSH of benzoic acid and *o*-, *m*- and *p*-nitrobenzoic acid.

RESULTS AND DISCUSSION

α-, β- and γ-CDs are natural macrocyclic polymers of glucose containing different D(+)-glucopyranose units, which contain cavities of different sizes. CDs were used as additives to the leading electrolyte in order to modify the effective mobilities of eighteen benzoic acid derivatives. The relative step heights (RSH) were calculated with the equation

$$\mathrm{RSH} = \frac{h_x - h_L}{h_s - h_L} \tag{1}$$

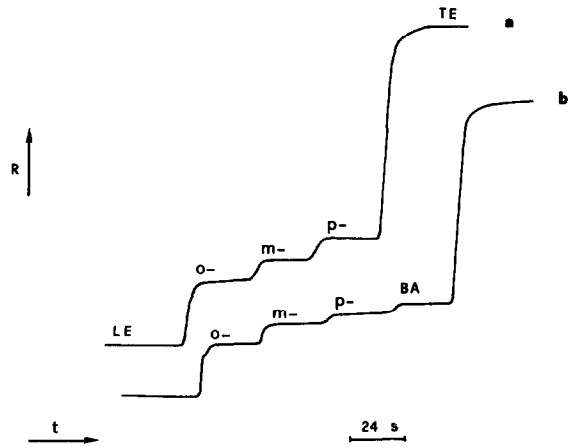

Fig. 2. Isotachophoretic separation of (a) *o*-, *m*- and *p*-nitrobenzoic acid with electrolyte system A (leading electrolyte containing 12 m*M* β-CD) and (b) mixtures of benzoic acid (BA) and its nitro derivatives (*o*-, *m*- and *p*-) with electrolyte system A (leading electrolyte containing 12 mM α-CD).

where h is the step height in cm and x, s and L are the examined anion, the reference compound (MES) and the leading electrolyte, respectively.

The experiments were carried out by adding the appropriate amount of CDs to the leading electrolyte at pH 5.1. In the absence of CDs, all isomers, except hydroxybenzoic acids, showed similar mobilities in aqueous solution at this pH.

Fig. 1 shows the effect of α-, β- and γ-CD concentration on the effective mobilities of nitrobenzoic acid isomers in aqueous solutions. The effective mobility sequence was always *p-* < *m-* < *o-*, which indicates that *p*-nitrobenzoic acid fits closely in the cavity of all the CDs. The strongest inclusion effect was observed between the *para* isomer and β-CD. *o*-Nitrobenzoic acid undergoes a negligible reduction in effective mobility on increasing the CD concentration in the leading electrolyte. It seems that, owing to steric hindrance, the *ortho* isomer forms weak host–guest complexes. Good resolution of the nitrobenzoic acid isomers was achieved by using 12 m*M* α-CD and 12 m*M* β-CD, as illustrated in Fig. 2.

It must be noted that β-CD, although the best resolving agent for the three isomers, was not able to separate benzoic acid in mixtures with substituted isomers. An inclusion effect was observed only at high concentration of γ-CD with *meta* and *para* isomers. In Fig. 3 the effect of α-, β- and γ-CD concentration on the effective mobilities of *o-*, *m-* and *p*-aminobenzoic acid is shown. The complexation order for CDs with the *para* isomer was α- > β- > γ-CD. The RSH of the *para* isomer as a function of the α-CD concentration was higher than that of other anions. It seems that *p*-aminobenzoic acid forms the strongest host–guest complex, probably owing to hydrogen bonding with hydroxyl groups of the CD. The effect of CDs on the mobilities of *ortho* and *meta* isomers seems not to be influenced, while β-CD was found to be highly selective at concentrations below 12 m*M*. Fig. 4 shows the sep-

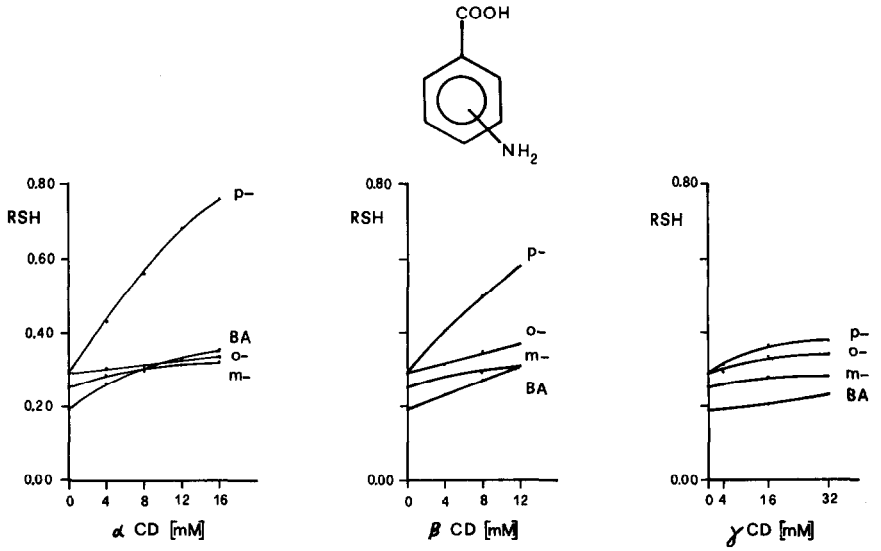

Fig. 3. Effect of α-, β- and γ-CD on the RSH of benzoic acid (BA) and its amino derivatives (*o-*, *m* and *p-*).

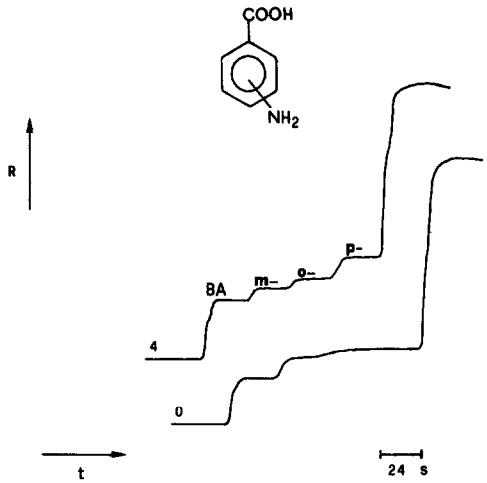

Fig. 4. Isotachopherogram of the separation of a mixture of benzoic acid and its amino derivatives (*o*-, *m*- and *p*-). Electrolyte system A [leading elecrolyte without CD (0) and 4m*M* β-CD (4)].

aration of benzoic acid and its amino derivatives and Fig. 5 shows the effect of CD concentration on the RSH values of hydroxybenzoic acid isomers.

The behaviour of the hydroxybenzoic acids is similar to that of the amino derivatives. Better selectivity in the presence of α-CD was observed. The higher mobility of the *para* isomer may be attributed to the lower donor–acceptor proton property of the OH group in comparison with the amino group. The complexing effect of α-, β- and γ-CDs added to the leading electrolyte on the effective mobility of the halogenobenzoic acids studied was generally noticeable only for *para* and *meta* isomers. Good resolution of mixtures of chloro- and bromobenzoic acids and benzoic

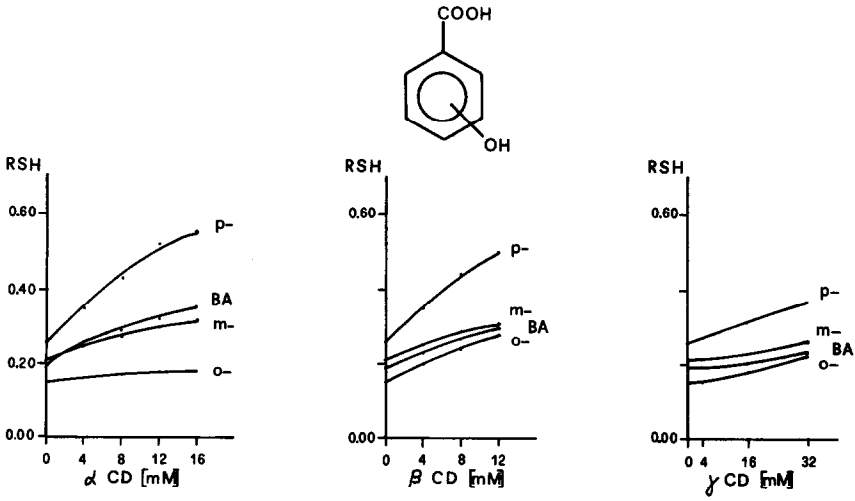

Fig. 5. Effect of α-, β- and γ-CD on the RSH of benzoic acid and its hydroxy derivatives (*o*-, *m*- and *p*-).

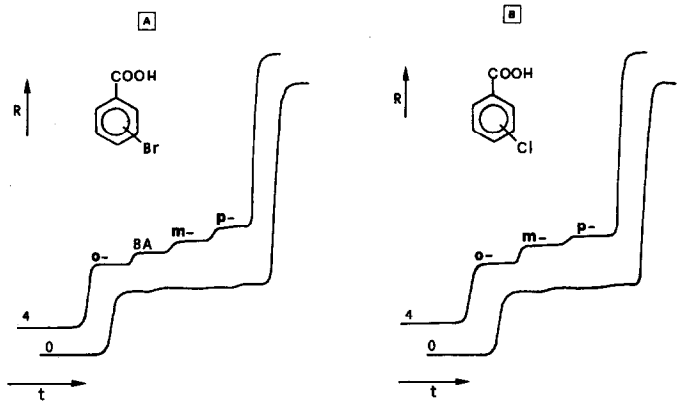

Fig. 6. (A) Isotachophoretic separation of a mixture of benzoic acid (BA) and its bromo derivatives. Electrolyte system B [water–methanol (80:20)]; 0 = without β-CD; 4 = in the presence of 4 m*M* β-CD. (B) Isotachophoretic separation of a mixture of *o*-, *m*- and *p*-chlorobenzoic acid. Electrolyte system B [water–methanol (80:20)]; 0 = without β-CD; 4 = in the presence of 4 m*M* β-CD.

acid was shown in the presence of α-CD. However, *m*- and *p*-fluorobenzoic acids did not separate. Our results for the halogen derivative confirm the data reported recently by Snopek *et al.*[8].

In order to improve the selectivity for halogenobenzoic acids aqueous–organic systems were used as electrolytes, water–methanol and water–ethanol mixtures being successful.

As shown in Fig. 6, the best results were obtained with water–methanol (80:20, v/v) containing β-CD (4 m*M*). This system allowed the complete separation of bromobenzoic acids and benzoic acid, and of chlorobenzoic acids, which otherwise could not be resolved. Experiments with fluorobenzoic acids did not yield satisfactory results.

We conclude that the addition of CDs to the leading electrolyte in capillary isotachophoresis improves the selectivity for structural isomers and permits their separation. The host–guest complexation is influenced not only by the hydrophobic effect originating from the CD cavity, but also by the dimensions of the substituent groups and their ability to form hydrogen bonds. Therefore, the nature of the solvent plays a very important role. In water–alcohol mixtures a general decrease in the complexation effect was noted, probably owing to the competition between the alcohol and the anions. The use of such mixtures increases the selectivity and permits the resolution of chloro- and bromobenzoic acid structural isomers.

γ-CD was found to give a noticeable complexing effect with the derivatives studied only at relatively high concentrations. Attempts to resolve the derivatives at such high concentrations of γ-CD were not succesful, however.

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