Fresenius J Anal Chem (2001) 369 : 666–669

SPECIAL ISSUE PAPER

Martin Pumera · Ivan Jelínek · Jindřich Jindřich

Determination of cyclodextrins and their derivatives by capillary electrophoresis with indirect UV and conductivity detection

Received: 27 September 2000 / Revised: 3 January 2001 / Accepted: 5 January 2001

Abstract A fast and simple capillary electrophoretic method suitable for the determination of native α-, β-, γ-cyclodextrins, their randomly substituted *tert*-butyl derivatives (average degree of substitution 3.8 – 4.4), heptakis (2,6-di-*O*-methyl)- and heptakis (2,3,6-tri-*O*-methyl)-β-cyclodextrin was developed. Naphthyl-2-sulfonic acid (2-NSA), 3-iodobenzoic acid (3-IBA) and (1*S*)-1-phenylethylamine (PHEA) were tested as selective complex forming and UV absorbing background electrolyte additives. The composition of optimized background electrolyte for the separation of uncharged cyclodextrins and their derivatives was: 15 mM 3-iodobenzoic acid titrated with tris[hydroxymethyl]aminomethane to pH 8.0, 5% (v/v) of acetonitrile. A complete resolution of mono-2-*O*-, mono-3-*O*- and mono-6-*O*-carboxymethyl-β-cyclodextrin regioisomers was achieved in the optimized background electrolyte system: 40 mM PHEA titrated with 2-[N-morpholino]ethanesulfonic acid to pH 5.6. In addition to indirect UV detection a contactless conductometric detector was successfully utilized.

1 Introduction

Cyclodextrins (CDs) are cyclic oligosaccharides composed of D(+)glucopyranose units with the shape of a torus. Due to their unique ability to form inclusion complexes with numerous compounds CDs are frequently applied in various fields of chemistry. Besides natural cyclodextrins a growing number of semi-synthetic derivatives and copolymers has been prepared and is already commercially available. Many of them found their use in analytical chemistry as structural and chiral selectors with new properties given by the type and number of substituents [1–3]. Besides CDs and their derivatives CD modified stationary phases and pseudophases have been successfully utilized in chromatography and electrophoresis. A growing interest in cyclodextrin chemistry stimulates the development of reliable, selective and sensitive analytical methods suitable for their determination [4, 5].

CDs are difficult to determine as they are uncharged and possess no appreciable spectral and electrochemical properties. Furthermore, the reactivity of these compounds is rather unspecific resulting in difficult labeling properties. Methods previously used for the determination of CDs in mixtures include paper chromatography [6], thin-layer chromatography [7], liquid chromatography [8–10] and gas chromatography [11]. In general, poor sensitivity and selectivity and long time of analysis are characteristic for these chromatographic assays.

Several attempts to utilize capillary electrophoresis (CE) for the determination of CDs have been made. Nardi et al. [12] demonstrated the separation of α-, β- and γ-CD in the mixture using benzoate as a selective complex forming and UV absorbing constituent of the background electrolyte (BGE). Analogously, Penn et al. [13] separated α-, β-, γ-CD and β-CD methylderivatives with different degrees of substitution using 2-aminonaphthalene-6-sulfonic acid as a complex forming and fluorescent additive. Fang et al. [14] separated α-, β-, γ-CD and mono-2-O-hydroxypropyl-β-CD and β-CD in ionized form using strongly alkaline buffer. Although the use of CE represents a suitable alternative to chromatographic methods, described assays are still far from optimum from the point of simplicity, sensitivity and selectivity.

The aim of this work was to develop an alternative for the assay of CD based on CE, with improved sensitivity and selectivity towards α-, β-, γ-CD, their uncharged methyl, and tert.butyl derivatives, and a new method to prepare regioselective mono-*O*-carboxymethyl derivatives of β-CD. Alternative complex forming and UV-absorbing background electrolyte additives, ensuring im-

M. Pumera (✉) · I. Jelínek
Department of Analytical Chemistry, Charles University,
Faculty of Science, Albertov 2030,
CZ-128 43 Prague 2, Czech Republic
e-mail: mpumera@email.cz

J. Jindřich
Department of Organic Chemistry, Charles University,
Faculty of Science, Albertov 2030,
CZ-128 43 Prague 2, Czech Republic

proved resolution and better sensitivity, have been tested. Both indirect photometric and conductometric detection techniques have been utilized and compared.

2 Experimental

2.1 Materials

The substances of α-CD, β-CD, γ-CD, heptakis (2,6-di-*O*-methyl)-β-CD (DM-β-CD), heptakis (2,3,6-tri-*O*-methyl)-β-CD (TM-β-CD), 2-[N-morpholino]ethanesulfonic acid (MES), tris[hydroxymethyl]aminomethane (TRIS), benzoic acid (BA), 3-iodobenzoic acid (3-IBA) and (1S)-1-phenylethylamine (1S-FEA) were of the highest grade commercially available obtained from Sigma-Aldrich (St. Louis, MO, USA). Randomly substituted *tert*-butyl cyclodextrins [15], t*ert*-butyl-α-CD (TB-α-CD; substitution degree (NMR) 3.8), *tert*-butyl-β-CD (TB-β-CD; substitution degree 4.5), *tert*-butyl-γ-CD (TB-γ-CD; substitution degree 4.4) and regioselective [16] mono-2-*O*-carboxymethyl-β-CD (2-*O*-CM-β-CD), mono-3-*O*-carboxymethyl-β-CD (3-*O*-CM-β-CD) and mono-6-*O*-carboxymethyl-β-CD (6-*O*-CM-β-CD) were prepared and characterized by NMR at the Department of Organic Chemistry, Charles University, Prague, Czech Republic. Deionized water was obtained from a Milli-Q water purification system (Millipore, Bedford, MA, USA).

2.2 Instrumentation and used experimental conditions

Capillary electrophoresis was performed on a Crystal CE system (ATI Unicam, Great Britain) with a laboratory made dual photometric-contactless conductometric detector for capillary electrophoresis [17] in fused silica capillary tube 63.0 cm × 75 μm i.d. (Composite Metal Services Ltd., UK). The distance to the detector was 50.0 cm. Sample solutions were injected at a pressure 500 Pa for 12 s. The separation voltage varied from 12 kV to 30 kV, which typically generated a capillary current of about 9.2–66.4 μA. All measurements were made at the temperature 30 °C. Detailed composition of background electrolyte systems is specified in the following text.

3 Results and discussion

3.1 Separation of uncharged CDs and their derivatives

The basic approach for the separation of uncharged cyclodextrins and their derivatives, utilizing complex forming buffer additive, has been taken from [12]. Based on the preliminary knowledge of stability constants with cyclodextrins, alternative complex forming and UV absorbing agents [4, 18], naphthalene-2-sulfonic acid (2-NSA) and 3-iodobenzoic acid (3-IBA), were tested.

3.1.1 The influence of 2-NSA

The use of 2-NSA as background electrolyte additive makes it possible to complex γ-CD more effectively than previously used benzoic acid [12] and shift its peak from the region of injection peak. The order of effective mobilities (μ_{CDB}-) of α-, β- and γ-CD inclusion complexes was always $|\mu_{\beta\text{-CDB}}| > |\mu_{\gamma\text{-CDB}}| > |\mu_{\alpha\text{-CDB}}|$ in BGE containing 2-NSA titrated with TRIS to pH 7.8. Significant increase of electrophoretic mobilities with increasing 2-NSA concentration indicates the formation of stable β- and γ-CD complexes, while the mobility of the very weak α-CD complex remains almost unchanged. The problem of extremely low mobility of α-CD due to weak complex formation has been solved by the addition of benzoic acid as a second additive with higher affinity towards α-CD. The formation of more stable and probably less soluble complexes of β- and γ-CD with 2-NSA results in significant tailing of the observed peaks. In order to solve this problem the addition of acetonitrile to the background electrolyte was tested and its concentration optimized from the point of view of peak symmetry and maximum resolution of migrating complexes. Based on the factorial design optimization procedure [19] the following separation conditions were optimum: 15 mM 2-NSA, 5 mM BA titrated by TRIS to pH 7.8 with 10% (v/v) ACN. The corresponding electropherogram of the α-, β- and γ-CD mixture in optimized BGE is shown in Fig. 1.

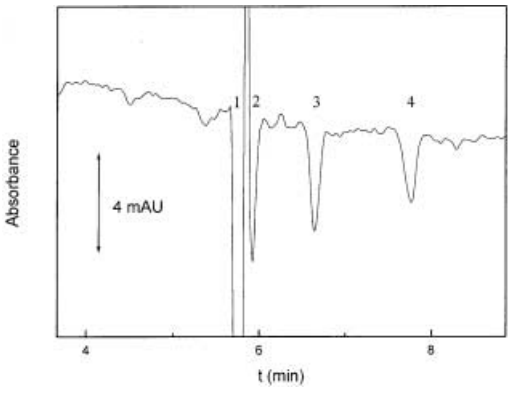

Fig. 1 The separation of a model mixture of α-, β- and γ-CD (3.3×10^{-3} M each) in BGE containing 15 mM 2-NSA, 5 mM BA and 10% ACN. Separation voltage 30 kV, I = 66.4 μA, photometric detection at 254 nm; *1* solvent peak, *2* α-CD, *3* γ-CD, *4* β-CD

3.1.2 The influence of 3-IBA

Based on [20], 3-IBA was selected as a complex forming and UV absorbing background electrolyte additive ensuring effective and selective complex formation of α-, β- and γ-CD. The order of effective mobilities (μ_{CDB}-) of α-, β- and γ-CD inclusion complexes was always $|\mu_{\alpha\text{-CDB}}| > |\mu_{\beta\text{-CDB}}| > |\mu_{\gamma\text{-CDB}}|$ in BGE containing 3-IBA titrated with TRIS to pH 8.0. Significant increase of electrophoretic mobilities with increasing 3-IBA concentration indicates the formation of sufficiently stable α-, β- and γ-CD complexes. From the above experiments, the concentration of 3-IBA 15 mM was estimated as optimal ensuring sufficient resolution and high sensitivity of indirect UV detection. Undesirable peak tailing due to lower solubility of formed complexes was eliminated by the addition of ACN to background electrolyte. Corresponding electropherograms in BGE without ACN (trace A) and with ACN added in concentrations 5 – 15% (v/v) (traces B, C, D) are shown in Fig. 2. With increasing ACN concentration the observed peaks loose their asymmetry. The con-

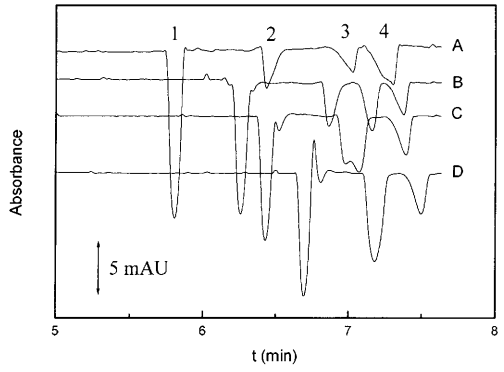

Fig. 2 The separation of a model mixture of α-, β- and γ-CD (3.3×10^{-3} M each) in BGE containing 15 mM 3-IBA and 0% ACN (trace A), 5% ACN (trace B), 10% ACN (trace C) and 15% ACN (trace D). Separation voltage 15 kV, I = 9.2 μA, photometric detection at 254 nm; *1* solvent peak, *2* γ-CD; *3* β-CD; *4* α-CD

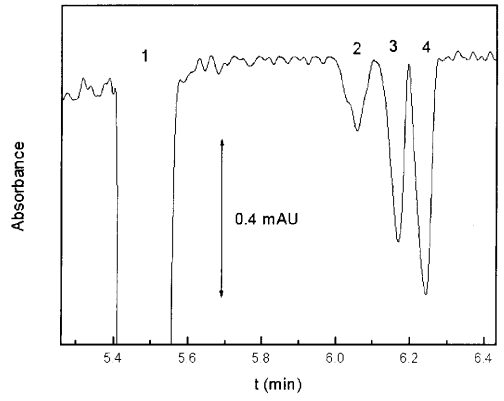

Fig. 3 The separation of a model mixture of TB-α-, TB-β- and TB-γ-CD (8.0×10^{-4} M each) in BGE containing 15 mM 3-IBA and 5% ACN. Separation voltage 15 kV, I = 9.2 μA, photometric detection at 254 nm; *1* solvent peak, *2* TB-γ-CD; *3* TB-β-CD; *4* TB-α-CD

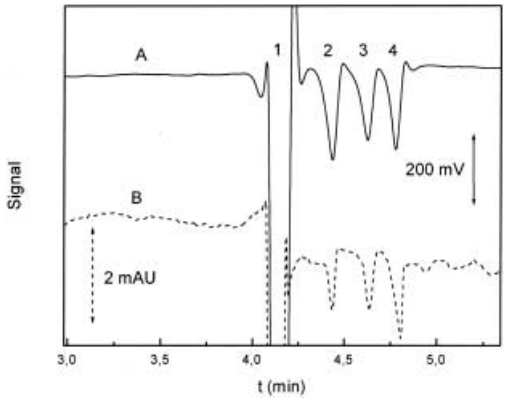

Fig. 4 The separation of a model mixture of DM-β-CD, TM-β-CD and β-CD (1.0×10^{-3} M each) in BGE containing 15 mM 3-IBA and 5% ACN. Separation voltage 15 kV, I = 9.2 μA, photometric detection at 254 nm; frequence of conductivity detector, 200 kHz. Trace *A* – conductivity detection, trace *B* – UV-Vis detection; *1* solvent peak, *2* TM-β-CD; *3* DM-β-CD; *4* β-CD

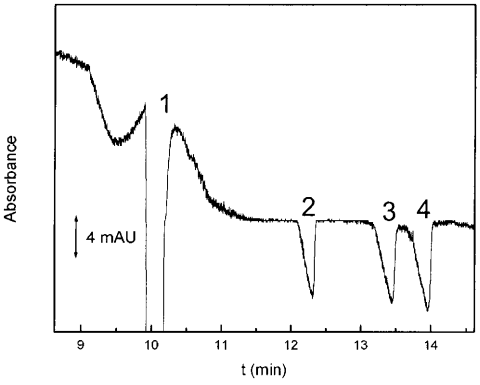

Fig. 5 The separation of a model mixture of 2-*O*-CM-β-CD, 3-*O*-CM-β-CD and 6-*O*-CM-β-CD (2.0×10^{-3} M each) in BGE containing 40 mM PHEA. Separation voltage 15 kV, I = 51.3 μA, photometric detection at 210 nm; *1* solvent peak, *2* 3-*O*-CM-β-CD *3* 2-*O*-CM-β-CD, *4* 6-*O*-CM-β-CD

centration 5% ACN is considered as optimal. Higher studied concentrations led to a lost of resolution between β- and γ-CD complexes due to a decrease of the bindings constants caused by competition in the complexation ACN/3-IBA with CDs.

Optimized BGE, consisting of 15 mM 3-IBA titrated with TRIS to pH 8.0, 5% (v/v) ACN added, was successfully used for the determination of other uncharged CD derivatives. Complete separation of TB-α-CD, TB-β-CD and TB-γ-CD (Fig. 3) and β-CD, DM-β-CD and TM-β-CD (Fig. 4) was achieved.

3.2 Separation of charged CD derivatives

Determined 2-*O*-CM-β-CD, 3-*O*-CM-β-CD and 6-*O*-CM-β-CD regioisomers show only minor differences in pK values, therefore, their electrophoretic separation without the use of selective complex forming agent is impossible. In order to achieve maximum mobility differences between the anionic CD derivatives determined and the complex forming agent, cationic PHEA was selected as a buffer additive. The order of effective mobilities (μ_{CDA}) of 2-*O*-CM-β-CD, 3-*O*-CM-β-CD and 6-*O*-CM-β-CD inclusion complexes was always $|\mu_{6\text{-}O\text{-CM-}\beta\text{-CDA}}| > |\mu_{2\text{-}O\text{-CM-}\beta\text{-CDA}}| > |\mu_{3\text{-}O\text{-CM-}\beta\text{-CDA}}|$ in BGE containing PHEA titrated with MES to pH 5.6. As a compromise between achieved resolution and sensitivity of indirect UV detection the concentration 40 mM of PHEA was selected as optimum. The corresponding electropherogram showing complete resolution of all three isomers in optimized BGE (40 mM PHEA titrated with MES to pH 5.6) is shown in Fig. 5.

3.3 Detection

The values of detection limits (L_D; signal to noise ratio of 3) for determined CDs and their derivatives in optimized background electrolyte systems are summarized in Table 1. The data were computed for both used detection tech-

Table 1 Detection limits (signal to noise ratio of 3) of studied CDs and their derivatives in optimized systems

Substance	I[a]		II[b]		III[c]		IV[d]	
	UV-Vis detection	Conductivity detection	UV-Vis detection	Conductivity detection	UV-Vis detection	Conductivity detection	Fluorescence detection	Amperometric detection
α-CD	1.5×10^{-3}	4.8×10^{-4}	2.6×10^{-5}	4.6×10^{-5}	–	–	6.2×10^{-5}	2.0×10^{-6}
β-CD	1.1×10^{-3}	4.3×10^{-4}	3.1×10^{-5}	5.2×10^{-5}	–	–	2.4×10^{-6}	1.6×10^{-6}
γ-CD	1.2×10^{-3}	6.8×10^{-4}	8.3×10^{-5}	3.6×10^{-5}	–	–	2.4×10^{-5}	1.0×10^{-6}
TB-α-CD	1.3×10^{-3}	2.3×10^{-3}	2.4×10^{-4}	4.6×10^{-4}	–	–	–	–
TB-β-CD	1.8×10^{-3}	2.8×10^{-3}	2.4×10^{-4}	5.3×10^{-4}	–	–	–	–
TB-γ-CD	2.3×10^{-3}	3.6×10^{-3}	5.2×10^{-4}	2.8×10^{-4}	–	–	–	–
TM-β-CD	4.1×10^{-4}	4.8×10^{-5}	2.2×10^{-4}	2.1×10^{-5}	–	–	–	–
DM-β-CD	2.6×10^{-4}	3.4×10^{-5}	1.4×10^{-4}	6.3×10^{-5}	–	–	–	–
2-CM-β-CD	–	–	–	–	2.3×10^{-4}	2.0×10^{-4}	–	–
3-CM-β-CD	–	–	–	–	1.8×10^{-4}	1.6×10^{-4}	–	–
6-CM-β-CD	–	–	–	–	2.8×10^{-4}	2.6×10^{-4}	–	–

[a] BGE containing 15 mM 2-NSA, 5 mM BA and 10% ACN. Separation voltage 30 kV, I = 66.4μA
[b] BGE containing 15 mM 3-IBA and 5% ACN. Separation voltage 15 kV, I = 9.2 μA
[c] BGE containing 40 mM PHEA. Separation voltage 15 kV, I = 51.3 μA
[d] Detection limits of fluorescence detection were obtained from [13], detection limits of amperometric detection were obtained from [14]

niques, indirect UV detection and contactless conductivity detection. From the calibration data it is possible to conclude that contactless conductivity detection is frequently more sensitive than indirect UV detection. For example, a slope of determination of α-CD in 3-IBA system was 1.9×10^3 (mAU s mol^{-1} L) for photometric detection and $3{,}05 \times 10^5$ (mV s mol^{-1} L) for contactless conductivity detection in the concentration range $(2–10) \times 10^{-5}$ mol L^{-1}. Moreover, the limits of detection for α-, β- and γ-CD (estimated from the signal to noise ratio of 3) were significantly lower than those presented in [12], comparable to the values obtained with fluorescence detection [13], but higher than the values obtained with amperometric detection [14], see Table 1.

4 Conclusion

Capillary electrophoresis proved to be a fast and efficient method for the determination of CDs, their uncharged and charged derivatives. The selection of suitable complex forming additive significantly affects both the resolution and sensitivity attainable with indirect UV detection. In addition to 2-NSA, 3-IBA proved to be an effective complex forming and UV absorbing agent making it possible to completely resolve α-, β- and γ-CDs, their methyl and *tert*-butyl derivatives. Cationic PHEA was used as a selective complex forming and UV absorbing agent suitable for the resolution of anionic 2-*O*-CM-β-CD, 3-*O*-CM-β-CD and 6-*O*-CM-β-CD regioisomers.

It was experimentally proved that a contactless conductivity detector represents a promising alternative to indirect UV detection making it possible to achieve at least comparable or even higher sensitivity measurements and lower detection limits for both uncharged and charged CDs and their derivatives.

Acknowledgements This study was supported by University Development Fund (grant 1788/2000), grant Agency of Charles University 22/1998/B, grant from Grant Agency of the Czech Republic (grant. 203/00/1564) and research project J13/98:113100002.

References

1. Furuike T, Aiba S (1999) Chem Lett: 69–70
2. Szente L, Szejtli J (1999) Advan Drug Delivery Rev 36: 17–28
3. Uekama K, Irie T (1987) Cyclodextrins and Their Industrial Uses, de Santé, Paris
4. Szejtli J (1998) Chem Rev 98: 1743–1753
5. Pumera M, Jelínek I, Jindřich J, Coufal P, Horský J (2000) J Chromatogr A 891: 201–206
6. Cramer F, Steinle D (1955) Justus Liebigs Ann Chem 595: 81–89
7. Wiedenhof N (1964) J Chromatogr 15: 100–102
8. Lammers JNJJ (1969) J Chromatogr 41: 462–466
9. Zsadon B, Otta KH, Tuedoes F, Szejtli J (1979) J Chromatogr 172: 490–492
10. Koizumi K, Kubota Y, Okada Y, Utamara T (1985) J Chromatogr 341: 31–41
11. Beadle JB (1969) J Chromatogr 42:201–206
12. Nardi A, Fanali S, Foret F (1990) Electrophoresis 11: 774–776
13. Penn SG, Chiu RW, Monning CA (1994) J Chromatogr A 680: 233–241
14. Fang Y, Gong F, Fang X, Fu C (1998) Anal Chim Acta 369: 39–45
15. Pitha J, Jindřich J (1988) US Patent Appl 09, 316, 378
16. Jindřich J, Horský J, Jelínek I (1997) Chem Listy 91: 356
17. Chvojka T, Jelínek I, Opekar F, Štulík K (2001) A dual photometric-contactless conductometric detector for capillary electrophoresis, Anal Chim Acta (in press)
18. Abou-Hamdan A, Bugnon P, Saudan C, Lye P, Merbach A (2000) J Am Chem Soc 122: 592–602
19. Rogan MM, Altria KD, Goodal DM (1994) Chromatographia 38: 723–729
20. Pumera M, Matalová R, Jelínek I, Jindřich J, Jůza J, Comparison of association constants of cyclodextrins and their tert.-butyl derivatives with halogenbenzoic acids and acridine derivatives, Molecules, accepted for publication