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Microemulsion electrokinetic chromatography: comparison with micellar electrokinetic chromatography

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ABSTRACT

The fundamental characteristics of microemulsion electrokinetic chromatography (MEEKC) were studied in comparison with micellar electrokinetic chromatography (MEKC). A microemulsion consisting of heptane–sodium dodecyl sulphate–butanol–buffer (pH 7.0) (0.81:1.66:6.61:90.92) was mainly employed. The separation selectivity of MEEKC was compared with that of MEKC with SDS micelles by using three different test mixtures. The microemulsion showed a stronger affinity to non-polar compounds than the SDS micelle. The migration-time window in MEEKC was easily extended owing to an increase in the electrophoretic mobility of the microemulsion by increasing the SDS fraction in the microemulsion. The efficiency in MEEKC was also compared with that in MEKC. The plate heights in MEEKC were higher than, but less than double, those in MEKC. The effect of microheterogeneity was not significant but the effect of sorption–desorption kinetics seemed more serious in MEEKC than in MEKC.

INTRODUCTION

Electrokinetic chromatography (EKC) [1] requires two phases, between which analytes are distributed. One phase, which is named separation carrier, must have an electrophoretic mobility to migrate at a velocity different from that of the surrounding medium; the other phase is generally an aqueous phase which is the solvent of the separation carrier. The carrier must interact with the analyte, and the aqueous phase must be electrically conductive and preferably transparent to UV radiation.

Ionic micelles are the most widely accepted carriers and the technique is named micellar EKC (MEKC) [2,3]. An advantage of MEKC is the easy availability of various surfactants, because different micelles show different selectivity. For example, bile salt micelles give significantly different selectivity from the sodium dodecyl sulphate (SDS) micelle [4,5]. Bile salts are also useful for enantiomeric separation owing to their chirality [6–8]. The other advantage of MEKC is easy modification of the aqueous phase, which is effective for selectivity manipulation.

Cyclodextrin (CD) derivatives having ionic groups and polymer ions have also been successfully employed in CDEKC [9] and ion-exchange EKC [10], respectively. CDEKC is useful for the separation of isomers of aromatic compounds and enantiomers [1,9]. A disadvantage of CDEKC is the difficulty of the preparation of CD derivatives useful for CDEKC. CD-modified MEKC (CD–MEKC), which employs a micellar solution containing a neutral CD, is easier to perform than CDEKC, because various CDs or CD derivatives are commercially available, but CD–MEKC provides a similar selectivity to CDEKC [11,12]. Ion-exchange EKC has limited applications.

Recently, Watarai [13,14] reported the use of an

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oil-in-water (o/w) microemulsion as a separation carrier in EKC. He showed that the microemulsion works similarly to the ionic micelle for the separation of neutral analytes and that the migration-time window is easily extended by changing the composition of the microemulsion. The migration-time window is defined as the possible range of the migration time of neutral analytes and is limited between the migration time of the bulk solution (t_o) and that of the micelle or of the microemulsion (t_m). A wider migration-time window leads to better resolution, although the total separation time becomes longer. Watarai [13,14] also observed no serious band broadening in spite of the larger size of the microemulsion compared with the micelle.

Microemulsions (o/w) prepared by mixing oil, water, a surfactant and a cosurfactant such as a medium alkyl-chain alcohol are transparent and thermodynamically stable if the composition is properly chosen. The structure of the o/w microemulsion is similar to that of the micelle, except that the microemulsion has a core of a minute droplet of an oil. The surfactant and the cosurfactant are located on the surface of the oil droplet to stabilize the droplet, as shown in Fig. 1.

This study was performed to evaluate the fundamental characteristics of microemulsion EKC (MEEKC) compared with MEKC, especially from the viewpoint of selectivity and efficiency. In this paper, preliminary results are outlined.

EXPERIMENTAL

EKC was performed with a P/ACE System 2000 (Beckman, Palo Alto, CA, USA) or a laboratory-built capillary electrophoresis (CE) instrument. The latter instrument was essentially the same as described previously [2] and consisted of a high-voltage d.c. power supply of a Matsusada Precision Devices HCZE30PN0.25-LDSW (Kusatsu, Shiga, Japan) or a Bertan High Voltage Series 230-30R (Hicksville, NY, USA) and a Jasco (Tokyo, Japan) UVIDEC-100-V spectrophotometric detector modified to accommodate a capillary for on-column detection. For detection, absorption was measured at 214 nm with the P/ACE System 2000 and at 210 nm with the Jasco detector. Chromatograms were recorded with a Shimadzu (Kyoto, Japan) Chromatopac C-R3A or C-R6A data processor. Plate numbers were calculated from peak area, migration time and peak height as described previously [15].

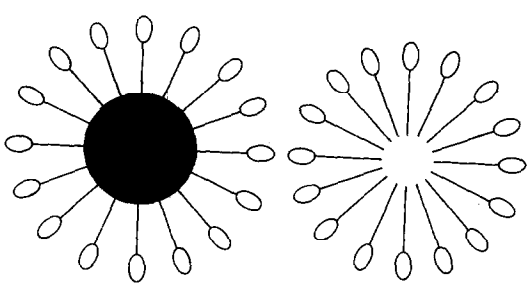

Fig. 1. Schematic illustration of the o/w microemulsion and micelle.

A fused-silica capillary of 50 μm I.D. and effective length 30 or 50 cm was used without any special wall treatment. Sample solutions were injected into an end of the capillary by the pressure injection (0.5 p.s.i., 1 s) method (P/ACE System 2000) or by the siphoning method (laboratory-built instrument). The temperature of the capillary was controlled at 35°C in the P/ACE System 2000, and the capillary was cooled with a small fan at ambient temperature in the laboratory-built instrument.

All the reagents and samples were of analytical-reagent grade. A microemulsion was prepared by mixing heptane (0.81%), SDS (3.31%), butanol (6.61%) and 100 m*M* borate–50 m*M* phosphate buffer (pH 7.0) (89.28%), according to the method reported by Watarai [13], although Watarai used water instead of the buffer solution and adjusted the pH with a carbonate–hydrogencarbonate buffer. In most instances a microemulsion consisting of a half fraction of SDS (60 m*M*) was used. SDS solutions were prepared with the same borate–phosphate buffer (pH 7.0) as were employed to prepare the microemulsion or 100 m*M* N,N-bis(2-hydroxyethyl)-2-aminoethanesulphonic acid (BES)–100 m*M* sodium hydroxide buffer (pH 7.0). The borate–phosphate buffer was prepared by mixing 25 m*M* sodium tetraborate and 50 m*M* sodium dihydrogenphosphate solutions in the appropriate ratio to give the desired pH, as described previously [2]. The BES buffer was similarly prepared from 100 m*M* BES and 100 m*M* sodium hydroxide solutions.

RESULTS AND DISCUSSION

Separation selectivity

Three examples of MEEKC separations of some test mixtures are given in Figs. 2–4 together with MEKC separations with the SDS micelle. All the sample zones migrated from the positive to the negative electrode. Both the microemulsion and the micelle had negative charge and hence migrated toward the positive electrode by electrophoresis. Therefore, the results indicate that the electroosmotic flow was stronger than the electrophoretic migration of either the microemulsion or the micelle. Although timepidium bromide was conveniently used as a tracer of the SDS micelle [16], it did not seem to be a correct tracer of the microemulsion, because phenanthrene and *p*-amylphenol migrated more slowly than timepidium bromide. Phenanthrene and *p*-amylphenol, which migrated at the same velocity, are electrically neutral, and therefore the slower velocity means that they are incorporated more into the microemulsion.

The capacity factor, k', defined as the ratio of the total moles of an analyte in the microemulsion or micelle to those in the surrounding aqueous phase, can be calculated according to [3]

$$k' = \frac{t_R - t_0}{t_0(1 - t_R/t_m)} \quad (1)$$

where t_R, t_0 and t_m are the migration times of the analyte, aqueous phase and microemulsion or micelle, respectively. The migration time of the aqueous phase was assumed to be equal to that of methanol. Timepidium bromide was used as a tracer of the micelle and phenanthrene was assumed to be a tracer of the microemulsion, as mentioned above, although the mixtures shown in Figs. 2–4 did not contain phenanthrene.

The capacity factor values are given in Tables I and II for the solutes shown in Figs. 2 and 4. It should be noted that the conditions employed to obtain the capacity factors in MEEKC were slightly different, mainly in the ambient temperature, from those in Figs. 2A–4A. Therefore, some discrepancies were observed between Fig. 2A and Table I and Fig. 4A and Table II. As can be seen in Table I, MEEKC gave substantially higher k' values than MEKC, except for toluene. The elution orders of toluene and 2-naphthol were reversed between MEEKC and MEKC, as shown in Fig. 2. The higher k' value of toluene in MEEKC suggests that the microemulsion has a stronger affinity for non-polar

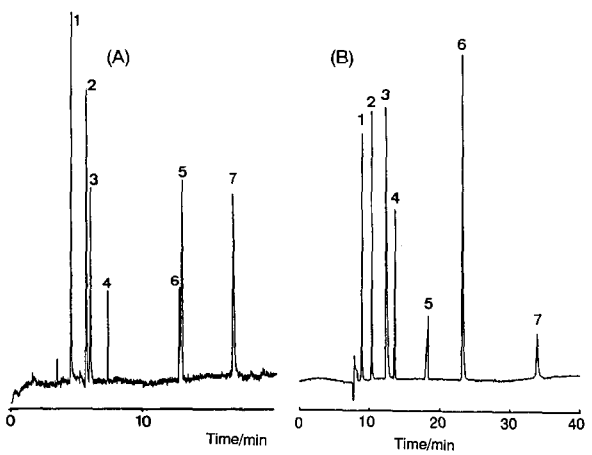

Fig. 2. (A) Microemulsion electrokinetic chromatogram and (B) micellar electrokinetic chromatogram of a test mixture. 1 = Resorcinol; 2 = phenol; 3 = *p*-nitroaniline; 4 = nitrobenzene; 5 = toluene; 6 = 2-naphthol; 7 = timepidium bromide. (A) Capillary, 50 cm × 52 μm I.D. (30 cm to the detector); separation solution, heptane–SDS–butanol–borate–phosphate buffer (pH 7.0) (0.81:1.66:6.61:90.92); applied voltage, 15 kV; current, 48 μA; temperature, ambient. (B) Capillary, 57 cm × 52 μm I.D. (50 cm to the detector); separation solution, 50 m*M* SDS in borate–phosphate buffer (pH 7.0); applied voltage, 10 kV; current, 24 μA; temperature, 35°C.

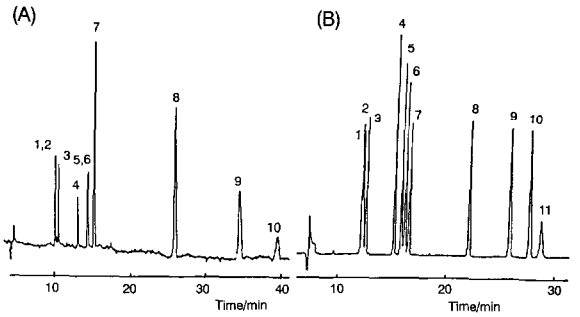

Fig. 3. Comparison between (A) MEEKC and (B) MEKC. 1 = *o*-Cresol; 2 = *m*-cresol; 3 = *p*-cresol; 4 = 2,6-xylenol; 5 = 2,3-xylenol; 6 = 3,4-xylenol; 7 = 2,4-xylenol; 8 = *p*-propylphenol; 9 = *p*-butylphenol; 10 = *p*-amylphenol; 11 = timepidium bromide. (A) Capillary, 48.2 cm × 52 μm I.D. (28.2 cm to the detector); separation solution, 79 m*M* heptane–60 m*M* SDS–874 m*M* butanol in borate–phosphate buffer (pH 7.0); applied voltage, 13 kV; current, 33 μA; temperature, ambient. (B) Current, 29 μA; other conditions as in Fig. 2B.

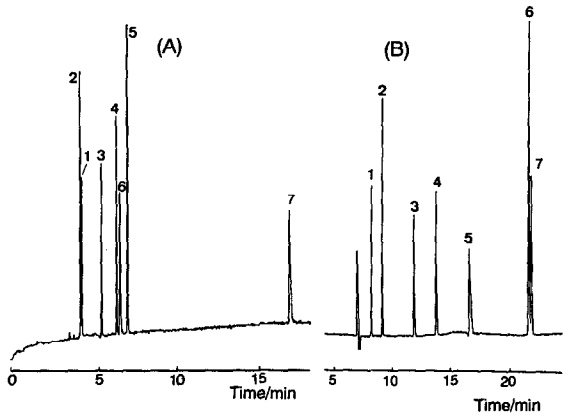

Fig. 4. Separation of cold medicine ingredients by (A) MEEKC and (B) MEKC. 1 = Acetaminophen; 2 = caffeine; 3 = guaiphenesin; 4 = ethenzamide; 5 = isopropylantipyrine; 6 = trimetoquinol; 7 = timepidium bromide. (A) Conditions as in Fig. 2A. (B) Separation solution, 50 m*M* SDS in BES buffer (pH 7.0); current, 19 μA; other conditions as in Fig. 2B.

compounds than the SDS micelle. For a strict comparison, the capacity factor or preferably the distribution coefficient at the same temperature should be employed as described below, because both the capacity factor and distribution coefficient are temperature dependent. However, the capacity factors in Tables I and II are still helpful for the purpose of qualitative comparison.

TABLE I

COMPARISON OF CAPACITY FACTORS OF TEST SAMPLES

Solute	k'	
	Microemulsion (MEEKC)[a]	Micelle (MEKC)[b]
Resorcinol	0.36	0.18
Phenol	0.76	0.44
p-Nitroaniline	0.94	0.87
Nitrobenzene	1.38	1.22
Toluene	6.19	2.89
2-Naphthol	6.19	6.10

[a] Separation solution, buffer (pH 7.0)–butanol–SDS–heptane (90.92:6.61:1.66:0.81, w/w); applied voltage, 15 kV; current, 37 μA. Other conditions as in Fig. 2.

[b] Separation solution, 50 m*M* SDS in borate–phosphate buffer (pH 7.0). Conditions as in Fig. 2.

TABLE II

COMPARISON OF CAPACITY FACTORS OF COLD MEDICINES

Solute	k'	
	Microemulsion (MEEKC)[a]	Micelle (MEKC)[b]
Acetaminophen	0.20	0.28
Caffeine	0.20	0.52
Guaiphenesin	0.62	1.52
Ethenzamide	1.00	2.59
Trimetoquinol	1.38	194
Isopropylantipyrine	1.68	5.66

[a,b] See Table I.

The capacity factor is also described by

$$k' = K(V_m/V_{aq}) \quad (2)$$

where K is the distribution coefficient and V_m and V_{aq} are the volumes of the microemulsion or the micelle and of the aqueous phase, respectively. The ratio V_m/V_{aq} is called the phase ratio. Therefore, it is more reasonable to discuss the relative affinity of the analyte between the microemulsion and micelle in terms of the distribution coefficient K rather than the capacity factor k', because K does not depend on the phase ratio. The volume of the micelle is easily calculated from the concentration of the micelle, which is equal to the difference between the surfactant concentration and the critical micelle concentration, and the partial specific volume, but the volume of the microemulsion is difficult to calculate precisely. In this study, the volume of the microemulsion is evidently larger than that of the SDS micelle by a factor of more than at least three. Therefore, the distribution coefficients of the analytes given in Table I are probably smaller for the microemulsion than for the SDS micelle.

It is apparent from Fig. 3 that selectivity is higher in MEKC than in MEEKC for isomeric cresols and xylenols. This may be due to the difference in surface structures between the microemulsion and micelle and also the core structures. The surface of the micelle will be more rigid than that of the microemulsion, because the surfactant molecules aggregate tightly to form the micelle, whereas the surfac-

tant will be on the surface of th oil droplet of heptane together with butanol in the microemulsion. The rigid surface structure will be capable of recognizing differences in the molecular structures of the isomers. On the other hand, it will be easier for the solute to penetrate into the core oil in the case of microemulsions.

The difference in selectivity between MEEKC and MEKC is also evident in Fig. 4. The capacity factors of the cold medicines are lower than those in MEKC, as given in Table II. This is also explained in terms of the polarity of the analytes. Because the core of the microemulsion is heptane and hence non-polar, the relatively polar cold medicines are not incorporated strongly by the microemulsion. In other words, the analytes do not adsorb on the surface of the microemulsion but are rather incorporated into the core of the droplet, because the capacity factors are much smaller than those of MEKC. This explanation is consistent with a lower selectivity among isomeric cresols or xylenols in MEEKC compared with MEKC, as mentioned above. Different elution orders are also observed between MEEKC and MEKC as shown in Fig. 4. The highest k' value of isopropylantipyrine is probably due to its non-polar character compared with trimetoquinol, although the latter shows a stronger affinity than isopropylantipyrine to the SDS micelle.

Migration-time window

The resolution equation in EKC is [3]

$$R_s = \frac{\sqrt{N}}{4}\left(\frac{\alpha - 1}{\alpha}\right)\left(\frac{k'_2}{1 + k'_2}\right)\left[\frac{1 - t_0/t_{mc}}{1 + (t_0/t_m)k'_1}\right] \quad (3)$$

where R_s is the resolution, N is the plate number and α is the separation factor, which is equal to k'_2/k'_1. The last term on the right-hand side originates from the contribution of the limited migration-time window between t_0 and t_{mc}. The smaller the migration time ratio, t_0/t_m, the higher is the resolution. To decrease t_0/t_m, it is necessary either to reduce the electroosmotic velocity or to increase the electrophoretic mobility of the micelle or microemulsion, as is easily predicted from the equation

$$\frac{t_0}{t_m} = \frac{v_m}{v_{eo}} = \left[1 + \frac{\mu_{ep}(m)}{\mu_{eo}}\right]E \quad (4)$$

where v_m is the migration velocity of the microemulsion or micelle, v_{eo} is the electroosmotic velocity,

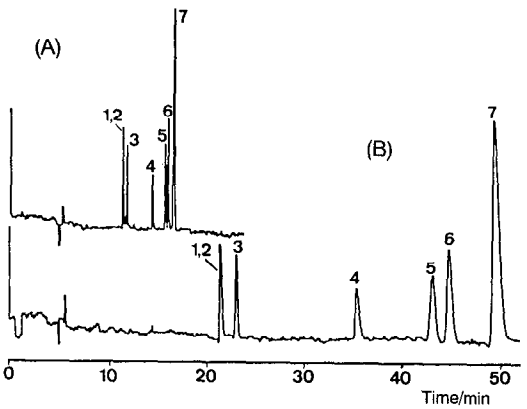

Fig. 5. Effect of the SDS fraction of the microemulsion on resolution. Peak numbers as in Fig. 3. (A) Conditions as in Fig. 3A. (B) Separation solution, 70 mM heptane–120 mM SDS–874 mM butanol in borate–phosphate buffer (pH 7.0); other conditions as in (A).

$\mu_{ep}(m)$ and μ_{eo} are electrophoretic mobilities of the microemulsion or micelle and the electroosmotic mobility, respectively, and E is the electric field strength. It should be noted that the ratio $\mu_{ep}(m)/\mu_{eo}$ is usually less than zero and greater than -1 in MEEKC and MEKC. Under the conditions of this study, $\mu_{ep}(m)$ is negative and μ_{eo} is positive. In MEKC, it is generally difficult to change $\mu_{ep}(m)$ freely over a wide range. Only when a high concentration of urea was added to micellar solutions has a decrease in t_0/t_m due to an increase in $\mu_{ep}(m)$ been reported [17]. Therefore, to extend the migration-time window in MEKC, the electroosmotic velocity must be reduced by adding an organic solvent [18,19] or by using a coated capillary [20].

Watarai [13] mentioned that t_0/t_m is easily decreased in MEEKC by increasing the volume fraction of the organic components. Fig. 5 demonstrates the effect of the SDS fraction on the migration times of analytes. The electroosmotic velocities were almost identical between the two chromatograms in Fig. 5, although they were slightly different between Figs. 3A and 5A even under the same experimental conditions. Fig. 5 clearly shows that doubling the SDS fraction increased dramatically the migration times of all the analytes or increased the electrophoretic mobility of the microemulsion without affecting the electroosmotic velocity. The enhanced resolution in Fig. 5B compared with Fig. 5A is apparent. This suggests that the migration-time

window can possibly be manipulated by changing the SDS fraction of the microemulsion without affecting the selectivity significantly. This is an obvious advantage of MEEKC over MEKC.

Band broadening

Band broadening in MEKC has been discussed in detail by Sepaniak and Cole [21] and Terabe *et al.* [15]. The total band broadening in the capillary is described as the sum of plate heights generated by five factors:

$$H_{tot} = H_l + H_m + H_{aq} + H_T + H_{ep} \quad (5)$$

where H_{tot} is overall plate height, and H_l, H_m, H_T and H_{ep} are plate heights generated by longitudinal diffusion, sorption–desorption kinetics in micellar solubilization, intermicelle mass transfer in the aqueous phase, radial temperature gradient effect on the electrophoretic velocity of the micelles and dispersion of electrophoretic mobilities of the micelles, respectively. Among these five factors, H_l, H_m and H_{ep} were found to contribute significantly to band broadening [15]. The same discussion will also be applicable to MEEKC.

The longitudinal diffusion decreases with an increase in the migration velocity or applied voltage, whereas H_m and H_{ep} increase linearly with increase in migration velocity [15]. The relative significance of each contribution depends on the capacity factor and v_{eo}: H_l is the most significant factor when both k' and v_{eo} are low; H_{ep} becomes serious when k' is large and v_{eo} is high; H_m contributes considerably when k' is medium and v_{eo} is high. The plate height H_{ep} is due to the microheterongeneity of the micellar size or, more correctly, to the difference in mobilities of the micelles [15].

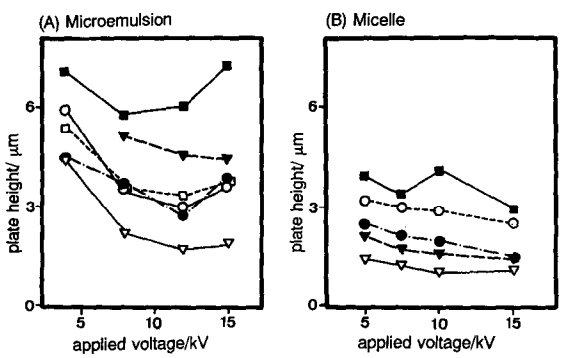

Fig. 6. Dependence of plate heights on the applied voltage in (A) MEEKC and (B) MEKC. □ = Resorcinol; ■ = phenol; ○ = *p*-nitroaniline; ● = nitrobenzene; ▼ = toluene; ▽ = 2-naphthol. (A) Conditions as in Fig. 2A except for applied voltages. (B) Conditions as in Fig. 4B except for applied voltages.

The dependence of plate height on the applied voltage is illustrated in Fig. 6 for the compounds shown in Fig. 2. The band broadening in MEKC is also shown in Fig. 6 for comparison. The most interesting point of band broadening in MEEKC is the contribution of the H_{ep} term compared with MEKC. The total plate heights in MEEKC were roughly twice those in MEKC, as shown in Fig. 6. The plate heights in MEKC shown in Fig. 6B can be explained by the major contribution of H_l. However, the results shown in Fig. 6A for MEEKC are not easily explained. In the region below 10 kV H_l was the main contributor and above 10 kV sorption–desorption kinetics seemed to be the major cause of band broadening. The microheterogeneity did not seem very significant, because 2-naphthol, which had a higher capacity factor, showed the lowest plate height among the solutes. The results in Fig. 6A are preliminary, and temperature was not controlled. To clarify the mechanism of band broadening in MEEKC, a more detailed study is necessary.

CONCLUSIONS

Although only preliminary results on MEEKC are given in this paper, MEEKC seems to be of comparable utility to MEKC. The efficiency of MEEKC is slightly lower than that of MEKC, but the migration-time window is easily manipulated to enhance the resolution. The separation selectivity of MEEKC may be affected by the character of the core oil of the microemulsion. The stability of the microemulsion and the reproducibility of migration times were not extensively studied in this work. The composition of the microemulsion containing 1.66% SDS, which was mostly used in this work, was not very stable and hence the reproducibility was probably worse than in MEKC with SDS.

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