Enantioseparation of racemic compounds in capillary electrophoresis using cationic single isomer cyclodextrin

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Method Article

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Abstract

Capillary electrophoresis \(CE) is a relatively new separation technique that provides rapid analysis with high efficiency and resolution due to the use of electric field and a variety of selective modes. Cyclodextrins are the most commonly utilized chiral selectors added into the background electrolyte to perform enantioseparation. This protocol describes an example of CE enantioseparation using a recently reported chiral selector mono-6-deoxy-6-\(3R,4R-dihydroxypyrrolidine)-β-CD chloride \(dhypy-CDCl).

Introduction

Enantioseparation has attracted considerable attention in biological science and pharmaceutical industry because of the different physiological activity and pharmacological effects of different enantiomers1. Amongst many analytical techniques, capillary electrophoresis \(CE) is a relatively new separation technique that provides rapid analysis with high efficiency and resolution due to the use of electric field and a variety of selective modes2. Cyclodextrins are the most commonly utilized chiral selectors added into the background electrolyte to perform enantioseparation. Besides neutral CDs derivatives, the synthesis and application of charged CDs derivatives have become attractive due to their good water solubility and excellent enantiorecognition abilities towards counter-charged racemic analytes3,4. Previously, our group has developed a series of positive changed single isomer CD selectors which afforded good enantioseparation abilities to anionic and ampholytic acids by CE5,6,7. This protocol describes an example of CE enantioseparation using a recently reported chiral selector mono-6-deoxy-6-\(3R,4R-dihydroxypyrrolidine)-β-CD chloride \(dhypy-CDCl)8.

Reagents

dhypy-CDCl was synthesized according to literature8 Sodium hydroxide \(NaOH) was purchased from Sigma-Aldrich \(138701) Sodium phosphate monobasic dehydrate \(NaH2PO4•2H2O) was purchased from Sigma-Aldrich \(71500) HPLC-grade methanol \(MeOH) was purchased from Sigma-Aldrich \(34860) HPLC-grade acetonitrile \(ACN) was purchased from Sigma-Aldrich \(34998) Ultra-pure water was prepared on a Arium 611VF water system \(Sarorius Stedim Biotech, Germany) 2-\(3-Chlorophenoxy)propionic acid \(3-ClPOP A) was purchased from Sigma-Aldrich \(233013) Dansyl-DL-valine cyclohexylammonium salt \(Dns-Val) was purchased from Sigma-Aldrich \(D1131)

Equipment

Beckman P/ACE MDQ CE system \(Fullerton, CA, USA), equipped with a 50 cm × 50 μm I.D. uncoated fused-silica capillary. The applied voltage was 15 kV \(normal polarity mode). Detection of analytes was carried out simultaneously at four channels 191, 214, 254 and 280 nm at 25 ºC. 32 Karat Software \(Version 5.0) \(Beckman Coulter, Fullerton, CA, USA) was used for data acquisition and system control.

Procedure

Running buffer preparation 1] Add a certain amount of NaH2PO4•2H2O into ultra-pure water to prepare 50 mM NaH2PO4 stock solutions. 2] Adjust the pH of NaH2PO4 solutions using 1 M NaOH until the desired pH \(generally 6~9) was obtained. 3] Add a certain amount of CD selector into the NaH2PO4 solution to obtain the desired CD concentration \(generally 0~30 mM). 4] Add a certain amount of organic modifier \(MeOH or ACN) \(generally 0~20%, vol/vol) into the solutions from Step 3. 5] Transfer 1.5 mL solutions into two 2 mL-vials 6] Degas the buffer before use. Sample preparation 1] Add a certain amount of racemic analytes into 50/50 \(vol/vol) methanol/water mixture to form stock solutions of 50 μg•mL-1. 2] Transfer 1.5 mL stock solutions into 2 mL-vials. 3] Degas the analyte solution before use. Capillary conditioning 1] Flush the capillary using 1 M NaOH solution for 30 min. 2] Flush the capillary using 0.1 NaOH solution for 30 min. 3] Flush the capillary using ultra-pure water for 30 min. 4] Flush the capillary using running buffer for 15 min. CE enantioseparation operation 1] Put the cartridge with fused-silica capillary on the CE equipment and put the two buffer vials and one analyte vial in the sample trays. 2] Set sample injection by pressure at 0.5 psi for 4 s. 3] Set the separation voltage as 15 kV. 4] Start injection and separation. 5] Collect separation data and stop running. 6] Flush the capillary using 1 M NaOH solution for 4 min. 7] Flush the capillary using ultra-pure water for 4 min. 8] Flush the capillary using running buffer for 4 min 9] Start next run.

Timing

Running buffer and sample preparation—approximately 2 h. Capillary condition—approximately 2 h. CE enantioseparation—approximately 10~30 min for each run.

Anticipated Results

Enantioseparation results 3-ClPOP A and Dns-Val were well resolved at 5 mM CD concentration. Decreased buffer pH prolonged the migration of 3-ClPOP A and increased the resolution \(Figure 1). The influence of ACN proportion in running buffer on the enantioseparation of Dns-Val was shown in Figure 2.

References

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Figures

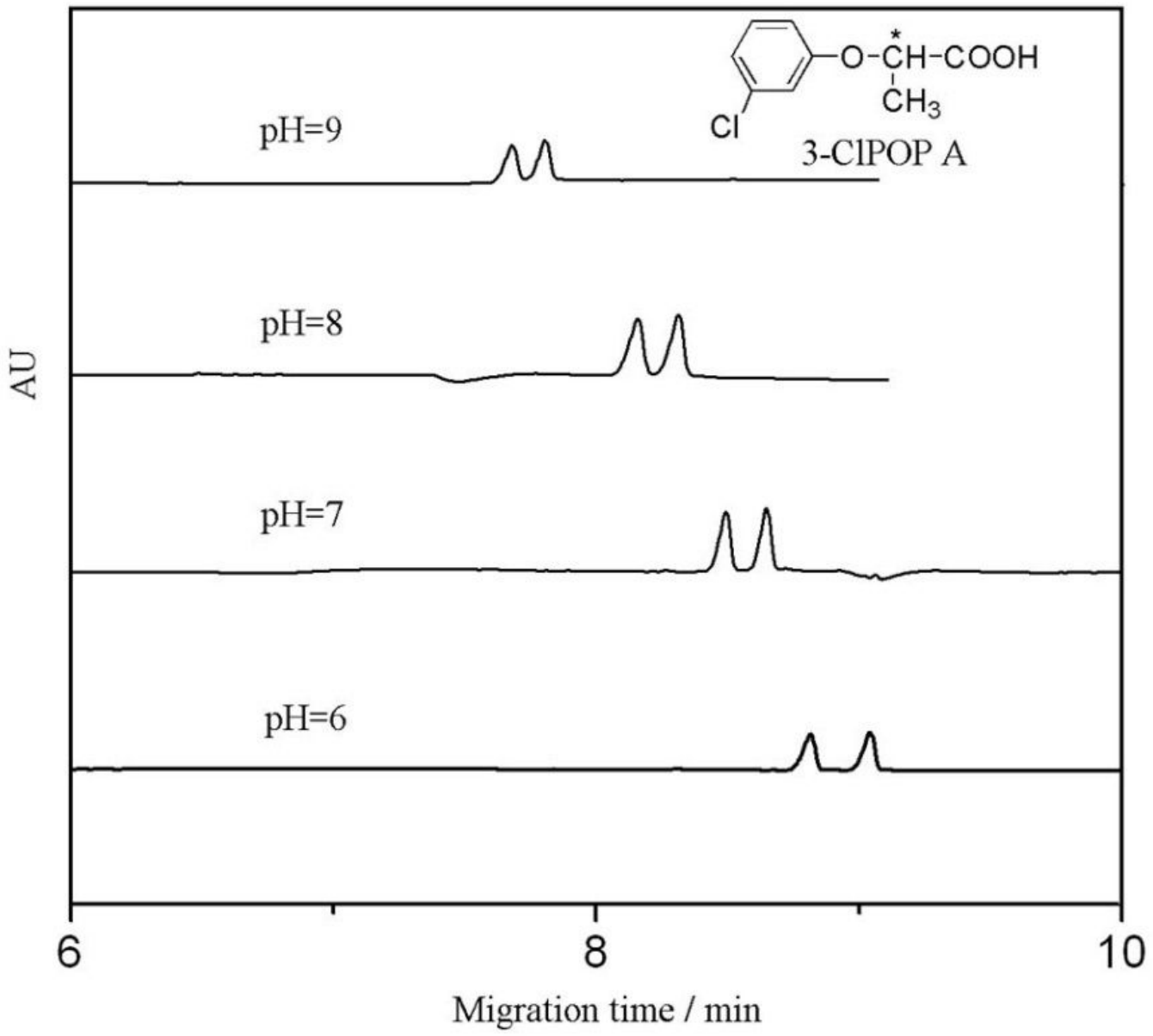

Figure 1

Enantioseparation of 3-ClPOP A with different buffer pH. Conditions: 5 mM dhypy-CDCl; 50 mM phosphate buffer; temperature 25 °C; applied voltage 15 KV.

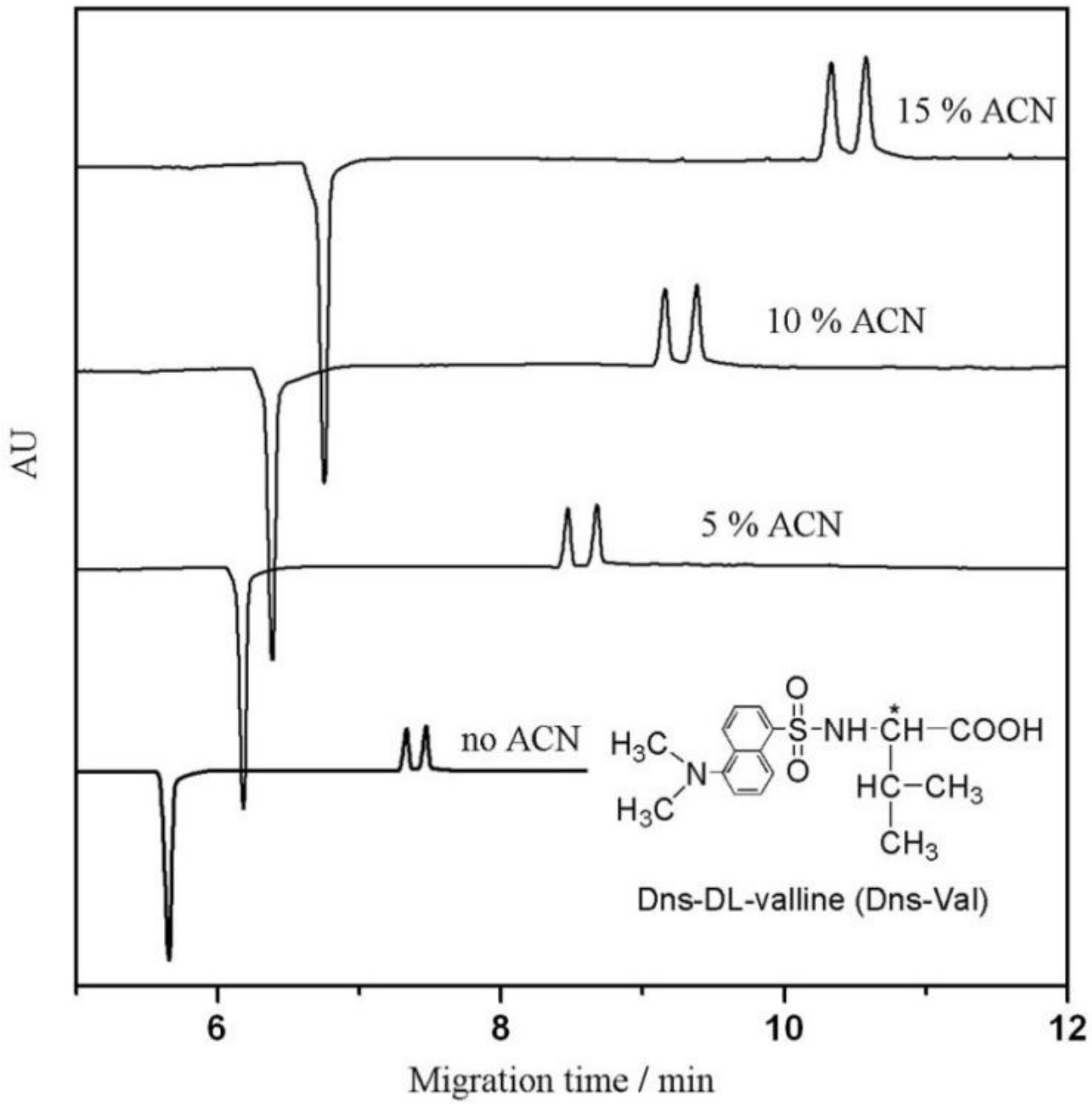

Figure 2

Influence of the organic modifier (ACN as the organic modifier). Conditions: 50 mM phosphate buffer; buffer pH=6; temperature 25 °C; applied voltage 15 KV.