Journal of Chromatography, 515 (1990) 221–226
Elsevier Science Publishers B.V., Amsterdam

CHROMSYMP. 1757

Enantiomeric resolution by micellar electrokinetic chromatography with chiral surfactants

KOJI OTSUKA*

Department of Industrial Chemistry, Osaka Prefectural College of Technology, Saiwai-cho, Neyagawa, Osaka 572 (Japan)

and

SHIGERU TERABE[a]

Department of Industrial Chemistry, Faculty of Engineering, Kyoto University, Sakyo-ku, Kyoto 606 (Japan)

ABSTRACT

Enantiomeric resolution of phenylthiohydantoin (PTH)-amino acids by micellar electrokinetic chromatography using chiral surfactants was studied. As a chiral surfactant, digitonin, which is a non-ionic compound, was used with anionic sodium dodecyl sulphate (SDS) to form mixed micelles. Under acidic conditions (pH 3.0), PTH derivatives of six amino acids (tryptophane, norleucine, norvaline, valine, α-aminobutyric acid and alanine) were separated from each other and optically resolved with a 25 m*M* digitonin–50 m*M* SDS solution, although a long separation time was required. The use of an anionic chiral surfactant, sodium N-dodecanoyl-L-valinate, was also examined under neutral conditions. In this instance, the same enantiomers as above except PTH-DL-Ala were resolved in a shorter time.

INTRODUCTION

Electrokinetic chromatography (EKC)[1] is a recently developed high-resolution separation method which is a branch of high-performance capillary electrophoresis (HPCE)[2–4] based on chromatographic principles using homogeneous solutions. EKC has the unique characteristic that both neutral analytes and charged solutes can be separated electrophoretically. Among various modes of EKC, micellar EKC (MEKC)[5,6], which uses micellar solutions of ionic surfactants, has become the most popular method for separating small neutral molecules and the fundamental characteristics of MEKC have been published[6,7].

Optical resolution of chiral compounds is a major application of chromatography, and a number of studies on chiral separations by chromatographic techniques have appeared. Zare and co-workers first reported the resolution of racemic mixtures

[a] Present address: Department of Material Science, Himeji Institute of Technology, 2167 Shosha, Himeji, Hyogo 671-22, Japan.

0021-9673/90/$03.50

of dansylated DL-amino acids by EKC with copper(II)–L-histidine[8] or copper(II)–aspartame complexes[9]. Recently, papers on optical resolution by MEKC without any additives such as metal ions have been published, chiral micelles of various bile salts[10,11] and a mixed micelle of an N-acyl-L-amino acid with sodium dodecyl sulphate (SDS)[12] being used to achieve chiral recognition.

In this paper, preliminary results on the enantiomeric resolution of phenylthiohydantoin-DL-amino acids (PTH-DL-AA) by MEKC with chiral surfactants are described. As a chiral surfactant we first used digitonin, which is a glycoside of digitogenin. As digitonin is electrically neutral, SDS was added to digitonin solutions to form mixed micelles having negative charges, and some PTH-DL-AA were successfully separated from each other and optically resolved. As another chiral surfactant, sodium N-dodecanoyl-L-valinate was used without making mixed micelles, and some PTH-DL-AA were also separated and optically resolved.

EXPERIMENTAL

Digitonin, the structure is shown in Fig. 1, was purchased from Wako (Osaka, Japan), and it is soluble in water. Although the digitonin used was of analytical-reagent grade, its purity was not examined. Sodium dodecyl sulphate (SDS) was obtained from Nacalai Tesque (Kyoto, Japan) and sodium N-dodecanoyl-L-valinate (SDVal) from Ajinomoto (Tokyo, Japan). All the reagents were used as received. PTH-DL-AA, obtained from Wako, were used as acetonitrile solutions with concentrations of *ca.* 1 mg/ml. Chromatographic solutions were prepared by dissolving digitonin and SDS or SDVal in 50 m*M* phosphate buffers adjusted to an appropriate pH. Sudan IV was obtained from Eastman Kodak (Rochester, NY, U.S.A.).

An untreated fused-silica capillary tube (Scientific Glass Engineering, Ringwood, Victoria, Australia) (630 mm × 0.05 mm I.D.) was used as a separation column, in which 490 mm from the injection end UV absorption was measured by a Jasco (Tokyo, Japan) UVIDEC-100-III spectrophotometric detector. As a regulated high-voltage power supply, an HepLL-30P0.08-LS (Matsusada Precision Devices,

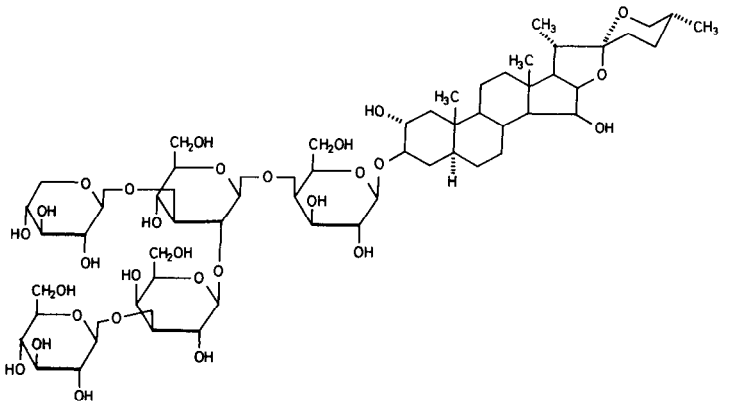

Fig. 1. Structure of digitonin.

Kusatsu, Shiga, Japan) was used. For data processing, a Chromatopac C-R3A (Shimadzu, Kyoto, Japan) was used. All experiments were carried out in the constant-voltage mode at ambient temperature.

RESULTS AND DISCUSSION

Digitonin–SDS solutions

First, we used a 25 m*M* digitonin–50 m*M* SDS solution of pH 7.0. Under these conditions, no enantiomeric resolution was achieved for any PTH-DL-AA. In this instance the electroosmotic velocity was large compared with the electrophoretic velocity of the digitonin–SDS mixed micelle, and hence the migration-time range was narrow and sufficient resolution could not be attained[6].

To suppress the electroosmotic flow and to extend the migration-time range, we used an acidic micellar solution (pH 3.0). The effects of pH on electrokinetic velocities, such as the electroosmotic velocity, electrophoretic velocity of the micelle and migrating velocity of the micelle, and on the chromatographic parameters in MEKC in the presence of SDS solutions have been reported previously[13]. Under the acidic conditions, PTH derivatives of six amino acids [tryptophane (Trp), norleucine (Nle), norvaline (Nva), valine (Val), α-aminobutyric acid (Aba) and alanine (Ala)] were separated from each other and each PTH-DL-AA was optically resolved as shown in Fig. 2, where a 25 m*M* digitonin–50 m*M* SDS mixed micellar solution of pH 3.0 was used, although a long separation time was required. Peak assignment of each enantiomer was not achieved. Here, the direction of migration of the mixed micelle was towards the positive electrode and was the opposite of the electroosmotic flow. As for other PTH derivatives, methionine was optically resolved, but serine was not resolved. The effect of concentrations of digitonin and SDS will be reported elsewhere.

Capacity factors, $\tilde{k}'$, defined as in previous papers[5,6], for each peak in Fig. 2, are

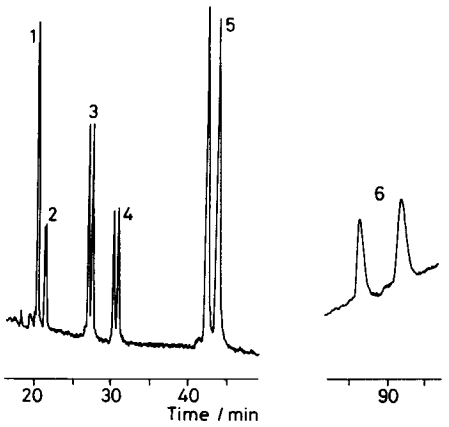

Fig. 2. Micellar electrokinetic chromatogram of six PTH derivatives of DL-amino acids. Corresponding amino acids: 1 = Trp; 2 = Nle; 3 = Nva; 4 = Val; 5 = Aba; 6 = Ala. Micellar solution, 25 m*M* digitonin–50 m*M* SDS (pH 3.0); separation tube, 630 × 0.05 mm I.D.; length of the tube used for separation, 490 mm; total applied voltage, 20 kV; current, 34 μA; detection wavelength, 260 nm; temperature, ambient.

TABLE I

CAPACITY FACTORS, SEPARATION FACTORS AND RESOLUTION OF SOME PTH DERIVATIVES OF DL-AMINO ACIDS

Conditions as in Fig. 2 (for digitonin–SDS) and Fig. 3 (for SDVal).

Solute	*Digitonin–SDS*				*SDVal*			
	$\tilde{k}'_1$	$\tilde{k}'_2$	α	R_s	$\tilde{k}'_1$	$\tilde{k}'_2$	α	R_s
Trp	38.8	44.5	1.15	0.54	2.4	3.7	1.56	8.01
Nle	18.3	20.5	1.12	0.83	5.3	8.1	1.54	8.58
Nva	5.2	5.5	1.06	1.49	0.6	0.8	1.46	5.28
Val	3.9	4.1	1.04	1.53	0.8	0.9	1.17	3.14
Aba	2.3	2.4	1.04	1.92	0.4	0.5	1.08	0.85
Ala	1.3	1.3	1.03	3.20				

given in Table I. In this instance, methanol and Sudan IV were used to measure the migration times of the aqueous phase (or the bulk solution), t_0, and of the micelle, t_{mc}, respectively. Here, t_0 and t_{mc} were found to be 24 and -19.5 min, respectively (the sign of the migration time was discussed previously[13]: a positive value means that the migration direction is from the positive to the negative electrode, and *vice versa*). As shown in Table I, the larger is $\tilde{k}'$, the shorter the migration time becomes[13]. As all the PTH-DL-AA separated in Fig. 2 are derivatized from monoaminomonocarboxylic acids and hence have no charge under the experimental conditions used, the calculated capacity factors can be considered as actual values. The validity of the calculation of $\tilde{k}'$ was confirmed as follows: three alkyl *p*-hydroxybenzoates, in which the carbon number, n of the alcohol residue ranged from 2 to 4, were injected under the same conditions as given in Fig. 2 and logarithms of the observed $\tilde{k}'$ values were plotted against n. A good linear relationship was observed and, therefore, the values of $\tilde{k}'$ calculated seem to be reliable. Methyl *p*-hydroxybenzoate ($n = 1$) was not detected owing to its very long migration time.

The separation factor, α, which is equal to $\tilde{k}'_2/\tilde{k}'_1$, and the resolution, R_s, between enantiomers for each PTH derivative are also given in Table I. In MEKC, resolution is related to the chromatographic parameters through[6]

$$R_s = \frac{\sqrt{N}}{4} \cdot \frac{\alpha - 1}{\alpha} \cdot \frac{\tilde{k}'_2}{1 + \tilde{k}'_2} \cdot \frac{1 - (t_0/t_{mc})}{1 + (t_0/t_{mc})\tilde{k}'_1} \quad (1)$$

where N is the theoretical plate number and R_s given in Table I was calculated by eqn. 1. It should be noted that in eqn. 1 (and also in the calculation of the separation factor) the capacity factors are taken as $\tilde{k}'_1 > \tilde{k}'_2$ in the case when the electroosmotic flow and the migration direction of the solute are opposite. For the case in Fig. 2, maximum resolution should be attained for the solute of $\tilde{k}' = -t_{mc}/t_0$ or 0.81 (ref. 13), provided that N and α are constant, although the solute having that $\tilde{k}'$ value will not migrate in the capillary. The fact that R_s for the pairs of Val, Aba and Ala becomes larger in that order, whereas the α values remain almost constant, clearly shows the effect of the product of the last two terms in eqn. 1, because its value increases in that order.

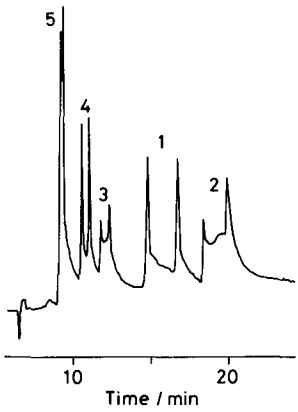

Fig. 3. Micellar electrokinetic chromatogram of five PTH derivatives of DL-amino acids. Micellar solution, 20 m*M* SDVal (pH 7.0); current, 38 μA; other conditions and peak numbers as in Fig. 2.

SDVal solutions

The use of SDVal in MEKC for chiral separations has been reported by Dobashi *et al.*[12]. In their study, SDS was added to SDVal solutions to form mixed micelles, and SDVal–SDS comicellar solutions containing methanol were also employed. In this study, however, we used SDVal solutions without any other surfactants or organic modifiers. Separation was carried out under neutral conditions or pH 7.0, where the SDVal micelle has negative charges.

As shown in Fig. 3, five pairs of enantiomers described above but not PTH-DL-Ala were resolved using a 20 m*M* SDVal solution in a much shorter time than in Fig. 2. For all peaks, especially Nva and Nle, much worse peak shapes than in Fig. 2 were observed. Unfortunately, the reason has not been clarified and is being investigated. Washing the capillary with sodium hydroxide solution, methanol or acetone was examined but no improvement in peak shape was obtained.

For the chromatogram in Fig. 3, the capacity factor for each peak, the separation factor and the resolution between each pair of enantiomers were calculated and are given in Table I. The reproducibility of the chromatogram for the sample shown in Fig. 3 was not good, especially with regard to baseline fluctuation and peak shape.

CONCLUSIONS

In conclusion, enantiomeric resolution by MEKC using chiral surfactants without any additives such as metal ions or chelating reagents was successfully achieved, although the resolution needs to be improved and the separation time reduced. The mixed micelle of digitonin, a neutral chiral surfactant, with anionic SDS can be used under acidic conditions, and SDVal, an anionic chiral surfactant, is also found to be useful in neutral solutions. Application of these MEKC systems to other chiral compounds is being investigated.

ACKNOWLEDGEMENTS

The authors are grateful to Dr. Akio Ishiwata of Ajinomoto for kindly providing SDVal. The authors also thank Mr. Tsutomu Mukai for technical assistance. This work was supported in part by a Grant-in-Aid for Scientific Research (No. 01750727) from the Ministry of Education, Science and Culture, Japan.

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