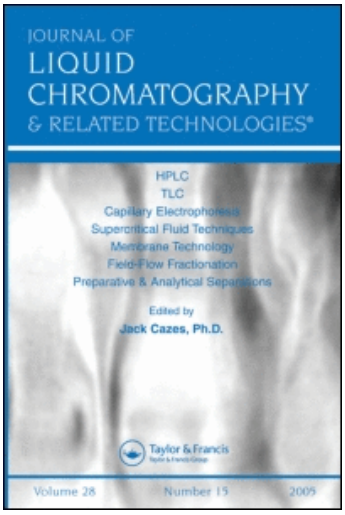

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ENANTIOMERIC SEPARATION BY CYCLODEXTRIN-MODIFIED MICELLAR ELECTROKINETIC CHROMATOGRAPHY USING BILE SALT

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ABSTRACT

A method for enantiomeric separation by cyclodextrin-modified micellar electrokinetic chromatography (MEKC) with bile salt micelles is described. The enantiomeric resolution was achieved with a mixed sodium taurodeoxycholate (TDC)-β-cyclodextrin (CD) solution (pH 7-9) utilizing a bare capillary. In sodium dodecyl sulfate (SDS)-CD solutions, similar result were not obtained. The effect of varying concentrations of TCD and β-CD and pH on the selectivity and resolution were also briefly investigated.

INTRODUCTION

Cyclodextrins are neutral polymers of glucose with a shape of a truncated cone and a cavity able to form inclusion complexes with several kinds of chemical compounds. Due to their properties CDs are frequently applied in different fields, e.g. in analytical chemistry for resolution of structural, positional and enantiomeric isomers [1-3]. Bile salt micelles possess a flexible structure of helical aggregates [4] and the lateral surface of the helix comprises the bile salt steroidal backbone with the angular methyl and hydroxyl groups protruding outside and inside the helix, respectively. Bile salt micelles have been ultilized for the separation of highly hydrophobic compounds

[5] and for separation of positional and enantiomeric isomers [6-8]. The mechanism of chiral separation by bile salt micelles is unknown, it is suggested that the enantiomeric resolution may be related to the relative planar structure of bile salt micelle [9].

MEKC is a relatively new analytical technique for separation of structural, positional and enatiomeric isomers [10-11]. The advantages of MEKC arise from its high separation efficiency, good reproducibility, low comsuption of either electrolytes and/or samples and speed of analysis. However, enatiomeric separation is still not quite as efficient by MEKC methods. CD modified MEKC for enantiomeric resolution have been previously reported by several research groups [12-14]. Much attention has been devoted to the improvement in the enantiomeric resolution of species by the CD-MEKC method.

This paper deals with the resolution of racemic dansyl-amino acids by bile salt micellar EKC using β-CD in a pH range 6-9. These racemic compounds could not be resolved by methods using CDs or MEKC using bile salt micelles and CD-SDS under similarly experimental conditions. The effect of concentration of CD and TDC (the bile salt) and pH on the selectivity and resolution are presented. The solubility of CDs was significantly improved in bile salt micelle solutions, which also provided the solutes with a longer and greater differential migration time compared to solutions lacking the micelles, hence improving resolution of these racemic compounds.

EXPERIMENTAL

Chemicals

Dansyl-DL-leucine, dansyl-DL-norleucine, dansyl-DL-threonine and dansyl-DL-tryptophan, β-CD, TDC (taurodeoxycholic acid, sodium salt) and SDS were purchased from Sigma (St. Louis, MO). All other chemicals were of analytical grade from Fisher (Springfield, NJ) or Aldrich (Milwaukee, WI). The run buffer solutions were prepared in doubly deionized water and filtered through a 0.4 μm membrance.

Apparatus

CE separation was performed on a in-house-built instrument consisting of an acrylic box designed with a safety-interlocked door to prevent operator contact with a 30 kV high-voltage power (Glassman High Voltage, Whitehouse Station, NY), which

was connected to the buffer reservoirs with platinum electrodes to effect CE separation. For on-line UV absorption detection, a detection window was made by burning off a small section of the polyimide coating at 75 cm from the anodic end of the electrophoretic capillary (100 cm x 75 μm I.D. x 365 μm O.D.) purchased from Polymicro Technologies (Phoenix, AZ). Absorption of analytes which migrate pass the detection window was measured using a Spectra-100 UV-Vis detector (Spectra Physics, San Jose, CA).

Procedures

New capillaries were conditioned with 20 mM borate-H_3PO_4 buffer for 12 h. before use. The capillary was washed between runs with a 20 mM borate-H_3PO_4 buffer. Sample injection was set for electrokinetic injection (10 kV) for a 2 s injection time and detection was measured at 230 nm.

RESULTS AND DISCUSSION

Figure 1 shows that four isomers of dansyl-amino acids were well resolved in 20 minutes using a TDC solution with β-CD as the run buffer modifier on a bare silica capillary. At pH above 7, electroosmosis is stronger than under acidic conditions and the elctroosmotic flow (bulk flow) will migrate toward the cathode. CD is a neutral polymer with a hydrophilic outside surface, and interacts with the micelle weakly, if at all. Thus, CD in the micellar solution may behave as another phase in addition to the micelle and migrate with an identical velocity (v_{CD}) of the electroosmotic flow (v_{eo}). Assuming the amount of solute in the aqueous medium is negligible, an ionized solute will only distribute itself between the micelle and CD. Utilizing the assumption that the electrophoretic velocity of micelle (v_{mc}) and v_{CD} are constant even incorporated solutes [15], the observed electrophoretic velocity of the solute in the CD-micellar solution, v^*_e, is given by

$$v^*_e = \frac{n_{CD}}{n_{mc}+n_{CD}} v_{CD} + \frac{n_{mc}}{n_{mc}+n_{CD}} v_{mc} \qquad (1)$$

where $v^*_e = v_{eo} - v_{obs}$ is the difference between electroosmotic velocity (v_{eo}) and migration velocity (v_{obs}) of solute in the CD-micellar solution, n_{CD} and n_{mc} are the molar fractions of the solute in CD and the micellar phase, respectively. The capacity

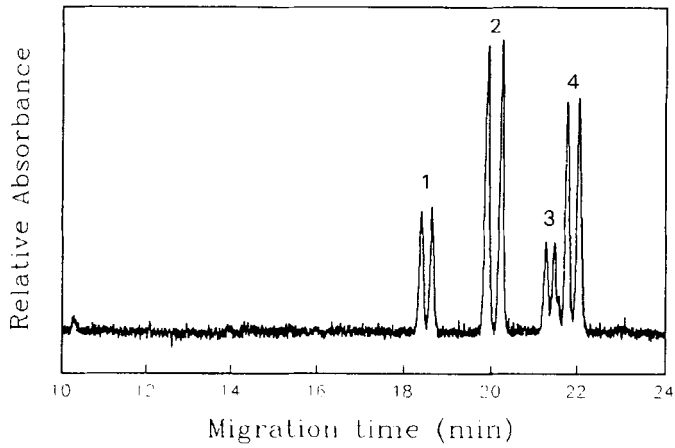

Figure 1. MEKC electropherogram of the enantiomeric separation. 1-0.62 μM of Dns-DL-threonine; 2-1.25 μM of Dns-DL-leucine; 3-0.37 μM of Dns-DL-tryptophan and 4-1.04 μM of Dns-DL-norleucine. Conditions: 40 mM β-CD - 80 mM TDC in 10 mM borate-H_3PO_4 buffer (pH = 7.8); capillary length = 100 cm (80 cm to the detector); electrokinetic injection 15 kV for 5 s; applied voltage = 20 kV; λ = 230 nm.

factor; k' is defined by [16]

$$k' = \frac{n_{mc}}{n_{CD}} \qquad (2)$$

and subsitution of Eqn.(2) into Eqn.(1) gives

$$k' = \frac{v^*_e - v_{CD}}{v_{mc} - v^*_e} \qquad (3)$$

The migration times, capacity factors, and resolutions of the four dansyl-amino acid isomers are summarized in Table I.

In comaprison to Figure 1, Figure 2 shows that enantiomeric separation of the solutes was unsuccessful in CD or CD-SDS solutions (Figure 2 (a) and 2 (c)). In the CD solution, the migration of solutes incorporated by CD was dependent on the charge of solute. Unlike in acidic conditions the electroosmosis dominates the electrophoresis, therefore the slight difference of electrophoretic velocities between incorporated and free solutes can not resolve the enantiomeric isomers. In the CD-

TABLE 1[α]

Amino Acid	t_L (min)	t_D (min)	k'_1	k'_2	α	R_s
Dns-DL-Threonine	18.40	18.65	5.38	5.07	1.06	1.47
Dns-DL-leucine	19.90	20.22	3.95	3.74	1.06	1.78
Dns-DL-tryptophan	21.20	21.42	3.23	3.14	1.03	1.38
Dns-DL-norleucine	21.70	22.00	3.03	2.92	1.04	1.58
Dns-DL-norvaline[β]	21.20	21.45	3.56	3.43	1.04	1.49

[α] Conditions are the same as in figure 1. t_o = 11.20 min and t_{mc} = 14.60 min. Injected a mixed sample solutions excepted Dns-DL-norvaline. t_o was measured by using methanol and t_{mc} was measured by using Sudan III. [β] measured on injections of the single sample solution. t_0 = 11.45 min and t_{mc} = 14.90 min.

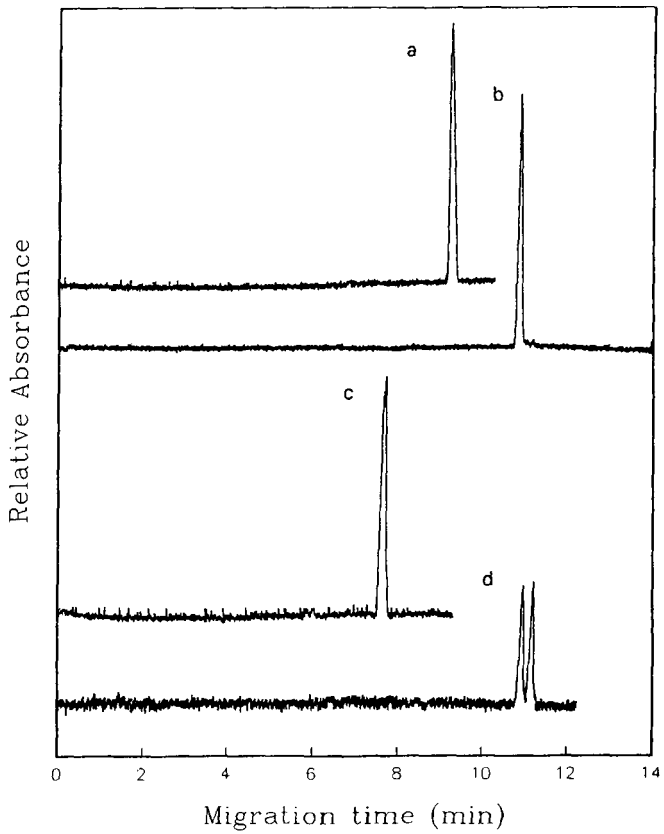

Figure 2. MEKC electropherograms of 1.2 μM of Dns-DL-norvaline in 1- 40 mM β-CD - 50 mM SDS solution; 2- 3% butanol - 80 mM TDC solution; 3- 10 mM β-CD and 4- β-CD - 80 mM TDC solution in 10 mM borate-H_3PO_4 buffer (pH= 7.8). Capillary length = 75 cm (50 cm to detector); electrokinetic injection 10 kV for 3-5 s; applied voltage = 15 kV.

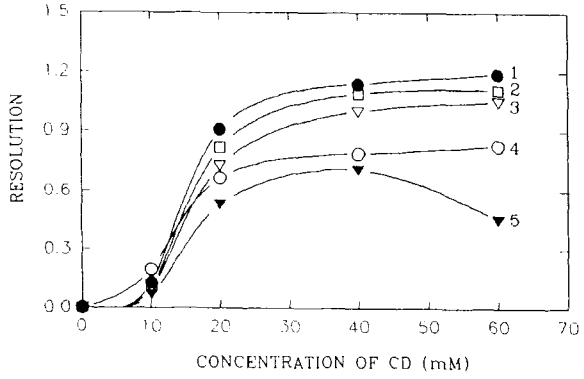

Figure 3. Effect of concentration of β-CD on resolution. Conditions are the same as in Figure 2, 60 mM TDC. 1- Dns-DL-leucine; 2- Dns-DL-norleucine; 3- Dns-DL-norvaline; 4- Dns-DL-threonine and 5- Dns-DL-tryptophan.

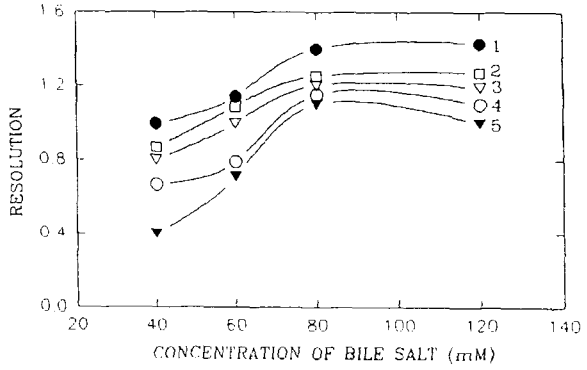

Figure 4. Effect of concentration of TCD on resolution. Conditions are the same as in Figure 2, 40 mM β - CD. 1- Dns-DL-leucine; 2- Dns-DL-norleucine; 3- Dns-DL-norvaline; 4- Dns-DL-threonine and 5- Dns-DL-tryptophan.

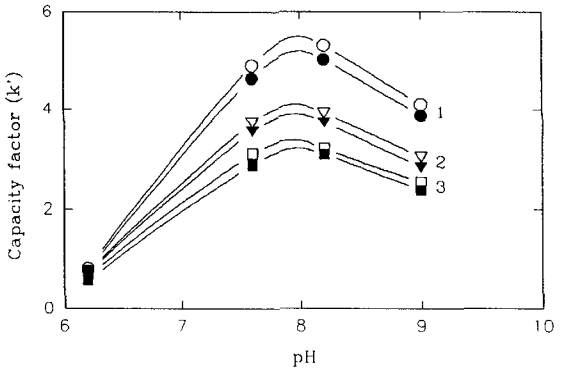

Figure 5. The influence of pH on the capacity factor. Conditions were the same as in Figure 1. 1- Dns-DL-threonine; 2- Dns-DL-leucine and Dns-DL-tryptophan.

SDS solution, it is proposed that the exchange of solutes between CD and SDS was too slow. If the solute retentivity of SDS, the nonchiral micelle, is stronger than CD, then the selectivity of the solutes is low. It is not suprising that the bile salt micelle is incapable of resolving the negatively charged chiral molecules, the same results as previously reported (Figure 2 (b)) [17]. Figures 3 and 4 show that resolution initially increases with the concentration of bile salt and CD, but begins to decrease at concentrations above 80 mM for the micelle solution and 40 mM for CD solution. The optimum concentrations of CD and the micelle were 40 mM and 80 mM, repectively. Similarly, figure 5 shows the optimum pH to be ~ 8 for the separation.

REFERENCES

1. W. L. Hinz, D. Y. Pharr, Z. S. Fu and W. G. Burkert, Anal. Chem., 61, 422 (1989)
2. S. J. Fanali, J. Chromatogr., 474, 441 (1989)
3. T. W. Ward and D. W. Amstrong, J. Liq. Chromatogr., 9, 407(1986)
4. M. C. Carey and D. M. Small, Arch. Intern. Med., 130, 506 (1972)
5. H. Nishi, T. Fukuyama and M. Matsuo, J. Chromatogr., 513, 278 (1990)
6. H. Nishi, T. Fukuyama and M. Matsuo, Anal. Chem. Acta, 236, 281(1990)
7. R. O. Cole, M. Sepaniak, J. Gorse and K. Oldigers, J. Chromatogr.,557, 113 (1991)
8. N. Wu, M. Lin, G. E. Barker and C. W. Huie, J. Chromatogr., submitted for publication

9. Y. Ishihama and S. Terabe, J. Liq. Chromatogr., 16, 933(1993)
10. S. A. Cohen, P. A. Paulus and B. L. Karger, Chromatographia, 24 , 15 (1987)
11. K. Otsuka and S. Terabe, J. Chromatogr., 515,221(1990)
12. S. Terabe, Trends in Anal. Chem., 129 (1989)
13. H. Nishi and M. Mastsuo, J. Liq. Chromatogr., 16, 973 (1993)
14. K. Otsuka and S. Terabe, J. Liq. Chromatogr., 16, 949(1993
15. K. Otsuka and S. Terabe, J. Chromatogr., 348, 39 (1985)
16. S. Terabe, Y. Miyashita, O. Shibata, E. R. Barnhart, L. R. Alexander, D. G. Patterson, B. L. Karger, K. Hosoya and N. Tanaka, J. Chromatogr.,516, 23 (1990)
17. R. O. Cole and M. Sepaniak, LC-GC, 10, 380 (1992)

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