Chinese Chemical Letters Vol. 10, No. 4, pp. 303–306, 1999

Chiral Separation of N-Benzoyl Phenylalanine Methyl Ester by Nonaqueous Capillary Electrophoresis

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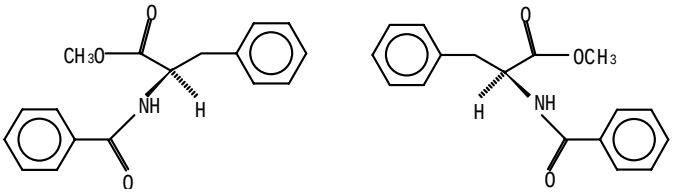
Abstract: A successful chiral separation of N-benzoyl phenylalanine methyl ester has been achieved by nonaqueous capillary electrophoresis (NACE) using β-CD as chiral selector in formamide (FA). Some experimental parameters influencing the chiral separation such as concentration of β-CD, ionic strength and apparent pH (pH*) are discussed.

Keywords: Nonaqueous capillary electrophoresis; chiral separation; N-benzoyl phenylala-nine methyl ester.

The separation of optical isomers is a rapidly growing area in capillary electrophoresis (CE), and most chiral separation by CE have been performed in aqueous background electrolyte. Organic solvents such as methanol and acetonitrile have been applied as buffer modifiers at concentration typically not higher than 40% in order to increase hydrophobic analyte solubility and to improve selectivity, resolution, or to alter the electroosmotic flow (EOF) and electrophoretic mobility of analyte. But there have been only a few reports on the chiral separation using pure nonaqueous media in CE[1,2,3,4].. These reports showed that NACE has some advantages over aqueous chiral separation. Firstly, it allows the chiral separation of analytes that are insoluble in water. Secondly, in contrast to the poor aqueous solubility of β-CD (0.018 mol/L) in water, more than 0.7 mol/L can be dissolved in FA[3]. This can be a significant advantage because the selectivity is strongly related to the concentration of chiral selector. Additionally, NACE has more parameters such as dielectric constant and viscosity of the solvent that could be adjusted to achieve better separation.

N-Benzoyl phenylalanine methyl ester is an intermediate product in synthesizing pharmaceutics. So it is very important to separate the enantiomers and determine the ratio of the optical isomers in order to control the asymmetry synthesis procedure. The chemical structure of the sample is shown in **Figure 1**. Because of its poor solubility in water, the enantiomers could not be analyzed by CE in aqueous system. In this paper the chiral separation of N-benzoyl phenylalanine methyl ester was performed in FA using β-CD as chiral selector, and acceptable resolution was obtained by this method.

Figure 1. Chemical structures of N-benzoyl phenylalanine methyl ester



R **S**

Experimental

All separations were performed using a HP^{3D}CE system (Hewlett-Packard, USA) with air cooling. An uncoated fused silica capillary with 50 μm I.D. × 375 μm O.D. (Yongnian Optical Fiber Factory, Hebei, P. R. China) was used. The total length of the capillary was 50 cm, and 41.5 cm to the detector. The temperature of the capillary was maintained at 25°C. The applied voltage was 30 kV to enhance resolution and shorten the time of electrophoresis. Sample was injected by applying a pressure of 5000 Pa for 5 s. The UV absorption was monitored at 260 nm with a diode array detector.

N-Benzoyl phenylalanine methyl ester was synthesized in our lab. β-CD was purchased from TCI (Tokyo, Japan). FA, NaCl, and acetic acid (HAc) were of analytical grade. The sample was dissolved in FA containing 0.06 mol/L NaCl and the final concentration of the sample was 1 mg/ml.

The separation was carried out in FA containing various concentrations of NaCl (0.03-0.07 mol/L), various concentrations of β-CD (0-0.12 mol/L) and various amounts of HAc (0-15%) to achieve the best separation. The electrolyte solution was degassed in an ultrasonic bath before use. The capillary was washed with FA for 5 min and then with the background electrolyte for 5 min between runs.

Result and discussion

Figure 2 shows the dependence of resolution (Rs) on β-CD concentration. The resolution between two enantiomers was calculated by the equation: $Rs=1.18\ (t_1-t_2)/(W_{h1}+W_{h2})$, where t_1 and t_2 are the migration times of the two enantiomers, respectively, W_{h1} and W_{h2} are the peak widths at the half height of the peak. It can be seen that the resolution increases as the concentration of β-CD is increased until about 0.1 mol/L where the resolution reaches a maximum of 1.51. The high optimum concentration of the chiral selector indicates that the binding constant of the sample and β-CD is low according to the equation: $C_{opt}=1/(K_1K_2)^{1/2}$

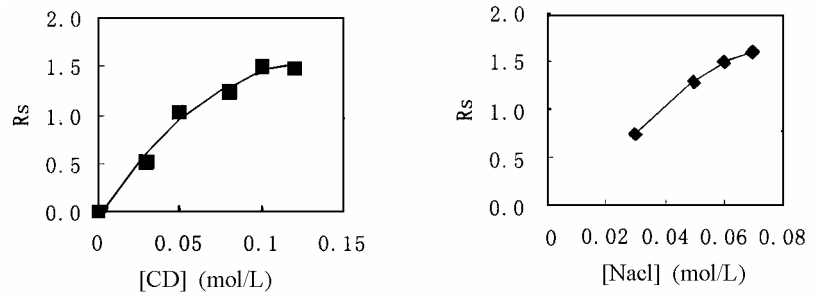
The increase of ionic strength upon increasing the concentration of NaCl decreases the zeta potentials of the capillary wall and the analyte ion (ξ_{wall} and ξ_{ion} respectively), as a result, both the electroosmotic and electrophoretic mobility (μ_{eo} and μ_{ep} respectively)

are affected by it according to the equations: $\mu_{eo}=\varepsilon\xi_{wall}/4\pi\eta$; $\mu_{ep}=\varepsilon\xi_{ion}/6\pi\eta$, which is the same as reported in the aqueous buffer.[5]

Figure 3 demonstrates the influence of ionic strength on chiral separation. The decrease in μ_{eo} and μ_{ep} due to higher NaCl concentration causes an increase in chiral resolution. However, there is little improvement of resolution after the concentration of NaCl reaches 0.06 mol/L. On the other hand, Joule heat effect which is caused by higher current will increase at higher ionic strength. Consequently, 0.06 mol/L NaCl was chosen as the optimum concentration.

Figure 2. Influence of β-CD concentration on chiral separation. Background electrolyte: 0.06 mol/L NaCl in FA with 10% HAc.

Figure 3. Influence of NaCl concentration on chiral separation. Background electrolyte 0.1 mol/L β-CD in FA with 10% HAc.



Ionic strength is one factor that will affect EOF, but high ionic strength will cause Joule heat effect that will have negative effect on separation. It has been recognized that pH also has an influence on the EOF and can change the charge status of a solute in aqueous system. We use pH* instead of pH in nonaqueous system because the knowledge about acid-base chemistry in organic media is very limited. Aparent pH was adjusted by adding different amounts of HAc to the background electrolyte. **Figure 4** illustrates the influence of various amounts of HAc on chiral separation. Both the migration time and chiarl separation increase greatly with increasing amount of HAc. No chiral separation could be observed if no HAc was added to the background electrolyte, and the resolution reached the highest when 10% HAc was added but at the sacrifice of longer electrophoresis time. **Figure 5** is a typical electrophoregram of the chiral separation of the sample. The effect of aparent pH on chiral separation could be explained by the following: since the adding of HAc decreases the electrophoretic mobilities of the enantiomers and EOF, the enantiomers interact with chiral selector in a longer time; the change in aparent pH will also change the charge status of the enantiomers, thus enhance the interaction between the enantiomers and the chiral selector. Both of the two factors lead to the increase in resolution.

Conclusion

A NACE method for chiral separation of N-benzoyl phenylalanine methyl ester is reported. Under the optimum condition of 0.1 mol/L β-CD,

Figure 4. The dependence of resolution on various amounts of HAc. Background electrolyte: 0.1 mol/L β-CD, 0.06 mol/L NaCl in FA.

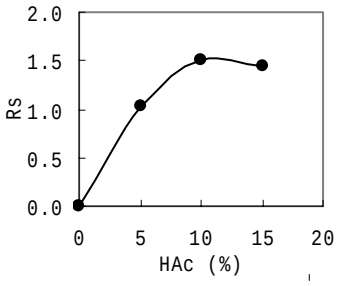
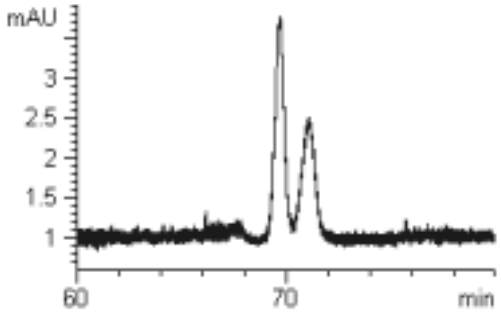


Figure 5. Chiral separation of N-benzoyl phenylalanine methyl ester. Background electrolyte: 0.1 mol//L β-CD, 0.06 mol/L NaCl, 10% HAc in FA. See text for other conditions



0.06 mol/L NaCl and 10% HAc in FA, the enantiomers could be baseline separated. Because of the low binding constant of the analyte with β-CD, higher resolution could only be obtained by adding HAc and adjusting the ionic strength to control EOF and electrophoretic mobility. The results indicate the potential of organic solvent for chiral separation by CE.

Acknowledgments

We would like to express our gratitude to Hewlett Packard for offering the HP^{3D}CE system. The study is supported by NSFC, Grant No. 29875001.

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Received 23 November 1998