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Capillary Electrophoresis Separation of the Six Pairs of Chiral Pharmaceuticals Enantiomers

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Under the given voltage, temperature, wavelength, electrolyte concentration and pH value, the capillary electrophoresis was used in this study to separate six pairs of enantiomers (3-quinine alcohol, tamsulosin, *etc*.) simultaneously, three types of cyclodextrin being the chiral separation selectors. In order to increase the separation capabilities, the electrolyte was modified by polyethylene glycol 4000, the addition of which greatly improved the chiral separation of the enantiomers. The parameters of chiral separation capabilities were also investigated, including resolutions, theoretical plates, capacity factors, apparent mobilities and retention times. This experiment proves this method a success and that the polyethylene glycol 4000 plays an important role in chiral separation when β-cyclodextrin exists.

Key Words: Capillary electrophoresis, Polyethylene glycol 4000 (PEG 4000), Cyclodextrin, Enantiomeric separation.

INTRODUCTION

The biological activities of chiral pharmaceuticals primary rests with stereoisomers, owing to the existence of pharmacological, toxicological absorption, distribution, metabolism and excretion differences between stereoisomers and chiral consideration which are now integral parts of drug research and development and of the regulatory process. To improve drug activities and reduce side-effects, it is necessary to develop chiral separation research method of drug enantiomers for drug production and quality control. Therefore, chiral drugs separation and determination in pharmaceutical analysis will provide powerful means for pharmacology and toxicology research[1,2]. Among the chromatographic methods so far developed, high-performance liquid chromatography (HPLC) methods are widely employed for the assays of drug isomers in pharmaceutical preparations and biological fluids[3], despite expensive commercial chiral columns[4-7] and low efficiency in the chiral stationary phases in the chiral chromatographic separation process during which most components need to be operated cumbersomely for pre-column derivatization and the objectivity of the results could be disturbed by artificial errors in improper intermediate process. The separation efficiency of the chiral mobile-phase method is also low, the stabilities and reproducibilities are rather poor. Capillary electrophoresis represents a powerful separation technique that is successfully utilized for separation of optical isomers as reported in many review papers[8-10]. The main advantages of this technique are high efficiency of separations, possibility to use new and inexpensive selectors, easy and fast method for development.

In order to study the stereoselectivity of human erythrocyte acetylcholinesterase and plasma butyrylcholinesterase[11], R-3-quinine alcohols were tested as substrates and/or as inhibitors of the cholinesterases. The chiral separation of 3-quinine alcohol by capillary electrophoresis has not been reported in recent literatures.

5-[2-(R,S)-aminopropyl]-2-methoxybenzene sulfonamide is the intermediate of tamsulosin hydrochloride treating the symptoms of an enlarged prostate. Enantiomeric impurities in tamsulosin hydrochloride synthesis process as: tamsulosin intermediate and chiral by-products can't be determined simultaneously in previous research[9].

2-Arylpropionic acids represent an important group of non-steroidal antiinflammatory drugs. All are chiral and with the exception of ibuprofen and naproxen, are marketed as racemates. Chiral separation of ibuprofen and naproxen in the presence of other chiral disruptors has not been reported yet[12].

The aim of this study was to develop a relatively rapid, precise and sensitive method to separate the six chiral pharmaceuticals[13] (Fig. 1) by capillary electrophoresis using underivatized cyclodextrin as chiral selectors[14,15]. The condition of buffer concentration, pH, chiral selector has effect on the enantiomeric separation. The optimized method is a useful example of the performance possible with an optimized chiral capillary electrophoresis method for enantiopurities determinations.

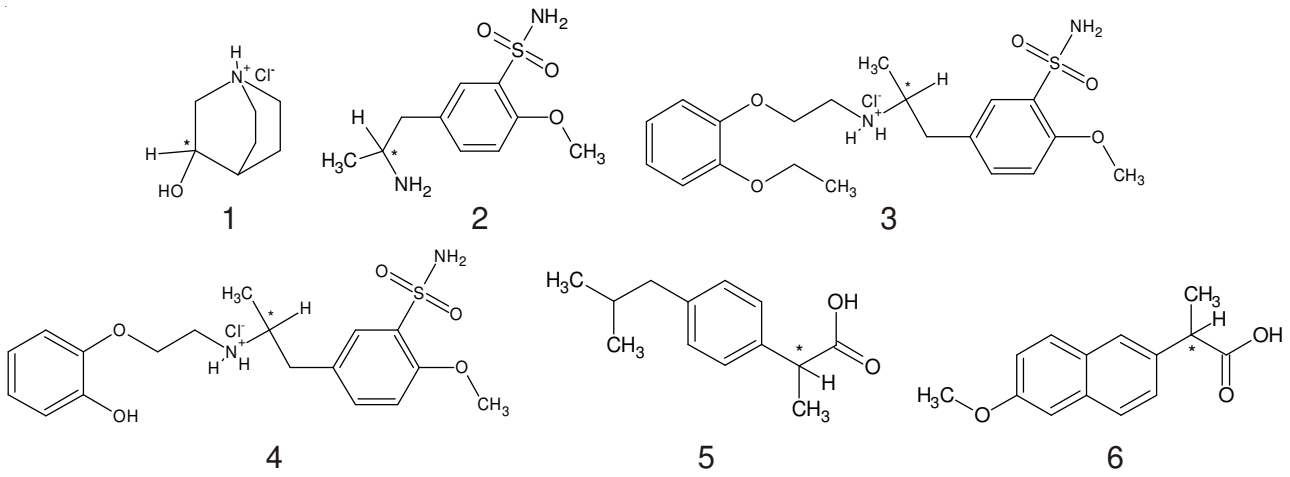

Fig. 1. Structure of chiral pharmaceuticals and their intermediates; **1**: R, S-3-quinine alcohol (QA), **2**: Tamsulosin intermediate (TI, 5-(2-(R, S)-aminopropyl) -2-methoxbenzene sulfonamide); **3**: Tamsulosin hydrochloride (TH, 5-[2-(R,S)-2-{[2-(2-ethoxyphenoxy)ethyl]amino}propyl]-2-methoxy-benzene-sulfonamide hydrochloride); **4**: chiral by-product(CP, 5-[2-(R,S)-2-{[2-(2-phenoxy)ethyl]amino}propyl]-2-methoxy-benzene sulfonamide hydrochloride); **5**: ibuprofen (BF); **6**: naproxen

EXPERIMENTAL

3-Quinine alcohol (QA), tamsulosin intermediate (TI), tamsulosin hydrochloride (TH), ibuprofen (BP), naproxen (NP) (North China Pharmaceutical Group Preparations Ltd., China), α-cyclodextrin (α-CD, bio basic Inc, separate loading, Canada, Lot: LJ0520B1008Y), β-CD (Tianjin Bodi Chemical Co. Ltd., China), γ-cyclodextrin (Fluka Chemika, Switzerland), 3-hydroxymethyl-amino methane (Tris, Chengdu Kelong Chemical Reagent Co. Ltd., China), polyethylene glycol 4000 (PEG 4000, Chengdu Kelong Chemical Reagent Co. Ltd., China), phosphoric acid, sodium hydroxide, benzoic acid and methanol. All of the chemicals used were of analytical grade purity. Buffer and sample solutions were prepared by using deionized water (resistivity = 18.2 mΩ) obtained from a Milli-Q system (Millipore, USA).

The instruments for the experiment are: P/A CE™ MDQ capillary electrophoresis system (Beckman Coulter, Fullerton, CA, USA); diode array detector; uncoated (inner wall) fused silica capillary (inner diameter: 75 μm, total length: 50 cm, effective length: 40 cm, Hebei Rui Feng HPLC Components Co. Ltd., Yongnian, China); 32 Karat electrophoresis analysis software; PHS-3C desktop type pH meter (precision: 0.01 pH unit, Shanghai Yi-lun Environmental Science and Technology Corporation, China); electronic balance (Sartorius, precision: 0.0001 g).

The capillary was rinsed with 0.1 NaOH solutions for 5 min, then with water (5 min) and 0.1 mol/L HCl (5 min) and again with water (5 min). Finally, the capillary was rinsed with working electrolyte containing chiral selector for 15 min. These procedures were performed at the beginning of every working day during the experiment.

The solutions of chiral drug enantiomers were prepared by deionized water. All buffer solutions (*Tris*-H_3PO_4), freshly prepared weekly and stored in a refrigerator previously, were filtered through a membrane filter (0.22 μm).

RESULTS AND DISCUSSION

The optimization of the electrophoretic separation was based on the production of acceptable peak shape, resolution and retention time. Electrophoretic factors potentially affecting these responses including separation temperature, diode array detector wavelength, electrolyte concentration, pH, content of various modifiers, type of chiral selectors (cyclodextrin, CD), organic additives as well as separation voltage were considered for optimization.

Effect of analytical voltage: The electrophoretic velocities were directly proportional to the electric field strength. The effect of the analytical voltage in the range of 8-25 kV was examined. The limiting factor here is Joule heating. The results showed that the resolution of enantiomers was decreased with increasing the voltage. Contrariously, the peaks were diffused longitudinally with the increasing of the voltage. Hence, the distortion of electrophoretic peak was due to longitudinal diffusion in very low voltage (8 kV). Based on literature sources[8,16,17] and previous experience, the optimal voltage was set at 10 kV, which ensured short retention time, acceptable current generated and good resolution.

Effect of capillary temperature on separation: The mobility of analytes can be changed in different temperature of capillary. The reduction of resolution at higher temperature was due to two factors: 1. The decrease in the formation constant of the analyte-chiral selector complex during the chiral separation; 2. The increase of the solute diffusion. Apparently, the complex-formation interaction of the drug with buffer was exothermic reaction and lower temperature supported formation of the complex. The effect of chiral separation on different temperature was demonstrated in Fig. 2. The retention times and resolutions were increased with the decreasing of the separation temperature. Furthermore, chiral compounds can be racemized at higher temperature for a longer time. In order to ensure the objectivity of measurement, that temperature was controlled at a lower level. Therefore, the capillary temperature was maintained at 15 ± 0.5 ºC (lowest operating temperature) to optimize the analysis of the racemic drugs.

Choice of detection wavelength: The electrophoretograms at different wavelengths (214, 254, 280 and 320 nm) were shown in Fig. 3. The detection was performed by diode array detector detector. According to the selection, the major related

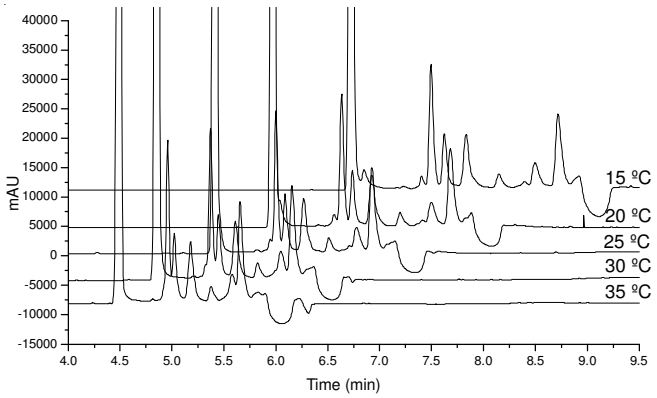

Fig. 2. Electrophoretogram of six pairs of enantiomeric complexes in different temperatures

factors of wavelengths choice are sensitivity, background noise and baseline. High sensitivity, low background noise and smooth baseline were indicated in the electrophoretogram at 214 nm. Fig. 3 showed the enantiomers with phenyl group, whose analytes molecular structures had been shown in Fig. 1, have much stronger ultraviolet absorption; while 3-quinine alcohol has enough absorption response values when a short wavelength of 214 nm was adopted. Therefore, the best wavelength of operation was 214 nm.

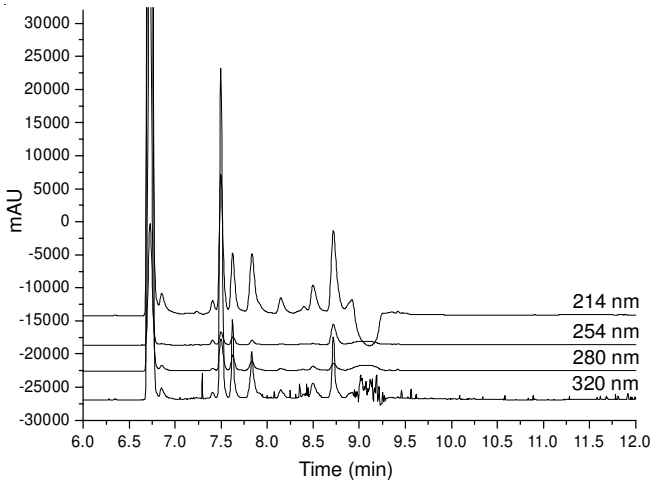

Fig. 3. Electrophoretogram of six pairs of enantiomeric complexes in different wavelength

Effect of buffer concentration: The chiral separations were performed in *tris*-phosphate buffer. The effect of the *tris*-phosphate concentration in the buffer solution on the retention time of racemic drugs is demonstrated in Fig. 4. The influence of buffer concentration on the generation of electric current was tested. The values of generated current were increased with the increasing of buffer concentration. The chiral separations were achieved with 42.86-100 mM *tris*-phosphate. In order to prevent the generation of extensive Joule heat, the 71.43 mM phosphate buffer was chosen as the optimal concentration. The use of Tris, a co-ion of low mobility, also resulted in good peak symmetry for ionic analyte, low current (13.70-17.80 μA) and high efficiency (*ca*. 150,000 theoretical plates per capillary in the low concentration range).

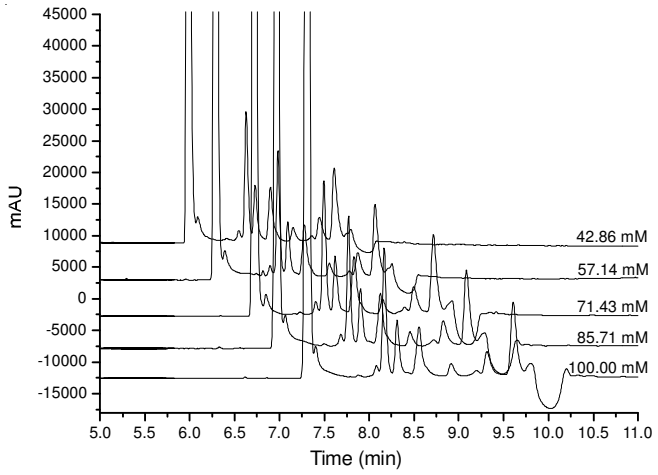

Fig. 4. Electrophoretogram of six pairs of enantiomeric complexes in different concentrations of buffer

Effect of buffer pH: The buffer pH has complicated influence on retention times. Effect of the buffer pH on the retention times of the drug embraces a number of aspects such as the degree of protonation of the nitrogen atom of the drug and ionization amplifier effect of the acid radical ion, compatibility of the protonated drug molecule with the buffer. This electrophoretic behaviour of the analyte is probably related to the change of the EOF. EOF at pH > 3.5 became considerable therefore short retention times were obtained. Accordingly, the retention times were prolonged with the pH decreasing in the range of 6.25-10.99. During pH optimization, it was found that resolution decreased at low and high pH. As the pH rose above 6.25, the separation improved, but the overall resolution was still inadequate even at the most favourable pH of 8.40.

Effect of the chiral selectors: Under the optimal separation conditions of voltage, separation temperature, buffer concentration and acidity, for further optimization of separation, the effect of different types of cyclodextrin (CD) and modifiers in the capillary electrophoresis electrolyte was investigated. The enantioseparation mechanisms involved the formation of some inclusion complexes which might be unstable at a pH lower than 3.5. Furthermore, the drug molecule protonation degree is smaller at a higher pH which may cause better fitting of the analyte molecule into the cyclodextrin cavities of the chiral selectors resulting in faster migration of the negatively charged complex formed.

Among the various conventional chiral selectors, β-cyclodextrin is one of the most frequently utilized cyclodextrin. The optimum resolution for analytes was adopted by the addition of β-cyclodextrin. In contrast, poor resolution was obtained with the addition α-cyclodextrin and γ-cyclodextrin in electrolyte. Interestingly, these experiments also demonstrated that the addition of PEG 4000 to the electrolyte containing β-cyclodextrin considerable improved the separation. However, PEG 4000 has no significant influence on enantioseparation capabilities in electrolyte containing α-cyclodextrin and γ-cyclodextrin (data was not shown). Typical electropherograms showing the effect of the addition of PEG 4000 to the electrolyte on separation are depicted in Fig. 5. The best results were obtained at appropriate concentrations of PEG 4000 and the separations became reproducible.

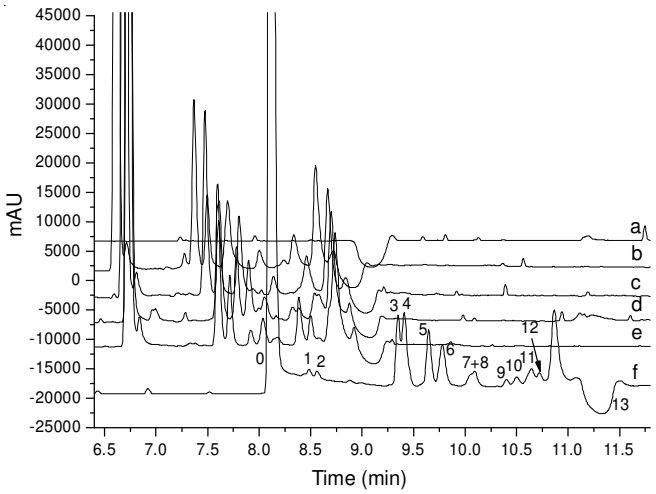

Fig. 5. Electrophoretogram of six pairs of enantiomeric complexes in different chiral selectors; **a**: *Tris*-H_3PO_4 (Blank solvent)) ; **b**: *Tris*-H_3PO_4; **c**: *Tris*-H_3PO_4 + α-CD; **d**: *Tris*-H_3PO_4 + β-CD; **e**: *Tris*-H_3PO_4 + γ-CD; **f**: *Tris*-H_3PO_4 + β-CD + PEG4000 ; **0**: non-chiral external standard (benzoic acid); **1,2**: R,S-3-quinine alcohol; **3,4**: R,S-Tamsulosin intermediate (5-(2-aminopropyl)-2-methoxy-benzene sulfonamide); **5,6**: R,S-Tamsulosin hydrochloride; **7,8**: R,S-chiral by-products; **9,10**: R,S-ibuprofen; **11,12**: R,S-naproxen; **13**: solvent peak (reversed)

Under the best conditions for electrophoresis, good separation effect can still be seen although there are slight fluctuations in the baseline. Except chiral by-products (7, 8), all the other enantiomers had a certain degree of separation. Although tamsulosin intermediate did not meet the baseline separation, the separation efficiency can be improved, to some degree, by decreasing its sample injection volume. Because of the diversities of the sample components, the baseline separation was not achieved in several enantiomers, which suggests some further researches to get the better separation result.

Ultimately, the optimum capillary electrophoresis conditions for the separation were determined to be: fused silica capillary: 50 cm (40 cm at detection window) × 75 μm id (375 μm od); buffer solution (pH = 8.40) composed of: 71.43 mM Tris-H_3PO_4, a small amount of methanol, β-CD and PEG 4000; separation voltage: +10 kv; injection pressure: 0.5 psi; injection time: 5.0 sec; and separation temperature: 15 ºC; Fig. 7 shows the typical electropherogram from the capillary electrophoresis separation of a standard mixture solution of each of the examined chiral drugs under these conditions.

Capacity assessment of enantiomeric separation: Retention times of chiral enantiomers were shown in Table-1. The retention time was prolonged slightly with the addition of chiral selectors in buffer. Nevertheless, the retention time was markedly prolongated by adding PEG 4000 in that such macromolecule as PEG 4000 can increase potential electrolyte viscosity coefficient and hence EOF could be decreased significantly. The different values of retention times were increased significantly by using β-CD and PEG 4000.

The effect of the five separation systems on the values of enantiomeric drugs resolutions and theoretical plates is demonstrated in Fig. 6. As can be seen, the influences of separation systems on resolutions and theoretical plates were dramatic. Generally, the resolutions and theoretical plates of electrolyte containing β-CD and PEG 4000 were higher than others.

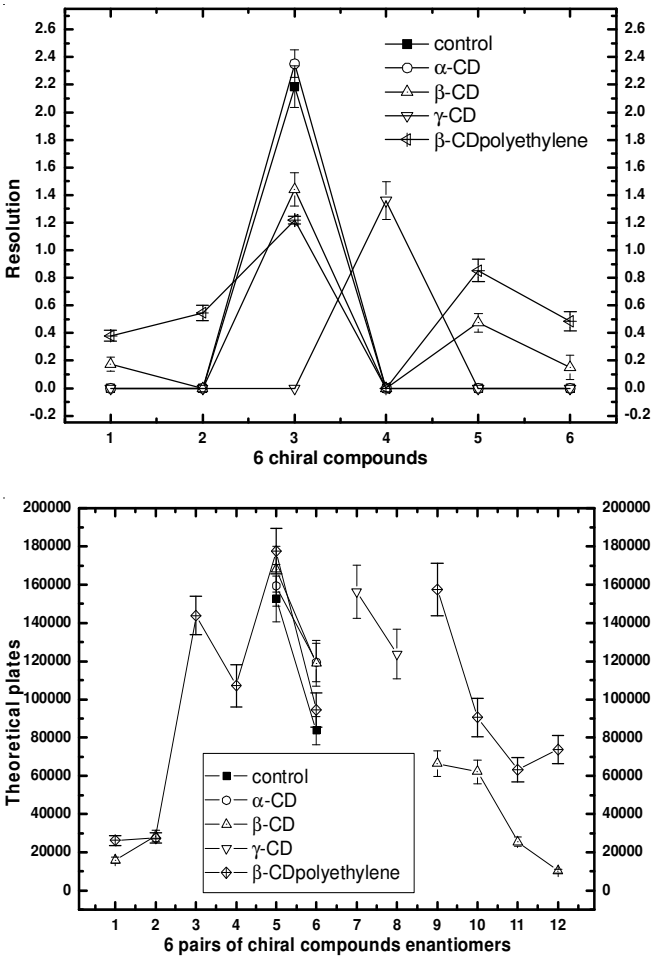

Fig. 6. Resolutions and theoretical plates of six pairs of enantiomeric complexes; Graph represent means ± SD (n = 5). Significantly lower ($p < 0.05$) than the corresponding control value, as obtained by Student's t test

The effect of the five separation systems on the values of enantiomeric drugs apparent mobilities and capacity factors is demonstrated in Fig. 7. The enantiomeric drugs apparent mobilities were decreased, capacity factors were increased by the addition of α-CD, β-CD[18] and γ-CD in electrolyte. The CD may attach to some extend onto the capillary wall due to non-specific adsorption, can reduce the electro-osmotic flow of the capillary inner wall. However, the influence of adding CD merely on apparent mobilities and capacity factors were not significant. Dramatic change of apparent mobilities and capacity factors were achieved by using PEG 4000. The application of PEG 4000 in electrolyte reduced the apparent mobilities by more than 0.00014 and the capacity factors increased about 1600000 correspondingly.

Function of PEG 4000 as modifier: The developed method was used for separating six kinds of enantiomers. During the separation, PEG 4000 was used as buffer modifier. Excellent chiral separation modifier as PEG 4000 has several properties including stable structure, high hydrophilicity, molecular group without strong UV absorption and compatibility of the methylenes and ether linkages in PEG 4000 with that of the chiral selector. When the buffer components - PEG 4000, CD and *Tris*- were at high concentrations, the electrolyte viscosity coefficient was high. Furthermore, dynamic spatial

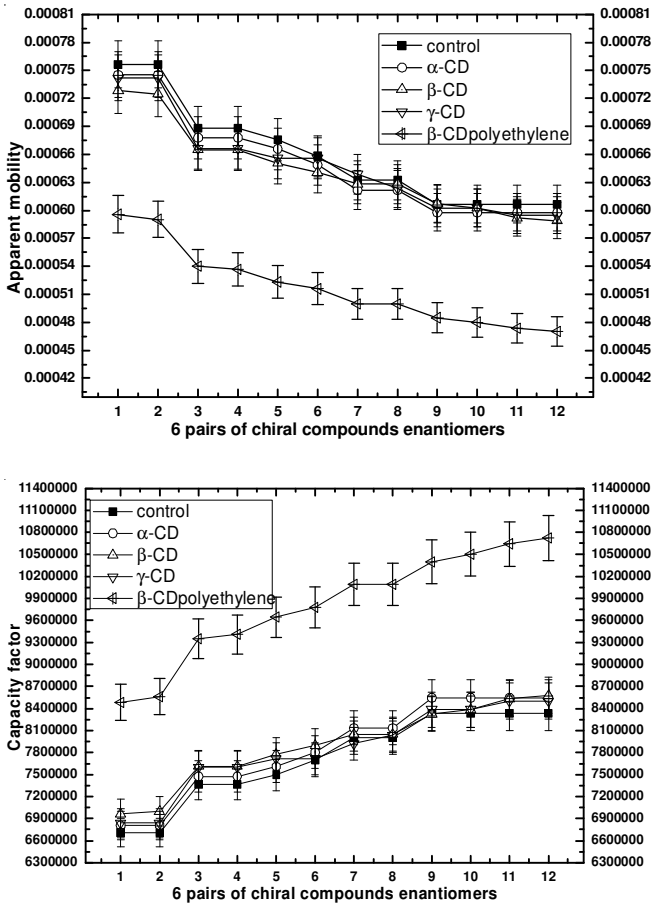

Fig. 7. Apparent mobilities and capacity factors of six pairs of enantiomeric complexes; Graph represent means ±SD (n = 5). Significantly lower ($p < 0.05$) than the corresponding control value, as obtained by Student's t test

net structure was performed and water, metal ions, acid radical ion and other inorganic small molecules can freely flow through the net. So the influence of electrostatic interactions among the analytes and the chiral selector on chiral separation was performed efficiently.

The mechanisms of PEG 4000 in the donors in capillary electrophoresis using polyethers (PEG 4000) as modifier was shown in Yuklhho Esaka's research[19-21] previously. The intramolecular hydrogen bond formation seems to compete with the interaction with PEG, resulting in a reduction of K compared with that of the former.

The hydrogen-bonding complex formation constant (K) is given by eqn. (1):

$$K = \frac{x}{1-x} \times [PEG] \quad (1)$$

where, x denotes the fraction of the analyte bound to PEG. The quantity [PEG] is the concentration of PEG (or of the polyether segment).

This result would not be interpreted simply in terms of the hydrogen bonding interaction which suggests the occurrence of some additional weak interaction, most probably hydrophobic interaction. However, in this case, the mechanisms of PEG 4000 in chiral separation were sophisticated under the existence of chiral selector. Further research will focus on the mechanisms of PEG 4000 and β-CD in chiral separation.

Conclusion

Three types of cyclodextrin chiral selectors were used to separate six chiral enantiomers, the separation efficiency of β-CD was better than that of a, γ-CD's. The separation efficiency was improved greatly by using PEG 4000 as the modifier. The results demonstrate that the capillary electrophoresis method is a useful, simple and rapid technique for the assay of QA, TI, TH, CP, BF and NP in pharmaceuticals. In addition, the proposed method is also applicable to the chiral purity control.

In conclusion, the study has shown that the optimized capillary electrophoresis method could be used successfully for the determination with a shorter analysis time compared to HPLC methods that had been reported. Another method to separate the six pairs of enantiomers was successfully achieved through suitable assessments of resolution, theoretical plate number, the apparent electrophoretic mobility and capacity factor. Therefore, it can be concluded that this capillary electrophoresis method using PEG 4000 as modifier in electrolyte is suitable for the routine assay and enantiopurities determination of chiral drugs as is mentioned above.

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