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Note

Use of cyclodextrins in isotachophoresis

III. Purity control of naftidrofuryl hydrogenoxalate and some of its synthesis intermediates

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The ability of cyclodextrins (CDs) to interact with various types of compounds, according to the sizes and shapes of the molecules, has enabled a wide range of analytical applications especially in chromatographic separations, where the selective inclusion interaction is increased by a multiple chromatographic process[1,2].

Some applications, which confirmed the possibility of using CDs in isotachophoresis (ITP), were recently presented[3–5]. Two important facts emerged from these experiments. The formation of the inclusion complex between the CD and the host molecule is a dynamic and very fast process which generally does not affect the steady-state formation in ITP, and the molecules of CDs are not ionized at the commonly used pH ranges and thus act as a quasi-stationary phase with negligible migration velocity.

Naftidrofuryl, a frequently used drug with significant antisclerotic effect, is a type of organic compound which can easily form an inclusion complex, preferentially with β-CD. From its structural formula (Table I) we may presume that β-CD is able to interact preferentially with the apolar part of the molecule formed by the naphthyl group. The use of 1-(chloromethyl)naphthalene (Weyl), containing as much as 7% of 2-(chloromethyl)naphthalene, as an initial substance in the synthesis, results in the formation of positional isomers (Table I). Each isomer pair, either with anionic or cationic character, may serve as an excellent sample for the study of equatorial and axial complex formation with β-CD in ITP (Fig. 1).

We now describe ITP measurements on model mixtures of isomer pairs which serve to confirm theoretically predicted stability differences between equatorial and axial inclusion complexes[6], and demonstrate a successful ITP determination and purity control of naftidrofuryl and its polar intermediates.

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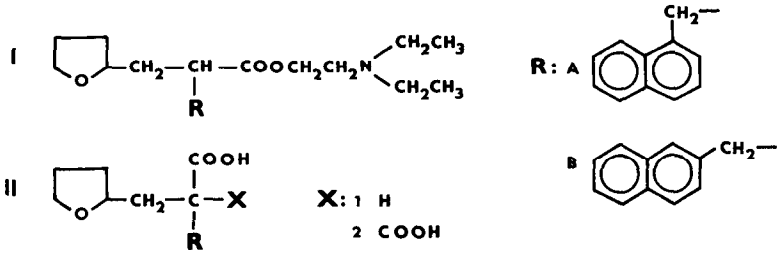
TABLE I

STRUCTURAL FORMULAE OF THE COMPOUNDS INVESTIGATED



Compound	*Abbreviation*	*Name*
I-A	1-NHF	3-(1′-Naphthyl)-2-(2″-tetrahydrofurfuryl)propionate 2‴-N,N-diethylaminoethyl ester (Naftidrofuryl)
I-B	2-NHF	3-(2′-Naphthyl)-2-(2″-tetrahydrofurfuryl)propionate 2‴-N,N-diethylaminoethyl ester
II-A-1	1-NPA	3-(1′-Naphthyl)-2-(2″-tetrahydrofurfuryl)propionic acid
II-B-1	2-NPA	3-(2′-Naphthyl)-2-(2″-tetrahydrofurfuryl)propionic acid
II-A-2	1-NMA	2-(2′-Tetrahydrofurfuryl)-2-(1″-naphthylmethyl)malonic acid
II-B-2	2-NMA	2-(2′-Tetrahydrofurfuryl)-2-(2″-naphthylmethyl)malonic acid

EXPERIMENTAL

Chemicals

Redistilled water was used in the preparation of the electrolyte solutions and of the solutions of the model mixtures. All chemicals were of the highest quality commercially available and were used without any purification: 37% hydrochloric acid, 70% perchloric acid, acetic acid, sodium acetate, histidine (His), 4-morpholinoethanesulphonic acid (Mes), tris(hydroxymethyl)aminomethane (Tris) (Merck, Darmstadt, F.R.G.); 6-aminocaproic acid (EACA) (Sigma, St. Louis, MO, U.S.A.); hydroxypropylmethylcellulose (HPMC) (Dow Chemical, Midland, MI, U.S.A.); β-cyclodextrin (Chinoin, Budapest, Hungary).

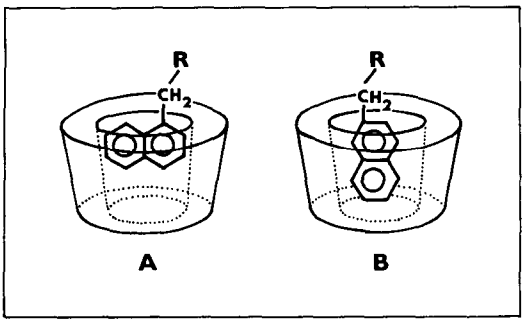


Fig. 1. (A) Equatorial inclusion of a 1-substituted naphthalene; (B) axial inclusion of a 2-substituted naphthalene.

The solutes were obtained from VÚFB (Prague, Czechoslovakia). Their formulae and abbreviations are given in Table I. Standard solutions of 1- and 2-naphthyl isomers of naftidrofuryl hydrogenoxalate (1-NHF and 2-NHF) were prepared by dissolving them in water; the synthetic intermediates 1- and 2-NPA, 1- and 2-NMA were dissolved in about 5 m*M* Tris to 10 m*M*.

Methods

ITP experiments were performed with a Tachophor 2127 (LKB, Bromma, Sweden) equipped with a conductivity detector and a poly(tetrafluoroethylene) (PTFE) capillary. Injections were made with a 10-μl Hamilton syringe. For conditions see Table II.

The concentration of the standard solutes 1-NHF and 2-NHF investigated was determined accurately by non-aqueous titration at a tungsten electrode with a graphite reference electrode and Titroprocessor 636 equipment (Metrohm, Switzerland).

All computations were made on a DS-15 Data Station (Varian, Australia).

TABLE II

ELECTROLYTE SYSTEMS AND CONDITIONS FOR ITP

Leading electrolyte (LE):	I, 5 m*M* sodium acetate including 0.2% HPMC with acetic acid to pH 5.50
	II, 5 m*M* hydrochloric acid including 0.2% HPMC with His to pH 5.00
Terminating electrolyte (TE):	I, 10 m*M* EACA
	II, 5 m*M* MES
Capillary: 400 mm × 0.5 mm I.D.	
Temperature: 18°C	
Detection: conductivity	
Current: anionic mode, 100 μA for 10 min; for detection, 50 μA	
cationic mode, 150 μA for 8 min; for detection, 50 μA	

RESULTS AND DISCUSSION

All the compounds studied are able to form relatively strong inclusion complexes with β-CD, due to the presence of a geometrically advantageous naphthyl group in their molecules. The optimum concentration of β-CD in the leading electrolyte (LE), for all measurements in the cationic and anionic modes, is about 0.5 m*M*. At higher β-CD concentrations, the mobility of the solutes in the ITP steady-state becomes too low and they migrate in the terminator. Lower β-CD concentrations lead to a lost of resolution between 1- and 2-isomers.

Fig. 2 shows the cationic ITP separation of a model mixture of 1-NHF and 2-NHF in electrolyte system I with 0.5 m*M* β-CD added to the LE. The resolution is achieved of both positional isomers, which differ only in the orientation of the naphthyl group. Completely separated and stable zones with sharp boundaries could be seen on the isotachophoregram. The observed relative step heights of 1-NHF and 2-NHF in the same electrolyte system but without β-CD are given in Table III. It can be concluded that without β-CD, the isomers are not separated and remain as a mixed zone.

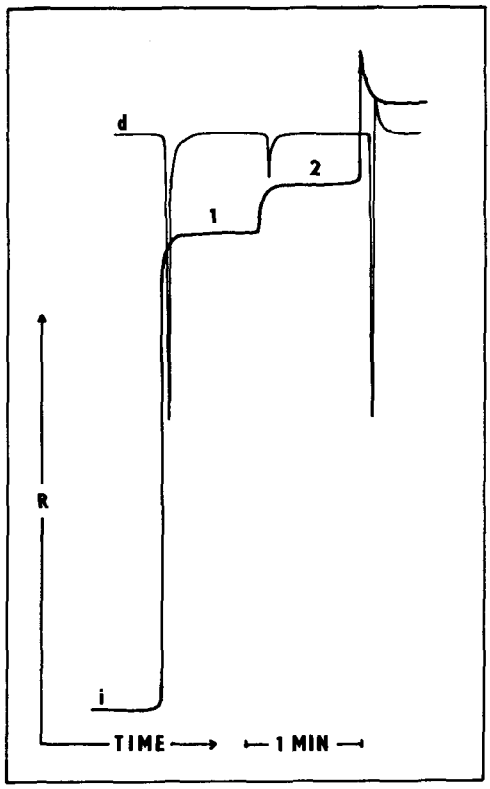


Fig. 2. Isotachophoretic separation of a 1:1 mixture of 1-NHF (1) and 2-NHF (2) in electrolyte system LE I with 0.5 m*M* β-CD and TE I. Injected volume: 5 μl. R = Detector response; i = conductivity signal; d = differentiated conductivity signal.

The anionic mode separation of naftidrofuryl intermediates 1- and 2-NMA, 1- and 2-NPA, expressed also in terms of the $(h_i)_{rel}$ values, is illustrated in Table III. The presence of 0.5 m*M* β-CD in the LE again leads to significant structural differentiation of the positional isomers, while in the system without β-CD resolution is not achieved. The zone boundaries between the pairs of isomers are not as sharp as in the cationic mode, substantially affecting the sensitivity of the method.

TABLE III

RELATIVE HEIGHTS, $(h_i)_{rel}$, OF SOLUTES IN LE WITHOUT (A) AND WITH 0.5 m*M* β-CD (B)

$(h_i)_{rel} = (h_i - h_l)/(h_t - h_l)$ where h_i, h_l and h_t = heights for sample, LE and TE respectively.

Isomer	*NHF* *LE I, TE I*		*NMA* *LE II, TE II*		*NPA* *LE II, TE II*	
	A	*B*	*A*	*B*	*A*	*B*
1-	0.780	0.781	0.622	0.616	0.733	0.784
2-	0.781	0.863	0.625	0.750	0.736	0.853

The cationic system (LE-I and TE-I), modified with β-CD, was used for quantitation of 2-NHF in real naftidrofuryl samples. The calibration results are presented in Table IV. The linearity and negligible *A* coefficient (intercept) of the calibration graph indicate suitable ITP conditions for the quantitative analysis. The differences between the calibration slopes for 1-NHF and 2-NHF are not statistically significant and thus it is possible to use calibration parameters for 1-NHF for the evaluation of the content of 2-NHF. The sensitivity of the ITP determination, dependent mainly by the sharpness of the zone boundaries, was tested on model mixtures with different ratios of the two isomers. These measurements showed that it is possible to detect even 0.1% of 2-NHF in the presence of 99.9% of the 1-isomer. The determination was verified on eleven different samples of naftidrofuryl, but only one sample contained 0.5% of 2-NHF as an impurity.

TABLE IV

CALIBRATION DATA

Calculations were made at $\alpha = 0.05$; number of calibration points, $n = 6$.

	1-NHF	*2-NHF*
Injected amount	0.811–4.866 μg (1–6 μl)	0.814–4.884 μg (1–6 μl)
Standard deviation, *s*	0.288	0.325
Intercept, *A*	−3.30 ± 0.744 s	0.88 ± 0.840 s
Slope, *B*	13.18 ± 0.236 s μg^{-1}	13.04 ± 0.265 s μg^{-1}
Correlation coefficient, *R*	0.9999	0.9999

CONCLUSIONS

The ITP resolution of pairs of isomers, using β-CD as an LE additive in both the cationic and anionic modes, confirms theoretically predicted differences between the stability of equatorial and axial inclusion complexes. It can be concluded from the separation pattern that axial 2-isomer–β-CD complexes are more stable than equatorial 1-isomer–β-CD ones.

β-CD, as a complex-forming agent, may be used in the cationic or anionic ITP mode. The experiments revealed comparable stereospecific differentiation of positional isomers, irrespective of their charge sign.

The ITP determination of 2-NHF in naftidrofuryl described is of practical analytical significance. The method enables an accurate and fast purity control without any sample pre-treatment.

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