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Micellar electrokinetic chromatography using high-molecular surfactants
Use of butyl acrylate–butyl methacrylate–methacrylic acid copolymers sodium salts as pseudo-stationary phases

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Abstract

Butyl acrylate–butyl methacrylate–methacrylic acid copolymers sodium salts (BBMA), high-molecular surfactants ($M_r \approx 40\,000$), were utilized in micellar electrokinetic chromatography. Non-ionic test solutes were successfully separated with a 2% BBMA solution in a borate–phosphate buffer (pH 8.0). BBMA showed significantly different selectivity for naphthalene derivatives in comparison with sodium dodecyl sulfate. The capacity factors were proportional to the concentration of BBMA, and the critical micelle concentration was found to be substantially close to zero, suggesting that one BBMA molecule forms one micelle. Effects of the pH, the composition and the molecular mass of BBMA were studied.

1. Introduction

Micellar electrokinetic chromatography (MEKC) [1–4], which uses an ionic micellar solution as the separation solution, is a mode of capillary electrophoresis (CE). CE is a separation technique of ionic analytes only, whereas MEKC is capable of separating both ionic and non-ionic analytes. Almost all advantages of CE apply to MEKC as well and many applications of MEKC separations have been reported [3,4].

The MEKC separation is based on the differential partitioning of an analyte between the micelle, which is a pseudo-stationary phase, and the surrounding aqueous phase, and therefore the choice of surfactants and modifiers of the aqueous phase is important for manipulating separation selectivity [5]. The effect of surfactant structure on selectivity has been discussed elsewhere [5]. It is generally recognized that different surfactants show different selectivity. In particular, the polar group of the surfactant affects selectivity more significantly than the hydrophobic group. Most surfactants have a long alkyl chain as a hydrophobic group but some have different structures: semiplanar structures such as bile salts or multiple-chain structures such as lecithins or some synthetic surfactants [6]. These surfactants are known to have some advantages over the single-alkyl-chain surfactants: bile salts have low solubilizing capability [7–9] and can recognize chirality [10–14]; a

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double-chain surfactant has shown significantly different selectivity [6].

The micelle is in equilibrium with the monomeric surfactant, whose concentration is called critical micelle concentration (CMC) and is constant irrespective of the concentration of the surfactant. Since the micelle works as the pseudo-stationary phase in MEKC, the volume of the micelle, V_{mc}, is directly related to the capacity factor, k', 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 excluding the volume of the micelle. The volume of the micelle is given as

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

where $\bar{v}$ is the partial specific volume of the surfactant forming the micelle and C_{srf} is the concentration of the surfactant. CMC depends on experimental conditions such as temperature, salt concentration and other additives.

When a high voltage is applied across the capillary length, the temperature inside the capillary will rise due to Joule heating even with a thermostated capillary [15–18]. The temperature rise of the running solution inside the capillary probably causes a change in CMC, the distribution coefficient and hence the capacity factor in addition to the viscosity. Therefore, the effect of the temperature rise on the migration time will be more serious in MEKC that in capillary zone electrophoresis (CZE).

High-molecular surfactants called oligo-soaps or poly-soaps are oligomers of monomeric surfactants or polymers that show surface active properties as a whole. The high-molecular surfactant is considered to form the micelle from a single molecule, which may be called a *molecular micelle*. The CMC can be zero or meaningless. Therefore, we can expect a constant concentration of the micelle for the high-molecular surfactant irrespective of the experimental conditions. Although the micelle formed from low-molecular surfactants exists in a dynamic equilibrium and has a limited life time less than 1 s, the molecular micelle is stable. Therefore, the high-molecular surfactant is expected to show different characteristics for the use in MEKC.

The size of the micelle has a distribution, which contributes to the band broadening in MEKC [19]. The effect of the size distribution on efficiency is significant only for analytes having a large capacity factor [19]. The effect of the size distribution can be leveled out by the dynamic exchange of the micellar size in the case of low-molecular surfactants. The distribution of the micellar size of the high-molecular surfactant will be wider than that of the low-molecular surfactant and may adversely affect efficiency of MEKC. However, the high-molecular surfactant will have other advantages over the low-molecular surfactant: a high content of organic solvent will not break down the micelle; very low concentrations of the micelle will be available; no monomeric surfactant that does not contribute to the separation is present.

So far, only one high-molecular surfactant has been reported for MEKC; Palmer et al. [20,21] synthesized undecylenate oligomer by polymerizing micellized sodium 10-undecylenate in aqueous solution. The oligomer was successful for the separation of hydrophobic compounds with relatively high concentrations of acetonitrile.

Butyl acrylate–butyl methacrylate–methacrylic acid copolymers sodium salts (BBMA) are a group of high-molecular surfactants, whose molecular structure is shown in Fig. 1. We tried to utilize BBMA as a pseudo-stationary phase for MEKC [22]. This paper describes some characteristics of BBMA as the pseudo-stationary phase in MEKC. Some other natural or synthetic high-molecular surfactants were also examined for use as pseudo-stationary phases in MEKC.

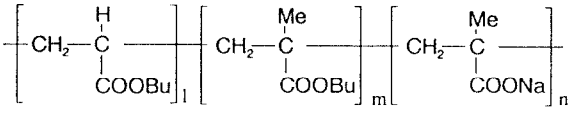

Fig. 1. Molecular structure of BBMA. Me = Methyl; Bu = butyl.

2. Experimental

2.1. Reagents

BBMAs were supplied by Dai-ichi Kogyo Seiyaku (Kyoto, Japan) as aqueous solutions. Most of this work was performed with a grade of BBMA, which was a 23% aqueous solution having a viscosity of 170 cP at 25°C. The molecular mass of the BBMA was about 40 000 from gel permeation chromatography (GPC) using standard polyethylene glycols. Since BBMA contained a minor amount of low-molecular components, it was purified by a reprecipitation method as follows: a portion of the BBMA solution was mixed with 50 portions of acetone; a precipitated polymer was separated by decantation and dried in vacuo at room temperature. Thus purified BBMA was used in this work if it is not mentioned otherwise. Three grades of BBMA having a different content of methacrylic acid (MAA) were used: 50, 40 and 30% of MAA with the same composition of butyl acrylate–butyl methacrylate. BBMAs having different viscosities are also employed: 3300, 250 and 60 cP.

Alginic acid, carboxymethylcellulose sodium salt and poly(N-vinyl-2-pyrrolidone) were purchased from Tokyo Kasei Kogyo (Tokyo, Japan). SDS and Chitosan were from Nacalai Tesque (Kyoto, Japan). Other reagents were of analytical grade and water was purified with a Milli-Q system.

Phenanthrene was used as a tracer of the micelle. Sample solutes were dissolved in 25% aqueous methanol, which was also a marker of the electroosmotic flow.

2.2. Apparatus

MEKC was performed with a Bio-Rad BioFocus 3000 (Hercules, CA, USA) using a fused-silica capillary of 36.5 cm (32 cm to the detector) × 50 μm I.D. obtained from Polymicro Technologies (Phoenix, AZ, USA). The temperature of the capillary was thermostated at 30°C. Samples were injected by the pressurization method and detected at 210, 250 and 280 nm simultaneously under the multi-wavelength mode. The electropherograms were recorded at 210 nm.

GPC was carried out with a Shimadzu LC-9A liquid-delivery pump (Kyoto, Japan) and a Shodex RI SE-51 refractive index (RI) detector (Tokyo, Japan) using Tosoh (Tokyo, Japan) TSK-gel G3000SW (60 cm × 8 mm I.D.) and G2000SW (60 cm × 8 mm I.D.) columns in series at room temperature. A sodium chloride solution (50 m*M*) containing 20% acetonitrile was employed as a mobile phase.

3. Results and discussion

3.1. GPC of BBMA

A gel permeation chromatogram of BBMA is shown in Fig. 2. The main peak was eluted early and assigned to BBMA. The peak is relatively sharp and hence the molecular mass will not be widely distributed. The weak negative peak was due to the sample solvent, water. The low and broad peak between the two peaks was considered to show a low-molecular compound. Almost the same chromatogram was observed for

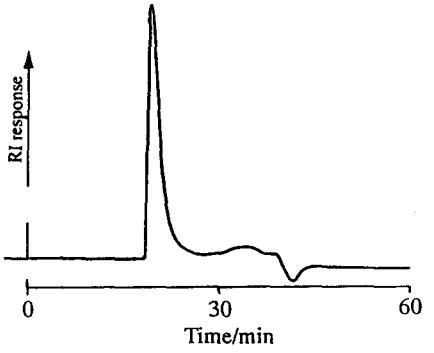

Fig. 2. Gel permeation chromatogram of BBMA. Column, TSK-gel G3000SW + G2000SW; mobile phase, 50 m*M* NaCl containing 20% acetonitrile; flow-rate, 1 ml min^{-1}; temperature, ambient; detector, refractometer.

the purified BBMA as described in the Experimental section.

3.2. Separation by MEKC with BBMA and other high-molecular surfactants

MEKC separations of three test mixtures, benzene derivatives, cold medicines, and naphthalene derivatives, are shown in Fig. 3, obtained with unpurified BBMA. The benzene derivatives were successfully separated, as shown in Fig. 3A, and the migration order was the same as that obtained with SDS [2]. The separation of the cold medicines was not very successful, as shown in Fig. 3B. The capacity factors were too small for the cold medicines but the migration order was the same as that observed with SDS. The naphthalene derivatives were well resolved, as shown in Fig. 3C. The migration order of the

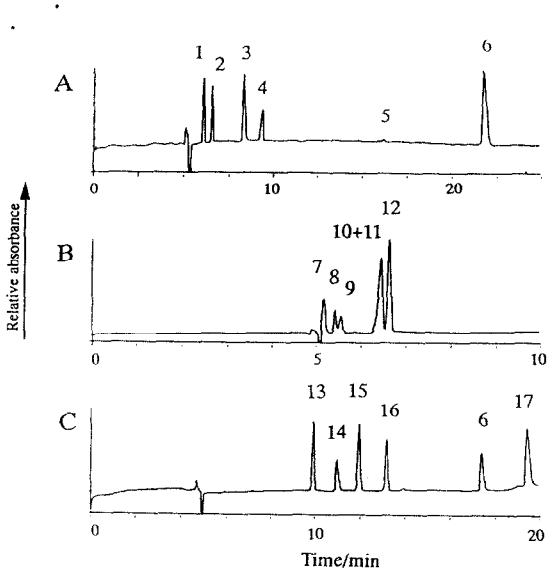

Fig. 3. MEKC separations of benzene derivatives (A), cold medicines (B) and naphthalene derivatives (C) using BBMA. Peaks: 1 = resorcinol; 2 = phenol; 3 = *p*-nitroaniline; 4 = nitrobenzene; 5 = toluene; 6 = 2-naphthol; 7 = acetaminophen; 8 = caffeine; 9 = guaifenesin; 10 = ethenzamide; 11 = isopropylantipyrine; 12 = trimetoquinol; 13 = 1-naphthalenemethanol; 14 = 1,6-dihydroxynaphthalene; 15 = 1-naphthylamine; 16 = 1-naphthaleneethanol; 17 = 1-naphthol. Conditions: capillary, 36.5 cm (32 cm to the detector) × 50 μm; running solution, 2% unpurified BBMA in 50 m*M* phosphate–100 m*M* borate buffer (pH 8.0); applied voltage, 20 kV; detection wavelength, 210 nm.

naphthalene derivatives was significantly different from that obtained with SDS. Efficiency was slightly lower than that usually obtained with low-molecular-mass surfactants, but it was still high enough for most purposes. The high efficiency shown in Fig. 3 suggests that the distribution of the molecular mass of BBMA does not cause a serious loss of efficiency.

The separation selectivity was extremely different especially for naphthalene derivatives in comparison with that observed using SDS. In particular, it is interesting that 1-naphthol migrated much slower than 1-naphthalenemethanol or 1-naphthaleneethanol. This has also been observed with a double-chain surfactant, 5,12-bis(dodecylmethyl) - 4,7,10,13 - tetraoxa - 1,16 - hexadecanedisulfonate (DBTD) [6]. The cold medicines had lower capacity factors than those with SDS, which is probably due to the difference in the polar group of the surfactants: a carboxyl group in BBMA, whereas a sulfate group in SDS. A similar difference in selectivity has also been observed between SDS and sodium trioxyethylene alkyl ether acetate (ECT), which has a carboxyl group [23]. Timepidium bromide, which is often used as a good tracer of the SDS micelle [24], migrated faster than Sudan IV and phenanthrene, which had the same migration times. Therefore, timepidium bromide cannot be used as a tracer of the BBMA micelle.

Solutions (2%) of alginic acid and carboxymethylcellulose in the phosphate–borate buffer (pH 8.0) were viscous and not suitable for use in MEKC. A 0.5% solution of chitosan in 0.33 *M* phosphoric acid was employed in MEKC. No resolution was obtained for the test mixtures but only a broad single peak was observed by applying −5 kV. Polyvinylpyrrolidone solution (2%) in the buffer (pH 7.0) was not effective for the separation of the test mixtures. Only a single peak was observed at about 10 min by applying 10 kV (40 μA).

3.3. Effects of concentration of BBMA and pH on migration time

The dependence of the capacity factors of naphthalene derivatives on the concentration of

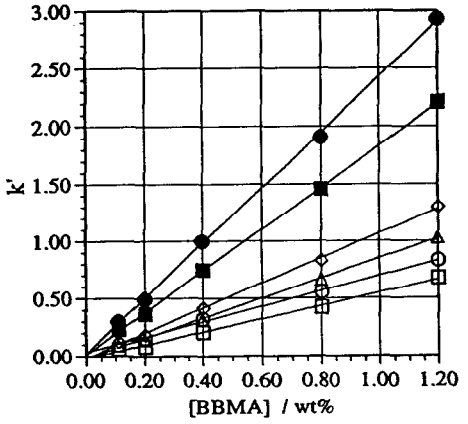

Fig. 4. Dependence of the capacity factor (k') on the concentration of BBMA. Solutes: □ = 1-naphthalenemethanol; ○ = 1,6-dihydroxynaphthalene; △ = 1-naphthylamine; ◇ = 1-naphthaleneethanol; ■ = 2-naphthol; ● = 1-naphthol. Conditions as in Fig. 3 except for the concentration of BBMA.

BBMA is shown in Fig. 4. The capacity factors were proportional to the BBMA concentration and the plotted line for each analyte passed the origin closely when it was extrapolated. The results clearly demonstrate that the CMC of BBMA is virtually zero, as deduced from Eq. 2. Thus, the micelle of BBMA can be assumed to be formed from one molecule by considering the molecular mass of BBMA.

The dependence of the migration-time window (t_{mc}/t_0, where t_{mc} and t_0 are migration times of the micelle and the aqueous phase) on pH is given in Fig. 5. The concentration of BBMA was 0.17%, because the solubility of BBMA was low at low pH. The migration-time window became wider with increasing pH values and it was almost constant between pH 7 and 9, although the results were not shown in Fig. 5. BBMA precipitated below pH 4 probably due to the decrease of the surface charge. The capacity factors of the naphthalene derivatives decreased with an increase in pH as shown in Fig. 6, which means that the solubilizing power of BBMA is reduced probably due to the increased surface charge or ionization of the carboxyl group. The results strongly suggest that although the solutes are neutral, the surface charge of the micelle significantly affects the distribution coefficient.

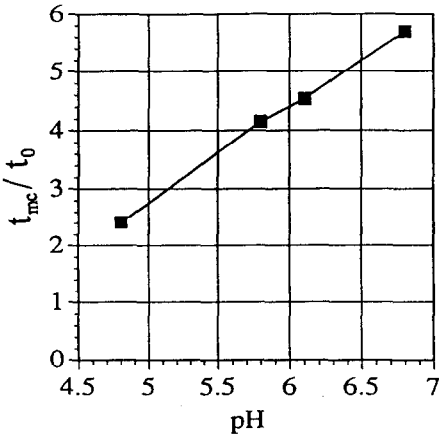

Fig. 5. Dependence of the separation window (t_{mc}/t_0) on pH. Running solution was 0.17% BBMA in 50 mM phosphate buffer. Other conditions as in Fig. 3.

3.4. Effects of the composition and molecular mass of BBMA on the separation

Three BBMAs having different contents of MAA were employed to study the effects of the composition on separation. Fig. 7 shows the separations of the naphthalene derivatives at pH 8.0 and Fig. 8 gives the dependence of their capacity factors on the MAA content. The migration-time window increased with an increase in the MAA content, as is clearly seen from Fig. 7, which was ascribed to an increase of

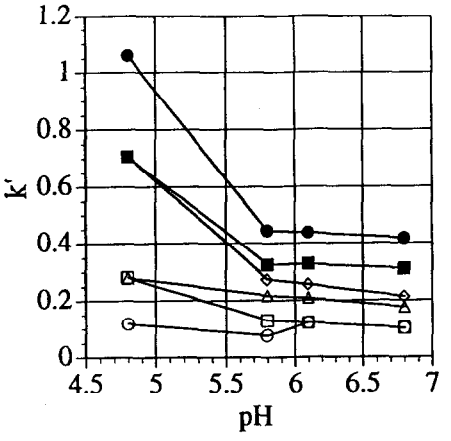

Fig. 6. Dependence of the capacity factors (k') of naphthalene derivatives on pH. Solutes and conditions as given in Fig. 4.

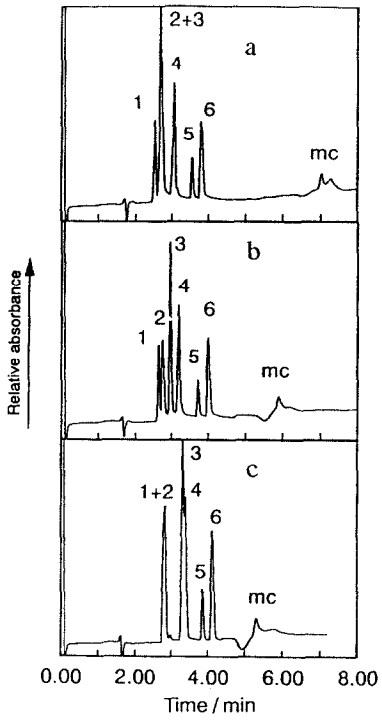

Fig. 7. Separation of naphthalene derivatives using BBMAs having different contents of MAA: (a) 50%, (b) 40% and (c) 30%. Peaks: 1 = 1-naphthalenemethanol; 2 = 1,6-dihydroxynaphthalene; 3 = 1-naphthylamine; 4 = 1-naphthaleneethanol; 5 = 2-naphthol; 6 = 1-naphthol. Running solution, 2% BBMA in a 100 m*M* borate–50 m*M* phosphate buffer (pH 8.0). Other conditions as in Fig. 3.

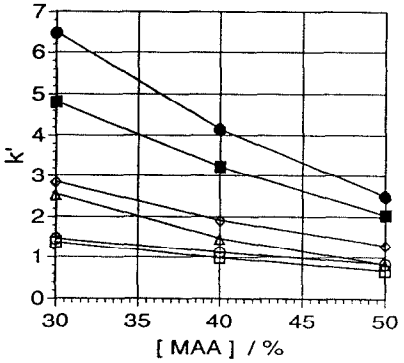

Fig. 8. Dependence of the capacity factor (k') of naphthalene derivatives on the content of MAA in BBMA. Solutes as in Fig. 4; conditions as in Fig. 7.

the surface charge owing to the increased number of carboxyl groups. The capacity factors decreased with an increase of the MAA content. The dependence of the capacity factor on the content of MAA was very similar to that on the pH described above. Both dependencies can be superficially explained in terms of surface charge. However, it should be mentioned that the electroosmotic flow is independent of the MAA content, whereas it is significantly dependent on the pH in the acidic region. Therefore, the use of BBMAs having different MAA contents is more advantageous than varying the pH to manipulate the migration-time window or capacity factor. One more disadvantage of the pH change is that BBMA tends to precipitate below pH 5.

Three BBMAs with different viscosities but same composition were employed to see the effect of molecular mass on the separation of naphthalene derivatives. The three BBMAs gave almost the same chromatograms for the naphthalene derivatives, which suggests that the solubilizing power and the electrophoretic mobilities of the BBMAs are independent of the molecular mass provided the composition is unchanged. Therefore, we can conclude that the molecular mass distribution will not be critical for the reproducibility of the migration time and selectivity, although a wider distribution of molecular mass values may cause lower efficiency.

4. Conclusions

BBMA has been found to be a useful high-molecular surfactant for the use in MEKC and has some advantages over low-molecular surfactants: zero CMC or molecular micelle; different selectivity; and possible manipulation of the migration-time window and capacity factor by changing the pH or content of MAA. The molecular micelle is characteristic of high-molecular surfactants and ensures the constant concentration of the micelle irrespective of the conditions. Although its constant concentration was not confirmed in this study, the advantage of constant concentration will be taken to produce

highly reproducible migration time data in a further work. The molecular micelle will be stable in a running solution containing a high concentration of an organic solvent. Since BBMA has carboxyl groups, it does not dissolve in acidic solution below pH 4. However, other high-molecular surfactants having phosphate or ammonium groups are expected to be usable in a wider pH range, and the use of such high-molecular surfactants is under investigation.

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