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Selected applications of cyclodextrin selectors in capillary electrophoresis

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ABSTRACT

Through the use of α-, β-, γ- and heptakis(2,6-di-O-methyl)-β-cyclodextrin as stereospecific selectors or electrolyte modifiers, both in capillary zone electrophoresis and isotachophoresis, selected model isomeric compounds (including optical isomers) were resolved. Soluble alkylhydroxyalkylcellulose derivatives were further added to the cyclodextrin-modified background electrolytes under study. Their presence was found to be essential, as demonstrated by improvements in both enantioselectivity and separation efficiency. The results obtained in both electrophoretic modes, under optimized conditions, are compared and discussed.

INTRODUCTION

Cyclodextrin-based complexation phenomena have recently received considerable attention in chromatography and electrophoresis. Through their use, both migration rates and the separation efficiency for various isomeric and structurally related compounds are known to be affected. The corresponding gains in resolution of isomers translate directly to both qualitative and quantitative aspects of such separations. These beneficial phenomena are due to the remarkable capacity of cyclodextrins (CDs) to include selectively, under appropriate experimental conditions, a wide variety of guest neutral molecules or ions within their hydrophobic cavity. Alternatively, additional stereospecific conditions can often be created. During the last decade, most known properties of CDs have been generally explored in analytical electromigration methods.

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The first successful uses of CDs and/or their derivatives were reported in isotachophoresis (ITP) of anionic species [1], positional isomers [2] and enantiomeric pairs [3], while other forms of capillary electrophoresis (CE) have also been explored for similar purposes [4–7]. The applications of cyclodextrins in CE have recently been reviewed [8,9].

The purpose of this paper is to demonstrate and compare the merits of ITP and other CE-based procedures with certain selected positional isomers (benzene- and naphthalene-based structures) and the enantiomers of chloramphenicol, thioridazine and ketotifen drugs. The roles of a modifier type and its concentration in the separation effect are emphasized.

EXPERIMENTAL

Distilled water, deionized with a Laboratory Water System XLDRO 1002 apparatus (Liquipure Europe, UK), was used for the preparation of the electrolyte solutions. All chemicals were of the highest quality commercially available: sodium acetate (NaAc), acetic acid (HAc), citric acid (CAc), hydrochloric acid and phosphoric acid (Merck, Darmstadt, Germany); β-alanine (β-Ala), methylhydroxyethylcellulose 3000 or 30 000 (MHEC), methylhydroxypropylcellulose 15 000 (HPMC) and hydroxyethylcellulose (HEC) (Serva, Heidelberg, Germany); 2-(N-morpholino)ethanesulfonic acid (MES), tris(hydroxymethyl)aminomethane (Tris), N-tris(hydroxymethyl)methylglycine (Tricine), α-cyclodextrin (α-CD), β-cyclodextrin (β-CD), γ-cyclodextrin (γ-CD), heptakis(2,6-di-O-methyl)-β-cyclodextrin (DM-β-CD), methanol and Amberlite MB-3 (Sigma, St. Louis, MO, USA); and 6-aminocaproic acid (EACA), benzoic acid (B), the positional isomers of iodobenzoic acid, the isomers of methoxyphenylacetic acid and naphthyl phosphate (Aldrich, Milwaukee, WI, USA). The samples of all drugs and their structurally related enantiomers and/or racemates were obtained from the Research Institute for Pharmacy and Biochemistry (Prague, Czechoslovakia).

Cyclodextrins and hydroxyalkylcellulose solutions were purified using Amberlite MB-3 mixed-bed ion-exchange resin. Stock solutions of analytes were prepared by dissolving each substance in methanol (*ca.* 1 mg ml^{-1}) and were stored in the dark under refrigeration. The structural formulae, names and abbreviations used for the analytes are given in Fig. 1 and Table I.

Methods

The isotachophoretic experiments were performed with a Tachophor 2127 (LKB, Bromma, Sweden) equipped with a conductivity detector and a poly(tetrafluoroethylene) capillary of 0.5 mm I.D. Samples were injected with a 10-μl microsyringe (Hamilton, Bonaduz, Switzerland). Additional operating conditions are given in the captions.

The experiments performed in the capillary zone electrophoretic (CZE) mode were made with a laboratory-made system similar to that described by Jorgenson and Lukacs [10]. The high-voltage power supply (0–30 kV) was a product of Spellman High Voltage Electronics (Plainview, NY, USA). Each end of the fused-silica capillary was placed in a small glass reservoir containing the appropriate platinum electrode connected to the power supply. A Jasco UVIDEC-100-IV detector from Japan

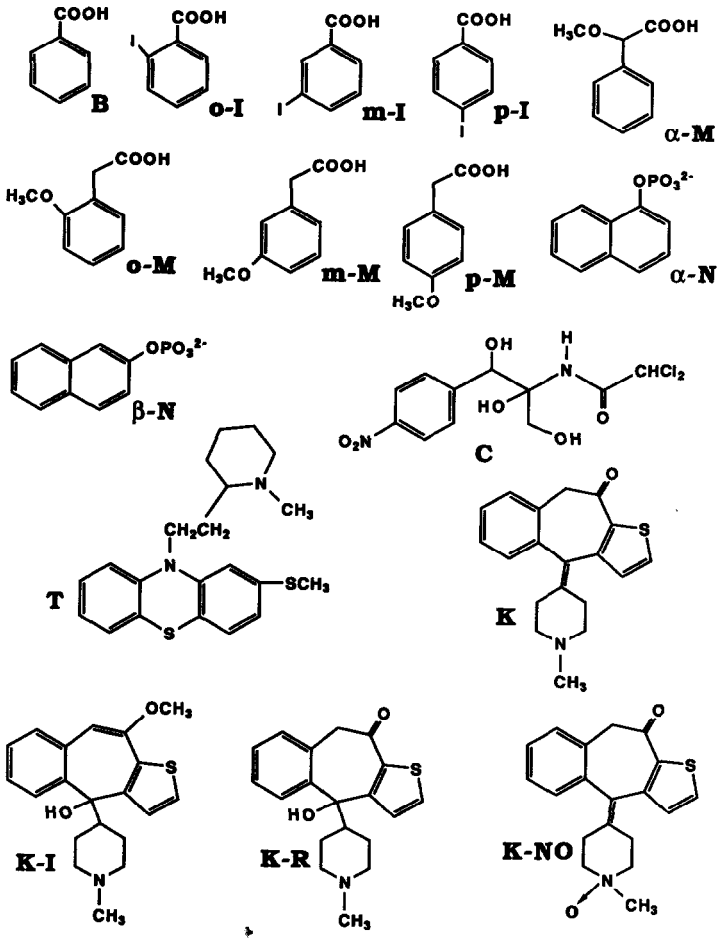

Fig. 1. Analyte structures. For abbreviations, see Table I.

Spectroscopic (Tokyo, Japan) operated at 254 nm was used throughout. An on-line optical detection cell was prepared by removing the polyimide coating from a short segment of the fused-silica capillary. Finally, the system, excluding the detector, was enclosed in a Plexiglas box with an interlock system to protect the operator.

Capillaries (49 μm I.D. × 186 μm O.D.) were purchased from Polymicro Technologies (Phoenix, AZ, USA). The capillary lengths ranged from 50 to 65 cm. The columns were pretreated overnight with 85% phosphoric acid diluted (1:1) with water. The capillary was further rinsed with water (5 min), followed by equilibration with a running buffer (5 min) by means of a vacuum procedure and then electroosmotic pumping for *ca.* 30–45 min at the beginning of each working day. The capillary was rinsed with a running buffer each time (before a run) for 1–2 min. Hydrodynamic sample introduction was utilized in all experiments.

TABLE I

ANALYTE NAMES AND ABBREVIATIONS

Abbreviation	Name
B	Benzoic acid
C	Chloramphenicol; *threo*-2,2-dichloro-N-[β-hydroxy-α-(hydroxymethyl)-β-(4-nitrophenyl)-ethyl]acetamide
o-I	*o*-Iodobenzoic acid; 2-iodobenzoic acid
m-I	*m*-Iodobenzoic acid; 3-iodobenzoic acid
p-I	*p*-Iodobenzoic acid; 4-iodobenzoic acid
K	Ketotifen; 4-(1-methylpiperidylidene)-4H-benzo[4,5]cyclohepta[1,2-*b*]thiophen-10(9H)-one
K-I	A synthesis intermediate of K; 10-methoxy-4-(1-methyl-4-piperidyl)-4H-benzo[4,5]cyclohepta[1,2-*b*]thiophen-4-ol
K-NO	N-Oxide of K; 4-(1-methylpiperidylidene-N-Oxide)-4H-benzo[4,5]cyclohepta[1,2-*b*]-thiophen-10(9H)-one
K-R	A compound structurally related to K; 4-(1-methyl-4-piperidyl)-4H-benzo[4,5]-cyclohepta[1,2-*b*]thiophen-10(9H)-one
α-M	α-Methoxyphenylacetic acid; O-methylmandelic acid
o-M	*o*-Methoxyphenylacetic acid; 2-methoxyphenylacetic acid
m-M	*m*-Methoxyphenylacetic acid; 3-methoxyphenylacetic acid
p-M	*p*-Methoxyphenylacetic acid; 4-methoxyphenylacetic acid
α-N	α-Naphthyl phosphate; 1-naphthyl phosphate
β-N	β-Naphthyl phosphate; 2-naphthyl phosphate
T	Thioridazine; 10-[2-(1-methyl-2-piperidyl)ethyl]-2-(methylthio)phenothiazine

RESULTS AND DISCUSSION

Separation of positional isomers

Owing to varying interactions with their hydrophobic cavities, cyclodextrins possess the remarkable capability of separating certain types of aromatic positional isomers. The overall molecular shape, determined by different substitutions on the aromatic ring structure, is a major factor affecting the migration order of these solutes.

As a model system, positional isomers of iodobenzoic acids were chosen to be separated by ITP. The objective was to compare separations of a mixture of *o*-I, *m*-I, *p*-I and B (Table I) carried out in conventional electrolyte systems with the separations accomplished in the CD-modified systems. Resolution was observed as a function of a CD concentration for a given sample mixture. Fig. 2 shows the correlation between the relative step-height, $(h_i)_r$, of the analytes and the concentration of α-CD in the leading electrolyte (LE). The $(h_i)_r$ value is used here as a solute migration characteristic and is defined by

$$(h_i)_r = h_i - h_L/h_T - h_L \quad (1)$$

where h_i, h_L and h_T are the step-heights of sample, leading electrolyte (LE) and terminator (TE) ions, respectively. Resolution of isomers could not be achieved in a slightly acidic non-modified LE [see ITP record (b) in Fig. 2]. Addition of α-CD resulted in a significant resolution improvement, as illustrated in ITP record (a). With an in-

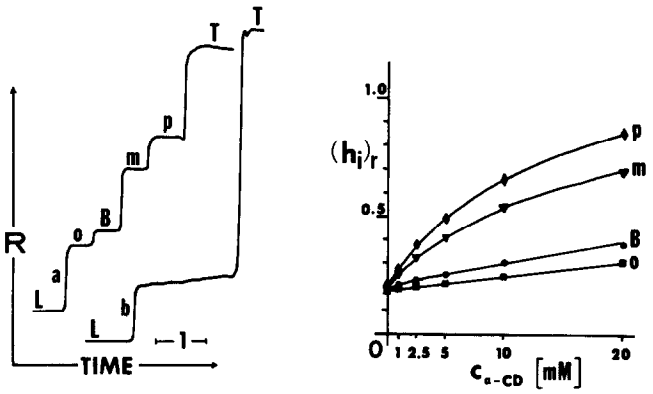

Fig. 2. Effect of α-CD on the ITP separation and $(h_i)_r$ values of (o) *o*-, (m) *m*- and (p) *p*-iodobenzoate and benzoate (B) anions. Concentration of α-CD in LE: record (a) 10 m*M*; (b) 0 m*M*. LE, 5 m*M* HCl + EACA to pH 4.70 + 0.2% HPMC; TE, 5 m*M* MES (pH 5.50); capillary, 400 cm; current, 100 μA (9 min), for detection 50 μA. *R* = conductivity response; L = leading zone; T = terminating zone; $c_{\alpha\text{-CD}}$ = concentration of α-CD in LE; 1 = 1 min.

creased α-CD concentration in LE (in the range 0–20 m*M* α-CD), the separation further improved.

Further separation possibilities with positional isomers are demonstrated under CZE conditions with methoxyphenyl acetates (Fig. 3) and naphthyl phosphates (Fig. 4). In both instances, overlapping peaks of these isomers are shown while using unmodified background electrolytes (BE) in Fig. 3a and Fig. 4 (record 0). Two additives, α-CD and β-CD, proved to be effective and suitable for resolution of both isomeric types: methoxyphenylacetate ions were successfully separated by a β-CD-modified BE (Fig. 3b), while naphthyl phosphates migrated apart owing to α-CD. By increasing the CD concentration, it was possible to achieve a better separation of each isomer type, as seen from the comparison of electropherograms 10 and 20 in Fig. 4.

The remaining questions concern the type of interaction between the model

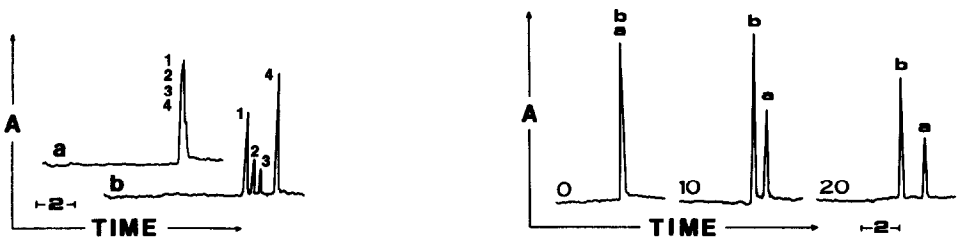

Fig. 3. CD-based CZE separation of (1) *o*-, (3) m-, (4) *p*- and (2) α-methoxyphenylacetates. Record (a) without CD; (b) with 10 m*M* β-CD. BE, 50 m*M* Tris + 50 m*M* Tricine (pH 8.06) + 0.1% MHEC; voltage, 16 kV; current, 7 μA; capillary, 50 cm (35 cm to the detector). *A* = response of UV detector (254 nm); 2 = 2 min.

Fig. 4. CD-based CZE separation of (a) α- and (b) β-naphthylphosphate anions. Record 0, without CD, 10 and 20 with 10 and 20 m*M* α-CD, respectively. BE, 50 m*M* Tris + Tricine to pH 8.50 + 0.1% MHEC; capillary, 50 cm (35 cm to the detector); voltage, 20 kV; current, 9 μA. *A* = response of UV detector (254 nm); 2 = 2 min.

solutes and the CDs. Generally proposed separation mechanisms based on the differences between the stabilities of equatorial and axial CD inclusion complexes for 1- and 2-naphthyl derivatives [11,12] do not seem applicable in this case, as the molecular dimensions of α-CD and the solutes under study are not compatible.

Chiral separations

Separation of enantiomers has traditionally been viewed as one of the more difficult problems in separation science. Such isomeric pairs are not easily separated from each other by any separation method, as their Gibbs free energy differences (ΔG) are zero. In order to enhance the ΔG values between enantiomers, the formation of diastereomers, or at least diastereomeric complexes, has often been advocated. Cyclodextrins (chiral compounds themselves) have been proposed in various separation techniques to cover a reasonable range of optical isomers.

Our ITP and CZE experiments with CD-modified electrolytes confirm the general importance of the cyclodextrin cavity size [8]. In addition, chemical modification of the hydroxyl groups on the cavity collar and the pH value of the buffer system used for the chiral separation may also play significant roles.

In certain instances, enantioselectivity and the separation efficiency can also be influenced by adding soluble alkylhydroxyalkylcellulose derivatives to the CD-modified electrolytes. The effect of MHEC on the CD-based chiral separation of the isomers of chloramphenicol is shown in Fig. 5. In this instance, complete baseline resolution of the enantiomers was achieved only in the electrolytes containing MHEC (Fig. 5a *vs.* b). In various applications of pharmaceutical interest, a complete sep-

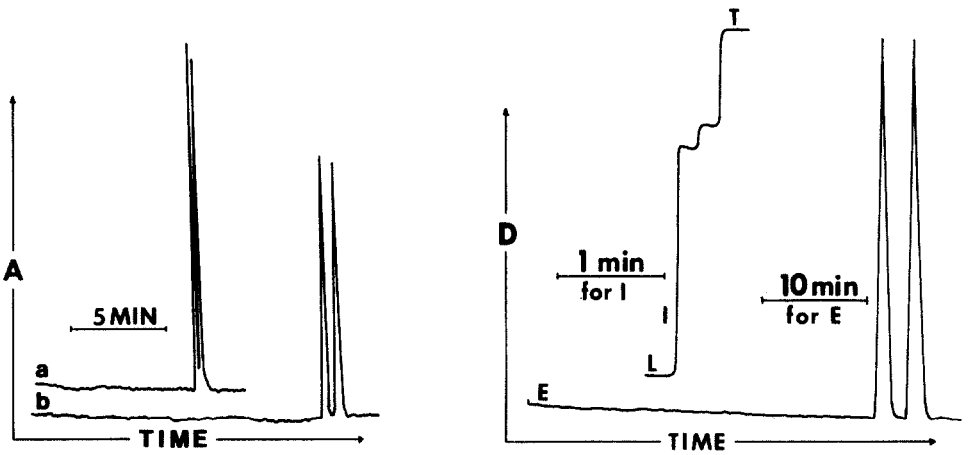

Fig. 5. Influence of MHEC on the chiral separation of chloramphenicol drug enantiomers with cyclodextrins. Record (a) without MHEC; (b) with 0.1% MHEC. BE, 20 m*M* Tris + CAc to pH 3.50 + 10 m*M* DM-β-CD; capillary, 65 cm (45 cm to the detector); voltage 18 kV; current, 6 μA. *A* = response of UV detector (254 nm).

Fig. 6. (I) ITP and (E) CZE separations of thioridazine enantiomers. ITP conditions: capillary, 22 cm; LE, 10 m*M* NaAc + HAc to pH 5.47 + 0.08% HEC + 5 m*M* γ-CD; TE, 10 m*M* β-Ala; current, 100 μA (13 min), 50 μA for detection. L = leading zone; T = terminating zone. CZE conditions: capillary, 65 cm (45 cm to the detector); BE, 20 m*M* Tris + H_3PO_4 to pH 2.50 + 5 m*M* γ-CD; voltage, 18 kV; current, 14 μA. D = response of the conductivity detector (for I) and UV detector (254 nm) (for E).

aration is often necessary for micropreparative recovery and characterization of the chiral metabolites.

Another class of optical isomers resolvable by both CZE and ITP are compounds with a tricyclic (aromatic and heterocyclic) structure. The ITP and CZE separations of a chiral phenothiazine drug, thioridazine, with the aid of γ-CD, are shown in Fig. 6. Both methods appear to offer a very good separation of enantiomers, but the advantage of ITP separation is that the separation time is at least three times shorter.

Separation of the optical isomers of ketotifene (K) and the enantiomers of structurally related compounds (K-I, K-R and K-NO) is illustrated in Figs. 7 and 8. Fig. 7 shows the effectiveness of ITP and CZE for the resolution of K enantiomers by β-CD-modified buffers. No optimum conditions were found for the ITP separation of K and K-I from each other in one analytical run. This separation is important from the standpoint of monitoring the chiral metabolism and the synthesis of ketotifen. Only under CZE conditions with a γ-CD-modified BE (Fig. 8a) did such a complete separation become feasible.

A well-known limitation of ITP is the requirement to find an appropriate terminating electrolyte. For example, the separation of components with relatively low net electrophoretic mobilities appears tedious. We experienced difficulties in finding appropriate conditions for optimum ITP migration of K-NO for this reason. While K-R had a suitable electrophoretic mobility, no chiral resolution was obtained with ITP. This was probably due to excessive and ineffective complexation with γ-CD in the ITP measurements. However, in CZE with the buffers containing small amounts of γ- or β-CD, all analyte enantiomers were easily separable (Fig. 8).

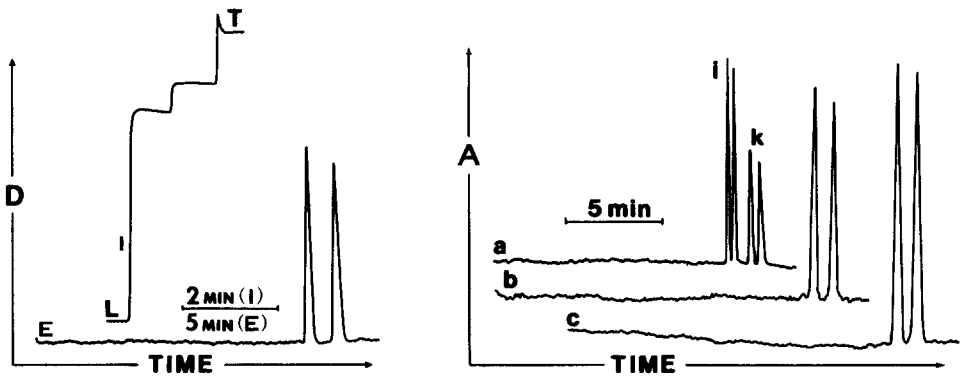

Fig. 7. (I) ITP and (E) CZE separations of ketotifen enantiomers. ITP conditions: capillary, 37 cm; LE, 5 m*M* NaAc + HAc to pH 5.50 + 4 m*M* β-CD + 0.2% HEC; TE, 10 m*M* β-Ala; current, 150 μA (9 min), 50 μA for detection. L = leading zone and T = terminating zone. CZE conditions: capillary, 65 cm (45 cm to the detector); BE, 20 m*M* Tris + CAc to pH 3.50 + 10 m*M* β-CD + 0.05% MHEC; voltage, 24 kV; current, 9 μA. D = response of the conductivity detector (for I) and UV detector (254 nm) (for E).

Fig. 8. CZE chiral separation of ketotifen (K) and its synthesic intermediate, K-I (i) [record (a)] and structurally related compounds, K-R [record (b)] and N-oxide, K-NO [record (c)]. Capillary, 65 cm [50 cm (a) and 45 cm (b and c) to the detector] (a) BE, 20 m*M* Tris + CAc to pH 3.75 + 10 m*M* γ-CD; voltage, 24 kV; current, 7.5 μA. (b) BE, 20 m*M* Tris + CAc to pH 3.50 + 20 m*M* γ-CD; voltage, 24 kV; current, 7 μA. (c) BE, 20 m*M* Tris + H_3PO_4 to pH 2.50 + 10 m*M* β-CD; voltage, 18 kV; current, 12.5 μA. *A* = response of UV detector (254 nm).

CONCLUSIONS

It appears that the complexation of various solutes with CDs may occur by slightly different mechanisms. Minor changes in the molecular shape of the solutes as determined by different substitutions, or even a variable space orientation of a substituent group, may give rise to different solute–CD complex stabilities and net electrophoretic mobilities. While much remains to be elucidated about such interactions, CD additives in both ITP and CZE have become useful in the separation of close isomers.

Both electromigration techniques employed here have distinct advantages and disadvantages. For example, ITP appears more suitable than CZE for the determination, or at least preconcentration, of minute components in a large excess of other mixture components. Unlike in CZE, it is feasible to work with larger sampling volumes in ITP. However, CZE is definitely preferable wherever high separation efficiencies are required. In addition, this mode of operation is also advantageous when working within a wide range of operating conditions, *e.g.*, in micellar electrokinetic chromatography.

Whereas the use of CD additives in electrophoresis is relatively recent, the results obtained for isomeric compounds in this laboratory and others rival the best separations reported using other separation methods.

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REFERENCES

1 M. Tazaki, M. Takagi and K. Ueno, *Chem. Lett.*, (1982) 639.
2 M. Tazaki, T. Hayashita, T. Y. Fujino and M. Takagi, *Bull. Chem. Soc. Jpn.*, 59 (1986) 3469.
3 J. Snopek, I. Jelinek and E. Smolkova-Keulemansova, *J. Chromatogr.*, 438 (1988) 211.
4 S. Terabe, H. Ozaki, K. Otsuka and T. Ando, *J. Chromatogr.*, 322 (1985) 211.
5 A. Guttman, A. Paulus, A. S. Cohen, N. Grinberg and B. L. Karger, *J. Chromatogr.*, 448 (1988) 41.
6 S. Fanali, *J. Chromatogr.*, 474 (1989) 441.
7 J. Liu, K. A. Cobb and M. Novotny, *J. Chromatogr.*, 519 (1990) 189.
8 J. Snopek, I. Jelinek, and E. Smolkova-Keulemansova, *J. Chromatogr.*, 452 (1988) 571.
9 J. Snopek and E. Smolkova-Keulemansova, in D. Duchene (Editor), *New Trends in Cyclodextrins and Derivatives*, De Sante, Paris, 1991, in press.
10 J. W. Jorgenson and K. Lukacs, *Anal. Chem.*, 58 (1981) 1928.
11 J. Szejtli, *Cyclodextrins and Their Inclusion Complexes*, Akadémiai Kiadó, Budapest, 1982.
12 K. Harata and H. Uedaira, *Bull. Chem. Soc. Jpn.* 48 (1975) 375.