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Chiral interactions in capillary zone electrophoresis: Computer simulation and comparison with experiment

Chiral interaction in capillary electrophoresis can be modeled using pK values, mobilities of analytes, and their formation constants with the chiral selector. An existing steady-state simulation program for CE (HPCESIM) was recently extended with a chiral submenu involving the chiral parameters listed above. These were experimentally determined in both our laboratories for mandelic acid and terbutaline using hydroxypropylated β-cyclodextrin as chiral selector. A comparison was made between both sets of parameters and between experimental electropherograms and those obtained from simulation. Error analysis of the results indicate the sensitivity of the obtained results.

1 Introduction

Chiral separation has proven to be a useful tool for the separation of optical isomers, especially with application to chiral drugs [1–3]. The main advantage of CE over HPLC is the ease of method development. This method development is mainly based upon theoretical models describing the mobility of optical isomers as a function of several physical and chemical parameters, such as the pH of the background electrolyte (BGE), the concentration of the chiral selector in the BGE, and the equilibrium constant of complex formation between the chiral selector and both optical isomers. Unfortunately, it is still difficult to predict optimum separation conditions, since hardly any data is available about the magnitude of the formation constants. Using recently developed training software for chiral separation in CE [4], it is possible to simulate the separation of both acidic and basic optical isomers. Although this software is mainly intended for training purposes, in cases where sufficient data is available about the analyte-selector equilibria, the program is also suitable for method development of chiral separations in CE. In the current study, we selected an acidic and a basic compound and we experimentally determined all parameters which are relevant for complex formation: pK, values, mobilities of the solutes, and their formation constants with the chiral selector. Two cyclodextrins were chosen in order to distinguish the three different mechanisms of selective complex formation, as proposed by Rawjee et al. [5, 6]. Experimental results were compared with computer simulations using the experimentally determined chiral parameters listed above.

2 Materials and methods

2.1 Apparatus

All analyses were performed on a capillary electrophoresis system, P/ACE 2200 (Beckman, Fullerton, CA).

2.2 Methods

β-Cyclodextrin (β-CD) used in Eindhoven was obtained from Fluka (Buchs, Switzerland). Hydroxypropyl β-CD (HP-β-CD; used in both laboratories) was obtained from Cyclolab (Budapest, Hungary). The degree of hydroxypropyl substitution was 6.5. Terbutaline was obtained from Sigma (St. Louis, MO, USA). Racemic mandelic acid and the pure optical isomers of mandelic acid were obtained from Fluka. All chemicals were of analytical grade. All solutions were prepared in demineralized water. For the determination of the pK, of mandelic acid, BGEs were used with a pH between 2.54 and 3.22. These BGEs were prepared by adjusting the pH of a 10 mM NaOH solution with formic acid. Similarly, BGEs for the determination of the pK, of terbutaline were prepared by adjusting the pH of a 10 mM HCl solution with Tris(hydroxymethylamino)methane (Tris) up to pH values between 8.22 and 8.96. To determine the formation constants of the dissociated mandelic acid, the protonated terbutaline and the nondissociated forms of these analytes, 10 mM NaCl/formate, pH 2.98, and 10 mM Cl-/Tris, pH 8.70, were used. β-CD was dissolved in these BGEs at concentrations of 2.5, 5, 10, and 15 mM. HP-β-CD was used at concentrations of 5, 10, 20, and 40 mM. Prior to use, these solutions were filtered with disposable 0.45 μm pore size filters.

3 Results and discussion

3.1 Determination of the pK, and mobility of mandelic acid and terbutaline

In order to determine the pK, of mandelic acid and terbutaline, the effective mobility, μeff, of these analytes was determined as a function of the pH of the BGE. In Eindhoven, mobilities were determined by the dual-marker method of Williams et al. [7], whereas mobilities in
Table 1. Relevant chiral parameters of mandelic acid and terbutaline

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mandelic acid</th>
<th>Terbutaline</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \mu_0 )</td>
<td>( 10^{-9} \text{ m}^2/\text{V/s} )</td>
<td>–28.7 (0.1)</td>
</tr>
<tr>
<td>( pK_a )</td>
<td>–</td>
<td>2.94 (0.02)</td>
</tr>
<tr>
<td>( RM )</td>
<td>–</td>
<td>0.62 (0.15)</td>
</tr>
<tr>
<td>( K_1 )</td>
<td>–</td>
<td>198 (5)</td>
</tr>
<tr>
<td>( \Delta K_1 )</td>
<td>%</td>
<td>0</td>
</tr>
<tr>
<td>( K_2 )</td>
<td>–</td>
<td>55.8 (7.8)</td>
</tr>
<tr>
<td>( \Delta K_2 )</td>
<td>%</td>
<td>0</td>
</tr>
</tbody>
</table>

*Standard deviations are given in parentheses.*

The differences in the \( pK_a \) values, obtained in the two laboratories, are smaller than 1%. The difference between our experimentally obtained data and the data found in literature is substantial, especially for mandelic acid (2.93 versus 3.34). Literature values, however, are extrapolated to an ionic strength of \( I = 0 \text{ mM} \), whereas our \( pK_a \) values were determined at \( I = 10 \text{ mM} \). Obviously, the use of the literature value of the \( pK_a \) of mandelic acid would have introduced a major mistake in the determination of the formation constant of nonionic mandelic acid with cyclodextrins. This stresses the importance of determining the \( pK_a \)s, using identical experimental conditions (temperature, ionic strength) as those applied for the determination of the formation constants.

### 3.2 Determination of formation constants

Formation constants for terbutaline and mandelic acid were determined using native \( \beta-CD \) as well as \( HP-\beta-CD \). Initial experiments pointed out that only the modified cyclodextrin was able to resolve the optical isomers of both racemic analytes whereas native \( \beta-CD \) showed only enantioselectivity towards the terbutaline enantiomers. For this reason, only \( HP-\beta-CD \) was used in both laboratories, while the determination of formation constants using \( \beta-CD \) was only performed in Eindhoven. In order to determine the formation constants of nonionic interaction \( (K_0) \) and ionic interaction \( (K_1) \), the effective mobilities of terbutaline and mandelic acid enantiomers were determined at different CD concentration levels, using a high and a low pH BGE. For the determination of \( K_1 \) of terbutaline, a 10 mM sodium/formate buffer of pH 2.98 was applied. For the determination of \( K_1 \) of mandelic acid, a 10 mM hydrochloric acid/Tris buffer, pH 8.70, was used. Under these experimental conditions, mandelic acid and terbutaline could be regarded as a strong acid and base, respectively. The same BGEs could be used for the determination of the \( K_1 \) values of both racemic analytes, since the pH values of these buffers are close to the \( pK_a \) values of mandelic acid \( (pK_a = 2.93) \) and terbutaline \( (pK_a = 8.68) \). Consequently, at these pH values, both ionic and nonionic interaction occur. The \( K_0 \) values could be determined by plotting the ratio of the mobility difference and the concentration of chiral selector versus the effective mobility according to Eq. (2) [8]. This equation is only valid for strong acids and bases. Using the specified experimental conditions (mandelic acid at pH 8.70 and terbutaline at pH 2.98) the separands are treated as such.

\[
\frac{\mu_1 - \mu_{eff}}{\mu_1} = K_1 (\mu_{eff} - \mu_0)
\]

where \( \mu_1 \) and \( \mu_0 \) are the mobilities of the analyte-CD complex, and of the free enantiomer (equal to \( \mu_1 \) for strong ions), respectively and \( \mu_1 \) is the CD concentration. This equation is derived from the simple model of Wren and Rowe [9] which describes the mobility of enantiomers as a function of the CD concentration and formation constant. It is assumed that the analytical CD concentration is practically the same as the concentration of the CD-buffer complexes. The existence of the CD-buffer complex does not inhibit the validity of the model [5], but the \( K_1 \) values found may depend on the
Table 2. Relevant chiral parameters of mandelic acid and terbutaline interacting with HP-β-CD: a comparison between data from Eindhoven and Vienna*

<table>
<thead>
<tr>
<th></th>
<th>Mandelic acid</th>
<th>Terbutaline</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Eindhoven</td>
<td>Vienna</td>
</tr>
<tr>
<td>( \mu_0 ) (10^{-9} \text{m}^2/\text{V.s})</td>
<td>-28.7 (0.1)</td>
<td>-29.8 (0.1)</td>
</tr>
<tr>
<td>( \mathcal{p} K_1 )</td>
<td>2.94 (0.02)</td>
<td>2.92 (0.04)</td>
</tr>
<tr>
<td>( RM )</td>
<td>0.76 (0.12)</td>
<td>0.79 (0.09)</td>
</tr>
<tr>
<td>( K_1 ) %</td>
<td>127 (4)</td>
<td>143 (5)</td>
</tr>
<tr>
<td>( \Delta K_1 ) %</td>
<td>6 (1)</td>
<td>13 (2)</td>
</tr>
<tr>
<td>( K_2 ) %</td>
<td>24.6 (2.3)</td>
<td>25.9 (2.4)</td>
</tr>
<tr>
<td>( \Delta K_2 ) %</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

a) Standard deviations are given in parentheses

where \( K_{AV} \) is the average formation constant of the \((R, S)\) enantiomers. In order to determine the \( K_i \) values of both analytes, the data was fitted according to the theoretical model of Rawjee et al. [5, 6]. In this model it is assumed that the \( pK \) values of the two isomers in the analyte-selector complexes are identical, which is not obvious for all cases [10].

The model describes the effective mobility of the enantiomers as a function of \( K_1, K_2 \), the acid and base dissociation constant (\( K_a \) and \( K_b \)), and the mobility of the analyte-selector complex. These relations can be linearized according to Reijenga et al. [4]:

For weak acids:

\[
(1 + RM K_2 C_i) \frac{\mu_c}{\mu_0} - 1 - K_2 C_i - [H_2O^+] / K_a = K_1 C_i \frac{[H_2O^+]}{K_a}
\]

(5)

For weak bases:

\[
(1 + RM K_2 C_i) \frac{\mu_c}{\mu_0} - 1 - K_2 C_i - [OH^-] / K_b = K_1 C_i \frac{[OH^-]}{K_b}
\]

(6)

Figure 3A shows the left-hand side of Eq. (3) plotted versus \( C_i \), so that the slope equals \( K_1[H_2O^+]/K_a \), resulting in the nonionic complex formation constants for mandelic acid enantiomers using the two different CDs. Analogously, \( K_1 \) values for terbutaline could be determined by plotting the left-hand side of Eq. (4) versus CD concentration. This is shown in Fig. 3B for both CDs. All forthcoming results are listed in Table 1 and 2. All parameters determined experimentally were obtained from linearized graphs. Error analysis of the scatter of these graphs readily yielded standard deviations of both slope and intercept, resulting in standard deviations of mobilities and \( K_i \) values. These are tabulated along with their average values. The number of determinations used in the regression analyses were 8–12 for the \( pK \) determination and 6–8 for the determination of the formation constants.

Several observations regarding lab-to-lab differences can be made. In view of calibrating the pH meter with pH references, an error of 0.02 is a fair estimate. This corresponds to the error in the \( pK \) or \( K_i \) thus determined. In the determination of the \( K_i \) values using Eq. (2), the error in \( C_i \) is neglected. Small differences in effective mobility are measured, accounting for the large standard deviation of the \( K_i \) values (around 10%). If, for the determination of the \( K_i \) values, using Eqs. (3) or (4), we look
Figure 3. Determination of $K_1$ for (A) mandelic acid and (B) terbutaline enantiomers. (O), L(+)-mandelate, (△), D(-)-mandelate. Other details as in Fig. 2.

at regression statistics only, scatter alone yields a lower relative standard deviation than obtained for $K_2$. This does not seem entirely correct, as the values for $K_1$, $K_2$, $[H^+]$, $[OH^-]$, and $K_1$ are included as well. Having used averaged values for these, their effect on the standard deviation is lost as well, so the standard deviation of $K_1$ is thus underestimated. For example, consider the right-hand side of Eqs. (3) or (4). The error in the $K_1$ or $K_2$ is 4.5% (except where the error in pH is 0.04, then it is 9%). The regression error is in the range of 2–3%. The error in $[H^+]$ or $[OH^-]$ is around 5%. As a result, a more realistic relative standard deviation of $K_1$ would be around 5–10%.

Native β-CD did not show any selectivity towards the enantiomers of mandelic acid. The enantiomers of terbutaline could be separated at low pH, where the basic compound is fully protonated. No resolution was obtained at high pH (pH = pK_a). This kind of separation is referred to as a Type II separation [5, 6] or ionoselective separation [11]. HP-β-CD was the more efficient selector. It allowed the separation of the enantiomers of both mandelic acid and terbutaline. The enantiomers of mandelic acid could be separated at low pH, but not at high pH, indicating that the nondissociated acid interacts stereoselectively with the modified CD. This kind of separation is referred to as a Type I separation [5, 6] or desionoselective separation [11]. Chiral interaction, resulting in optical resolution, between the enantiomers of terbutaline and HP-β-CD was observed at both low and high pH. Both the nonionic as well as the protonated terbutaline interact stereoselectively with the chiral selector. This kind of separation is referred to as a Type III separation [5, 6] or duoselective separation [11]. Biggin et al. [11] recently showed the experimental evidence for the existence of duoselective enantiomer separations of weak acids. The migration order of the enantiomers was determined by spiking the racemic sample with pure standards. The L(+)-isomer of mandelic acid migrated slower than the D(-), and therefore $K_{L}>K_{D}$. This confirms earlier experiments of Nardi et al. [12]. Fanali [13] showed that (+)-terbutaline has the highest affinity towards the CD cavity. This was verified for native β-CD, heptakis(2,6-di-O-methyl)-β-CD and heptakis(2,3,6-tri-O-methyl)-β-CD using a BGE at low pH (pH 2.5).

Using the experimentally obtained parameters presented in Table 2, we simulated the chiral separation of man-
delic acid and terbutaline enantiomers, using HP-β-CD at a concentration of 40 mM, using the low pH BGE. This was carried out using the steady-state simulation program for CE [14, 15], recently extended with a submenu for chiral interactions [4]. The simulations using the parameters obtained in Eindhoven are shown in Fig. 4A, the simulation using parameters obtained in Vienna are shown in Fig. 4B, and the actual separations (performed in Eindhoven) are shown in Fig. 4C. Reasonable coincidence is found between both simulated electropherograms and the experimentally obtained electropherograms. The selectivities calculated using the “Vienna” simulation match the selectivity of terbutaline enantiomers (0.077 vs. 0.073), whereas the selectivity for mandelic acid enantiomers is overestimated (0.082 vs. 0.039). The “Eindhoven” simulation exactly matches the experimentally obtained selectivity for mandelic acid enantiomers (0.039). This simulation, however, overestimated the selectivity for terbutaline enantiomers (0.104 vs. 0.073). The simulated efficiencies are overestimated in all cases, resulting in much higher resolutions than those calculated from Fig. 4C. A possible explanation for these high efficiencies can be an incorrect modeling of the “stacking” process by the simulation algorithm, occurring after the injection of the sample.

4 Concluding remarks
In conclusion, the accuracy and precision of the determination of chiral complex equilibrium constants in CE are limited. This is especially so in the case of the relatively small K values in the examples chosen, because calculations are based on determinations of very small mobility differences. In addition, small errors in pH, [H+] RM values propagate in the calculation of the final K values. Considering these relatively large standard deviations, reasonable agreement was found between the values of the chiral parameters determined in both laboratories. Agreement between simulations based on the two different sets of parameters and between simulation and experiment were quite satisfactory.

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5 References