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Chiral separation of dioxypromethazine enantiomers by capillary electrophoresis using β-cyclodextrin as a chiral selector

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Abstract

A method was developed to separate dioxypromethazine enantiomers using capillary electrophoresis. The chiral separation was accomplished by the addition of β-cyclodextrin (β-CD) that was used as a chiral selector to the buffer. The effects of pH, β-CD concentration, electrolyte concentration and methanol concentration on the chiral separation are investigated in detail. A model was proposed to explain the effects of pH and β-CD concentration in buffer on the chiral separation. A diode array detector was employed for the detection of enantiomers, which obtained three-dimensional electropherograms of enantiomers.

Keywords: Enantiomer separation; Dioxypromethazine

1. Introduction

Stereochemistry can have a significant effect on the biological activity and the side effects of a drug, different from those of the optically pure drugs. Therefore, the development of a chiral separation method for the determination of optical purity is desirable in many areas [1].

In the more conventional chromatographic procedures such as high-performance liquid chromatography [2], gas chromatography [3] and thin-layer chromatography [4], chiral separations were achieved by the use of chiral additives in the mobile phase or the use of a chiral stationary phase.

Capillary electrophoresis provided a highly efficient separation method for chiral compounds by the use of a chiral selector in the running buffer. These chiral selectors used in chiral separation mainly include bile salts [5], chiral surfactants [6], chiral crown ethers [7], cyclodextrin (CD) derivatives [8–10] and so on, some of which have been successfully applied in the conventional chromatographic procedures mentioned above. In the chiral separation for capillary electrophoresis, CDs have been used extensively as chiral selectors. The chiral separation was usually achieved by the addition of CD to the running buffer. CDs are cyclic oligosaccharides with truncated cylindrical molecular shapes. They have particular names: α-CD, β-CD and γ-CD for those having six, seven and eight glucopyranose units, respectively. Since their inside surfaces are hydrophobic, CDs tend to form inclusion complexes that fit their cavities by hydrophobic interaction. The size of the cavity differs significantly among α-CD, β-CD and γ-CD. Some researchers [11–13] investigated the mechanism of chiral separation using CDs as selectors. It was generally agreed that chiral separation was achieved based on the difference in

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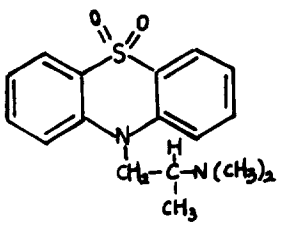

Fig. 1. Structure of dioxypromethazine.

inclusion complex formation constants between a pair of enantiomers and CDs.

Dioxypromethazine enantiomers are basic drugs, whose structure is shown in Fig. 1 and which have a cough-easing function. It is a significant ingredient of some drugs and exists as a pair of enantiomers. In the present work, capillary electrophoresis was used for the separation of dioxypromethazine enantiomers using β-CD as a chiral selector. The effects of pH, β-CD concentration, electrolyte concentration and methanol concentration on the chiral separation of dioxypromethazine enantiomers were investigated in detail.

2. Theory

In the chiral separation of basic (or acidic) enantiomers by capillary electrophoresis using a CD as a chiral selector, both the pH and the CD concentration in the buffer have significant effects on the mobility of the individual enantiomers and the chiral separation efficiency. Rawjee et al. investigated the electrophoretic behavior of acidic enantiomers in buffer containing CD and proposed a model to explain the separation mechanism of acidic enantiomers [12]. With reference to their work, we propose the following model to simulate the effects of pH and CD concentration on the separation of basic enantiomers, on the basis of both protonation equilibria and complexation equilibria.

In the buffer, basic enantiomers, such as *R*-dioxypromethazine (R) and *S*-dioxypromethazine (S) undergo basic dissociation according to the following equations:

$$R + H_2O - HR^+ + OH^-$$

$$K_b^R = [HR^+][OH^-]/[R] \quad (1)$$

$$S + H_2O = HS^+ + OH^-$$

$$K_b^S = [HS^+][OH^-]/[S] \quad (2)$$

The complexations of R, S, HR^+ and HS^+ with CD will be expressed:

$$R + CD = RCD \quad K_{RCD} = [RCD]/[R][CD] \quad (3)$$

$$S + CD = SCD \quad K_{SCD} = [SCD/[S][CD] \quad (4)$$

$$HR^+ + CD = HRCD^+$$

$$K_{HRCD} = [HRCD^+]/[HR^+][CD] \quad (5)$$

$$HS^+ + CD = HSCD^+$$

$$K_{HSCD} = [HSCD^+]/[HS^+][CD] \quad (6)$$

The mass balance equations of R and S related species are in terms of their analytical concentrations C_R and C_S:

$$C_R = [R] + [HR^+] + [RCD] + [HRCD^+] \quad (7)$$

$$C_S = [S] + [HS^+] + [SCD] + [HSCD^+] \quad (8)$$

The respective mole fractions of the positively charged species HR^+, $HRCD^+$, HS^+, and $HSCD^+$ are:

$$\alpha_{HR^+} = [HR^+]/C_R \quad (9)$$

$$\alpha_{HRCD^+}[HRCD^+]/C_R \quad (10)$$

$$\alpha_{HS^+} = [HS^+]/C_S \quad (11)$$

$$\alpha_{HSCD^+} = [HSCD^+]/C_S. \quad (12)$$

An analytical expression can be obtained from Eqs. 1–6 for the species concentrations $[HR^+]$, $[HS^+]$, $[HRCD^+]$ and $[HSCD^+]$, as well as [R], [S], [RCD], and [SCD]. Substitution of these expressions into Eqs. 7–12 yields:

$$\alpha_{HR^+} = \frac{1}{1 + K_{HRCD^+}[CD] + (K_{RCD}[CD] + 1)[OH^-]/K_b^R} \quad (13)$$

$$\alpha_{HRCD^+} = \frac{K_{HRCD^+}[CD]}{1 + K_{HRCD^+}[CD] + (K_{RCD}[CD] + 1)[OH^-]/K_b^R} \quad (14)$$

$$\alpha_{Hs^+} = \frac{1}{1 + K_{HSCD^+}[CD] + (K_{SCD}[CD] + 1)[OH^-]/K_b^S} \quad (15)$$

$$\alpha_{HSCD^+} = \frac{K_{HSCD^+}[CD]}{1 + K_{HSCD^+}[CD] + (K_{SCD}[CD] + 1)[OH^-]/K_b^S}. \quad (16)$$

The effective mobilities of the two enantiomers can be expressed as the mole fraction-weighted ionic mobilities of the respective species [14]:

$$\mu_R^{eff} = \mu_{HR^+}^0 \alpha_{HR^+} + \mu_{HRCD^+}^0 \alpha_{HRCD^+} \quad (17)$$

$$\mu_S^{eff} = \mu_{HS^+}^0 \alpha_{HS^-} + \mu_{HSCD^+}^0 \alpha_{HSCD^+} \quad (18)$$

$$\Delta\mu = \mu_R^{eff} - \mu_S^{eff} \quad (19)$$

$\Delta\mu$ can be used as the window of optimizing the conditions of chiral separation. In fact, the ionic mobilities of the enantiomers in buffer are identical: $\mu_{HR^+}^0 = \mu_{HS^+}^0 = \mu^0$, $\mu_{HRCD^+}^0 = \mu_{HSCD^+}^0 = \mu_{CD}^0$. The basic dissociation constants of two enantiomers are also identical [13]: $K_b^S = K_b^R = K_b$. Since they mainly depend on the hydrophobicity of the hydrophobic part and the stereo-structure of the chiral part, the complexation constants of individual enantiomer related species can be considered as identical: $K_{HRCD^+} = K_{RCD} = K_R$, $K_{HSCD^+} = K_{SCD} = K_S$.

Substitution of Eqs. 13–18 and the hypothesis above into Eq. 19 yields:

$$\Delta\mu = \frac{[CD](K_R - K_S)(\mu_{CD}^0 - \mu^0)}{(1 + [OH^-]/K_b)(K_R[CD] + 1)(K_S[CD] + 1)} \quad (20)$$

Eq. 20 reflects the effects of pH and the concentration of CD on the effective mobility difference of the enantiomers. It is seen in Eq. 20 that $\Delta\mu$ increases with an increase in the hydrogen ion concentration of the buffer. If the pH of the buffer is low, that is $[OH]/K_b \ll 1$, Eq. 20 is simplified to:

$$\Delta\mu = \frac{[CD](K_R - K_S)(\mu_{CD}^0 - \mu^0)}{(K_R[CD] + 1)(K_S[CD] + 1)} \quad (21)$$

Eq. 21 is as same as Eq. 5 in Ref. [11]. In this case, the model proposed by Wren and Rowe can be used to express the mechanism of chiral separation.

3. Experimental

3.1. Instrumentation

A P/ACE System 5500 CE apparatus coupled with a diode array detector (Beckman Instruments, Fullerton, CA, USA) was used in capillary electrophoresis. A pH meter (Lezi instrument factory, Shanghai, China) was employed to measure the pH of the buffer. The uncoated capillary [57 cm (effective length 50 cm) × 75 μm I.D.) was provided by Beckman Instruments.

3.2. Reagents

Dioxypromethazine enantiomers were purchased from the Institute of Analysis and Testing for Drugs and Biological Reagents of China. β-CD was purchased from Sigma. Other chemicals were of analytical-reagent grade. The running buffer containing Tris and H_3PO_4 was formed by the addition of H_3PO_4 (80%) to Tris solution, in which H_3PO_4 was used for adjusting the pH of the running buffer. The sample solutions and buffer were filtered with a membrane filter of 0.45 μm pore size.

3.3. Capillary electrophoresis

Before injection of the sample, a new capillary was rinsed with 0.1 *M* NaOH, methanol, water and running buffer, respectively. Pressure injection was employed, in which the injection time was 5 s at 3447.4 Pa. The buffer was replaced after each run. A diode array detector, with scan wavelength from 190 nm to 360 nm, was used for the detection of analytes. Data were analyzed using System Gold software. The temperature was set at 25°C and

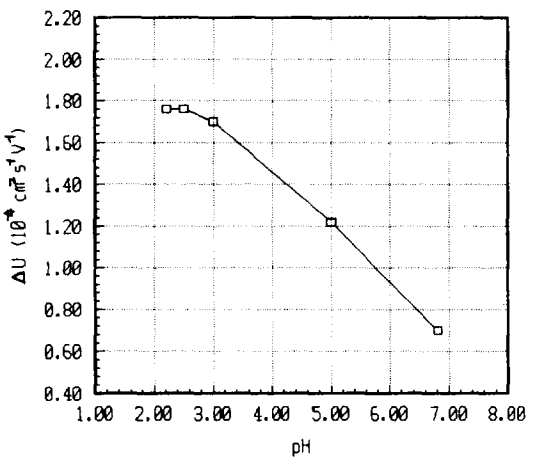

Fig. 2. Effect of pH of buffer on $\Delta\mu$. Electrophoretic conditions: buffer, 0.03 *M* Tris–H_3PO_4 containing 0.02 *M* β-CD; applied voltage, 20 kV; temperature, 25°C; detection wavelength, 228 nm; sample concentration, 50 μg/ml.

electrophoretic runs were performed under the conditions specified in the figures. Phenol was used as a neutral marker to monitor the electrosmotic flow. In the experiment, the difference in the mobility ($\Delta\mu$) of the enantiomers was used as the window for optimizing experimental conditions. The mobility difference was calculated according to the following equation:

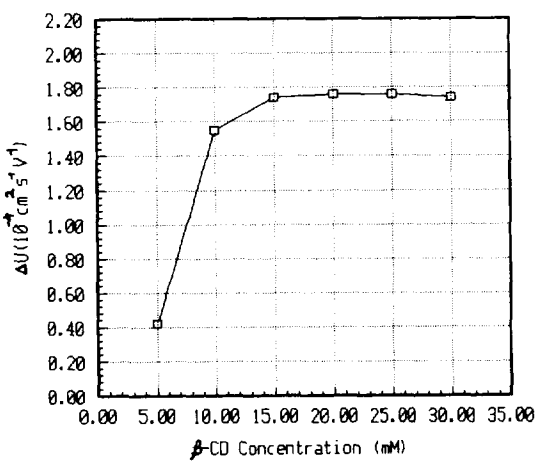

Fig. 3. Effect of β-CD concentration on $\Delta\mu$. Electrophoretic conditions: buffer, 0.03 *M* Tris–H_3PO_4 (pH 2.5); other conditions as in Fig. 2.

$$\Delta\mu = Il/V(1/t_R - 1/t_S) \quad (22)$$

Where L was the total length of capillary and l the effective length of capillary; t_R and t_S were the migration times of the enantiomers; V was voltage applied in electrophoresis.

4. Results and discussion

4.1. Effect of pH on $\Delta\mu$

Dioxypromethazine enantiomers are basic drugs which exist as positively charged ions in acidic buffer. The pH was the significant factor affecting the chiral separation. Fig. 2 shows the effects of buffer pH on $\Delta\mu$. It is shown in Fig. 2 that $\Delta\mu$ increased with a decrease in buffer pH. When the buffer pH was ca. 3, $\Delta\mu$ tended to a constant value. It is well known to us that the degree of protonation of dioxypromethazine enantiomers increases as the pH value of the buffer decreases, on the basis of the acid base equilibria of the R- and S-enantiomers. The effective mobilities are related to the degree of protonation of the dioxypromethazine enantiomers [13]. The effective mobility of basic compounds increases with an increase in the hydrogen ion concentration of the buffer, which leads to an increase in $\Delta\mu$. It was seen in Eq. 20 that $\Delta\mu$ increased with an increase in the hydrogen ion concentration (or decrease in the hydroxyl ion concentration) when the β-CD concentration was constant. Since K_b of dioxypromethazine enantiomers was in the 10^{-9} range, it was predicted by Eq. 20 that $\Delta\mu$ tended to a constant value when pH of the buffer reached ca. 3: ($[OH^-]<0.01K_b$, $1+ [OH^-]/K_b=1$), which was in good agreement with that in Fig. 2, providing strong support for the model proposed in Eq. 20. Additionally, the electrosmotic flow decreased with an increase in hydrogen ion concentration. The low pH of the buffer caused an improvement in the chiral separation.

4.2. Effect of β-CD concentration on $\Delta\mu$

The chiral recognition of β-CD is based on the difference in the formation constants of β-CD com-

plexes with enantiomers, which reflect the difference in hydrophobic complex-forming interaction between the CD cavity and the guest molecule. The β-CD concentration is an important factor affecting the chiral separation according to Eq. 21. Fig. 3 displays the effect of β-CD concentration on $\Delta\mu$. It is shown in Fig. 3 that $\Delta\mu$ increased with increasing β-CD concentration. When the β-CD concentration was higher than 15 mM, $\Delta\mu$ tended towards a constant value. On the basis of complexation equilibria between β-CD and enantiomers, the complex concentration increased with an increase in β-CD concentration. Moreover, it led to an increase in $\Delta\mu$, due to differences in the formation constants. In pH 2.5 buffer, dioxypromethazine enantiomers are protonized completely. Thus, the relationship between $\Delta\mu$ and CD concentration can be expressed as in Eq. 21. According to the chiral separation mechanism pro-

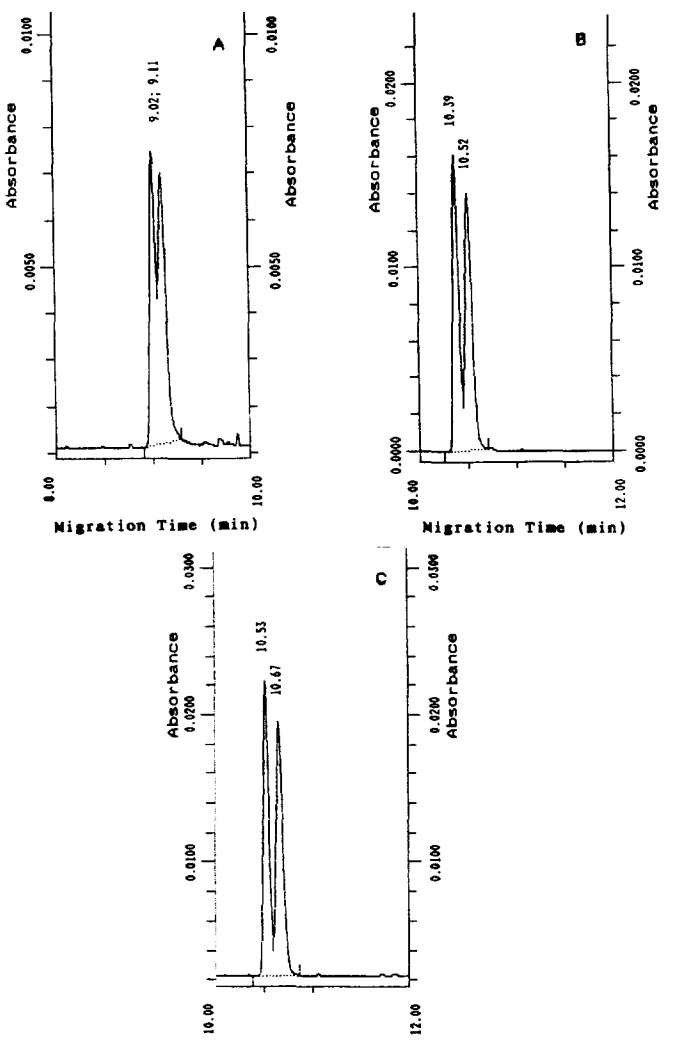

Fig. 4. Electropherograms of dioxypromethazine enantiomers in three different concentrations of electrolyte buffer. Electrophoretic conditions: (A) buffer, 0.02 M Tris–H_3PO_4; (B) buffer, 0.03 M Tris–H_3PO_4; (C) buffer, 0.04 M Tris–H_3PO_4 (pH 2.5); other conditions as in Fig. 2.

posed by Wren and Rowe [11], there was an optimum β-CD concentration for chiral separation. The optimum β-CD concentration can be predicted by Eq. 23 if K_R and K_S are known:

$$[CD]_{opt} = 1/(K_R K_S)^{1/2}. \quad (23)$$

It is shown in Fig. 3 that the optimum β-CD concentration was ranged from 15 m*M* to 20 m*M*.

4.3. Effect of electrolyte concentration on the chiral separation

Fig. 4 shows the electropherograms of enantiomers in three different electrolyte buffer concentrations. It is shown in Fig. 4 that the chiral separation of enantiomers improved with an increase in electrolyte concentration. This was because an increase in electrolyte concentration decreased the effects which were caused by the differences of the pH and electrolyte concentrations of the sample zone from that of the buffer. Moreover, the increase in electrolyte concentration made the electroomotic flow decrease, and led to an improvement in the resolution of enantiomers [14]. However, the current in electrophoresis increased with increasing electrolyte concentration of the buffer. When the Tris concentration was 40 m*M*, the current reached 65 μA. The high concentration of electrolyte in buffer was not beneficial to chiral separation, due to a Joule heating effect.

4.4. Effect of methanol on the chiral separation

Some researchers [8] reported that the addition of organic solvents to the buffer improved the separation efficiency of chiral compounds. However, we found that the addition of methanol to the buffer reduced the separation efficiency for dioxypomethazine enantiomers. Fig. 5 shows the electropherogram of enantiomers using 10% methanol in the buffer. In comparison with Fig. 4C, the separation efficiency in Fig. 5 is reduced significantly. The result was attributed to the fact that the solubility of β-CD in the buffer was reduced by the addition of methanol to the buffer, which led to lower stability of the enantiomer–β-CD complexes. We tried to investigate the effects of higher methanol concentrations in the chiral separation of enantiomers, but this investigation was discontinued because the solubility of β-CD in buffer containing methanol imposed restrictions on further increases in methanol concentration.

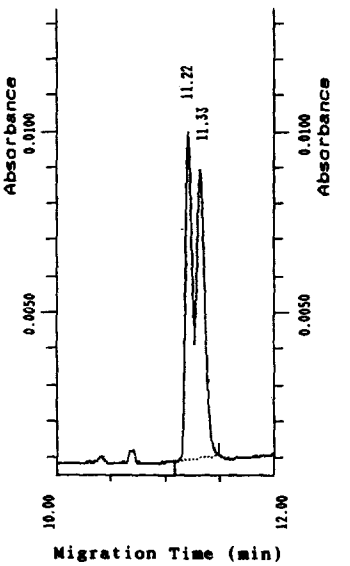

Fig. 5. Electropherogram of dioxypromethazine enantiomers in buffer containing methanol. Electrophoretic conditions: buffer, 0.03 *M* Tris–H_3PO_4–0.02 *M* β-CD containing 10% methanol (pH 2.5); other conditions as in Fig. 2.

4.5. Application of a diode array detector

The use of a diode array detector in capillary electrophoresis allows to obtain three-dimensional electropherograms of analytes, which can be used in the qualitative analyses of unknown samples. Fig. 6 shows a three-dimensional electropherogram of enantiomers. It is shown in Fig. 6 that the spectra of a pair of enantiomers are identical.

Acknowledgments

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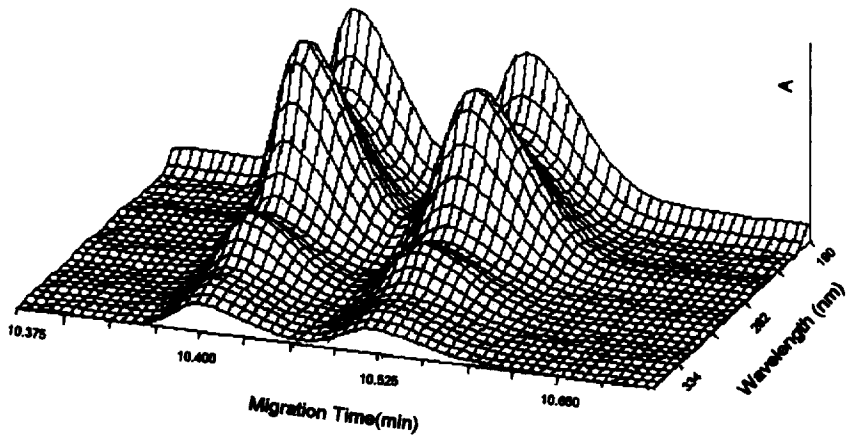

Fig. 6. Three-dimensional electropherogram of dioxypromethazine enantiomers. Electrophoretic conditions: buffer, 0.04 *M* Tris–H_3PO_4–0.02 *M* β-CD (pH 2.5); other conditions as in Fig. 2. Scan wavelength, 190–360 nm; scan time, 10.375–10.883 min.

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