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Enantioseparation of Pharmaceuticals by Capillary Electrophoresis Using Per-(6-sulfo-6-deoxy)-β-Cyclodextrin as Chiral Selector

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Four pairs of model enantiomers with different functional groups, phenylalanine, 1-phenylethanol, chlorpheniramine and promethazine, were separated by capillary electrophoresis using negative charged per-(6-sulfo-6-deoxy)-β-cyclodextrin as chiral selector. Baseline resolutions were achieved for all of the enantiomers under the optimal conditions of buffer pH, buffer concentration, per-(6-sulfo-6-deoxy)-β-cyclodextrin concentration and applied voltage.

Keywords: Chiral separation, Enantiomer, Capillary electrophoresis, Sulfonated β-cyclodextrin.

INTRODUCTION

Enantiomers of pharmaceuticals have distinct biological interactions and may show considerable differences in their pharmacological, pharmacokinetic or toxicological effects in biological environments[1]. The US Food and Drug Administration (FDA) therefore recommends the evaluation of the safety and effectiveness of each individual enantiomer, as well as their combination, if a new drug contains asymmetric centers[2]. Therefore, chiral separations have become an important part of the drug discovery and development processes[3].

Chiral separations in liquid phases for analytical purposes are dominated by high performance liquid chromatography (HPLC) and capillary electrophoresis (CE)[4]. Chiral separations are enabled by the use of either chiral stationary phases (CSPs) or chiral additives (CA). The interest in chiral separations by capillary electrophoresis is attributable to its experimental suitability. The small-diameter capillaries used for efficiently dissipate heat, allowing for the use of high voltages that result in rapid and efficient separations. That is especially attractive for chiral separations in which a values are very small and require many theoretical plates. The use of exotic and expensive chiral selectors becomes feasible because of the small quantities consumed in capillary electrophoresis[5].

By far the most extensively used chiral selectors in capillary electrophoresis are cyclodextrins and their derivatives. Cyclodextrins are nonionic, cyclic, chiral carbohydrates composed of glucopyranose units bound through α-(1, 4) linkages. The structure of a cyclodextrin is that of a truncated cone with a relatively hydrophobic interior and hydrophilic exterior. The larger opening of the cavity is lined with secondary hydroxyls and the smaller opening is lined with primary hydroxyls. Cyclodextrins are ideal as chiral selectors in capillary electrophoresis because of their ability to form host-guest complexes, also called inclusion complexes, with a wide variety of compounds[6,7]. Several factors contribute to the use of cyclodextrins as additives in the liquid phase separation techniques. They are extremely stable and do not appreciably absorb UV or visible light[8].

Derivatization of a cyclodextrin with ionic groups can also affect its buffer solubility and the electrophoretic mobility of the inclusion complexes. According to charge state, cyclodextrin derivatives can be classified as neutral cyclodextrin derivatives[9-11], negatively charged cyclodextrin derivatives[12-14] and positively charged cyclodextrin derivatives[15-17]. Comparing with neutral cyclodextrins, charged cyclodextrin derivatives can separate not only charged analytes but also uncharged analytes[18].

In this paper, four pairs of enantiomers involving different functional groups were chosen as model solutes and per-(6-sulfo-6-deoxy)-β-cyclodextrin (sulfonated β-CD) used as a chiral selector. Chiral separations of the enantiomers by capillary electrophoresis were accomplished after optimization of buffer pH and concentration, chiral selector concentration and applied voltage.

EXPERIMENTAL

The capillary electrophoresis experiments were performed on a CE-L1 instrument (CE Resources, Singapore) equipped with a Linear UV-VIS 200 detector (Alltech, Deerfield, IL, USA).

Electropherograms were recorded with the CSW (Chromatography Station for Windows) (DataApex, Prague, Czech Republic). Fused-silica capillaries of 60 cm total length and 50 cm effective length and of 375 μm O.D. and 50 μm I.D. were purchased from Yongnian Optical Fiber Factory (Hebei, China). Prior to each analysis, a new capillary was washed with 0.1 M NaOH for 10 min, water for 3 min and corresponding buffer for 10 min. Buffers were filtered through Nylon filters (Quandao Technical Company, Shanghai, China) with pore size of 0.45 μm. The detection wavelength was set at 214 nm.

tris(Hydroxymethyl)aminomethane (*tris*), Na_2HPO_4, NaH_2PO_4 and H_3PO_4 were purchased from Aladdin (Shanghai, China). Phenylalanine (Phe), 1-phenylethanol (1-PE), chlorpheniramine (CPA) and promethazine (PM) were products of TCI (Tokyo, Japan). Their structures are shown in Fig. 1. per-(6-sulfo-6-deoxy)-β-cyclodextrin was obtained from YingTeng Co. (Shanghai, China) (Firstly, per-(6-thio-6-deoxy)-β-cyclodextrin was obtained and then it was oxidated by hydrogen peroxide to per-(6-sulfo-6-deoxy)-β-cyclodextrin).

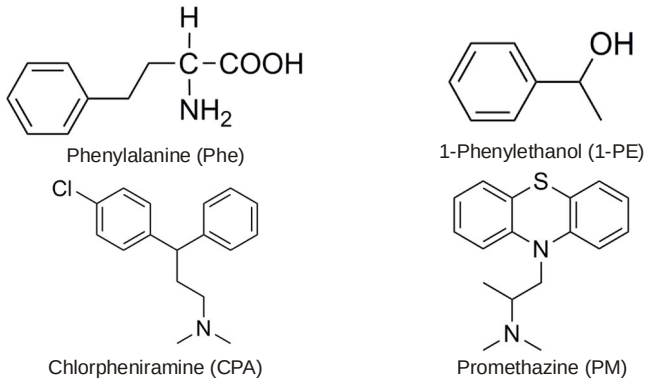

Fig. 1. Structures of phenylalanine, 1-phenylethanol, chlorpheniramine and promethazine

The Na_2HPO_4-NaH_2PO_4 buffer consisted of the same concentration of Na_2HPO_4 and NaH_2PO_4. *tris*-H_3PO_4 buffer was prepared by dissolving a certain amount of *tris* in distilled water and adjusted to appropriate pH with phosphoric acid. Working solutions of phenylalanine, 1-phenylethanol and promethazine were 0.5 mg/mL and chlorpheniramine was 0.2 mg/mL.

RESULTS AND DISCUSSION

Buffers selection: To ensure sufficient interaction between the solutes and the chiral selector, we tried to use acidic buffers for the enantiomer separation. In acidic buffers, the solutes bearing amino groups can be protonated and favorably interacted with negatively charged sulfonated β-cyclodextrin. The experiments indicated that all of the four solutes could be separated in either *tris*-H_3PO_4 or Na_2HPO_4-NaH_2PO_4 using sulfonated β-cyclodextrin as chiral selector. Occasionally, buffer of Na_2HPO_4-NaH_2PO_4 has been used due to its less UV absorption.

Effect of pH and buffer concentration: In capillary electrophoresis of weak acids or bases, pH of buffer can be a practical and complicating factor. pH of buffer can influence the electroosmotic mobility and/or electrophoretic mobility, consequently migration time. For example, 1-phenylethanol was an intrinsic neutral compound and its electrophoretic mobility did not change with pH of the buffer. But its effective electrophoretic mobility did change due to association with charged chiral selector sulfonated β-cyclodextrin. Finally, the migration time of 1-phenylethanol depended on the combination of the effective electrophoretic mobility and the electroosmotic mobility. In fact, the migration time of 1-phenylethanol increased due to the fact the effective electrophoretic mobility was greater than the electroosmotic mobility when buffer pH was above 3.

Resolution could be affected by pH of buffer as well. Fig. 2 showed the effect of buffer pH on resolution of four pair enantiomers. Resolutions of phenylalanine and chlorpheniramine were sensitive to pH 2-4 of buffer. For promethazine, resolution of the enantiomors basically remained unchanged in the pH range studied (pH = 1.9-4). For 1-phenylethanol, it was essentially neutral. As mentioned above, the chiral separation of 1-phenylethanol was preferably at low pH in terms of separation time.

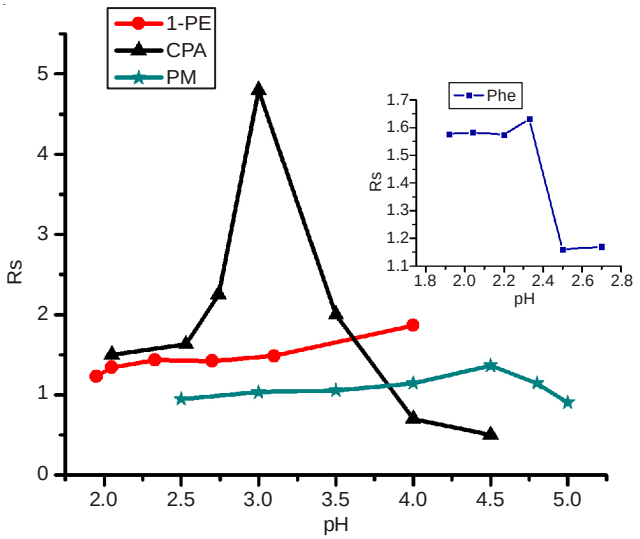

Fig. 2. Effect of buffer pH on the resolution

Buffer concentration would have effects on buffer capacity and on buffer conductivity. Fig. 3 examined the effect of buffer concentration on resolution. Lines in Fig. 3 showed a general trend. At low buffer concentrations, the resolutions of all enantiomers were slightly improved with increase of concentration. At about 50 mM, the resolutions reached the maxima. When the concentration continued to increase, the resolutions decreased due to the Joule heating and band broadening.

Effect of sulfonated β-cyclodextrin concentration: Concentration of the chiral selector, sulfonated β-cyclodextrin, would be a key factor affecting chiral resolution. As reported by Wren and Rowe[19], there would be the optimal concentration of the chiral selector which corresponds to the highest value of resolution of enantiomers. Resolutions of phenylalanine and 1-phenylethanol might have the maxima in about 60-70 mg/mL. The maximum of resolution of promethazine seemed not present in the concentration range studied. When concentration of sulfonated β-cyclodextrin was higher than 125 mg/mL, nevertheless, baseline resolution of two enantimoers of promethazine was obtained. The inset of Fig. 4 showed the effect of

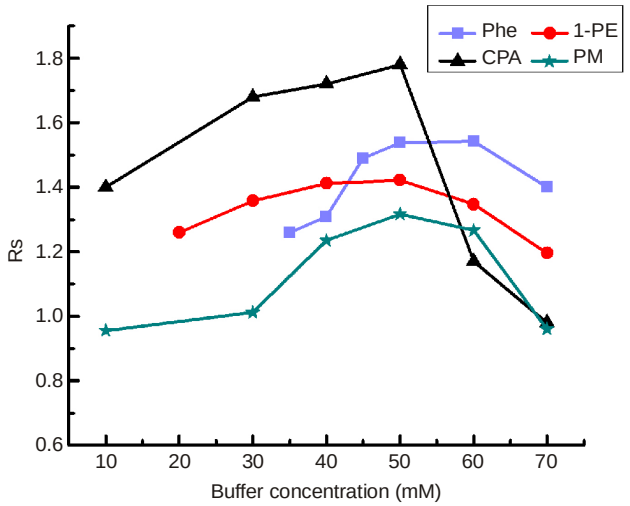

Fig. 3. Effect of buffer concentration on the resolution; Na_2HPO_4-NaH_2PO_4 for phenylalanine and 1-phenylethanol; *tris*-H_3PO_4 for chlorpheniramine and promethazine

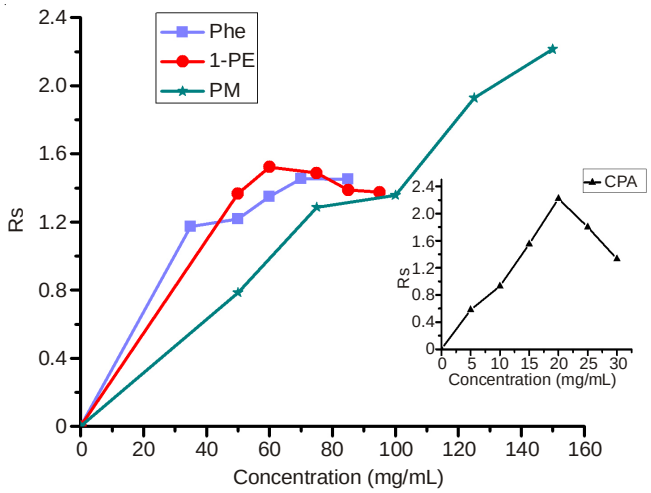

Fig. 4. Effect of sulfonated β-cyclodextrin concentration on the resolution

concentratin of sulfonated β-cyclodextrin on the resolution of chlorpheniramine enantimoers.

Effect of applied voltage: In capillary electrophoresis, migration time of a solute is generally decreased as applied voltage increases. Initially, applied voltage increases and the resolution becomes greater due to the fact that time for diffusion is limited. Applied voltage increases further and excessive Joule heating occurs. This causes band broadening and the results deteriorated resolution. As shown in Fig. 5(a) and (b), the three pairs of enantiomer resolution were not significantly lowered until 18 or -18 kV was applied. However, chiral resolution of chlorpheniramine was more sensitively affected by applied voltage.

Chiral separation of enantiomers under the optimal conditions: Under the optimal conditions, the four pairs of enantiomers were baseline resolved in capillary electrophoresis using sulfonated β-cyclodextrin as chiral selector (Fig. 6).

Conclusion

In acidic buffers, the solutes bearing amino groups could be protonated and favorably interacted with negatively charged sulfonated β-cyclodextrin. At the buffer concentration of about

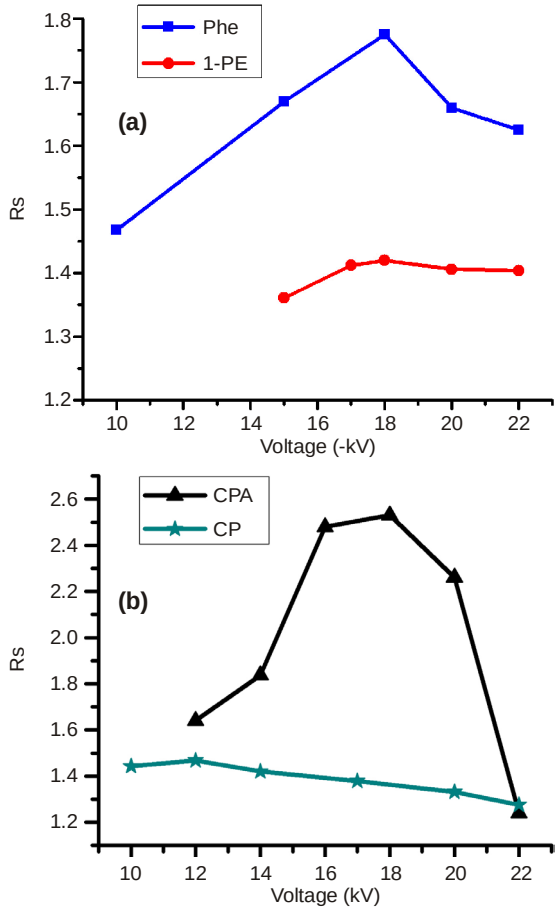

Fig. 5. Effect of applied voltage on the resolution; (a) applied negative voltage; (b) applied positive voltage

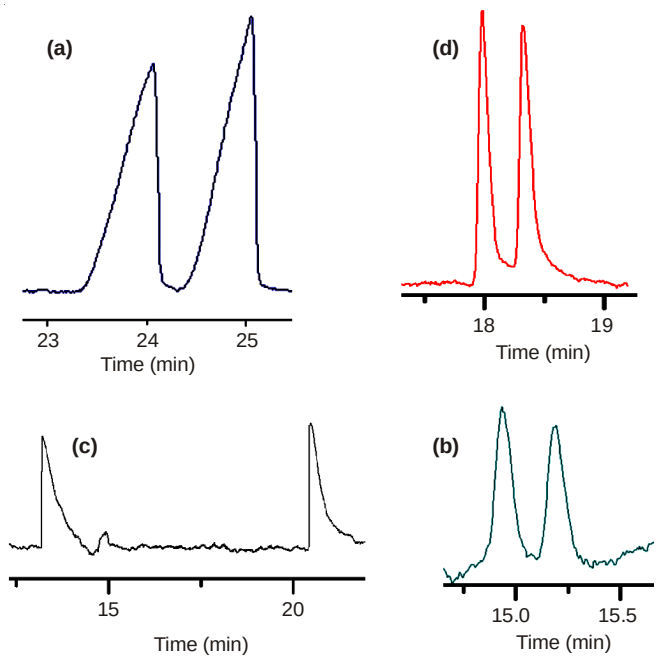

Fig. 6. Typical electropherograms of four pairs of enantiomers; Sulfonated β-cyclodextrin concentration were 70, 60, 20 and 125 mg/mL for phenylalanine, 1-phenylethanol, chlorpheniramine and promethazine, respectively; (a) Phenylalanine: Na_2HPO_4-NaH_2PO_4 50 mM (pH 2.3), Voltage -18 kV; (b) 1-Phenylethanol: Na_2HPO_4-NaH_2PO_4 50 mM (pH 3.1), Voltage-18 kV; (c) Chlorpheniramine: *tris*-H_3PO_4 50 mM (pH 3), Voltage 18 kV; (d) Promethazine: *tris*-H_3PO_4 50 mM (pH 4.5), Voltage 12 kV

50 mM, the resolutions reached the maxima. Under the optimal conditions, four pairs of model enantiomers involving different functional groups were baseline separated in short time by capillary electrophoresis using sulfonated β-cyclodextrin as a chiral selector.

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