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# Comparative studies on the enantioseparation of hydrobenzoin and structurally related compounds by capillary zone electrophoresis with sulfated β-cyclodextrin as the chiral selector in the presence and absence of borate complexation

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**Abstract**

Comparative studies on the enantioseparations of racemic hydrobenzoin, together with benzoin and benzoin methyl ether, in capillary zone electrophoresis using sulfated β-cyclodextrin (S-β-CD) as a chiral selector in the presence and absence of borate complexation were investigated. The influences of S-β-CD concentration on the enantioseparation of benzoins in a borate buffer and a phosphate background electrolyte and the influences of the concentration and the pH of borate buffer containing S-β-CD on the enantioseparation of hydrobenzoin were examined. The results indicate that, depending on the degree of strong borate complexation and comparatively weak CD complexation, the selectivity of the enantiomers of hydrobenzoin can be greatly reduced in a buffer system containing borate ions. Enantioseparation of hydrobenzoin is mainly governed by the interaction between hydrobenzoin–borate complexes and S-β-CD in a borate buffer, whereas enantioseparation of benzoins is primarily determined by CD complexation in a phosphate background electrolyte. Effective enantioseparations of benzoins were simultaneously achieved with addition of S-β-CD at a concentration greater than 3.0% (w/v) in a borate buffer and at a concentration greater than 2.5% (w/v) in a phosphate background electrolyte at pH 9.0.


*Keywords:* Enantiomer separation; Borate complexation; Cyclodextrin–borate complexation; Complexation; Hydrobenzoin; Benzoins; Cyclodextrins

## 1. Introduction

In capillary electrophoresis (CE), borate complexation has been frequently involved for the separation of compounds with diol structures [1,2]. The electrophoretic mobility of diol compounds can be greatly affected using a borate buffer at pH greater than 7, because negatively charged complexes are formed due to borate complexation [3]. It has been shown that the combination of CD and borate complexations is a useful approach for separation of nucleotide isomers [4], carbohydrates [5] and other vicinal diol compounds [6,7]. In the case of the separation of enantiomeric pairs of charged and neutral compounds, the use of cyclodextrins (CDs) as chiral selectors is the most common strategy employed [8–11]. The electrophoretic migration of the enantiomers may be modified through the complexation between the enantiomers of analytes and CDs. Thus CD complexation can lead to enantiomeric resolution, provided that there are differences in binding constants and/or differences in the mobility of the complexes formed between the enantiomers of an analyte and CDs [12,13].

For neutral analytes in the separation mode of capillary zone electrophoresis (CZE), enantioseparation is not achievable with neutral CDs unless the electrophoretic system is modified with the use of a buffer system containing CD which can form charged complexes with analytes [6,7], or with the addition of charged CDs in a buffer system [14–22].

Hydrobenzoin is a typical diol compound. Most of the work on the enantioseparation of hydrobenzoin and structurally related benzoin compounds have been scatteringly reported using a phosphate buffer. The enantiomers of benzoin were completely resolved using the single isomer heptakis(2,3-dimethyl-6-sulfate)-β-CD as a chiral resolving agent in a phosphate buffer containing methanol up to 50% [26]; the enantiomers of benzoin and benzoin

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methyl ether was partially resolved with a dual CD system composed of 20 mM mono(6-amino-6-deoxy)-β-CD (β-CD-NH2)/trimethyl-β-CD (TM-β-CD) in a phosphate buffer at low pH [19]. Enantioseparation of hydrobenzoin and benzoin was studied with 25 mM sulfated β-CD (S-β-CD) alone and with a mixture of 10 mM S-β-CD and a surfactant derived from an amino acid such as (*S*)-*N*-octoxycarbonylleucine in a phosphate-borate buffer at pH 8.8 [25]. However, the role of CD–borate complexation in the enantioseparation of hydrobenzoin with S-β-CD was not discussed at all by the authors. The enantiomers of hydrobenzoin and benzoin were baseline resolved using S-β-CD (2%) as a chiral selector in 10 mM phosphate buffer at pH 3.8 [16]. Unfortunately, no detailed information on the enantioseparation of hydrobenzoin with the use of S-β-CD was provided. To the best of our knowledge, so far, only one article concerning CD–borate complexation of hydrobenzoin appeared in the literature [7]. The enantiomers of hydrobenzoin were resolved using a borate buffer containing a relatively high concentration of β-CD 1.8% (w/v) or succinyl-β-CD 2.0% (w/v) at pH 9.3 [7]. Apparently, enantioseparation and migration behavior of hydrobenzoin using S-β-CD as a chiral selector in the presence of borate complexation need to be explored.

The aims of the present investigation are thus to explore the enantioseparation and migration behavior of hydrobenzoin and its structurally related compounds in CZE using S-β-CD as a chiral selector in a background electrolyte containing borate ions at an alkaline pH, to evaluate the effectiveness of enantioseparations of hydrobenzoin with S-β-CD in the presence and absence of borate complexation, and to study the influence of S-β-CD concentration on the enantioseparation and migration behavior of hydrobenzoin in CZE in the presence and absence of borate complexation. Furthermore, the role of CD–borate complexation between the borate complexes of hydrobenzoin and S-β-CD in the enantioseparation of hydrobenzoin is examined.

## 2. Experimental

### 2.1. Apparatus

All CE separations were performed on a Beckman P/ACE 5500 system 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 and a laser printer. 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

The three benzoins studied, (*R*,*R*)-(+)-hydrobenzoin, and S-β-CD were obtained from Sigma–Aldrich (St. Louis, MO, USA). 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 were prepared by dissolving analytes in a mixture of water–methanol (9:1). The pH of a phosphate background electrolyte was adjusted to the desired pH value by mixing various proportions of a certain concentration of 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 a certain concentration of 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 p.s.i. = 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. Mesityl oxide 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:

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

where $\mu_{ep}$ is the electrophoretic mobility of the analyte tested, $\mu$ the apparent mobility, $\mu_{eo}$ 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. Influence of S-β-CD concentration on enantioseparation of benzoins

#### 3.1.1. In the absence of borate complexation

Fig. 1 shows the structures of the three benzoins studied. Fig. 2 shows the variations of the electrophoretic mobility of the three benzoins studied as a function of S-β-CD concentration in the range 0.5–3.5% (w/v) using a phosphate background electrolyte at pH 9.0. As S-β-CD is composed of a number of randomly sulfate-substituted β-CD (typically with substitution 7–11 moles/mole β-CD), thus the concentration of S-β-CD is given by weight percentage (%), instead of mM. As expected, the measured electrophoretic mobility of these three benzoins increases (toward the anode) with increasing S-β-CD concentration because of the increasing mole fraction of the anionic complexes. 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 [23,24]. Nevertheless, the order of the electrophoretic mobility of the enantiomers of these three analytes should be the same as shown in Fig. 2. Therefore, the extents of the variation in the measured electrophoretic mobility of these three analytes increase relatively in the order hydrobenzoin < benzoin < benzoin methyl ether. As the interactions of benzoins with S-β-CD can be reflected from the extents of the variation of electrophoretic mobility, the results clearly indicate that the binding strength of these three benzoins to S-β-CD increases relatively in the order hydrobenzoin < benzoin < benzoin methyl ether. Evidently, the migration order and electrophoretic mobility of these three benzoins in a phosphate background electrolyte is primarily determined by CD complexation.

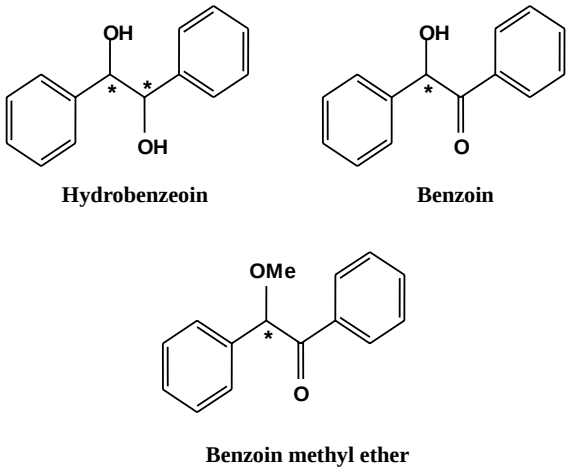


Fig. 1. The structures of the three benzoins studied (chiral centers denoted by asterisk).

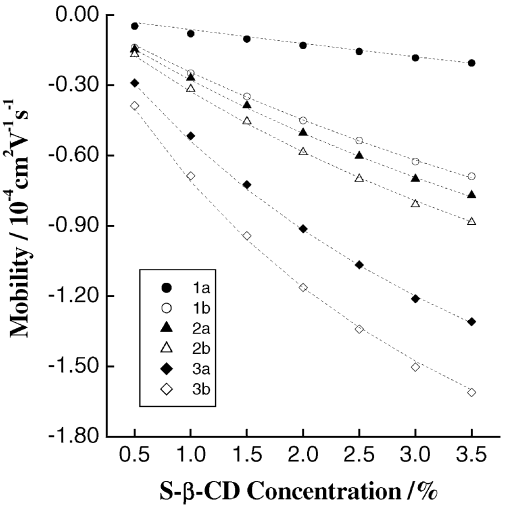


Fig. 2. Variations of the electrophoretic mobility of benzoins as a function of S-β-CD concentration in the range 0.5–3.0% using 50 mM phosphate background electrolyte at pH 9.0. Capillary, 57 cm × 50 μm i.d.; sample concentration, 20 μg/ml; detection wavelength, 214 nm; other operating conditions, 20 kV, 25 °C. Curve identification: 1, hydrobenzoin (●, ○); 2, benzoin (▲, △); 3, benzoin methyl ether (◆, ◇).

The separation window was markedly enlarged and the enantioseparability of these benzoins were remarkably enhanced as S-β-CD concentration increased from 0.5 to 3.5%. In fact, the enantiomers of hydrobenzoin, benzoin methyl ether, and benzoin could be resolved with addition of S-β-CD at concentrations above 1.0, 1.0 and 2.0%, respectively, in a phosphate background electrolyte (>15 mM) at pH 9.0. Moreover, effective enantioseparation of these three benzoins was achieved with addition of S-β-CD at concentrations above 2.5%. This is consistent with previous results [16,25,26].

On the other hand, the enantioselectivity which is defined as the ratio of the electrophoretic mobility of the two enantiomers of each individual analyte increases as S-β-CD concentration increases. As shown in Fig. 2, at a given concentration of S-β-CD, the enantioselectivity of hydrobenzoin is greater than that of benzoin methyl ether, which in turn is greater than that of benzoin in a phosphate background electrolyte. The enhancement of enantioselectivity of benzoins with increasing S-β-CD concentration can be ascribed to the increased mobility difference between the two enantiomers of each individual analyte and the decrease in the differences between the electroosmotic mobility and the average of the electrophoretic mobility of the two enantiomers [27].

#### 3.1.2. In the presence of borate complexation

Fig. 3 shows the variations of the electrophoretic mobility of benzoins as a function of S-β-CD concentration in the range 0.5–3.5% (w/v) using a borate buffer (50 mM) at pH 9.0. 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.

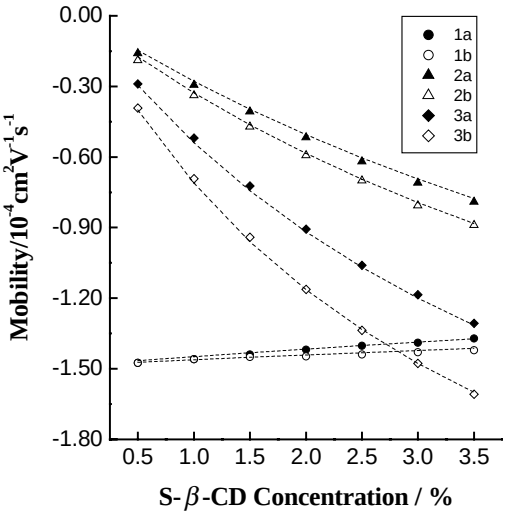


Fig. 3. Variations of the electrophoretic mobility of benzoins as a function of S-β-CD concentration using 50 mM borate buffer at pH 9.0. Other operating conditions and curve identification are the same as for Fig. 2.

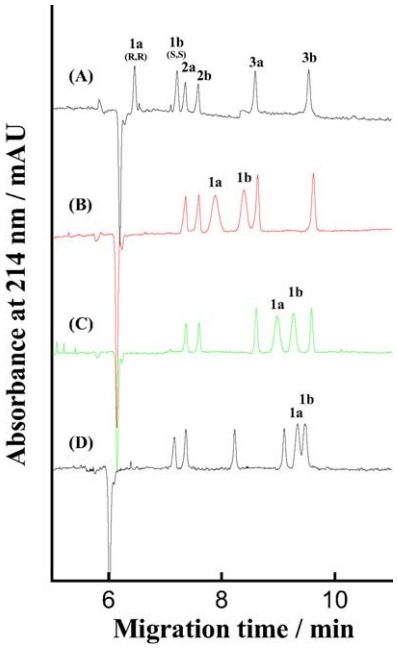


Fig. 4. Electropherograms of benzoins obtained with addition of 3.0% (w/v) S-β-CD in a phosphate-borate buffer (50 mM) at pH 9.0 at the borate/phosphate ratios of (A) 0%, (B) 10%, (C) 30%, (D) 100%. Other operating conditions are the same as for Fig. 2.

Apparently, the results reveal that the interactions of benzoin and benzoin methyl ether with borate buffer are very weak. However, as hydrobenzoin is a typical 1,2-diol compound which can form borate complexes with borate buffer, the migration behavior of hydrobenzoin in a borate buffer is very different from that in a phosphate background electrolyte. The electrophoretic mobility of hydrobenzoin varies from about $-0.10 \times 10^{-4}$ cm$^2$ V$^{-1}$ s$^{-1}$ in 50 mM phosphate background electrolyte to $-1.48 \times 10^{-4}$ cm$^2$ V$^{-1}$ s$^{-1}$ in 50 mM borate buffer containing 2.5% (w/v) S-β-CD at pH 9.0. Due to the presence of strong borate complexation, the mobility difference between the two enantiomers of hydrobenzoin decreases drastically in a borate buffer at pH 9.0 and the enantioselectivity of hydrobenzoin is greatly reduced. As shown in Fig. 3, the electrophoretic mobility of hydrobenzoin varies only to a small extent as S-β-CD concentration increases from 0.5 to 3.5% (w/v). This phenomenon is understandable because CD complexation between hydrobenzoin and S-β-CD is much weaker than borate complexation between hydrobenzoin and borate buffer, which plays a predominant role in manipulating the separation and migration behavior of hydrobenzoin in a borate buffer containing S-β-CD. Nevertheless, enantioseparation of hydrobenzoin is governed by CD–borate complexation between S-β-CD and hydrobenzoin–borate complex. It was found that the enantiomers of hydrobenzoin could be baseline resolved with addition of S-β-CD at concentrations greater than 3.0% (w/v) in 50 mM borate buffer at pH 9.0. Typical electropherograms of benzoins obtained with addition of S-β-CD in a borate buffer and of benzoins obtained in a phosphate background electrolyte at pH 9.0 as well are shown in Fig. 4D and 4A, respectively.

By spiking the (*R*,*R*)-enantiomer of hydrobenzoin, the first enantiomeric peak of hydrobenzoin was experimentally confirmed to be the (*R*,*R*)-enantiomer in both phosphate and borate background electrolytes. Evidently, the (*R*,*R*)-enantiomer of hydrobenzoin has a smaller binding strength with S-β-CD than the (*S*,*S*)-enantiomer. This is consistent with the results obtained by HPLC using β-CD as a chiral selector in a borate buffer at pH 8.3 [28]. Based on the structural similarity, it is reasonable to assign the first enantiomeric peaks of benzoin and benzoin methyl ether to the *R*-enantiomer.

### 3.2. Other factors affecting enantioseparation of hydrobenzoin involving CD–borate complexation

#### 3.2.1. Influence of borate concentration

The influence of borate concentration in the presence of S-β-CD on the enantioseparation of hydrobenzoin was examined. Fig. 5 shows the variation of the electrophoretic mobility of the enantiomers of hydrobenzoin as a function of borate concentration in the range 10–100 mM in the presence of 2.5% (w/v) S-β-CD at pH 9.0. For comparison, the variation of the electrophoretic mobility of hydrobenzoin as a function of borate concentration in the range 5–100 mM in the absence of S-β-CD is also included (Fig. 5A). As can be seen, the electrophoretic mobility (toward the anode) of hydrobenzoin increases quite drastically with increasing borate concentration from 10 (or 5 mM for hydrobenzoin in the absence of S-β-CD) to 30 mM, then increases very gradually from 30 to 100 mM. As the extent of the variation of the electrophoretic mobility as a function of borate concentration reflects the binding strength of the analyte to borate buffer, strong binding strength of hydrobenzoin to

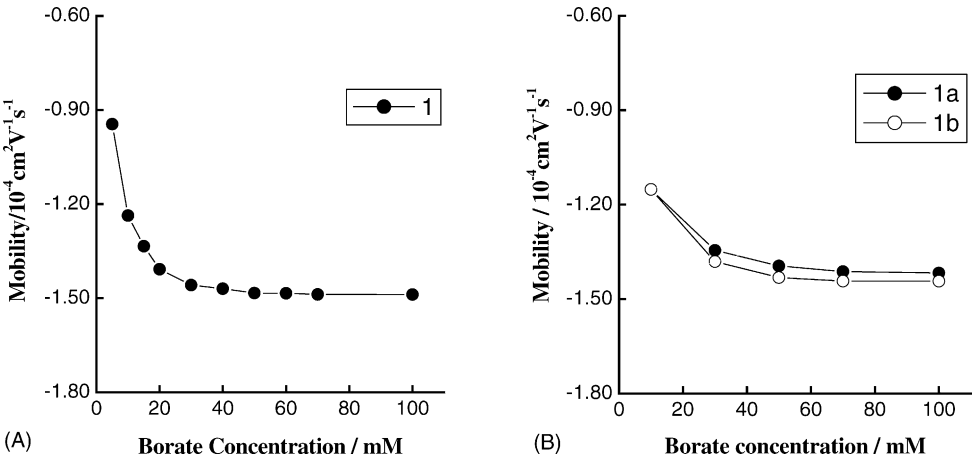


Fig. 5. Variations of the electrophoretic mobility of hydrobenzoin as a function of the concentration of borate buffer in the range 10–100 mM at pH 9.0 (A) in the absence of S-β-CD and (B) in the presence of 2.5% (w/v) S-β-CD. Other operating conditions and curves identification are the same as for Fig. 2.

borate buffer is indicative. In the presence of 2.5% (w/v) S-β-CD, the enantiomers of hydrobenzoin could be satisfactorily separated using a borate buffer at concentrations greater than 30 mM. It is also of interest to note that the measured effective mobility of the two enantiomers of hydrobenzoin decreased from $-1.48 \times 10^{-4}$ to $-1.39 \times 10^{-4}$ and $-1.44 \times 10^{-4}$ cm$^2$ V$^{-1}$ s$^{-1}$. This result indicates that the mobility of hydrobenzoin is not severely depressed by the increased ionic strength in a borate buffer with addition of 2.5% (w/v) S-β-CD.

### 3.2.2. Influence of borate/phosphate ratio of background electrolyte

The electrophoretic mobility of the enantiomers of hydrobenzoin can be varied by manipulating the composition ratio of a borate–phosphate buffer at a fixed concentration of the background electrolyte to obtain different extent of borate complexation. Thus the migration order of these three benzoins can be controlled. For illustration, electropherograms of benzoins obtained with addition of 3.0% (w/v) S-β-CD in a phosphate–borate buffer (50 mM) at the borate/phosphate ratios of 10 and 30%, together with 0 and 100%, at pH 9.0 are shown in Fig. 4. As illustrated, the enantioselectivity of hydrobenzoin was found to decrease as the borate/phosphate ratio of background electrolyte was increased. Apparently, the increased borate complexation is not in favor of enantioseparation of hydrobenzoin. Due to the presence of CD–borate complexation, broad enantiomeric peaks of hydrobenzoin were observed at the borate/phosphate ratio of 10% and 30%. However, the broadening of the enantiomeric peaks was considerably reduced at high borate/phosphate ratio of the buffer.

### 3.2.3. Influence of the pH of borate buffer

Depending on the structure of diol compounds, 1,2-diol compounds can form negatively charged complexes with borate buffer at pH > 7. In fact, the extent of borate complexation increases as buffer pH increases from 7 to a pH value in the range 9.0–9.7 [3,6,7]. Accordingly, the electrophoretic mobility of the enantiomers of hydrobenzoin, and the mobility difference between the two enantiomers as well, are expected to vary as the pH of the buffer increases. Fig. 6 shows such variations of the electrophoretic mobility of the enantiomers of hydrobenzoin in the pH range 7.0–9.1 using 50 mM borate buffer containing 3.0% S-β-CD. As can be seen, the electrophoretic mobility of the enantiomers of hydrobenzoin increases, while the mobility difference between the two enantiomers decreases, with increasing buffer pH. The results clearly demonstrate that the enantioselectivity and enantioseparation of hydrobenzoin decreases with increasing borate complexation. This phenomenon was not observed when enantioseparation of hydrobenzoin was conducted when using a phosphate background electrolyte containing S-β-CD at pH 9.0 because the differences in

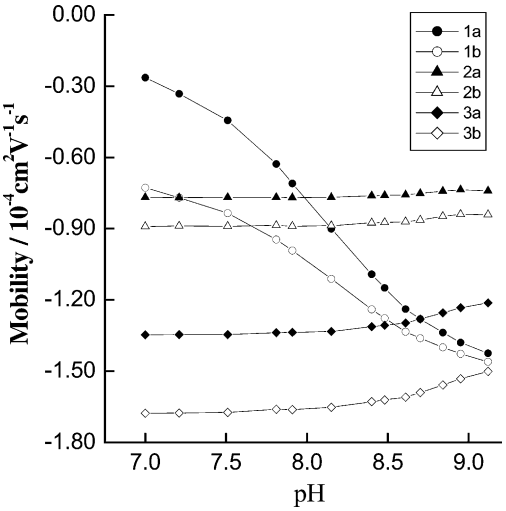


Fig. 6. Variation of the electrophoretic mobility of hydrobenzoin as a function of pH in the range 7.0–9.1 using a borate buffer (50 mM) containing S-β-CD 3.0% (w/v). Other operating conditions and curve identification are the same as for Fig. 2.

the electrophoretic mobility of benzoin and benzoin methyl ether are essentially pH independent in the pH range studied.

## 4. Conclusion

Effective enantioseparations of benzoins in CZE were achieved with addition of S-β-CD as a chiral selector in the presence and absence of borate complexation at pH 9.0. Due to a strong borate complexation, enantioselectivity of hydrobenzoin is considerably reduced with the use of a borate buffer containing S-β-CD. Enantioseparation of hydrobenzoin in a borate buffer containing S-β-CD is mainly governed by CD–borate complexation between hydrobenzoin–borate complex and S-β-CD, whereas enantioseparation of benzoins in a phosphate background electrolyte is primarily determined by CD complexation.

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