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# Chiral separations by capillary electrophoresis using cyclodextrin-containing gels

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ABSTRACT

Allyl carbamoylated β-cyclodextrin derivatives were synthesized to be used as chiral resolving agents when copolymerized with acrylamide to form gels suitable for enantiomer separations in capillary electrophoresis. Both solid and liquid gels have been produced by adjusting the concentrations of acrylamide, bis-acrylamide and the allyl cyclodextrin derivative. The liquid gels were free of the problems associated with solid gels: bubble formation, short lifetime and poor reproducibility. The chiral selectivity of the liquid cyclodextrin gels in the separation of dansylated amino acid enantiomers depended on the cyclodextrin concentration of the gel. The liquid gels were successfully used to separate the enantiomers of several chiral molecules.

## INTRODUCTION

Capillary electrophoresis (CE) has become a powerful analytical technique during the past decade and spun several variants of the original method ranging from free solution CE through micellar techniques to capillary gel electrophoresis [1–3]. Though capillary gel electrophoresis is mostly used to separate biopolymers based on their size differences [4], Guttman *et al.* [5] used the polyacrylamide gel matrix to support selective complexing agents, such as native cyclodextrins, to alter the separation selectivity of the CE system.

β-Cyclodextrin (β-CD) is a toroidally shaped oligosaccharide composed of seven glucose units which are connected through α-(1,4)-linkages. The inner surface of the hollow truncated cone is relatively hydrophobic, whereas the external surfaces are hydrophilic due to the presence of the secondary hydroxyl groups at the larger opening and the primary hydroxyl groups at the smaller opening of the cavity [6]. CDs have been successfully used for chiral separations in high-performance liquid chromatography [7], capillary gas chromatography [8,9], isotachophoresis [10–13], free-solution CE [14–17], micellar electrokinetic chromatography [18,19] and capillary gel electrophoresis [5]. To the best of our knowledge, no report has been published yet on the CE use of allyl carbamoylated β-CD-acrylamide copolymer gels as described here.

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## EXPERIMENTAL

Allyl carbamoylated β-cyclodextrin derivatives (ac-β-CD) were synthesized according to the general method described in ref. 20, with some modifications as follows. A 10 mmol portion of β-CD (American Maize-Products, Hammond, IN, USA) was dried overnight *in vacuo* at 60°C, and then dissolved in 150 ml of dry pyridine. 25 mmol of allyl isocyanate (Aldrich, Milwaukee, WI, USA) were added and the reaction mixture was stirred for three days at room temperature. Thin-layer chromatographic separations revealed the presence of mono-, di-, tri- and tetra-substituted ac-β-CD derivatives. No unreacted β-CD was detected at the end of the third day. The raw product was purified by repeated crystallization from acetone.

The ac-β-CD–acrylamide copolymer gels were

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prepared by dissolving known quantities of the β-CD derivative, acrylamide (Aldrich) and/or bisacrylamide (Aldrich) in a known volume of pH 8.3 0.1 *M* Tris–0.25 *M* boric acid buffer. The solutions were filtered through a FHLP04700 membrane (Millipore, Bedford, MA, USA) and degassed before polymerization. Polymerization was carried out by adding, N,N,N′,N′-tetramethylethylenediamine (TEMED) as catalyst, and ammonium persulfate as radical initiator [4], and the reaction mixture was left to stand overnight. When bisacrylamide was present, the result was a solid gel; when it was absent, the product was a liquid gel.

A CE system assembled from a Spectroflow 783 UV detector (ABI, San Jose, CA, USA) a Type PS/EH30R03.0 high-voltage power supply (Glassman, Whitehouse Station, NJ, USA) and a plexiglass safety box [2] was used for the measurements. A Chrom-1/AT analog/digital converter board (Keithley Metrabyte, Tauton, MA, USA), installed in a Vectra-30 IBM-compatible personal computer and controlled by a data acquisition program developed in our laboratory [21], was used to collect and analyze data. Separations were carried out in 100 μm I.D. fused-silica capillaries (Polymicro Technologies, Phoenix, AZ, USA). The dansylated amino acid and drug standards were obtained from Sigma (St. Louis, MO, USA). Their solutions were prepared with the same buffer used in the preparation of the gels. The concentrations of the sample solutions were: 1.2 m*M* dansyl-D,L-aspartic acid (Asp), 1.5 m*M* dansyl-D,L-α-amino-*n*-butyric acid (But), 1.5 m*M* dansyl-D,L-glutamic acid (Glu), 1.2 m*M* dansyl-D,L-leucine (Leu), 1.2 m*M* dansyl-D,L-methionine (Met), 1.2 m*M* dansyl-D,L-norleucine (Norleu), 1.6 m*M* dansyl-D,L-norvaline (Norval), 1.4 m*M* dansyl-D,L-phenylalanine (Phe), 1.6 m*M* dansyl-D,L-serine (Ser), 1.4 m*M* dansyl-D,L-threonine (Thr), 1.0 m*M* dansyl-D,L-tryptophan (Trp), 1.1 m*M* dansyl-D,L-valine (Val), 6.2 m*M* homatropine and 5.9 m*M* atropine.

## RESULTS AND DISCUSSION

Several gel filled capillaries were prepared by keeping the concentration of acrylamide and bisacrylamide (%T and %C) constant and varying the concentration of ac-β-CD. The observed chiral selectivity for the separation of the enantiomers of dansyl-D,L-phenylalanine increased from 1.04 to 1.21 as the concentration of ac-β-CD was increased from 20 to 40 m*M*. Fig. 1 shows a typical electropherogram for the separation of the enantiomers of dansyl-D,L-phenylalanine, obtained on a 5%T, 3.3%C and 40 m*M* ac-β-CD solid-gel filled capillary. Though chiral selectivity is high, separation efficiency is much lower than customary in CE. In addition, practical problems such as short lifetime due to bubble formation and gel extrusion at higher field strengths could not be eliminated easily and reliably [22]. Therefore, solid gels were abandoned in favor of the liquid gels, which were free of these problems.

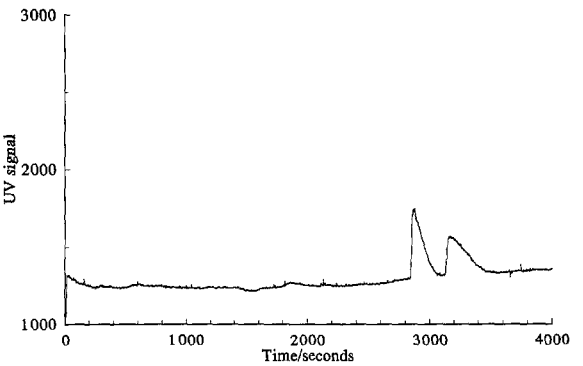


Fig. 1. Separation of the enantiomers of 1.1 m*M* dansyl-D,L-phenylalanine in a solid-gel filled capillary. Gel constituents: 5%T, 3.3%C and 40 m*M* ac-β-CD derivative in pH 8.3 0.1 *M* Tris–0.25 *M* boric acid buffer. Field strength: 95 V/cm. Electrokinetic injection at 16 V/cm for 5 s. Capillary dimensions: total length: 19 cm, injector to detector length: 12 cm, I.D.: 100 μm, O.D.: 170 μm. Detection wavelength: 255 nm.

Stable and reproducible liquid gels were obtained when the reaction mixture contained 1 to 2% (140 to 280 m*M*) acrylamide, 0 to 30 m*M* ac-β-CD, 4 to 10 μl/ml (26 to 66 m*M*) TEMED and 4 to 10 μl/ml of 10% (w/w) (1.8 to 4.4 m*M*) ammonium persulfate in the pH 8.3 Tris–boric acid buffer. To achieve complete copolymerization, the liquid gels were allowed to stand in the reaction vessel for at least 12 h before being transferred into the separation capillaries. Though viscous, the liquid gels could be transferred from the reaction vessels with microsyringes. Figs. 2 and 3 show typical electropherograms for the enantiomers of a few dansylated amino acids. Though the chiral selectivities are slightly lower than those obtained with the solid-gel filled capillaries, the separation efficiencies are much bet-

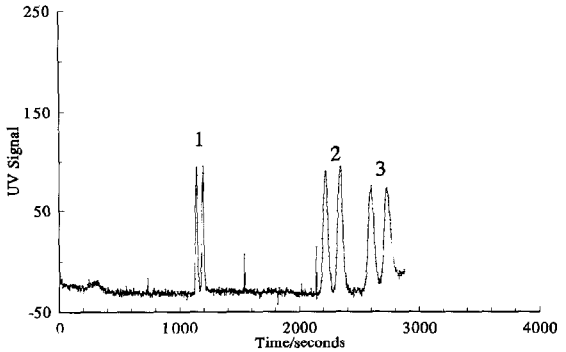


Fig. 2. Separation of the enantiomers of dansyl-D,L-aspartic acid, threonine and methionine in a liquid-gel filled capillary. Peaks: 1 = dansyl-D,L-aspartic acid; 2 = dansyl-D,L-threonine; 3 = dansyl-D,L-methionine. Gel constituents: 2% acylamide, 20 m*M* ac-β-CD derivative in pH 8.3 0.1 *M* Tris–0.25 *M* boric acid buffer. Field strength 89 V/cm. Electrokinetic injection at 15 V/cm for 1 s. Capillary dimensions: total length: 20 cm, injector to detector length: 13 cm, I.D. 100 μm, O.D. 170 μm. Detection wavelength: 255 nm.

TABLE I

SELECTIVITY AND PEAK RESOLUTION FOR THE SEPARATION OF THE ENANTIOMERS OF DANSYL-D,L-AMINO ACIDS

Conditions as in Fig. 2.

| Amino acid | Selectivity | Resolution |
|---|---|---|
| Asp | 1.046 | 1.5 |
| But | 1 | 0 |
| Glu | 1.031 | 1.0 |
| Leu | 1.127 | 2.5 |
| Met | 1.050 | 1.1 |
| Norleu | 1.095 | 2.1 |
| Norval | 1 | 0 |
| Phe | 1.052 | 1.1 |
| Ser | 1.033 | 0.8 |
| Thr | 1.055 | 1.3 |
| Trp | 1 | 0 |
| Val | 1.027 | 0.6 |

ter. Table I contains the selectivity and resolution data for the enantiomers of 12 dansylated amino acids. The separations were achieved with a 2% acrylamide and 20 m*M* ac-β-CD liquid gel, at a field strength of 89 V/cm. The enantiomers of three amino acids: tryptophan, norvaline and α-amino-*n*-butyric acid were not resolved.

Chiral selectivity and efficiency (*N*) were found to depend on the concentration of ac-β-CD in the copolymer: chiral selectivity increased, while *N* decreased with increasing concentration of ac-β-CD (Figs. 4 and 5). Presumably, both phenomena are brought about by the increased number of interactions between the solutes and the cyclodextrin moieties and the slow complexation kinetics, respectively. The opposing trends lead to resolution maxima at intermediate ac-β-CD concentrations, in agreement with the behavior observed in free solution systems [15].

The 2% acrylamide and 20 m*M* ac-β-CD liquid gels were successfully used for separation of the enantiomers of chiral drugs. However, the same gel

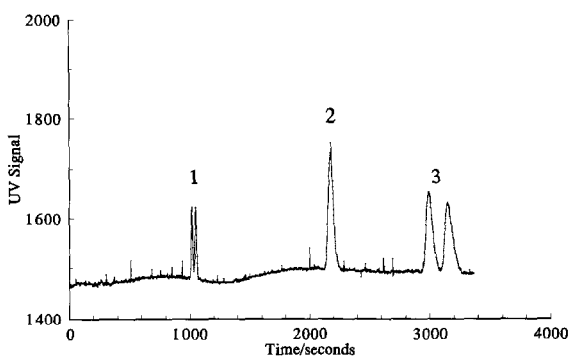


Fig. 3. Separation of the enantiomers of dansyl-D,L-glutamic acid, norvaline and phenylalanine in a liquid-gel filled capillary. Peaks: 1 = dansyl-D,L-glutamic acid; 2 = dansyl-D,L-norvaline; 3 = dansyl-D,L-phenylalanine. Conditions as in Fig. 2.

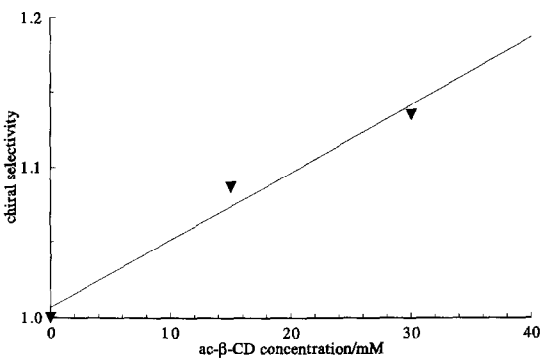


Fig. 4. Selectivity for the separation of the enantiomers of dansyl-D,L-leucine as a function of the ac-β-CD concentration of the liquid gel. Conditions as in Fig. 2, except field strength is 74 V/cm.

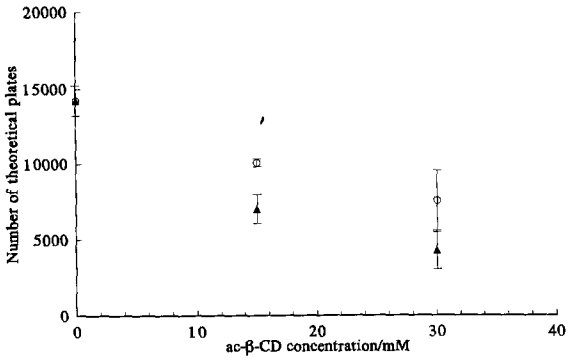


Fig. 5. Efficiencies (number of theoretical plates, $N$) calculated from the electropherograms of the enantiomers of dansyl-D,L-leucine as a function of the ac-$\beta$-CD concentration of the liquid gel. Conditions as in Fig. 4. ○ = More mobile enantiomer; ▲ = less mobile enantiomer.

does not always resolve the enantiomers of closely related chiral compounds. For example, baseline-to-baseline enantiomer separation could be obtained for homatropine (Fig. 6a), but no separation was seen for atropine (Fig. 6b). This indicates that the chiral resolving ability of CD must be tailored to match the structure of the solutes via modification of the secondary OH groups of the ac-$\beta$-CD molecule. Further work is in progress in our laboratory in this direction.

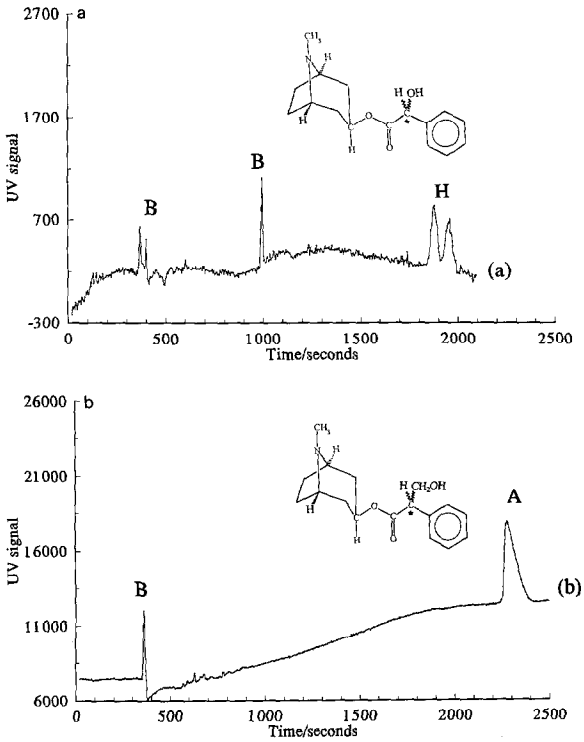


Fig. 6. Separation of the enantiomers of (a) homatropine and (b) atropine in a liquid-gel filled capillary. Peaks: B = unknown; H = homatropine; A = atropine. Conditions as in Fig. 2, except that field strength is 99 V/cm and detection wavelength is 210 nm.

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REFERENCES

1 J. W. Jorgenson, *Trends Anal. Chem.*, 3 (1984) 51.
2 J. W. Jorgenson and K. D. Lukacs, *Anal. Chem.*, 53 (1981) 1298.
3 M. J. Gordon, X. Huang, S. J. Pentoney and R. N. Zare, *Science (Washington, D.C.)*, 242 (1988) 224.
4 A. S. Cohen, A. Paulus and B. L. Karger, *Chromatographia*, 24 (1987) 15.
5 A. Guttman, A. Paulus, A. S. Cohen, N. Grinberg and B. L. Karger, *J. Chromatogr.*, 488 (1988) 41.
6 M. L. Bender and M. Komiyama, *Cyclodextrin Chemistry*, Springer, New York, 1st ed., 1978.
7 D. Armstrong, *Anal. Chem.*, 59 (1987) 84A.
8 E. Smolková-Keulemansová, *J. Chromatogr.*, 251 (1982) 17.
9 V. Schurig and H. P. Nowotny, *J. Chromatogr.*, 441 (1988) 155.
10 I. Jelínek, J. Snopek and E. Smolková-Keulemansová, *J. Chromatogr.*, 438 (1988) 211.
11 I. Jelínek, J. Snopek and E. Smolková-Keulemansová, *J. Chromatogr.*, 439 (1988) 386.
12 S. Fanali and M. Sinibaldi, *J. Chromatogr.*, 442 (1988) 371.
13 I. Jelínek, J. Dohnal, J. Snopek and E. Smolková-Keulemansová, *J. Chromatogr.*, 464 (1989) 139.
14 S. Terabe, *Trends Anal. Chem.*, 8 (1989) 129.
15 A. Fanali, *J. Chromatogr.*, 474 (1989) 441.
16 J. Snopek, H. Soini, M. Novotny, E. Smolková-Keulemansová and I. Jelínek, *J. Chromatogr.*, 559 (1991) 215.
17 M. Tanaka, S. Asano, M. Yoshinago, Y. Kawaguchi, T. Tetsumi, T. Shono, *Fresenius Z. Anal. Chem.*, 339 (1991) 63.
18 S. Terabe, H. Ozaki, K. Otsuka and T. Ando, *J. Chromatogr.*, 332 (1985) 211.
19 H. Nishi and M. Matsuo, *J. Liq. Chromatogr.*, 14 (1991) 973.

20 K. Fujimura, S. Suzuki, K. Hayashi and S. Masada, *Anal. Chem.*, 62 (1990) 2198.
21 Gy. Vigh, G. Quintero and Gy. Farkas, in Cs. Horváth and J. G. Nikelly (Editors), *Analytical Biotechnology*, American Chemical Society, Washington DC, 1990, p. 181.
22 I. D. Cruzado and Gy. Vigh, in A. Hedges (Editor), *Proceedings of the 6th International Symposium on Cyclodextrins*, Editions de Sante, Paris, 1992, in press.