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Note

Use of cyclodextrins in isotachophoresis

VIII. Two-dimensional chiral separation in isotachophoresis

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Many factors influence the separation efficiency of isotachophoresis (ITP)[1]. Modification of the leading electrolyte (LE) by non-polar complex-forming agents, cyclodextrins (CDs) proved to be an important method for the separation of different types of structurally related and/or isomeric compounds[2–9]. In previous contributions[10–12] we dealt with the use of CDs as enantioselective selectors added to the LE for the chiral separation of optical isomers in a device equipped with a one-column separation compartment. A device with coupled columns offers the possibility of performing one-run, two-step separations in two columns filled with LE of different composition[13].Mixtures of chiral substances of different structure usually cannot be resolved using one type of enantioselector only. In some instances it is possible to use mixtures of two or more selectors in the LE.

In this study we developed a two-dimensional chiral separation method that can be used with commercially available column-coupling equipment, both capillaries of which are filled separately with LE containing CDs as chiral selectors.

EXPERIMENTAL

Chemicals

Distilled water deionized with a Laboratory Water System XLDRO 1002 apparatus (Liquipure Europe, U.K.) was used for the preparation of the electrolyte solution and of the solutions of model compounds. All chemicals were of the highest quality commercially avialable: acetic acid, sodium acetate and oxalic acid were from Merck (Darmstadt, F.R.G.), β-alanine (β-Ala) from Serva (Heidelberg, F.R.G.); Natrosol 250 HR (hydroxyethylcellulose, HEC) from Hercules (Wilmington, DE, U.S.A.), Zerolit DM-F indicator from BDH (Poole, U.K.), γ-cyclodextrin (γ-CD) from Astec (Whippany, U.S.A.) and heptakis (2,6-di-O-methyl)-β-cyclodextrin (DM-β-CD) from Chinoin (Budapest, Hungary).

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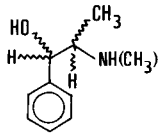
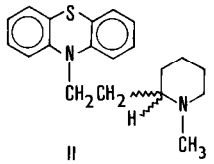
TABLE I

STRUCTURAL FORMULAE OF THE MODEL COMPOUNDS



I



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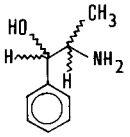

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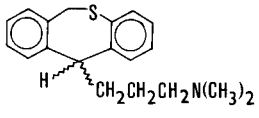

IV

Compound	*Name*
I, (+)- or (−)-	(+)- or (−)-pseudoephedrine; (1*S*,2*S*)- or (1*R*,2*R*)-2-methylamino-1-phenylpropanol
II, (+)- or (−)-	(+)- or (−)-thioridazine; (*R*,*S*)-10-[2-(1-methyl-2-piperidyl)ethyl]-2-methylthiophenothiazine
III, (+)- or (−)-	(+)- or (−)-norpseudoephedrine; (1*S*,2*S*)- or (1*R*,2*R*)-2-amino-1-phenylpropanol
IV, (+)- or (−)-	(+)- or (−)-hydrothiadene = (*R*,*S*)-11-(3-dimethylaminopropyl)-6,11-dihydrodibenzo(*b*,*e*)thiepin

Cyclodextrins and Natrosol 250 HR solutions were purified using Zerolit DM-F mixed bed ion-exchange resin. The solutes investigated were obtained from Research Institute for Pharmacy and Biochemistry (Prague, Czechoslovakia). Their formulae and numbering are given in Table I. Stock sample solutions were prepared by dissolving substances I and III in water (1 mg ml^{-1}) and substances II and IV in 5 m*M* oxalic acid (1 mg ml^{-1}) and were stored in dark bottles in a refrigerator.

Methods

Isotachophoretic experiments were performed using a ZKI-001 column-coupling isotachophoretic analyser (Institute of Radioecology and Applied Nuclear

TABLE II

ELECTROLYTE SYSTEMS AND CONDITIONS FOR ITP

Parameter	*Conditions*
Leading electrolyte	10 m*M* sodium acetate containing 0.08% HEC with acetic acid to pH 5.47
Terminating electrolyte	10 m*M* β-Ala
Capillary	1st column: 170 mm × 0.8 mm I.D. 2nd column: 170 mm × 0.3 mm I.D.
Current	In 1st column, 200 μA (800 s); for detection 100 μA In 2nd column, 50μA

Techniques, Plant for Development and Production of Nuclear Instruments, Spišská Nová Ves, Czechoslovakia) equipped with two polytetrafluoroethylene capillary columns with independent conductivity detectors and sample valve (volume 30 μl). The operating conditions are given in Table II.

The optical rotation of optically enriched mixtures of enantiomers were measured with a model 241 polarimeter (Perkin-Elmer, Norwalk, CT, U.S.A.). The ITP separation pattern of enantiomers of compounds II and IV was determined indirectly by comparison of ITP and optical rotation measurements for optically enriched samples obtained by selective precipitation of racemates with γ-CD on the micropreparative scale.

RESULTS AND DISCUSSION

The compounds in Table I were used for the preparation of four-component model mixtures (two pairs of appropriate enantiomers). Preliminary experiments indicated that I and III could be enantioselectively separated with DM-β-CD-modified LE and II and IV could be enantioselectively separated in γ-CD-containing LE.

The requirements for the chiral separation of solute mixtures of I + II and/or III + IV racemates are much more demanding on the experimental technique. It

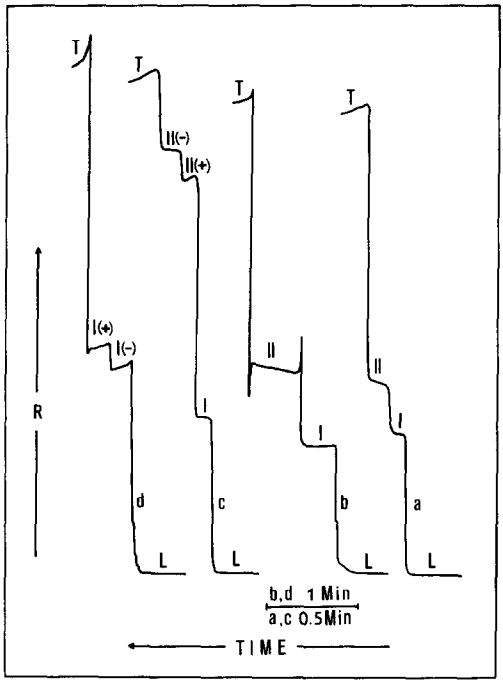

Fig. 1. ITP separation of the optical isomers of compounds I and II (a and b) without CD, (c) with 5 mM γ-CD and (d) with 10 mM DM-β-CD in the leading electrolyte with the two-column system (a and c) in the first capillary and (b and d) in the second capillary. R = response of detector; L and T = leading and terminating zones, respectively. Amount of sample introduced: (I) 1.07 μg and (II) 3.21 μg, in all measurements.

cannot be achieved in one column filled with a mixture of the two CDs at concentrations found to be most effective for the resolution of single racemates. The main problem results from the strong non-effective complexation[14] of II and IV, the DM-β-CD complexes of which are lost in the terminating electrolyte zone.

The most effective solution to this problem is to apply a two-column system in which each separation compartment is filled with one type of CD-modified LE. Practical examples of the two-dimensional chiral separation of mixtures of I and II and of III and IV racemates are illustrated in Figs. 1 and 2, respectively.

The non-chiral separation of the solutes in the two separation compartments filled with CD-free LE is shown in traces (a) and (b). The addition of γ-CD to the first and DM-β-CD to the second compartment, at the optimum concentrations given in the legends, leads to complete enantioselection in one experimental run. The complete chiral separation of solutes II and IV by means of γ-CD is shown in traces (c). Compounds I and III became almost uncomplexed and were completely unresolved. Different separation patterns were observed in the second compartment filled with DM-β-CD. All the compounds investigated were substantially complexed. The strong retardation of II and IV led to their disappearance in the zone of the terminat-

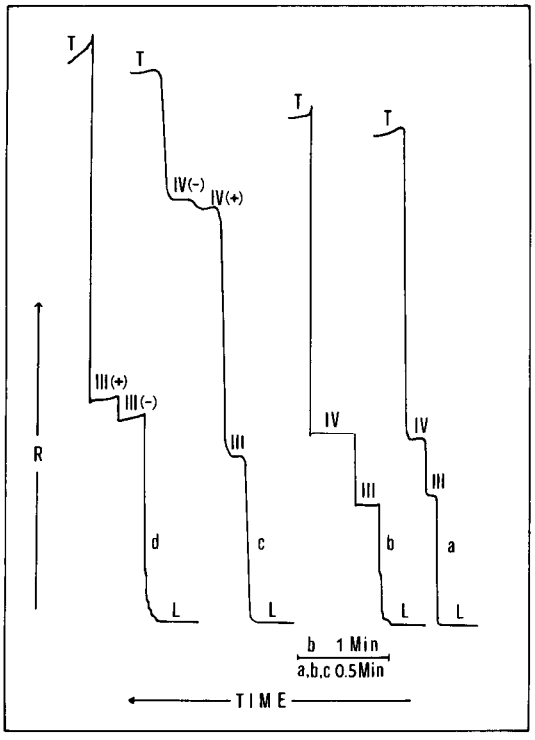

Fig. 2. ITP separation of the optical isomers of compounds III and IV (a and b) without CD, (c) with 15 mM γ-CD and (d) with 10 mM DM-β-CD in the leading electrolyte with the two-column system. Amount of sample introduced: (III) 0.86 μg in (a) and (b) and 0.66 μg in (c) and (d); (IV) 3.34 μg in (a) and (b) and 2.63 μg in (c) and (d). Other details as in Fig. 1.

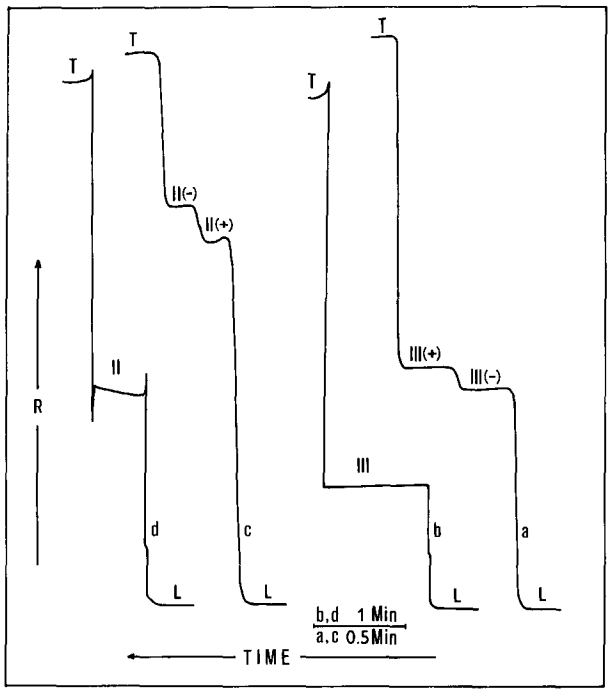

Fig. 3. Demonstration of instability of CD–enantiomer complexes of compounds II and III. The first capillary was filled with (c) 5 m*M* γ-CD and (a) 10 m*M* DM-β-CD modified LE, respectively. Amount of sample introduced: (II) 3.33 μg and (III) 3.00 μg. Other details as in Fig. 1.

ing electrolyte. In contrast, the interaction of solutes I and III with the CD led to a significant resolution of their optical isomers.

In order to examine the stability of the CD complexes formed, experiments were also carried out with the two-column system in which the first capillary was filled with CD-modified LE and the second did not contain CD in the LE. As illustrated in Fig. 3 and Table III, the CD complexes generally decompose during the migration

TABLE III

$(h_i)_{rel}$ VALUES OF COMPOUNDS I AND IV

$(h_i)_{rel} = (h_i - h_l) / (h_t - h_l)$, where h_i, h_l and h_t = step height of sample, leading electrolyte and terminating electrolyte, respectively.

Compound	*1st column (with CD)*		*2nd column (without CD)*
	(+)-Enantiomer	*(−)-Enantiomer*	*racemate*
I[a]	0.487	0.448	0.270
IV[b]	0.737	0.764	0.382

[a] LE with 10 m*M* DM-β-CD.
[b] LE with 15 m*M* γ-CD.

through the second CD-free capillary. This results in a lowering of the $(h_i)_{rel}$ values and combination of zones of the enantiomers resolved in the first column. It should be noted, however, that the fixed distance between the two detectors does not enable the half-life of decomposition of the CD complex to be determined accurately.

CONCLUSIONS

The two-dimensional ITP system described, using different types of CDs for modification of the LE, proved useful for the investigation of complicated mixtures of racemates. The main advantages are a shorter separation time comparing with two runs with a one-column system filled gradually with an enantioselector, lower consumption of the samples and chiral selectors and the possibility of using two interfering selectors in one two-step separation.

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