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# Chiral separation of pemoline enantiomers by cyclodextrin-modified micellar capillary chromatography

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**Abstract**

Micellar electrokinetic chromatography (MEKC) was successfully applied to the chiral separation with the addition of cyclodextrins (CDs) as chiral selector to running buffer. Chiral separation depended on the type of CDs. Mono-3-*O*-phenylcarbamoyl-β-CD was effective for the chiral separation of pemoline. We investigated the type and concentration of CD and other parameters such as buffer pH, the concentration of SDS and the effect of organic modifier. The conditions for enantiomeric separation of pemoline were as follows: 40 mmol/l borate buffer at pH 9.0 with 40 mmol/l SDS, 20 mmol/l mono-3-*O*-phenylcarbamoyl-β-CD and 10% 2-propanol. Baseline separation (Rs = 2.21) of pemoline can be achieved. 

*Keywords:* Capillary electrophoresis; Enantiomer separation; Cyclodextrin; β-Cyclodextrin derivative; Chiral drugs

## 1. Introduction

Micellar electrokinetic chromatography (MEKC) which was introduced by Terabe et al. [1] is a variation of capillary electrophoresis (CE), where separation is a function of the distribution of the solutes between a micellar phase, working as pseudo-stationary phase, and an aqueous mobile phase, which are moving at different velocities with a fused silica capillary.

MEKC, which was first developed for the separation of non-ionic compounds using the CZE technique [2], has many attractive advantages even for the separation of ionic compounds [3,4], e.g. water soluble vitamins [5]. The selectivity and peak shape was improved in comparison with CZE. An important advantage of MEKC over the other type of chromatography is that the micellar phase and the chiral selector can easily be changed.

Most separation studies on MEKC have been performed with sodium dodecyl sulphate (SDS) [4,6,7], some other surfactants and bile salts [8] have also been successfully employed.

In a chiral compound the enantiomers may have different pharmacokinetic properties such as absorption, distribution, matabolism and excretion quantitatively or qualitatively different pharmacological or toxicological effects [9]. Therefore, it is important to produce individual drug enantiomers.

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A prerequisite for the accurate study of stereoselective effects of the action of chiral drugs is the development of a versatile and accurate method for the resolution of enantiomers. The application of new types of chiral selectors with higher efficiency is of primary importance in this area at present.

In this paper, a new derivative of β-cyclodextrin mono-3-*O*-phenylcarbamoyl-β-CD which was improved as a good chiral selector [10] for many chiral drugs by us was used as a effective chiral selector for the separation of pemoline by cyclodextrins (CD)-MEKC mode.

Pemoline (2-imino-4-oxo-5-phenyloxazolidine) belongs to the groups of medicines called central nervous system stimulants, it is mainly used to treat children with attention-deficit hyperactivity disorder [11]. HPLC has been used for its bioassay [12]. However, it is difficult to develop chiral HPLC methods that are robust or rugged. The lack of robustness arises from changes in the properties of columns with time, poor column to column reproducibility and the relatively low efficiency obtained from most chiral column. In addition, CE separations have the advantages of shorter run times, greater resolution and smaller amounts of chiral additive. So far as we know, there is only one report [13] about the chiral separation of pemoline by CZE mode, the resolution is only 1.40. In addition, the proposed method was not fully validated, and no application was given.

The aim of the present work was to develop a MEKC method for the separation of pemoline. Simple and rapid MEKC methods were developed for the analysis of pemoline in a crude drug form (tablets). We carried out experiments investigating the best conditions for the separation of pemoline.

## 2. Experiment

### 2.1. Apparatus

The experiments were carried out on a laboratory-assembled CE apparatus, equipped with a multiwavelength UV detector. The UV signals were recorded at 214 nm. A fused silica capillary of 60 cm length (effective length 50 cm) and of 75 μm i.d. (Hebei Yongnian Optical Fiber Factory, China) was used as a separation tube. A high-voltage power supply that can provide voltage from 0 to 30 kV was used to drive the separation.

### 2.2. Chemicals and reagents

Racemic pemoline was purchased from Sigma (St. Louis, MO, USA). Crude drug of pemoline enantiomers was purchased from Shanghai drug store. Mono-3-*O*-phenylcarbamoyl-β-CD [10], which had been used for the separation of many drugs by us before, was synthesized in our laboratory. α-, β-, γ-CD, 2,6-di-*O*-methyl-β-CD (DM-β-CD) and Hydroxypropyl-β-CD (HP-β-CD) were obtained from Sigma. All organic solvents and other chemicals were of analytical grade. SDS, boric acid ($H_3BO_3$), Sodium hydroxide (NaOH) and disodium tetraborate ($Na_2B_4O_7{\cdot}10H_2O$) were obtained from Beijing Chemical Factory. Double distilled water was used for the preparation of all solutions. 0.25 μm pore size filters was used to filter all the solutions.

### 2.3. Capillary electrophoresis

A new capillary was conditioned by flushing successively with 1.0 mol/l NaOH (overnight), 0.1 mol/l NaOH (30 min) and then equilibrated with double distilled water and running buffer each for 30 min before use. Between each injection, the capillary was rinsed with 0.1mol/l NaOH (2 min), double distilled water (2 min) and with the respective running buffer (5 min). Samples were injected by an electrokinetic method at the anode, MEKC operations were run under constant voltage at ambient temperature.

### 2.4. Preparation of crude drug

Solution of the crude drug form (tablet) of pemoline was preprocessed as follows. First removed the sugar-coat of a tablet (0.1 g) sample, then weighed accurately, and put into 100 ml volumetric flask. Added 50 ml methanol to the volumetric flask. After shaking the mixture for 20 min, added methanol to scale. Then filtered and a

portion of the filtrate solution was diluted with the running buffer to 200 μg/ml before analyzed by CE.

*2.4.1. Standard solution*

Stock solution of pure racemic pemoline was prepared in methanol (1.0 mg/ml). It was diluted with the running buffer to appropriate concentrations before use.

## 3. Result and discussion

### *3.1. Effect of the type of chiral selector and CD concentration on chiral recognition*

In the inclusion–complexation mechanism, the compound fits the CD cavity with the whole molecule or with the hydrophobic part and, thus, the CD type has a very important role in the separation process. The hydrophobic interaction with the cavity alone is not sufficient to enable the separation of chiral drugs; weak bonds between substituent groups on the asymmetric center of analytes and secondary and/or primary groups of CD ring are responsible for chiral recognition. So the chiral separation using CDs derivatives is one of the challenging subject. Enantiomeric separation depends on the type of CD. In the experiment, six CDs: α-CD,β-CD,γ-CD, 2-HP-β-CD, DM-β-CD and mono-3-*O*-phenylcarbamoyl-β-CD were used as chiral selectors for separation of pemoline enantiomers. Experiments was performed with the BEG composed of 15 mmol/l chiral selector, 40 mmol/l SDS and 40 mmol/l borate at pH 9.0. However, the enantiomers can be separated only by mono-3-*O*-phenylcarbamoyl-β-CD. So, mono-3-*O*-phenylcarbamoyl-β-CD possessed stronger ability of chiral separation than the other CDs and CD derivatives.

The effect of concentrations of mono-3-*O*-phenylcarbamoyl-β-CD on chiral separation was investigated over the concentration range of 0–25 mmol/l. Fig. 1 shows the electropherograms of pemoline at different mono-3-*O*-phenylcarbamoyl-β-CD concentration. When the concentration of mono-3-*O*-phenylcarbamoyl-β-CD was increased, the resolution was improved and the migration time shortened, because a more stable inclusion complex of the solute with the CD is formed at higher CD concentration and it, therefore, migrates faster. When high concentrations of mono-3-*O*-phenylcarbamoyl-β-CD (25 mmol/l) was used, mono-3-*O*-phenylcarbamoyl-β-CD tended to be easily precipitated. This precipitation caused clogging of the capillary and the reproducibility dropped dramatically. Therefore, we chose 20 mmol/l as optimum concentration of mono-3-*O*-phenylcarbamoyl-β-CD in our study.

### *3.2. Effect of SDS concentration*

Although SDS was not regarded to attain enantiomer separation, enantioseparation was also affected by concentration of SDS. SDS monomers can have their hydrophobic tail concluded in the CD cavity along with the solute, This could change the nature of the solute and CD interaction and consequently the resolution.

Surfactants above their critical miceller concentration (CMC) are normally employed when enhanced selectivity of CE separation is needed.

The effect of concentrations of SDS on migration time and resolution were investigated over the concentration range of 0–50 mmol/l. The migration times increased greatly from 20.88 to 28.84 min when the concentrations of SDS in-

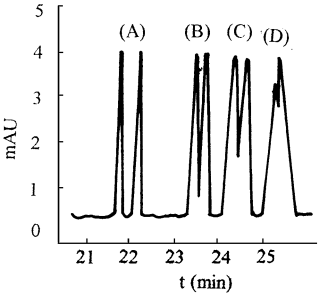


Fig. 1. Electropherograms of pemoline at different mono-3-*O*-phenylcarbamoyl-β-CD concentration. Electrophoretic conditions: 40 mmol/l borate (pH 9.0) containing different concentration of mono-3-*O*-phenylcarbamoyl-β-CD (A) 20 mmol/l (B) 15 mmol/l (C) 10 mmol/l (D) 5 mmol/l; 40 mmol/l SDS and 10% 2-propanol; applied voltage, 15 kV; temperature: 25 °C; detection wavelength 214 nm; injection: 15 kV per 5 s. Analyte concentration: 400 μg/ml.

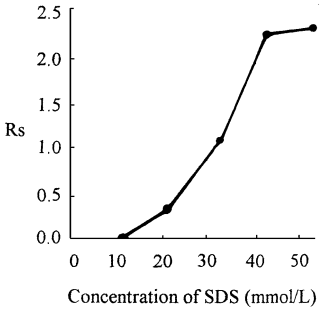


Fig. 2. Effect of SDS concentration on Rs. Electrophoretic conditions: 20 mmol/l mono-3-*O*-phenylcarbamoyl-β-CD; other conditions were the same as in Fig. 1.

creased from 0 to 50 mmol/l. It may be that the increased fraction of the solute partitioning into the SDS micellar phase at higher SDS concentrations delays the migration time of the solute. The increase in current was probably due to the increase in sodium ion concentration originated from the SDS. Fig. 2 shows the dependence of resolution (Rs) on SDS concentration. When the concentration of SDS is less than 10 mmol/l, pemoline cannot be separated. Pemoline can be best separated when the concentration of SDS was higher than 40 mmol/l.

### 3.3. Effect of buffer pH

pH is one of the most important parameters for improving selectivity in CE, because it affects both the charges of the analytes and the magnitude of the EOF. The effect of running buffer pH on resolution, which was studied in the pH range-

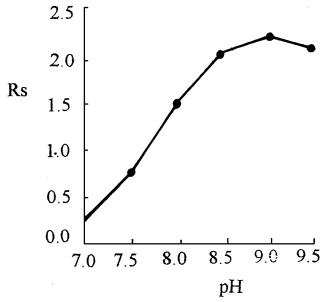


Fig. 3. Effect of buffer pH on Rs. Electrophoretic conditions: 40 mmol/l SDS; other conditions were the same as in Fig. 2.

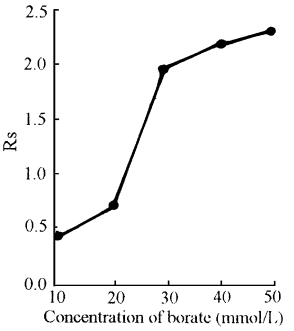


Fig. 4. Effect of borate concentration on Rs. The electrophoretic conditions were the same as in Fig. 3.

7.0–9.5, is shown in Fig. 3. From Fig. 3 we can see that the optimum pH in our experiments is pH 9.0.

### 3.4. Effect of buffer concentration

The effect of borate concentration on Rs was also investigated in the range of 10–50 mmol/l at pH 9.0. When the concentrations of borate were increased from 10 to 50 mmol/l, we observed an improvement of the enantiomeric resolution and a lengthening of the migration time. This is consistent with several publications that emphasize the use of high ionic concentration of buffer solutions to improve resolution and peak shape [14,15]. But high ionic concentration buffers increase current generation and may lead to Joule heating. Fig. 4 showed the effect of borate concentration on Rs. Borate buffer (40 mmol/l) was used in our experiments.

### 3.5. Effect of organic modifier

The important role of organic modifiers in CE enantioseparation was first reported by Fanali [14]. It has been considered that the organic modifier can have two roles: (1) improving the solubility of chiral substances. This effect diminishes the interaction of substances with the capillary wall and leads to decreased peak broadening. (2) decreasing the affinity of chiral substances for the hydrophobic cavity of chiral selectors. This effect decreases the interactions of chiral substances with the chiral selectors and leads to the

improvement of the peak shape but sometime accompanied by loss of selectivity.

The resolution in MEKC can be improved by modifying the buffer by adding some short-chain alcohols, which decrease the electroosmotic flow (EOF) and affinity of the hydrophobic solute for the micellar phase [15] [16]. The addition of urea is also known to improve the selectivity in CD-MEKC [17].

In our study, methanol, urea and 2-propanol were used as organic modifier. The concentration of each organic modifier were 5, 10 and 15%. Table 1 shows the effect of the content of organic modifier on the resolution and migration times. It can be seen that the migration times were longer for methanol and urea than 2-propanol. The effect with 2-propanol seems to be most pronounced.

### 3.6. Linearity and limits of detection

The calibration curves for pemoline showed good linearity in the range of 50–500 μg/ml ($r \geq 0.99$). The limit of detection was 10 μg/ml. The reproducibilities (R.S.D., $n = 6$) were 0.95 and 2.36%, respectively on the basis of migration time and peak area. The recoveries of pemoline enantiomers were 98.4, 97.9 and 101.3%.

Table 1
Influence of organic modifiers on the enantiomeric separation of pemoline

| Organic modifier | Content (%) | $t$ (min) | | Rs |
|---|---|---|---|---|
| | | $t_1$ | $t_2$ | |
| None | | 20.57 | 20.88 | 1.23 |
| Methanol | 5 | 22.86 | 23.20 | 1.30 |
| | 10 | 24.08 | 24.38 | 1.18 |
| | 15 | 25.22 | 25.38 | 0.92 |
| Urea | 5 | 24.30 | 24.62 | 1.29 |
| | 10 | 25.58 | 25.86 | 1.07 |
| | 15 | 26.42 | 26.63 | 0.96 |
| 2-Propanol | 5 | 21.38 | 21.73 | 1.25 |
| | 10 | 21.81 | 22.32 | 2.20 |
| | 15 | 23.45 | 23.45 | 1.34 |

Electrophoretic conditions: pH 9.0; other conditions were the same as in Fig. 4.

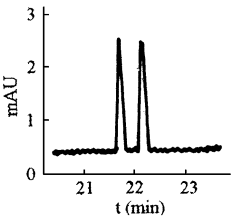


Fig. 5. Electropherograms of crude drugs. Electrophoretic conditions: 40 mmol/l borate (pH 9.0) containing different concentration of mono-3-*O*-phenylcarbamoyl-β-CD; 40 mmol/l SDS and 10% 2-propanol; applied voltage, 15 kV; temperature: 25 °C; detection wavelength 214 nm; injection: 15 kV per 5s. Analyte concentration: 200 μg/ml.

### 3.7. Analysis of crude drug of pemoline

Pemoline enantiomers in the crude drugs were analyzed using the MEKC method. Under the optimized conditions, the electropherogram is shown in Fig. 5. It can be seen that the electropherogram is simple, and no interference peak was observed.

### 3.8. Stability of sample solution

Solutions of pemoline enantiomers were stored for 4 months at 0 °C and analyzed again using the same MEKC method, almost the same results (migration times and peak areas) were obtained indicating its good stability.

## 4. Conclusions

Pemoline was better separated with mono-3-*O*-phenylcarbamoyl-β-CD modified MEKC (Rs = 2.21) than CZE mode (Rs = 1.40). A borate buffer that contained SDS micelles provided a suitable electrophoresis medium for the separation. Addition of an organic modifier to the separation solution can improve the enantiomeric separation. The simple direct determination of pemoline is a good alternative to CZE. Compared with HPLC, the process of optimization presented here is more efficient and economical. We could achieve accurate determination of pemoline enantiomers in crude drug separation.

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