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Microemulsion Capillary Electrophoresis

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Microemulsion capillary electrophoresis, which employed oil in water microemulsions as the electrophoretic media was demonstrated for the separation of ionic and non-ionic samples.

Capillary electrophoresis (CE) is one of the most attractive techniques in analytical separation which have been developed for the last decade. Although several modifications of the technique such as micellar electrokinetic chromatography,[1] isoelectric focusing[2] and gel electrophoresis[3] have been reported, there will be still room for new modifications.

In the present study, microemulsion capillary electrophoresis (MCE), as a new modification of CE, is proposed. Microemulsions are microheterogeneous liquids which have the structures such as oil in water, bicontinuous or water in oil dispersion system depending on the compositions. They have characteristic properties as solvent such as optical transparency, thermodynamic stability and high solubilization power. The oil in water microemulsions composed of water/sodium dodecyl sulfate(SDS)/1-butanol/heptane[4] was demonstrated here as a useful media for capillary electrophoresis of ionic and non-ionic samples. As for detection, both direct and indirect fluorescence methods were examined. The direction of the migration could be altered by changing the pH of the solution.

The microemulsions used were prepared by mixing the weighed amount of each component. Mostly studied composition here, as used in Figs. 1 and 2, was water(89.28%)/SDS(3.31%)/1-butanol(6.61%)/heptane(0.81%) by weight, which constructed the solution of negatively charged microdroplets of the organic components. SDS micellar solution of 0.1 M (1 M= 1 mol/dm^3) concentration was also studied for the comparison of the migration behavior. The pH of the solutions was controlled by 0.01 M phosphoric acid or 0.01 M carbonate-bicarbonate buffer. Sample solutions

were prepared at the concentration of 10^{-3} M in the buffer solutions.

The apparatus for the capillary electrophoresis was built with a dc-high voltage power supply (Glassman Model EG30R-1, +30--30 kV) and a fluorescence spectrophotometer (Hitachi 650-40). Fused silica capillary of 50 μm i.d. (SGE 10VS-050 ID) and 35 cm long was used. One end of the capillary was immersed, through the Teflon cap, into the 10 ml vessel containing 5 ml buffer which was placed in the cell compartment of the spectrophotometer. The cylindrical Teflon cap had a deep hollow in its side where the capillary was bared so as to be served for the on-column fluorimetric detection. Another end of the capillary was immersed in the buffer vessel placed outside of the cell compartment. Samples were injected by means of an electromigration method. The operation was carried out in the thermostated room at 25 ± 1℃ and the capillary was cooled by an additonal air-circulating system.

Figure 1 shows an example of the electrophoretic separation of some fluorescent aromatic compounds under the acidic (pH=3.0) conditions. In this case, all of the solutes including neutral and anionic ones migrated toward the anodic end, indicating that the electrophoretic migration of the anionic microemulsion droplets was superior to that of the electroosmotic flow which worked inversely on the migration of the droplets. The increasing order of the migration time, naphthalene< α- and β-naphthols< N-acetyl-α-naphthylamine, corresponds to the decreasing order of the hydrophobicity, suggesting that the solute better partitioning to the droplets migrates faster. The separation of α- and β-naphthols, which was failed in Fig. 1, was succeeded by the increase of the pH from 3.0 to 6.0, where in higher pH the electroosmotic flow was increased and the resolution was

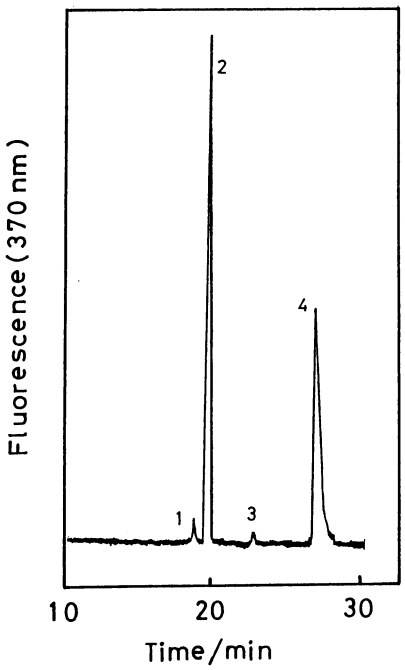

Fig. 1. Separation of aromatic compounds detected by the fluorescence of the solute ; Excitation at 290 nm, 3.0 kV, 20 μA, 10 s sampling, pH=3.0 ; 1, naphthalene, 2, α- and β-naphthols, 3, N-acetyl-α-naphthylamine, 4, 2-naphthol-6-sulfonate.

improved, although the migration time was also increased about 2.6 times. The fact that the anionic solute, 2-naphthol-6-sulfonate, migrated slower than the neutral ones may reflect the effect of the electroosmotic flow which retarded the migration of the anion dissolved in the continuous phase.

Under the alkaline (pH=9.5) conditions, the electroosmotic mobility of the solution became greater than the electrophoretic one of the droplets. Thus the migration of the solutes could be detected at the cathodic end. In Fig. 2, the separation of some ketones and β-diketones in the alkaline microemulsion is shown, which was detected as the negative peaks in the background fluorescence of naphthalene dissolved at the concentration of 10^{-3} M in the buffer solution. The naphthalene was considered completely dissolved in the microemulsion droplets. The overlap of the excitation or emission spectrum of naphthalene with the absorption spectra of the solutes lowered the background fluorescence. The migration order in Fig. 2 is thought to be governed by the hydrophobicity and acidity of the solutes and the electromobility of the anionic dissociation forms. The β-diketones containing trifluoromethyl group were completely dissociated at pH 9.5, and so the migration of them was expected to be affected by the adsorptivity on the microemulsion droplets and electrophoretic mobility of the β-diketonates, both making the migration slow. The increasing order in the capacity factor of the β-diketonates was close to that of the adsorption constants of them which were determined at the heptane/water interface,[5] not to that of the expected conductivity, hence the predominant effect of the interfacial adsorption was suggested.

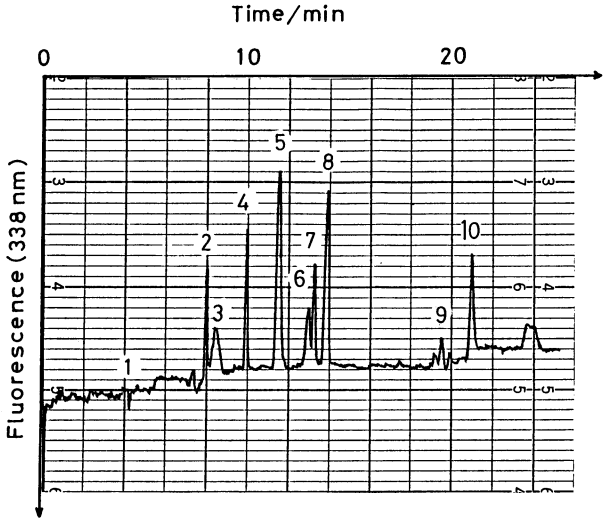

Fig. 2. Separation of ketones and β-diketones detected by the indirect fluorescence method ; Excitation at 279 nm, 10.0 kV, 40 μA, 5 s sampling, pH=9.5 ; 1, solvent peak, 2, 2-acetylthiophene, 3, acetylacetone, 4, acetophenone, 5, benzoylacetone, 6, pivaloyltrifluoroacetone, 7, 2-thenoyltrifluoroacetone, 8, benzoyltrifluoroacetone, 9, 2-naphthoyltrifluoroacetone, 10, system peak.

An advantage of MCE over the micellar electrokinetic

chromatography is the feasibility of wider range in the migration time between the solvent peak, t_0, and the system peak, t_{ME}, which are noted in Fig. 2. According to the discussion developed in the electrokinetic chromatography,[1,6] the resolutions of a pair of neutral solutes are strongly dependent on the ratio of t_0/t_{ME}, and the capacity factor has therefore the optimum value approximated by $(t_0/t_{ME})^{-1/2}$. The value of the ratio in micellar electrokinetic chromatography is at most 0.2-0.3, whereas that in MCE was readily decreased to less than 0.1 by increasing the volume fraction of the organic components. This is promising for the achievement of better resolution. Another difference between microemulsion and micellar systems is noticed in the migration behavior of anion. The migration order of the acetophenone and benzoyltrifluoroacetone in Fig. 2 was inversed in 0.1 M SDS system. This may be due to the difference in the interaction between benzoyltrifluoroacetonate and microemulsion droplets or micelles. The anion may penetrate slightly to the microemulsion droplets, but it will be repelled from the SDS micelle. The higher solubilization power of microemulsions might be another advantage affording the wider dynamic range in the sample concentration. The larger size of the microemulsion droplets than the micelle gave no serious broadening in the peaks on the electropherogram of MCE, because of the comparable time scale of aggregate formation and breakdown in both systems.[7]

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