Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography A, 1032 (2004) 227–235
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Enantioseparation of benzoins and enantiomer migration reversal of hydrobenzoin in capillary zone electrophoresis with dual cyclodextrin systems and borate complexation

Ching-Erh Lin*, Sheng-Li Lin, Wei-Ssu Liao, Yu-Chih Liu

Department of Chemistry, National Taiwan University, 1 Roosevelt Road Section 4, Taipei 10674, Taiwan

Abstract

Enantioseparations of racemic hydrobenzoin and structurally related compounds, including benzoin and benzoin methyl ether, in capillary zone electrophoresis (CZE) with dual cyclodextrin (CD) systems consisting of S-β-CD (mixed isomers) and a neutral CD, including β-CD and hydroxypropyl-β-CD (HP-β-CD), as chiral selectors in the presence of borate complexation at pH 9.0 were investigated. Effective enantioseparations of hydrobenzoin were achieved with addition of dual CD systems and also with neutral CDs in a borate buffer. The enantioseparation and migration behavior of hydrobenzoin in such an electrophoretic system are primarily governed by the interaction of the borate complex of hydrobenzoin with β-CDs. The CD complexations of both hydrobenzoin and the borate complexes of hydrobenzoin with β-CDs increase in the order S-β-CD < HP-β-CD < β-CD. As a result, enantioseparations of hydrobenzoin with the use of dual CD systems consisting of S-β-CD/β-CD and S-β-CD/HP-β-CD as chiral selectors are more advantageous than that with the use of S-β-CD alone. With these dual CD systems in the presence of borate complexation, the enantiomer migration reversal was observed for hydrobenzoin. The interactions of hydrobenzoin with neutral CDs and with S-β-CD exhibit the same chiral recognition pattern, but opposite effect on the mobility of the enantiomers. The (*S*,*S*)-enantiomer of hydrobenzoin was found to interact more strongly than the (*R*,*R*)-enantiomer with neutral CDs. For comparison, enantioseparation of hydrobenzoin, together with benzoin and benzoin methyl ether, with dual CD systems in a phosphate background electrolyte at pH 9.0 was also examined. The migration order and enantioselectivity of these three benzoins depend on the degree of CD complexations between benzoins and both S-β-CD and neutral CD in a phosphate background electrolyte. In addition, effective enantioseparations of hydrobenzoin were also achievable with addition of either β-CD at concentrations greater than 1.0 mM or HP-β-CD at concentrations exceeding 2.0 mM in a borate buffer at pH 9.0.

Keywords: Enantiomer separation; Complexation; Benzoins; Hydrobenzoin; Cyclodextrins

1. Introduction

In capillary electrophoresis (CE), effective separation of neutral compounds with diol structures can often be achieved using a borate buffer at pH greater than 7 because negatively charged complexes are formed due to borate complexation [1–5]. Cyclodextrins (CDs) are frequently used as electrolyte modifiers to affect the separation and selectivity of analytes with closely related structures [6–8]. Thus, the combination of CD complexation and borate complexation is a useful approach for separation of vicinal diol compounds [4,5,9–11]. Also, CDs are the most commonly used selectors for enantioseparation [6,7,12,13]. Effective enantioseparation of neutral analytes in capillary zone electrophoresis (CZE) can be achieved with charged CDs [14–21] or with dual CD systems consisting of a charged CD and a neutral CD [22–33] or two charged CDs [34].

Most of the works on the enantioseparation of hydrobenzoin, which is a typical diol compound, and structurally related benzoin compounds have been scatteringly reported using a phosphate background electrolyte containing charged CDs alone or mixed with neutral CDs. The enantiomers of benzoin were resolved using heptakis(2,3-dimethyl-6-sulfato)-β-CD as a chiral resolving agent in a phosphate buffer containing methanol up to 50% [35]. Enantioseparation of benzoin and benzoin methyl ether was attempted with the use of a dual CD system consisting of mono(6-amino-6-deoxy)-β-CD (β-CD-NH2) and trimethyl-β-CD in a phosphate buffer at low pH, but with little success [21]. Enantioseparation of hydrobenzoin and

* Corresponding author. Tel.: +886-223635357; fax: +886-223636359.
E-mail address: celin@ccms.ntu.edu.tw (C.-E. Lin).

0021-9673/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2004.01.011

benzoin could effectively be achieved with both sulfated β-CD (S-β-CD) and a mixture of S-β-CD and a surfactant derived from an amino acid in a phosphate–borate buffer at pH 8.8 [36]; the enantiomers of hydrobenzoin were resolved using S-β-CD as a chiral selector in a phosphate buffer at pH 3.8 [16]. However, no detailed information on the enantioseparation of hydrobenzoin with the use of S-β-CD was provided.

On the other hand, so far only one article on the enantioseparation of hydrobenzoin using a borate buffer appeared in the literature. The enantiomers of hydrobenzoin were resolved using a borate buffer containing relatively high concentration of β-CD (1.8% (w/v)) or succinyl-β-CD (2.0% (w/v)) at pH 9.3 [11]. As the use of dual CD systems consisting of two charged CDs or a charged CD and a neutral CD can offer possibilities for an enhancement of selectivity and resolution for some analytes in chiral CE [22–34], the use of a dual CD system as chiral selectors offers one additional approach to the study of enantioseparations and migration behavior of hydrobenzoin and structurally related benzoin compounds in the presence and absence of borate complexation.

The migration reversal of the enantiomers of analytes in CE has attracted the attention of a number of researchers [12,37–59]. In chiral CE, the reversal of the migration order of enantiomers is an important issue. This is particularly true when one enantiomer is considered as an impurity which has to be detected and the mobility difference between the enantiomeric impurity and the major component is small. For avoiding the minute peak of the impurity to be obscured by the tail of the peak of major enantiomeric component and lowering the detection limit of the enantiomeric impurity, it is desirable to change the migration order so that the minor enantiomeric impurity migrates ahead of the principal enantiomeric component in the electropherogram. Experimentally, several approaches have been used to achieve the enantiomer migration reversal. The simplest way for reversal of the migration order of enantiomers is to reverse the electroosmotic flow (EOF) by using a cationic surfactant [60], or a cationic CD [41] or even a neutral CD [43] as an electrolyte modifier in the buffer electrolyte and reverse the polarity of the electrodes. The migration orders of enantiomers can also be reversed by employing chiral micelles with opposite configuration [61], or using different CDs with opposite chiral recognition [38,42–50]. Moreover, with the use of charged CDs or even with neutral CDs where the charge of CD–analyte complexes can be altered by varying buffer pH the migration reversal of the enantiomers may also occur [38,45,51,52,54–58]. Furthermore, the occurrence of the reversal of the migration order of the enantiomers of analytes by simply varying CD concentration has been reported [37,39–41,52,62]. Therefore, with the use of dual CD systems consisting of two types of CDs with opposite chiral recognition, the migration order of the enantiomers can be reversed by varying the concentration of one type of CD in the presence of the other type of CD at a fixed concentration.

The present investigation is aimed to develop a CZE method for simultaneous enantioseparation of hydrobenzoin and its structurally related compounds in CZE using dual CD systems consisting of S-β-CD and a neutral CD as chiral selectors in a borate buffer and a phosphate background electrolyte at pH 9.0 and to study the influences of dual CD complexation on the enantioseparation and migration behavior of hydrobenzoin in CZE in the presence and absence of borate complexation. Furthermore, enantiomer migration reversal of hydrobenzoin in CZE with dual CD systems in the presence of borate complexation is studied.

2. Experimental

2.1. Apparatus

All CE separations were performed on a Beckman P/ACE 5500 equipped with a UV detector for absorbance measurements at 214 nm (Beckman Coulter, Fullerton, CA, USA). Uncoated fused-silica capillaries purchased from Polymicro Technologies (Phoenix, AZ, USA) were used. The dimensions of the capillary were 57 cm × 50 μm i.d. The effective length of the capillary was 50 cm from the injection end of the capillary. The CE system was interfaced with a microcomputer. System Gold software of Beckman was used for data acquisition. For pH measurements, a pH meter (Suntex Model SP-701, Taipei, Taiwan) was employed with a precision of ±0.01 pH unit.

2.2. Chemicals and reagents

Three benzoins studied, (*R*,*R*)-(+)-hydrobenzoin, HP-β-CD, and S-β-CD were obtained from Sigma–Aldrich (St. Louis, MO, USA). β-CD was purchased from Merck (Darmstadt, Germany). All other chemicals were of analytical grade. Deionized water was prepared with a Milli-Q system (Millipore, Bedford, MA. USA).

Standard solutions of benzoins at a concentration of 20 μg ml^{-1} were prepared by dissolving analytes in a mixture of a phosphate buffer solution containing 10% (v/v) methanol. The pH of a phosphate background electrolyte was adjusted to the desired pH value by mixing various proportions of 50 mM trisodiumphosphate solution with the same concentration of phosphoric acid. Similarly, the pH of a borate buffer was adjusted to the desired pH value by mixing various proportions of 50 mM sodium tetraborate solution with the same concentration of boric acid. All buffer solutions, freshly prepared weekly and stored in a refrigerator before use, were filtered through a membrane filter (0.22 μm).

2.3. Electrophoretic procedure

When a new capillary was used, the capillary was washed 30 min with 1.0 M NaOH solution, followed by 30 min

with deionized water at 25 °C. Before each injection, the capillary was prewashed for 3 min with running buffer and postwashed for 3 min with deionized water, 3 min with 0.1 M NaOH, and 5 min with deionized water to maintain proper reproducibility of run-to-run injections. Sample injections were done in a hydrodynamic mode over 5 s under a pressure of 1.0 p.s.i at 25 °C (1 psi = 6894.76 Pa). The measurements were run at least in triplicate to ensure reproducibility. An applied voltage of 20 kV for phosphate buffer was selected to keep the total current less than 90 μA. The detection wavelength was set at 214 nm. Peak identification was conducted by spiking with the analyte to be identified. Methanol was used as neutral marker. The relative standard deviation of migration time is less than 0.6% (n = 5).

2.4. Mobility calculations

The electrophoretic mobility of analytes was calculated from the observed migration times with the equation:

$$\mu_{ep} = \mu - \mu_{eo} = \frac{L_d L_t}{V}\left(\frac{1}{t_m} - \frac{1}{t_{eo}}\right) \quad (1)$$

where μ_{ep} is the electrophoretic mobility of the analyte tested, μ the apparent mobility, μ_{eo} the electroosmotic mobility, t_m the migration time measured directly from the electropherogram, t_{eo} the migration time for an unchanged solute, L_t the total length of capillary, L_d the length of capillary between injection and detection, and V is the applied voltage.

3. Results and discussion

3.1. Enantioseparation with dual CD systems involving S-β-CD and a neutral CD in the absence of borate complexation

3.1.1. S-β-CD/β-CD system

Fig. 1 shows the structures of the three benzoins studied. Fig. 2 shows the variation of the electrophoretic mobility of benzoins as a function of β-CD concentration in the range 0–3.0 mM in the presence of 3.5% (w/v) S-β-CD using a phosphate background electrolyte (50 mM) at pH 9.0. As S-β-CD is composed of a number of randomly sulfated-substituted β-CD (typically with substitution 7–11 mol/mol β-CD), thus the concentration of S-β-CD is given in % (w/v), instead of mM. As can be seen, at a given concentration of S-β-CD, the electrophoretic mobility of benzoin methyl ether and that of benzoin decrease considerably (toward the anode) with increasing β-CD concentration, whereas the electrophoretic mobility of hydrobenzoin decreases to a much less extent. It should be noted that the ionic strength of the background electrolyte increases with increasing the concentration of S-β-CD. The increased ionic strength of the background electrolyte depresses the mobility of the complexes, thus resulted in the lowering of the effective mobility to some extents in the

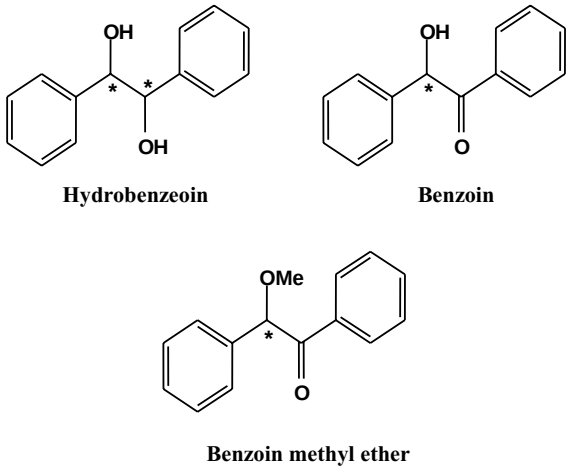

Fig. 1. The structures of the three benzoins studied (chiral centers denoted by asterisk).

presence of S-β-CD [63,64]. Nevertheless, the order of the electrophoretic mobility of the enantiomers of these three analytes should remain the same as shown in Fig. 2.

It is noted that the extents of the variation in the electrophoretic mobility of these three analytes increases in the order hydrobenzoin < benzoin < benzoin methyl ether. As the interactions of benzoins with β-CD can be reflected from the extents of the variation of the electrophoretic mobility, the results shown in Fig. 2 clearly indicate that the binding strength of these three benzoins to β-CD in a phosphate background electrolyte is in the order hydrobenzoin < benzoin < benzoin methyl ether. This is consistent with the result obtained as in the case of S-β-CD [65]. The results also reveal that entantioseparation of benzoins with this dual CD system in a phosphate background

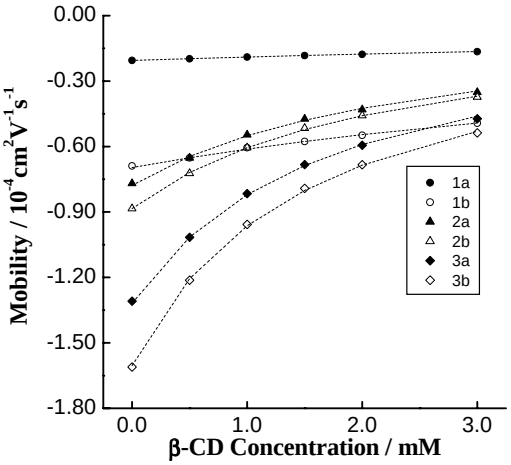

Fig. 2. Variations of the electrophoretic mobility of benzoins as a function of β-CD concentration in the range 0–3.0 mM in the presence of 3.5% (w/v) S-β-CD using 50 mM phosphate background electrolyte at pH 9.0. Capillary: 57 cm × 50 μm i.d.; sample concentration: 20 μg ml^{-1}; detection wavelength: 214 nm; other operating conditions: 20 kV, 25 °C. Curve identification: 1 = hydrobenzoin (●, ○); 2 = benzoin (▲, △); 3 = benzoin methyl ether (◆, ◇).

electrolyte containing 3.5% (w/v) S-β-CD becomes not favorable with addition of β-CD at concentrations greater than 1.5 mM because mobility of benzoin methyl ether and benzoin decreases quite drastically and the enantioselectivity (α), which is defined as the ratio of the electrophoretic mobility of the two enantiomers, decreases also with increasing β-CD concentration. For instance, the α value of benzoin decreases from 1.15 to 1.09 when β-CD concentration increases from 0 to 1.5 mM. In fact, the enantiomers of benzoin cannot be effectively resolved with this dual CD system when β-CD concentration exceeds 1.5 mM.

By spiking the (*R*,*R*)-enantiomer of hydrobenzoin, the first enantiomeric peak of hydrobenzoin was experimentally confirmed to be the (*R*,*R*)-enantiomer in a phosphate background electrolytes. Evidently, the binding strength of the (*R*,*R*)-enantiomer of hydrobenzoin with S-β-CD is weaker than that of the (*S*,*S*)-enantiomer. This is consistent with the results obtained by HPLC using β-CD as a chiral additive in the mobile phase made of a borate buffer at pH 8.3 [66]. Based on the structural similarity, it is reasonable to assign the first enantiomeric peaks of benzoin and benzoin methyl ether to the *R*-enantiomers in a phosphate background electrolyte.

3.1.2. S-β-CD/HP-β-CD system

Fig. 3 shows the variations of the electrophoretic mobility of benzoins as a function of HP-β-CD concentration in the range 0–3.0 mM in the presence of 3.5% (w/v) S-β-CD using a phosphate background electrolyte (50 mM) at pH 9.0. The trends in the variations of the electrophoretic mobility of the enantiomers of benzoins as a function of HP-β-CD concentration in a phosphate background electrolyte at pH 9.0 are similar to those observed as in the case of S-β-CD/β-CD system, but the electrophoretic mobility varies to a less extent. This is because the binding strength of benzoins to HP-β-CD is weaker than that of benzoins to β-CD. Similarly, the mobility of benzoin methyl ester and benzoin decreases considerably and the enantioselectivity of the two enantiomers of these two analytes decreases also with increasing HP-β-CD concentration. The α value of benzoin decreases from 1.15 to 1.06 when HP-β-CD concentration increases from 0 to 3.0 mM. Thus with this dual CD system, the separation window becomes narrow and the enantiomers of benzoin cannot be effectively resolved when HP-β-CD concentration exceeds 3.0 mM.

3.2. Enantioseparation with dual CD systems in the presence of borate complexation and enantiomer migration reversal of hydrobenzoin

3.2.1. S-β-CD/β-CD system

Fig. 4 shows the variations of the electrophoretic mobility of benzoins as a function of β-CD concentration in the range 0–1.5 mM using a borate buffer (50 mM) containing 3.5% (w/v) S-β-CD and β-CD at pH 9.0. The trends in the variations of the electrophoretic mobility of the enantiomers of benzoin methyl ether and benzoin are similar to those shown in Fig. 2. As in the case of S-β-CD [65], no significant differences in the variations of the electrophoretic mobility were observed for both benzoin and benzoin methyl ether in a borate buffer, as compared with those observed in a phosphate background electrolyte, because borate complexation between these two analytes and borate ions is very weak.

As shown in a previous paper [65], depending on the concentration of S-β-CD and the degree of relatively strong borate complexation, the electrophoretic mobility of the enantiomers of hydrobenzoin decreases to about $-1.48 \times 10^{-4}\,cm^2\,V^{-1}\,s^{-1}$. It is of interest to note that the measured effective mobility of the two enantiomers of hydrobenzoin decreased from $-1.48 \times 10^{-4}\,cm^2\,V^{-1}\,s^{-1}$ to -1.39×10^{-4} and $-1.44 \times 10^{-4}\,cm^2\,V^{-1}\,s^{-1}$ [65]. This result indicates that the mobility of hydrobenzoin is

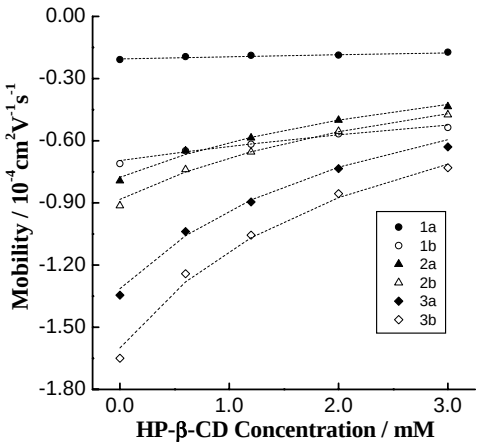

Fig. 3. Variations of the electrophoretic mobility of benzoins as a function of HP-β-CD concentration in the range 0–3.0 mM in the presence of 3.5% (w/v) S-β-CD using 50 mM phosphate background electrolyte at pH 9.0. Other operating conditions and curve identification as for Fig. 2.

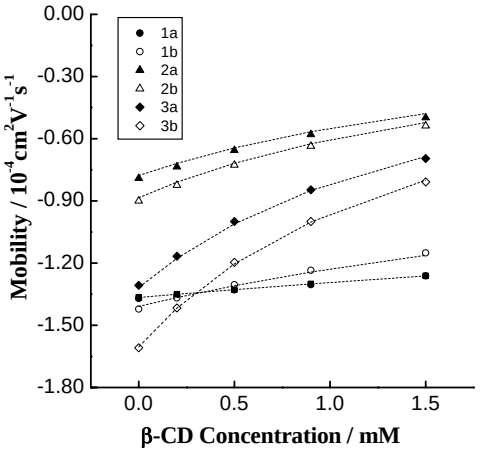

Fig. 4. Variations of the electrophoretic mobility of benzoins as a function of β-CD concentration in the range 0–1.5 mM in the presence of 3.5% (w/v) S-β-CD using 50 mM borate buffer at pH 9.0. Other operating conditions and curve identification are the same as for Fig. 2.

not severely depressed by the increased ionic strength in a borate buffer with addition of 2.5% (w/v) S-β-CD. By increasing β-CD concentration from 0 to 1.5 mM in the presence of 3.5% (w/v) S-β-CD, the migration order of the enantiomers of hydrobenzoin was found to reverse at about 0.3 mM. In the absence of β-CD, the first enantiomeric peak of hydrobenzoin appeared in the electropherogram was confirmed to be the (*R*,*R*)-enantiomer [65]. However, it was also confirmed that the (*R*,*R*)-enantiomer eluted after the (*S*,*S*)-enantiomer with addition of β-CD at concentrations greater than 0.3 mM in the presence of 3.5% (w/v) S-β-CD in a borate buffer at pH 9.0. Evidently, the interactions of hydrobenzoin with β-CD and with S-β-CD exhibit opposite effect on the mobility of the enantiomers. As the extent of the variation in the electrophoretic mobility of hydrobenzoin as a function of β-CD concentration in the presence of S-β-CD (3.5% (w/v)) is greater in comparison with that as a function of S-β-CD concentration, the results clearly indicate that the enantiomers of hydrobenzoin interacts more strongly with β-CD than with S-β-CD. Thus, chiral recognition of the enantiomer of hydrobenzoin with S-β-CD/β-CD system is predominantly governed by the interactions of the hydrobenzoin–borate complexes with β-CD when β-CD concentration is greater than 0.3 mM in the presence of S-β-CD (3.5% (w/v)). A schematic diagram illustrating different interactions with a dual CD system in a borate buffer leading to the separation of the enantiomers of an analyte is shown in Fig. 5.

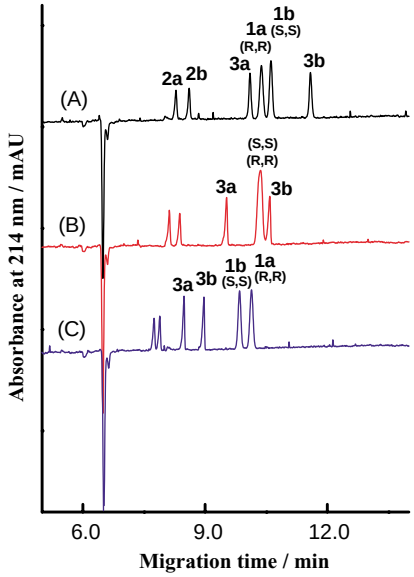

Fig. 6. Electropherograms of benzoins obtained with addition of β-CD at concentrations of (A) 0; (B) 0.3; and (C) 0.9 mM in the presence of 3.5% (w/v) S-β-CD in 50 mM borate buffer at pH 9.0. Other operating conditions as for Fig. 2.

The electropherograms of the enantiomers of hydrobenzoin, together with benzoin and benzoin methyl ether, obtained with addition of β-CD at concentrations of 0, 0.2, and 0.9 mM in the presence of 3.5% (w/v) S-β-CD in a borate buffer (50 mM) at pH 9.0 are shown in Fig. 6. As

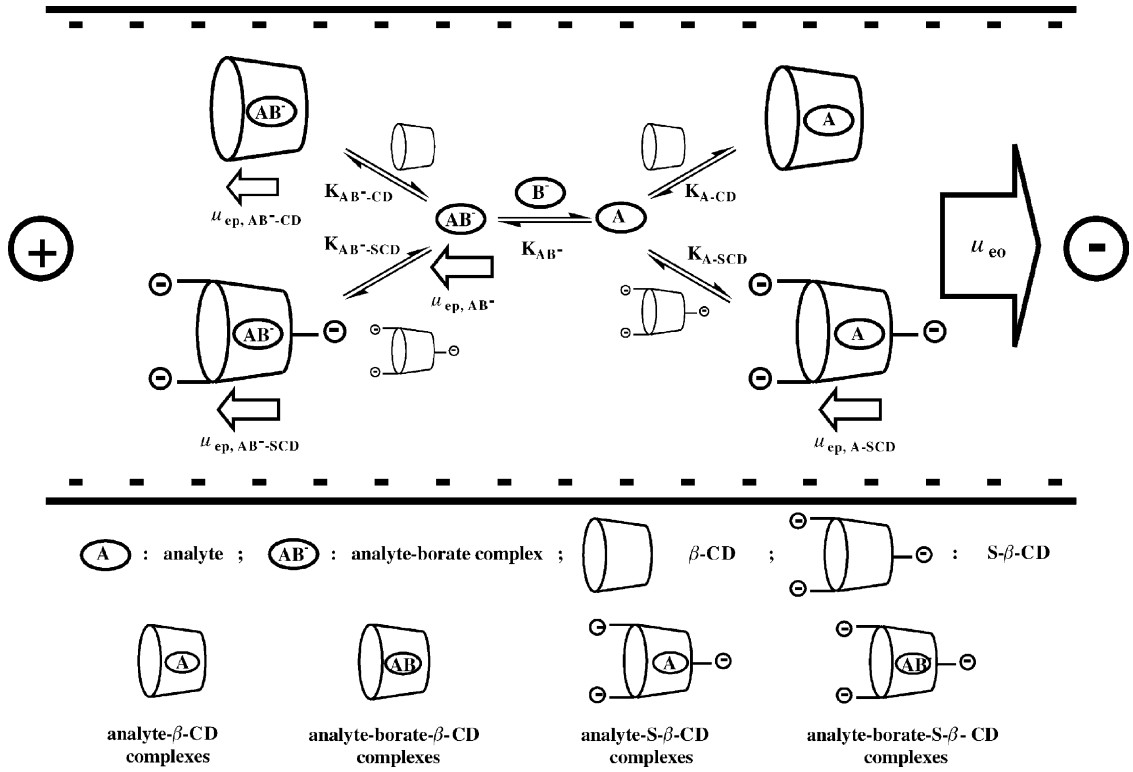

Fig. 5. A schematic diagram illustrating different interactions with a dual CD system in a borate buffer leading to the separation of the enantiomers of an analyte.

illustrated, enantioseparation of hydrobenzoin can be significantly enhanced with the use of a dual CD system consisting of S-β-CD and β-CD in a borate buffer, whereas the enantiomers of benzoin methyl ether and benzoin are also effectively separated. Evidently, due to stronger interaction of the enantiomers of hydrobenzoin with β-CD than with S-β-CD, enantioseparation of hydrobenzoin with the use of this dual CD system in a borate buffer is more advantageous than that with the use of S-β-CD alone. In fact, effective enantioseparation of benzoins was achieved with addition of β-CD optimally at concentrations in the range 0.9–1.5 mM in the presence of 3.5% (w/v) S-β-CD using a borate buffer at pH 9.0.

3.2.2. S-β-CD/HP-β-CD system

To add further support, a dual CD system consisting of S-β-CD and HP-β-CD is also considered. Fig. 7 show the variations of the electrophoretic mobility of benzoins as a function of HP-β-CD concentration in the range 0–3.0 mM in the presence of 3.5% (w/v) S-β-CD using a borate buffer (50 mM) at pH 9.0. As expected, the trends in the variations of the electrophoretic mobility of the enantiomers of benzoins are similar to those observed as in the case of S-β-CD/β-CD system, but the electrophoretic mobility varies to a less extent. This is because the binding strength of benzoins to HP-β-CD is comparatively weaker than that of benzoins to β-CD.

By varying HP-β-CD concentration, the enantiomer migration reversal was also observed for hydrobenzoin with this dual CD system. As a matter of fact, the migration order of the enantiomers of hydrobenzoin was reversed with addition of HP-β-CD at about 0.8 mM in the presence of 3.5% (w/v) S-β-CD. As in the case of S-β-CD/β-CD, the first enantiomeric peak of hydrobenzoin appeared in the electropherogram was confirmed to the (*R*,*R*)-enantiomer in the absence of HP-β-CD, but the (*R*,*R*)-enantiomer eluted after the (*S*,*S*)-enantiomer when HP-β-CD concentration greater than

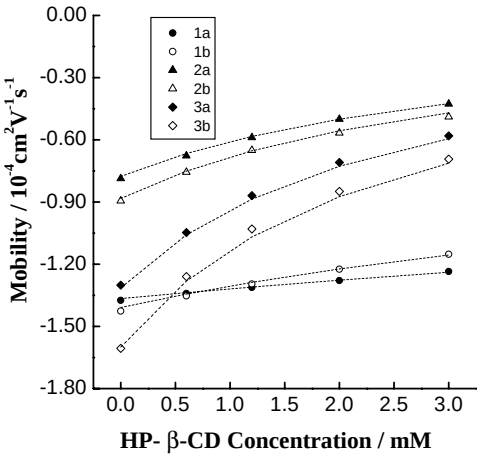

Fig. 7. Variations of the electrophoretic mobility of benzoins as a function of HP-β-CD concentration in the range 0–3.0 mM in the presence of 3.5% (w/v) S-β-CD using 50 mM borate buffer at pH 9.0. Other operating conditions as for Fig. 2.

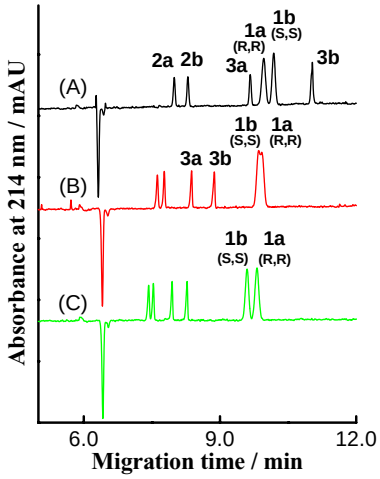

Fig. 8. Electropherograms of benzoins obtained with addition of HP-β-CD at concentrations of (A) 0; (B) 0.9; and (C) 2.0 mM in the presence of 3.5% (w/v) S-β-CD in 50 mM borate buffer at pH 9.0. Other operating conditions as for Fig. 2.

0.8 mM was used. To demonstrate the enantiomer migration reversal of hydrobenzoin, Fig. 8 shows the electropherograms of benzoins obtained with addition of HP-β-CD at concentrations of 0, 0.9 and 2.0 mM in the presence of 3.5% (w/v) S-β-CD in a borate buffer (50 mM) at pH 9.0. In fact, effective enantioseparation of benzoins was achieved with addition of HP-β-CD optimally at concentrations greater than 2.0 mM, but less than 3.0 mM, in the presence of 3.5% (w/v) S-β-CD using a borate buffer at pH 9.0.

3.3. Enantioseparation of hydrobenzoin with neutral CDs in the presence of borate complexation

3.3.1. Enantioseparation with β-CD

Due to borate complexation, enantioseparation of hydrobenzoin is achievable in a borate buffer containing neutral CDs. In this electrophoretic system, the interactions of hydrobenzoin with a neutral CD in the presence of borate complexation are still quite complicated because CD complexations between hydrobenzoin and neutral CDs and that between hydrobenzoin–borate complex and neutral CDs are involved. For a better understanding on the enantioseparations of hydrobenzoin with dual CD systems involving S-β-CD and a neutral CD, experiments similar to the ones conducted by Schmid et al. [11] were performed, but we focused our attention on the interactions of hydrobenzoin with β-CD at much lower concentrations (≤3.5 mM). Fig. 9A shows the variation of the electrophoretic mobility of hydrobenzoin as a function of β-CD concentration in the range 0–3.5 mM in a borate buffer at pH 9.0. The results indicate that baseline enantioseparation of hydrobenzoin can be achieved with addition of β-CD at a concentration as low as 1 mM (Fig. 10A). The concentration of β-CD for effective enantioseparation of hydrobenzoin is much lower than that reported previously [11].

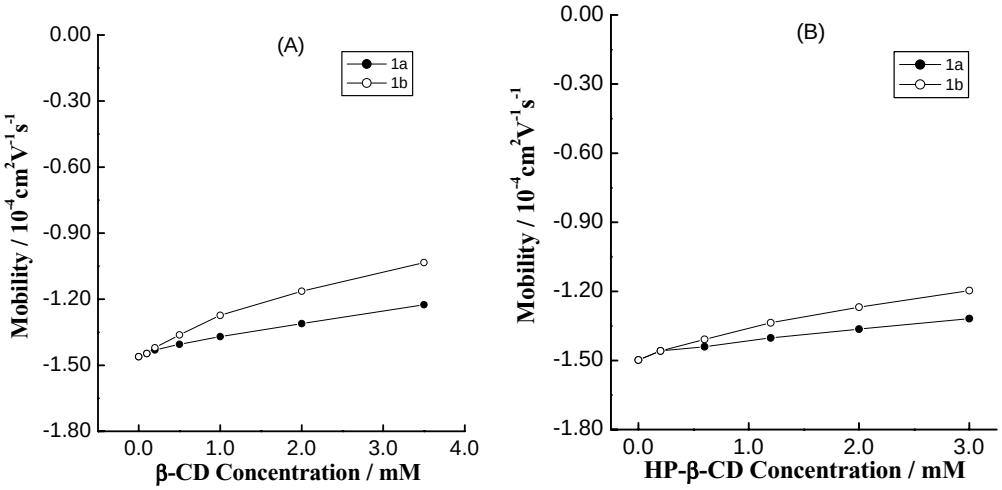

Fig. 9. Variations of the electrophoretic mobility of hydrobenzoin as a function of (A) β-CD concentration in the range of 0–3.5 mM and (B) HP-β-CD concentration in the range 0–3.0 mM using 50 mM borate buffer at pH 9.0. Other operating conditions as for Fig. 2.

3.3.2. Enantioseparation with HP-β-CD

Enantioseparation of hydrobenzoin with HP-β-CD as a chiral selector in a borate buffer at pH 9.0 was also examined. Fig. 9B shows the variations of the electrophoretic mobility of hydrobenzoin as a function of HP-β-CD concentration in the range 0–3.0 mM in a borate buffer at pH 9.0. Effective enantioseparation of hydrobenzoin can be achieved with addition of HP-β-CD at concentrations greater than 2.0 mM (Fig. 10B).

3.3.3. Evaluation of binding constants

In the presence of borate complexation, the interactions of hydrobenzoin with a neutral CD become complicated because, in addition to borate complexation, CD complexations of hydrobenzoin and hydrobenzoin–borate complexes

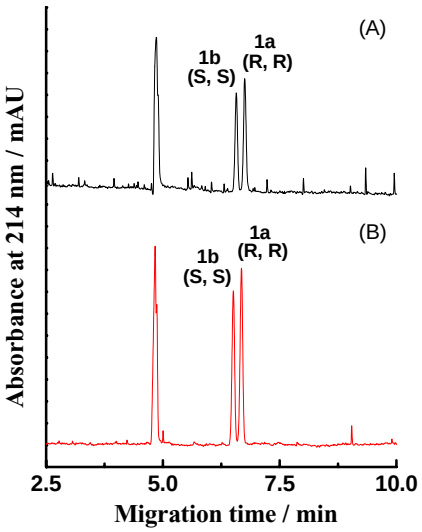

Fig. 10. Electropherograms of hydrobenzoin obtained in 50 mM borate buffer at pH 9.0 with addition of (A) 1.0 mM β-CD and (B) 2.0 mM HP-β-CD. Other operating conditions as for Fig. 2.

are involved. Thus, the effective electrophoretic mobility of hydrobenzoin can be described by the following equation

$$\mu_{\text{eff}} = \frac{K_{A\cdot B^-}[B^-]\mu_{A\cdot B^-} + K_{A\cdot B^-\cdot CD}K_{A\cdot B^-}[B^-][CD]\mu_{A\cdot B^-\cdot CD}}{1 + K_{A\cdot CD}[CD] + K_{A\cdot B^-}[B^-] + K_{A\cdot B^-\cdot CD}K_{A\cdot B^-}[B^-][CD]} \quad (2)$$

where $[B^-]$ and [CD] are the concentrations of borate ions and CD, respectively, $K_{A\cdot CD}$, $K_{A\cdot B^-}$ and $K_{A\cdot B^-\cdot CD}$ are the binding constants of hydrobenzoin to CD, to borate ions, and that of hydrobenzoin–borate complexes to CD, respectively, and $\mu_{A\cdot B^-}$ and $\mu_{A\cdot B^-\cdot CD}$ are the electrophoretic mobility of hydrobenzoin–borate complexes and the complexes formed between hydrobenzoin–borate complexes and neutral CD, respectively. In Eq. (2), the limiting mobility ($\mu_{A\cdot B^-\cdot CD}$) and binding constants ($K_{A\cdot CD}$ and $K_{A\cdot B^-\cdot CD}$) can be designated by the upper subscripts, (+) and (−) for the (*R*,*R*)- and (*S*,*S*)-enantiomers of hydrobenzoin, respectively.

The binding constants of each enantiomer of hydrobenzoin to neutral CD are evaluated based on the dependence of the effective electrophoretic mobility of each enantiomeric hydrobenzoin on the concentration of neutral CD by curve-fitting the experimental mobility data through the utilization of Microcal Origin software (version 6.0) according to Eq. (2) as described previously [62]. The trial values of $K_{A\cdot B^-}$ and $\mu_{A\cdot B^-}$, determined from the experimental mobility data of hydrobenzoin as a function of borate concentration, are 550 M^{-1} and -1.54×10^{-4} $cm^2\,V^{-1}\,s^{-1}$, respectively [65]. The trial values of $K_{A\cdot CD}$, determined from the experimental mobility data of hydrobenzoin as a function of β-CD concentration or HP-β-CD concentration at a given concentration of S-β-CD in a phosphate background electrolyte at pH 9.0, are 90 and 190 M^{-1} with β-CD and 60 and 150 M^{-1} with HP-β-CD for the (*R*,*R*)- and (*S*,*S*)-enantiomers of hydrobenzoin, respectively. The

trial value of $\mu_{A\cdot B^{-}\cdot CD}$ estimated from $\mu_{A\cdot B^{-}}$ according to Offord's equation [67] is about $-5.0 \times 10^{-5}\,cm^2\,V^{-1}\,s^{-1}$. In addition, $\mu^{(+)}_{A\cdot B^{-}\cdot CD} \cong \mu^{(-)}_{A\cdot B^{-}\cdot CD}$ is assumed. The mobility curve of each enantiomer of an analyte as a function of β-CD concentration is then simulated by adjusting the values of $K_{A\cdot B^{-}\cdot CD}$ and $\mu_{A\cdot B^{-}\cdot CD}$, together with $K_{A\cdot B^{-}}$, $K_{A\cdot CD}$ and $\mu_{A\cdot B^{-}}$, as parameters until the predicated mobility curve is best-fitted to the experimental data. The binding constants of the enantiomers of hydrobenzoin–borate complexes to β-CD evaluated are 105 and 280 M^{-1} for the (*R*,*R*)- and (*S*,*S*)-enantiomers, respectively, whereas the binding constants of the enantiomers of hydrobenzoin–borate complexes to HP-β-CD evaluated are 65 and 140 M^{-1} for the (*R*,*R*)- and (*S*,*S*)-enantiomers, respectively. The results confirm that the binding strength of hydrobenzoin with HP-β-CD is about one-half as large as that of hydrobenzoin with β-CD and the (*S*,*S*)-enantiomer of hydrobenzoin interacts more strongly with neutral CDs than the (*R*,*R*)-enantiomer of hydrobenzoin in a borate buffer.

4. Conclusion

In the presence of borate complexation at pH 9.0, effective enantionseparations of benzoins in CZE were more advantageously achieved with dual CD systems consisting of S-β-CD and a neutral CD than with S-β-CD alone as chiral selector. The enantiomers of hydrobenzoin could also be baseline separated with addition of a neutral CD in a borate buffer at pH 9.0. Enantioseparation of hydrobenzoin is mainly governed by CD–borate complexations in a borate buffer, whereas enantioseparation of benzoins is primarily determined by CD complexations in a phosphate background electrolyte. With dual CD systems, the complexation between borate complexes of hydrobenzoin and neutral CDs plays a predominant role in the enantioseparation and migration behavior of the enantiomers of hydrobenzoin with addition of neutral CDs at or greater than a certain concentration. By varying the concentration of neutral CDs, the enantiomer migration reversal of hydrobenzoin occurs owing to opposite effect on the mobility of the enantiomers with S-β-CD and neutral CDs in the presence of borate complexation.

Acknowledgements

We thank the National Science Council of Taiwan for financial support.

References

[1] S. Hoffstetter-Kuhn, A. Paulus, E. Gassmann, H.M. Widmer, Anal. Chem. 63 (1991) 1541.
[2] J.P. Landers, R.P. Oda, M.D. Schuchard, Anal. Chem. 64 (1992) 2846.
[3] A.B. Foster, Adv. Carbohydr. Chem. 12 (1957) 81.
[4] T. Tadey, W.C. Purdy, J. Chromatogr. B 657 (1994) 365.
[5] M. Stefansson, M. Novotry, J. Am. Chem. Soc. 115 (1993) 11573.
[6] S. Fanali, J. Chromatogr. A 875 (2000) 89.
[7] G. Vigh, A.D. SoKolowski, Electrophoresis 18 (1997) 2305.
[8] K.H. Chen, C.E. Lin, W.S. Liao, W.Y. Lin, Y.Y. Hsiao, J. Chromatogr. A 979 (2002) 399.
[9] W. Lindner, B. Bohs, V. Seidel, J. Chromatogr. B 697 (1995) 549.
[10] T. Jira, A. Bunke, M.G. Schmid, G. Gubitz, J. Chromatogr. A 761 (1997) 269.
[11] M.G. Schmid, K. Wirnsberger, T. Jira, A. Bunke, G. Gubitz, Chirality 9 (1997) 153.
[12] B. Chankvetadze (Ed.), Capillary Electrophoresis in Chiral Analysis, Wiley, Chichester, 1997.
[13] J. Landers (Ed.), Handbook of Capillary Electrophoresis, CRC Press, Boca Raton, FL, 1997, pp. 75–100.
[14] T. Schmitt, H. Engelhardt, Chromatographia 37 (1993) 475.
[15] B. Chankvetadze, G. Endresz, G. Blaschke, Electrophoresis 15 (1994) 804.
[16] A.M. Stalcup, K.H. Gahm, Anal. Chem. 68 (1996) 1360.
[17] S.R. Gratz, A.M. Stalcup, Anal. Chem. 70 (1998) 5166.
[18] R.J. Tait, D.O. Thompson, V.J. Stella, J.F. Stobaugh, Anal. Chem. 66 (1994) 4013.
[19] H. Nishi, S. Terabe, J. Chromatogr. A 694 (1995) 245.
[20] B. Chankvetadze, J. Chromatogr. A 792 (1997) 269.
[21] F. Lelievre, P. Gareil, V. Bahaddi, H. Galons, Anal. Chem. 69 (1997) 393.
[22] M. Fillet, P. Hubert, J. Crommen, J. Chromatogr. A 875 (2000) 123.
[23] S.I. Izumoto, H. Nishi, Electrophoresis 20 (1999) 189.
[24] S. Surapaneni, K. Ruterbories, T. Lindstrom, J. Chromatogr. A 761 (1997) 249.
[25] A.M. Abushoffa, M. Fillet, P. Hubert, J. Commen, J. Chromatogr. A 948 (2002) 321.
[26] M. Fillet, B. Chankvetadze, J. Crommen, G. Blaschke, Electrophoresis 20 (1999) 2691.
[27] M. Fillet, L. Fotsing, J. Crommen, J. Chromatogr. A 817 (1998) 113.
[28] F. Lelievre, P. Gareil, A. Jardy, Anal. Chem. 69 (1997) 385.
[29] K.H. Gahm, L.W. Chang, D.W. Armstrong, J. Chromatogr. A 759 (1997) 149.
[30] I.S. Lurie, J. Chromatogr. A 792 (1997) 297.
[31] I.S. Lurie, R.F. Klein, T.A. Dal Cason, M.J. LeBelle, R. Brenneisen, R.E. Weinberger, Anal. Chem. 66 (1994) 4019.
[32] M.J. Sepaniak, C.L. Copper, K.W. Whitaker, V.C. Anigbogu, Anal. Chem. 67 (1995) 2037.
[33] V.C. Anigbogu, C.L. Copper, M.J. Sepaniak, J. Chromatogr. A 705 (1995) 343.
[34] A.M. Abushoffa, M. Fillet, A.-C. Servais, P. Hubert, J. Crommen, Electrophoresis 24 (2003) 343.
[35] H. Cai, G. Vigh, J. Chromatogr. A 827 (1998) 121.
[36] W. Ding, J.S. Fritz, J. Chromatogr. A 831 (1999) 311.
[37] T. Schmitt, H. Engelhardt, J. High Resolut. Chromatogr. 16 (1993) 525.
[38] T. Schmitt, H. Engelhardt, J. Chromatogr. A 697 (1995) 561.
[39] M.E. Biggin, R.L. Williams, G. Vigh, J. Chromatogr. A 692 (1995) 319.
[40] S.A.C. Wren, J. Chromatogr. A 768 (1997) 153.
[41] H. Cai, T.V. Nguyen, G. Vigh, Anal Chem. 70 (1998) 580.
[42] B. Chankvetadze, G. Schulte, G. Blaschke, J. Chromatogr. A 732 (1996) 183.
[43] B. Chankvetadze, G. Schulte, G. Blaschke, J. Pharm. Biomed. Anal. 15 (1997) 1577.
[44] G. Schulte, B. Chankvetadze, G. Blaschke, J. Chromatogr. A 771 (1997) 259.
[45] B. Chankvetadze, G. Schulte, D. Bergenthal, G. Blaschke, J. Chromatogr. A 798 (1998) 315.
[46] B. Chankvetadze, G. Pintore, N. Burjanadze, D. Bergenthal, D. Strickmann, R. Cerri, G. Blaschke, Electrophoresis 19 (1998) 2101.

[47] B. Chankvetadze, G. Pintore, N. Burjanagze, D. Bergenthal, K. Bergander, J. Breitkreutz, C. Muhlenbrock, G. Blaschke, J. Chromatogr. A 875 (2000) 455.
[48] B. Chankvetadze, K. Lomsadze, D. Bergenthal, J. Breitkreutz, K. Bergander, G. Blaschke, Electrophoresis 22 (2001) 3178.
[49] B. Chankvetadze, I. Kartozia, N. Burjanadze, D. Bergenthal, H. Luftmann, G. Blaschke, Chromatographia 53 (2001) S290.
[50] B. Chankvetadze, N. Burjanadze, G. Blaschke, Electrophoresis 22 (2001) 3281.
[51] J. Li, K.C. Waldron, Electrophoresis 20 (1999) 171.
[52] A.M. Rizzi, L. Kremser, Electrophoresis 20 (1999) 2715.
[53] S. Sabah, G.K.E. Scriba, J. Chromatogr. A 822 (1998) 137.
[54] S. Sabah, G.K.E. Scriba, J. Chromatogr. A 833 (1999) 261.
[55] S. Sabah, G.K.E. Scriba, J. Chromatogr. A 894 (2000) 267.
[56] S. Sabah, G.K.E. Scriba, Electrophoresis 22 (2001) 1385.
[57] S. Sabah, F. SuB, G.K.E. Scriba, Electrophoresis 22 (2001) 3163.
[58] N. Sidamonidze, F. SuB, W. Poppitz, G.K.E. Scriba, J. Sep. Sci. 24 (2001) 777.
[59] A. Aumatell, R.J. Wells, J. Chromatogr. B 669 (1995) 331.
[60] X.W. Yao, D. Wu, F.E. Regnier, J. Chromatogr. 636 (1993) 21.
[61] K. Otsuka, S. Terabe, J. Chromatogr. 515 (1990) 221.
[62] C.E. Lin, W.S. Liao, K.H. Chen, Electrophoresis 24 (2003) 3139.
[63] W. Friedl, J.C. Reijenga, E. Kenndler, J. Chromatogr. A 709 (1995) 163.
[64] D.K. Maynard, G. Vigh, Electrophoresis 22 (2001) 3152.
[65] S.-L. Lin, C.-E. Lin, J. Chromatogr. A 1032 (2004) 215.
[66] M.G. Schmid, B. Harringer, G. Gubitz, K. Szabo, J. High Resolut. Chromatogr. 21 (1998) 414.
[67] R.E. Offord, Nature 5049 (1966) 591.