Chinese Chemical Letters Vol. 15, No. 1, pp 112– 114 , 2004
http://www.imm.ac.cn/journal/ccl.html

Carboxymethyl-β-cyclodextrin for Chiral Separation of Amino Acids Derivatized with Fluorescene-5- isothiocyanate by Capillary Electrophoresis and Laser-induced Fluorescence Detection

Yu Yun CHEN, Wei WANG, Wei Ping YANG, Zhu Jun ZHANG*

College of Chemistry and Materials Science, Shaanxi Normal University, Xi'an 710061

Abstract: A method using carboxymethyl-β-cyclodextrin (CM-β-CD) as selector for chiral separation of amino acids by capillary electrophoresis and laser-induced fluorescence detection was studied. Resolution was better than that obtained by β-CD or HP-β-CD.

Keywords: Chiral separation, amino acids, capillary electrophoresis, derivatization, CM-β-CD.

The chiral separation of amino acids is very important in pharmaceutics, biology and agriculture. In recent years, chiral capillary electrophoresis[1] has developed rapidly and become a very strong tool for separation of amino acid enantiomers. Compared to GC and HPLC, CE offers the advantages such as high separation efficiency and low costs.

A variety of derivatization reagents have been explored to achieve a better detection sensitivity of the enantioseparation of amino acids. Fluorescene-5-isothiocyanate(FITC) is an excellent one and the application of CE-LIF to the separation of FITC-amino acids are reported in a few instances[2-5]. Y. F. Cheng *et al.*[2] and Keita Takizawa *et al.*[3] separated 20 FITC-amino acids, but they had not carried out chiral separation. Yi Chen[4] *et al.* and G. N. Olafo *et al.*[5] chirally separated FITC-amino acids, but they both used binary selectors, and did not do quantitative analysis.

Many β-CD derivatives have been used as selectors in the separation of amino acid enantiomers, but CM-β-CD used as selector for amino acids separation had been reported very few. C.Perrin *et al.*[6] separated several amino acids using CM-β-CD as a selector.

The sensitivity of UV detection was low， at the same time the quantitative analysis has not been done.

In the present work, we used one selector (CM-β-CD) to separate the FITC-amino acid enantiomers and satisfactory results were obtained. The resolution of some FITC-amino acids such as D, L-β-phenylserine (Rs=15.28) and D, L-tryptophan (Rs=5.43) were higher than those reported in ref.[4], in which the resolutions were 1.90 and 4.84, respectively. We also determined the limits of detection. Other two selectors, β-CD and HP-β-CD, have also been investigated and compared with CM-β-CD. Influence of concentrations of CM-β-CD and borate on resolution were also studied and optimized.

* Email: zzj18@hotmail.com

Experimental

D, L-β-Phenylserine(Pser), D, L-leucine(Leu), D, L-tryptophan(Trp) , D, L-α-alanine (Ala) were of biochemical reagent grade from Shanghai Biochemical Work (Shanghai, China). D, L-methionine(Met), D, L-serine(Ser), D, L-valine(Val) were of biochemical reagent grade from Beijing Chemical work(Beijing, China). Borate and β-CD were both of analytical reagent grade purchased from Xi'an Chemical work(Xi'an, China). CM-β-CD and HP-β-CD were self-made. All solutions were prepared in double-distilled water.

The CE separations were made on a P/ACE 5000 system equipped with a laser-induced fluorescence detector (Beckman Instrument, Fullerton, CA, USA). The detector was carried out with excitation at 488 nm and emission at 520 nm. The uncoated fused-silica capillary of 47 cm× 75 μm I.D used was from Beckman Instrument (Beckman Instrument, Fullerton, CA, USA). The capillary temperature was maintained at 20℃. Samples were injected with pressure at 5 kg/cm^2 for 5 s and separated at 18 kV.

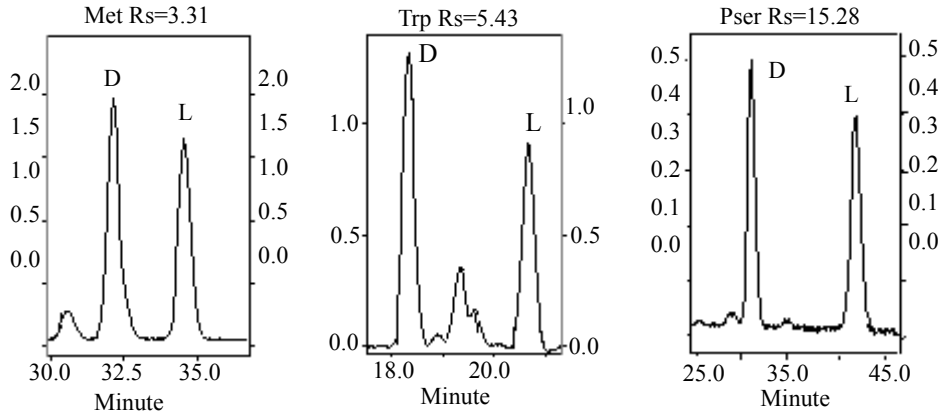
The solution of FITC was prepared by dissolving 3 mg FITC in 25 mL acetone (3.0×10^{-4} mol/L) and stored at −10℃. Amino acids were individually dissolved in 50 mmol/L borate at pH=9.0 to concentration level of 10^{-3} mol/L. Derivatization of individual amino acid was carried out by mixing 0.5 mL amino acid solution and 0.5 mL FITC solution and the mixture was kept in darkness overnight.

Results and Discussion

Different selectors have different separation selectivity for FITC-amino acids. The selectivity of the selectors β-CD, HP-β-CD and CM-β-CD were studied. **Figure 1** showed the optimum chiral separations obtained with CM-β-CD.

The influence of borate concentration was investigated by the addition of borate in the running buffer containing 5 mmol/L CM-β-CD. When the concentration of borate was higher than 50 mmol/L, the current and electrosmotic flow was too strong. So the suitable concentration was 50 mmol/L.

Figure 1 Chiral separation of some FITC-amino acids under optimized conditions



Buffer, 5 mmol/L CM-β-CD in 50 mmol/L borate at pH=9.0; capillary, washed between two successive runs with H_2O 1 min, 0.1 mol/L NaOH 1 min, H_2O 1 min and buffer for 2 min.

Conclusion

The concentration of CM-β-CD influenced the separation. As the concentration increased, the separation was better, but the migration time was longer. In order to get a good separation efficiency, the optimum concentration of CM-β-CD was 5 mmol/L.

In the optimized condition, chiral separation of three mixed FITC-amino acids, leucine, β-phenylserine and α-alanine was obtained. The detection limits were also investigated ($0.1\sim2.0\times10^{-8}$ mol/L). The results show different chiral selectors have different chiral selectivity.

Acknowledgment

This work was financially supported by the National Natural Science Foundation of China (No.20175039).

References

1. B. Chankvetadze, G. Blaschke, *J. Chromatogr. A*, **2001**, *906*, 309.
2. Y. F. Cheng, N. Dovichi, *Science*, **1988**, *242*, 562.
3. K. Takizawa, H. Nakamura, *Anal. Sci.*, **1998**, *14*, 925.
4. X. N. Lu, Y. Chen, *J. Chromatogr. A*, **2002**, *955*, 133.
5. G. N. Okafo, C. Bintz, S. E. Clarke, P. Camilleri, *J .Chem. Soc*., *Chem. Commun*., **1992**, 1189.
6. C. Perrin, M. G. Vargas, Y. Vander Heyden, M. Maftoub, D. K. Massart, *J. Chromatogr*. , **2000**, *883*, 249.

Received 20 December, 2002