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Micellar electrokinetic chromatography using high-molecular-mass surfactants: comparison between anionic and cationic surfactants and effects of modifiers

Hiroto Ozaki[a,*], Akinobu Ichihara[b], Shigeru Terabe[a]

[a]*Faculty of Science, Himeji Institute of Technology, Kamigori, Hyogo 678-12, Japan*

[b]*Dai-ichi Kogyo Seiyaku Co. Ltd., 55, Nishi-shichijo, Shimogyo-ku, Kyoto 600, Japan*

Abstract

Two high-molecular-mass surfactants, butyl acrylate–butyl methacrylate–methacrylic acid copolymer sodium salt (BBMA) and butyl methacrylate–methacryloyloxyethyltrimethylammonium chloride copolymer (BMAC), were utilized as new pseudo-stationary phases in micellar electrokinetic chromatography (MEKC). As with BBMA, BMAC was successfully employed as a pseudo-stationary phase, but reversal of the electroosmotic flow was observed. The capacity factors were proportional to the concentration of BMAC, and the critical micelle concentration was effectively zero. The effect of the addition of methanol and a non-ionic surfactant were studied in MEKC with BBMA. Chiral separation was studied by cyclodextrin (CD) modified MEKC with BBMA: enantiomers of dansylated-DL-amino acids were separated with 2% BBMA and 10 m*M* β-CD, giving larger separation factors than those obtained with 100 m*M* sodium dodecyl sulfate and 60 m*M* β-CD.

1. Introduction

Micellar electrokinetic chromatography (MEKC) [1–5], is a mode of capillary electrophoresis, where ionic micelles are used as pseudo-stationary phases. The separation by MEKC is based on the differential partitioning of analytes between the micelle and the surrounding aqueous phase. Therefore, it is important for manipulating resolution and selectivity in MEKC to select suitable surfactants and modifiers [6]. Different surfactants generally show different selectivity. Bile salts [7–9] and a double-chain surfactant [10], which have significantly different molecular structures compared to sodium dodecyl sulfate (SDS), have shown remarkably different selectivity in comparison with SDS.

In MEKC, the capacity factor, k', which is defined as the ratio of the number of the analyte molecules incorporated into the micelle to that in the aqueous phase, is related to the volume of the micelle, V_{mc}, through

$$k' = K(V_{mc}/V_{aq}) \quad (1)$$

where K is the distribution coefficient and V_{aq} is the volume of the aqueous phase. The volume of the micelle is given as

$$V_{mc} = \bar{\nu}(C_{srf} - \mathrm{CMC}) \quad (2)$$

where $\bar{\nu}$ is the partial specific volume of the surfactant forming the micelle, C_{srf} is the con-

* Corresponding author. Permanent address: Kaneka Techno Research Co. Ltd., 1-2-80, Yoshida-cho, Hyogo-ku, Kobe 652, Japan.

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centration of the surfactant, and CMC is the critical micelle concentration. CMC depends on temperature, salt concentration, and additives.

High-molecular-mass surfactants are oligomers of monomeric surfactants or the polymers which show surface active properties as a whole. Palmer et al. [11,12] reported on MEKC with an oligomer synthesized from undecylenate. We reported on MEKC with butyl acrylate–butyl methacrylate–methacrylic acid copolymer sodium salt (BBMA), whose structure is shown in Fig. 1A [13,14]. BBMA is not an oligomer of the surfactant but a polymer which shows surface active properties. BBMA showed significantly different selectivity for naphthalene derivatives in comparison with SDS [14]. The CMC of BBMA was found to be effectively zero [14]. It was suggested that the stability of the micelle concentration of BBMA was superior to other surfactants.

Cyclodextrin (CD) is a chiral compound and capable of recognizing the molecular chirality. Therefore, CD modified MEKC (CD–MEKC) is useful for separating enantiomers [15–18]. Chiral separation of dansylated-DL-amino acids (Dns-DL-AAs) by CD–MEKC with SDS has been described previously [19]. SDS or low-molecular-mass surfactant molecules are considered to be co-included into the cavity of CD with the analyte enantiomers. BBMA is expected to be too large to be included into the cavity of CD.

In this paper, we describe the use of a cationic high-molecular-mass surfactant, the effects of modifiers in MEKC with BBMA, and the chiral separation by CD–MEKC with BBMA. Butyl methacrylate–methacryloyloxyethyltrimethylammonium chloride (BMAC), as shown in Fig. 1B, is a cationic high-molecular-mass surfactant whose main chain is similar to that of BBMA. In MEKC with BBMA, methanol was used as a modifier of the aqueous phase and octaoxyethylene dodecanol [$(EO)_8R_{12}$], which is expected to form a mixed micelle, as a modifier of the micelle. CD–MEKC with BBMA was applied to separation of enantiomers of Dns-DL-AAs. Results of CD–MEKC with BBMA were compared with those using SDS.

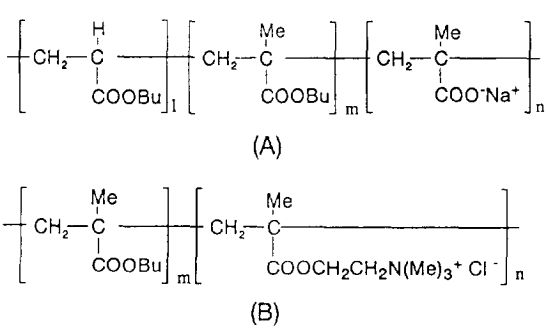

Fig. 1. Molecular structure of BBMA (A) and BMAC (B). Me = methyl; Bu = butyl.

2. Experimental

2.1. Reagents

BBMA and BMAC were supplied by Dai-ichi Kogyo Seiyaku (Kyoto, Japan). BBMA was provided as a 23% aqueous solution having a viscocity of 170 cP at 25°C. Since BBMA contains a minor amount of low-molecular-mass components, it was purified by the reprecipitation method with acetone [13,14]. BMAC was obtained as a 50% solution in water containing 40% 2-propanol and 10% methanol, and used without further purification. SDS, $(EO)_8R_{12}$, α-CD, β-CD and γ-CD were purchased from Nacalai Tesque (Kyoto, Japan). All other reagents were of analytical grade and water was purified with a Milli-Q system. All sample compounds, naphthalene derivatives and Dns-DL-AAs, were of analytical grade and used as received. Sample solutes were dissolved in about 25% aqueous methanol, which also served as a marker of the electroosmotic flow. Phenanthrene was used as a tracer of the micelle.

2.2. Apparatus

MEKC was performed with a Bio-Rad BioFocus 3000 CE System (Hercules, CA, USA) using a fused-silica capillary of 50 μm I.D. obtained from Polymicro Technologies (Phoenix, AZ, USA). The length of the capillary used in

MEKC was 36.5 cm (32 cm to the detector) or 54.5 cm (50 cm to the detector). The capillary was thermostated at 30°C and sample vials were at 15°C. Samples were injected by the pressurization method [2–5 s at 1 p.s.i. (6894.76 Pa)]. Separated zones were detected at 210, 250 and 280 nm simultaneously under the multi-wavelength mode and the electropherograms shown in this paper were recorded at 210 nm.

3. Results and discussion

3.1. Separation by MEKC with BMAC

A mixture of naphthalene derivatives was separated by MEKC with 2% BMAC and 20% 2-propanol in 100 m*M* borate–50 m*M* phosphate buffer (pH 7.0). BMAC was not soluble in an aqueous buffer without 2-propanol. Since the direction of electroosmotic flow was reversed, all solutes were injected at the negative end and migrated toward the positive electrode. Fig. 2 shows separations of naphthalene derivatives by MEKC with three different surfactants, BBMA, BMAC, and SDS. The migration order with BMAC was similar to that with BBMA, except for 1-naphthylamine and 1-naphthaleneethanol. However, it was significantly different from that with SDS. In Fig. 2A and B, the peaks with BMAC were just as sharp as with BBMA, suggesting that the adsorption of BMAC onto the capillary wall did not cause a serious loss of efficiency.

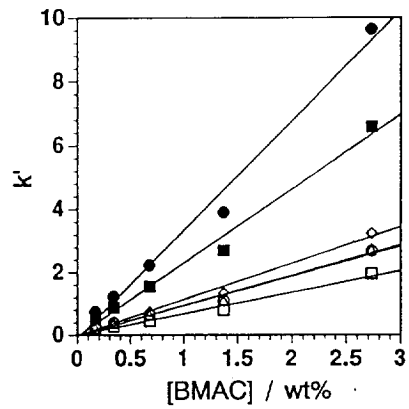

Fig. 3. Dependence of the capacity factor (k') on the concentration of BMAC. Solutes: □ = 1-naphthalenemethanol; ○ = 1,6-dihydroxynaphthalene; △ = 1-naphthylamine; ◇ = 1-naphthaleneethanol; ■ = 2-naphthol; ● = 1-naphthol. The conditions were the same as in Fig. 2B except for the concentration of BMAC.

Fig. 3 shows the dependence of the capacity factors of the naphthalene derivatives on the concentration of BMAC. The capacity factors were proportional to the BMAC concentration and all the plotted lines passed through or nearly through the origin. The CMC of BMAC was nearly zero as deduced from Eq. 2, therefore the

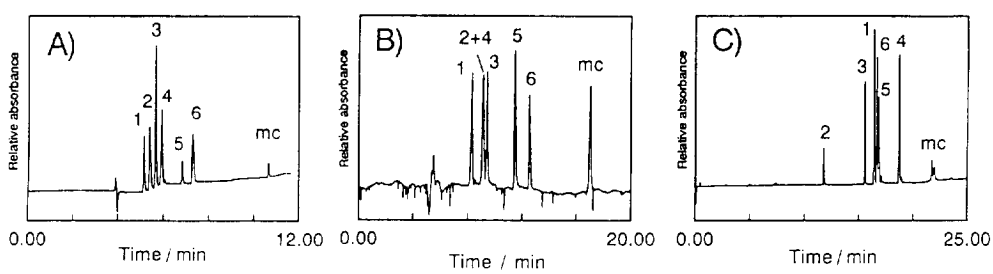

Fig. 2. MEKC separations of naphthalene derivatives with BBMA (A), BMAC (B), and SDS (C). Peaks: 1 = 1-naphthalenemethanol; 2 = 1,6-dihydroxynaphthalene; 3 = 1-naphthylamine; 4 = 1-naphthaleneethanol; 5 = 2-naphthol; 6 = 1-naphthol; mc = phenanthrene. Conditions: capillary, 36.5 cm (32 cm to the detector) × 50 μm I.D.; separation solution, (A) 2% BBMA in 50 m*M* phosphate–100 m*M* borate buffer (pH 8.0), (B) 2% BMAC and 20% 2-propanol in 50 m*M* phosphate–100 m*M* borate buffer (pH 7.0), (C) 50 m*M* SDS in 50 m*M* phosphate–100 m*M* borate buffer (pH 7.0); applied voltage (A) 15 kV, (B) 20 kV, (C) 20 kV; detection 210 nm.

micelle of BMAC was formed from one molecule.

3.2. Effects of additions of methanol and a non-ionic surfactant on the MEKC separation with BBMA

The effect of the pH, the compositon, and the molecular mass of BBMA on the MEKC separation have been reported previously [13,14]. The effect of modifiers of the aqueous phase on separation were investigated in this study. Fig. 4 shows the dependence of capacity factors of the naphthalene derivatives on the concentration of methanol. The capacity factors decreased with an increase in the methanol concentration. Fig. 5 shows the effect of methanol on the separation of the naphthalene derivatives using BBMA and SDS. The migration order of 1,6-dihydroxynaphthalene was altered by the methanol addition to the BBMA system as shown in Fig. 5B and that of 1-naphthol changed in the SDS system as in Fig. 5D. The capacity factors decreased by the methanol addition both to the BBMA and SDS systems.

Fig. 6 shows the dependence of the capacity factors on the concentration of $(EO)_8R_{12}$ added to the BBMA solution. The capacity factors increased with an increase in the amount of $(EO)_8R_{12}$ probably because of the increase in the micellar volume by the formation of a mixed

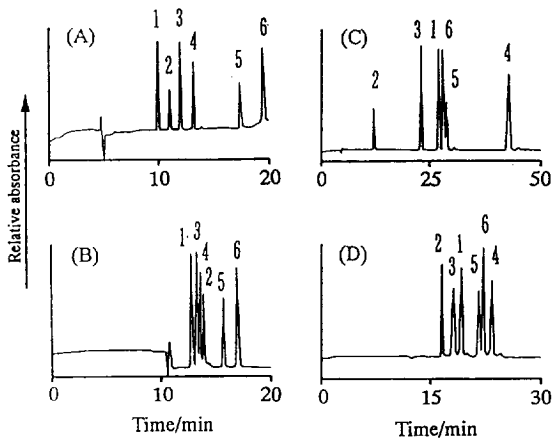

Fig. 5. Separation of the naphthalene derivatives with BBMA (A), BBMA and methanol (B), SDS (C), and SDS and methanol (D). The solutes are the same as in Fig. 2. Separation solution, (A) 2% BBMA in 50 m*M* phosphate–100 m*M* borate buffer (pH 8.0), (B) 40% methanol in the same BBMA solution as used in (A), (C) 50 m*M* SDS in 50 m*M* phosphate–100 m*M* borate buffer (pH 7.0), (D) 40% methanol in the same SDS solution as used in (C); applied voltage, 10 kV. The other conditions are the same as in Fig. 2.

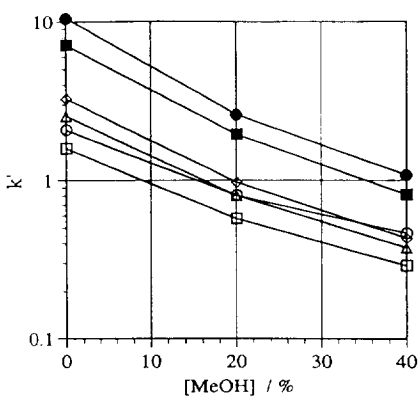

Fig. 4. Dependence of the capacity factors (k') of the naphthalene derivatives on the concentration of methanol. The solutes are the same as in Fig. 3. Separation solution, 2% BBMA and methanol in 50 m*M* phosphate–100 m*M* borate buffer (pH 8.0); applied voltage, 10 kV. The other conditions are the same as in Fig. 2.

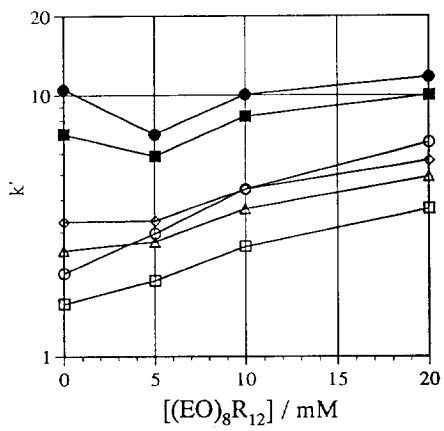

Fig. 6. Dependence of the capacity factors (k') of the naphthalene derivatives on the concentration of $(EO)_8R_{12}$. The solutes are the same as in Fig. 3. Separation solution, 2% BBMA and $(EO)_8R_{12}$ in 50 m*M* phosphate–100 m*M* borate buffer (pH 8.0); applied voltage, 10 kV. The other conditions are the same as in Fig. 2.

micelle [6,21]. In the case that mixed micelle is not formed and two kinds of micelles are formed, the capacity factors should decrease with an increase in the ammount of the non-ionic surfactant. Therefore, the results strongly suggest that $(EO)_8R_{12}$ formed a mixed micelle with BBMA. The migration order of 1,6-dihydroxynaphthalene was changed more significantly than the others with an increase in the concentration of $(EO)_8R_{12}$. The migration-time window became narrower with increasing non-ionic surfactant concentration because of the decrease in the surface charge density.

Fig. 7 shows the separation of the naphthalene derivatives by MEKC with three different mixed micelles containing $(EO)_8R_{12}$. It should be noted that the mixed micelle with BBMA shows a different selectivity compared to either the mixed micelles with SDS or with sodium laurate, which also have a carboxylate group as the polar group. The addition of a non-ionic surfactant to

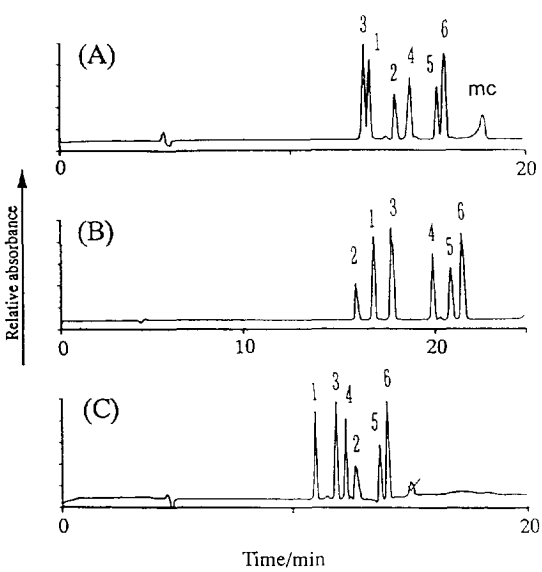

Fig. 7. Separations of the naphthalene derivatives using $(EO)_8R_{12}$ with sodium laurate (A), SDS (B), and BBMA (C). The solutes are the same as in Fig. 2. Separation solution, (A) 50 m*M* sodium laurate and 20 m*M* $(EO)_8R_{12}$ in 50 m*M* phosphate–100 m*M* borate buffer (pH 7.0), (B) 50 m*M* SDS and 20 m*M* $(EO)_8R_{12}$ in the same buffer as used in (A), (C) 2% BBMA and 20 m*M* $(EO)_8R_{12}$ in the same buffer as used in (A); applied voltage, 20 kV. Other conditions are the same as in Fig. 2.

SDS solutions resulted on the alteration of selectivity [5,21,22] as shown in Fig. 5C and Fig. 7B.

3.3. Chiral separation of Dns-DL-AAs by CD–MEKC with BBMA

Table 1 lists the results of the separation of enantiomers of ten Dns-DL-AAs by CD–MEKC with 10 m*M* β-CD and 2% BBMA. The results with 60 m*M* β-CD and 100 m*M* SDS [19] are also given in Table 1. The migration time of the BBMA micelle in the CD–BBMA system was assumed to be equal to that of phenanthrene observed with 2% BBMA in the absence of β-CD. BBMA was used without purification in these experiments. The separation solution used was clear at first, but a white precipitate was observed after a few hours. In Table 1, nine pairs of the ten enantiomeric pairs were successfully separated with the BBMA system and the separation factors of eight pairs of these were larger than those with the SDS system.

The migration times of different DNS-AAs were close each other in the BBMA system; from 10 to 11 min, except for those of Dns-DL-Asp with the SDS system, where the migration times of DNS-AAs were widely spread [19]. The migration order with the BBMA system was significantly different from that with the SDS system. It should be noted that Dns-DL-Phe migrated fastest of all and Dns-DL-Nle migrated faster than Dns-DL-Nva. This order is not consistent with the hydrophobicity of the analyte. The results suggest that Dns-DL-AAs are mainly solubilized by the BBMA micelle with the incorporation of the Dns-group into the hydrophobic core of the micelle.

Table 2 summarizes the results obtained with 2% unpurified or purified BBMA with 10 m*M* β-CD and 20% methanol, and the results from Ref. [19] with 60 m*M* β-CD, 100 m*M* SDS and 20% methanol. The separation solution prepared with unpurified BBMA generated a white precipitate after a day, but that with purified BBMA did not show any changes. In Table 2, all enantiomers were separated with both the BBMA systems and all separation factors were

Table 1
Separation of Dns-DL-AAs by CD–MEKC

Dns-DL-AAs	2% BBMA–10 m*M* β-CD			100 m*M* SDS–60 m*M* β-CD
	t_1/min	t_2/min	α^a	α^b
Phe	10.00	10.00	1.00	1.04
Leu	10.61	10.73	1.05	1.03
Nle	10.67	10.78	1.04	1.02
Trp	10.54	10.60	1.02	1.04
Met	10.67	10.76	1.03	1.00
Nva	10.70	10.87	1.07	1.02
Val	10.61	10.75	1.05	1.03
Ser	10.92	11.01	1.03	1.00
Thr	10.76	10.91	1.05	1.00
Asp	15.47	15.97	1.10	1.03

Conditions: capillary, 54.5 cm (50 cm to the detector) × 50 μm I.D.; separation solution, 2% unpurified BBMA and 10 m*M* β-CD in 50 m*M* phosphate–100 m*M* borate buffer (pH 8.0); applied voltage, 20 kV; detection wavelength, 210 nm.
[a] The migration time of the micelle was assumed to be equal to that of phenanthrene observed with 2% BBMA solution without β-CD.
[b] From Ref. 19.

larger than those with the SDS system. The purified BBMA clearly showed better resolution than the unpurified BBMA.

BBMA forms a molecular micelle which consists of one molecule, whereas SDS forms a micelle in the presence of the monomeric molecule whose concentration is equal to the CMC. The white precipitate mentioned above was probably a complex formed from β-CD and a minor component of BBMA which was removed by the purification, because the BBMA molecule should not form a complex with β-CD. The results of Table 1 show that BBMA was superior to SDS for the separation of enantiomeric Dns-

Table 2
Separation of Dns-DL-AAs by CD–MEKC with methanol

Dns-DL-AAs	2% unpurified BBMA –10 m*M* β-CD			2% purified BBMA –10 m*M* β-CD			100 m*M* SDS –60 m*M* β-CD
	t_1/min	t_2/min	α^a	t_1/min	t_2/min	α^b	α^c
Phe	13.03	13.25	1.05	10.46	10.61	1.06	1.03
Leu	13.52	13.80	1.07	10.87	11.08	1.08	1.03
Nle	13.57	13.80	1.05	10.84	11.01	1.06	1.02
Trp	13.77	13.84	1.02	11.31	11.36	1.02	1.01
Met	13.91	14.08	1.04	11.15	11.31	1.06	1.02
Nva	13.93	14.12	1.04	11.15	11.31	1.06	1.02
Val	14.09	14.35	1.06	11.59	11.79	1.07	1.03
Ser	14.35	14.53	1.04	11.73	11.89	1.24	1.02
Thr	14.68	14.98	1.07	11.90	12.14	1.08	1.04
Asp	25.67	27.08	1.30	21.49	22.50	2.15	1.05

Conditions are same as shown in Table 1 except for the addition of 20% methanol to the separation solution.
[a,b] See the footnote a and b in Table 1.
[c] From Ref. [19].

DL-AAs owing to the absence of the monomeric surfactant molecules which might be co-included with the enantiomer into the cavity of β-CD. Furthermore, the results of Table 2 show that the purified BBMA was superior to the unpurified BBMA because of the absence of the minor components which gave the precipitate. From Tables 1 and 2, the addition of methanol to the BBMA system increased the resolution except for Dns-DL-Nva in comparison with the results with SDS system [19]. Fig. 8 shows the example of the separation of enantiomers by CD–MEKC with purified BBMA. Enantiomers of timepidium bromide were successfully resolved under the same condition as in Fig. 8.

The other CDs were also employed together with purified BBMA. No enantiomer of the ten Ds-DL-AAs were separated with 10 m*M* α-CD and 2% BBMA. The separation solution did not generate any precipitate. Seven of the ten Dns-DL-AAs (Leu, Nle, Met, Nva, Val, Thr and Asp) were successfully separated with 10 m*M* γ-CD and 2% BBMA. The separation factors of three of the seven (Val, Thr and Asp) were larger, but those of the other four were less than those obtained with 60 m*M* γ-CD and 100 m*M* SDS [19]. The separation solution gave a white precipitate after few hours. It should be noted that γ-CD tends to generate the precipitate more than β-CD. These results suggest that in the BBMA system β-CD is most suitable for CD–MEKC separation of Dns-DL-AAs. In the SDS system γ-CD is more suitable than β-CD except for Trp, Ser and Asp [19]. With the purified BBMA, which will not generate precipitate with γ-CD, Dns-DL-AAs will be better separated with the BBMA and γ-CD system than with the SDS and γ-CD system.

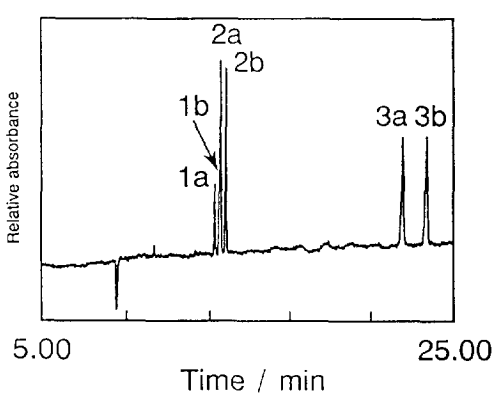

Fig. 8. Separations of Dns-DL-AAs by CD–MEKC with BBMA: 1a, 1b = Dns-DL-Phe; 2a, 2b = Dns-DL-Thr; 3a, 3b = Dns-DL-Asp. Separation solution, 10 m*M* β-CD, 2% BBMA and 20% methanol. Other conditions are the same as in Table 1.

4. Conclusions

BMAC, as BBMA, is found to be a useful high-molecular-mass surfactant for MEKC. BMAC gave a zero CMC, reversed direction of electroosmotic flow, and different selectivity. Additions of methanol or $(EO)_8R_{12}$ changed separation and selectivity in MEKC with BBMA. For the chiral separation of Dns-DL-AAs by CD–MEKC, BBMA was superior to SDS because of the absence of monomeric surfactant.

Acknowledgements

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