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# Control of selectivity in micellar electrokinetic chromatography by modification of sodium dodecyl sulfate micelles with organic hydroxy compounds

Shoichi Katsuta*, Tohru Tsumura, Koichi Saitoh, Norio Teramae

*Department of Chemistry, Faculty of Science, Tohoku University, Sendai 980-77, Japan*

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**Abstract**

Modification of micellar phases with solubilized solutes was examined to control the separation in micellar electrokinetic chromatography (MEKC). The addition of organic hydroxy compounds (1-hexanol, cyclohexanol and phenol) as modifiers to a micellar solution of sodium dodecyl sulfate specifically decreased the capacity factor of some aromatic analytes possessing hydrophilic functional groups. The effect of phenol was different in selectivity to that of 1-hexanol and cyclohexanol. The effects of these modifiers were mainly explained in terms of the saturation of the micellar palisade layer with the modifiers and the hydrogen-bond interaction between the modifier and analyte molecules in the micellar phase. Such micellar modification were applied to improve the MEKC separation.

## 1. Introduction

Micellar electrokinetic chromatography (MEKC) is a useful separation method which allows excellent separations of neutral compounds utilizing capillary electrophoretic techniques [1]. The separation of neutral compounds in MEKC is performed on the basis of their differential partitioning between an electroosmotically pumped aqueous phase and a slower moving, electrophoretically retarded, ionic micellar phase. Therefore, it is difficult in principle to separate compounds whose partition coefficients between the micellar and aqueous phases are close to each other. In such a case, selective control of their partition coefficients is necessary to improve the separation.

Several ionic surfactant micelles [2,3] and mixed micelles [4,5] have been used to optimize the selectivity of MEKC separation. The types of surfactants commercially available and efficient for MEKC [1] are, however, not numerous. The use of additives such as hydrophilic organic solvents (methanol [6], dimethylformamide [7], etc.) and hydrophilic solutes (urea [8], cyclodextrin [9], etc.) in the micellar solution has also been studied. These additives, except for cyclodextrin, are mainly used to enhance the solubility of hydrophobic analytes in the aqueous phase and usually decrease the partition coefficient of the analytes unselectively.

On the other hand, our preliminary study

* Corresponding author. Present address: Department of Chemistry, Faculty of Science, Science University of Tokyo, Kagurazaka, Shinjuku-ku, Tokyo 162, Japan

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indicated that the selectivity of MEKC separation is changed considerably by the addition of a small amount of cyclohexanol to the micellar solution [10]. This effect can probably be attributed to the changes in micellar properties with solubilized cyclohexanol. This concept, "modification of micellar phases with solubilized solutes," may widen the scope and the application range of MEKC because of the diversity of the solutes to be used as modifiers.

In this study, organic hydroxy compounds, 1-hexanol, cyclohexanol and phenol, were used as modifiers for sodium dodecyl sulfate micelles, and their effects on the MEKC separation of various neutral aromatic compounds were compared. These modifiers contain the same number of carbon atoms, but the structures of their hydrocarbon parts and the acidities of their hydroxyl groups are different. Cyclohexane was also examined as a modifier for comparison. The variation of the capacity factors of the aromatic analytes was evaluated, and the features and mechanism of the effect of micellar modification are discussed.

## 2. Experimental

### 2.1. Apparatus

The apparatus used was a Jasco (Tokyo, Japan) Model CE-800 capillary electrophoresis system with a 700 mm × 0.05 mm I.D. fused-silica capillary tube. Detection was performed by on-column measurements of UV absorption at a position 500 mm from the injection end of the capillary. A Shimadzu (Kyoto, Japan) Chromatopak CR-1A data processor was used for recording chromatograms.

### 2.2. Reagents

All reagents were of analytical-reagent grade and used as received. Sodium dodecyl sulfate (SDS) was obtained from Wako (Osaka, Japan). The modifiers, 1-hexanol, cyclohexanol, phenol and cyclohexane, were purchased from Tokyo Kasei Kogyo (Tokyo, Japan). The aromatic analytes used were benzene, chlorobenzene, *o*-cresol, *p*-cresol, 2-chlorophenol, 4-chlorophenol, 2-naphthol, nitrobenzene and acetophenone, which were purchased from Wako or Tokyo Kasei Kogyo. Water was doubly distilled and further purified using a Milli-Q Labo system (Millipore, Bedford, MA, USA).

### 2.3. Procedure

The fused-silica capillary was filled with pH 6.8 buffer solution (0.010 *M* sodium phosphate–0.0020 *M* sodium tetraborate) containing 0.075 *M* SDS and 0.050–0.10 *M* additive compound. Sample solutions containing $5 \cdot 10^{-4}$–$5 \cdot 10^{-3}$ *M* aromatic analytes, 10% (v/v) methanol and $3 \cdot 10^{-5}$ *M* Sudan III or Oil Yellow OB were introduced from the positive end of the capillary by siphoning. Separations were performed by applying 20 kV at 25 ± 1°C. The detector was operated at 250 or 260 nm. Before each run, the capillary was rinsed successively with acetone (5 min), 0.1 *M* sodium hydroxide (5 min), water (5 min) and running buffer (10 min).

## 3. Results and discussion

### 3.1. Characteristics of the effect of micellar modification with organic hydroxy compounds

Fig. 1 shows examples of chromatograms in the absence and presence of 0.10 *M* 1-hexanol in the micellar solution of 0.075 *M* SDS. The presence of 1-hexanol (only 1.4%, v/v) in the micellar solution has a considerable effect on the retention order of the analytes. The partition coefficient of 1-hexanol at a low concentration was reported to be $2.25 \cdot 10^3$ on the mole fraction concentration scale [11]. Therefore, it is estimated that about 70% of 1-hexanol in the micellar solution is distributed into the micellar phase at a surfactant concentration of 0.075 *M*; the number of moles of 1-hexanol in the micellar phase is comparable to that of the surfactant and the properties of the micelles should be modified with the solubilized hexanol. Substantial changes in the selectivity of separation were also ob-

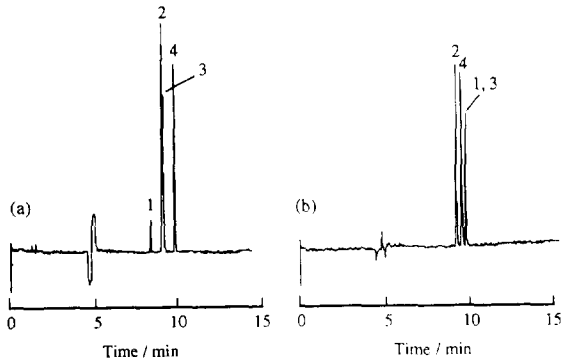


Fig. 1. Effect of the addition of 1-hexanol on the micellar electrokinetic chromatogram of aromatic compounds. Micellar solution: (a) 0.075 *M* SDS in phosphate–borate buffer (pH 6.8); (b) 0.075 *M* SDS and 0.092 *M* 1-hexanol in phosphate–borate buffer (pH 6.8). Peaks: 1 = benzene; 2 = nitrobenzene; 3 = *p*-cresol; 4 = acetophenone.

served in the presence of cyclohexanol and phenol.

In order to evaluate quantitatively the micellar modification effect on the partitioning of analytes, the capacity factor ($k'$), defined as the mole ratio of the analytes in the micellar phase to that in the aqueous phase, were calculated from the following equation [1]:

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

where $t_0$, $t_R$ and $t_{mc}$ denote the migration times of the aqueous solution, analyte and micelle, respectively. Here, $t_0$ and $t_{mc}$ were determined from the measurement of the migration time of methanol as the solute insolubilized in the micellar phase and that of Sudan III or Oil Yellow OB as the solute completely solubilized in it, respectively. Sudan III and Oil Yellow OB, which differ considerably in molecular size and structure, always gave an identical migration time, and this result supports the contention that both dyes are completely solubilized in the micellar phase.

The capacity factors of various aromatic compounds in the absence and presence of each modifier in 0.075 *M* SDS solution are summarized in Table 1. Here the modifier concentration was 0.1 *M*, except for cyclohexane, the concentration of which was 0.05 *M* because of its limited solubility. In Fig. 2, the dependence of the log $k'$ values of some analytes on the concentration of 1-hexanol, cyclohexanol and phenol in the micellar solution is shown.

The presence of 1-hexanol and cyclohexanol decreases considerably the capacity factors for acetophenone and nitrobenzene but not for *p*-cresol. In contrast, the presence of phenol, whose hydroxyl group is much more acidic than that of alcohols, decreases the capacity factor of *p*-cresol but does not change those of acetophenone and nitrobenzene. All the hydroxy modifiers, especially 1-hexanol, increase the capacity factors of analytes that possess no hydrophilic groups (benzene, toluene and chlorobenzene).

Table 1
Variation of capacity factors by the use of organic modifiers

| Solute | Log $k'$ without modifier | Increment of log $k'$ with modifier | | | |
|---|---|---|---|---|---|
| | | Cyclohexane | 1-Hexanol | Cyclohexanol | Phenol |
| Benzene | 0.22 | +0.04 | +0.14 | +0.06 | +0.01 |
| Nitrobenzene | 0.33 | 0.00 | −0.04 | −0.05 | −0.04 |
| *p*-Cresol | 0.34 | +0.01 | +0.02 | −0.01 | −0.16 |
| Acetophenone | 0.44 | 0.00 | −0.11 | −0.14 | −0.03 |
| Toluene | 0.65 | +0.04 | +0.15 | +0.06 | |
| Chlorobenzene | 0.76 | +0.04 | +0.16 | +0.05 | |
| 2-Naphthol | 1.05 | +0.01 | −0.03 | −0.05 | |

SDS concentration, 0.075 *M*; modifier concentration, 0.10 *M*, except for cyclohexane (0.050 *M*).

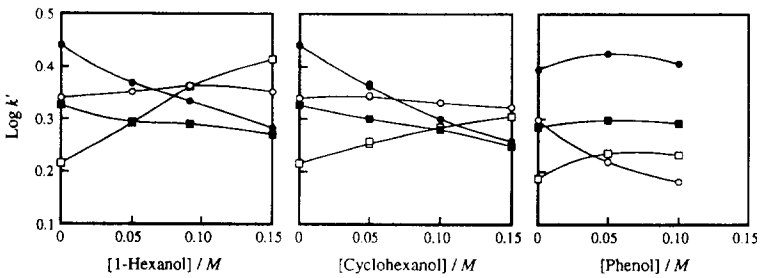


Fig. 2. Dependence of log $k'$ on the concentration of modifiers in the micellar solution (0.075 $M$ SDS, pH 6.8): □ = benzene; ■ = nitrobenzene; ○ = $p$-cresol; ● = acetophenone.

On the other hand, cyclohexane as the modifier has no effect on the capacity factors of most of the analytes, although those of benzene, toluene and chlorobenzene are slightly enhanced. It is apparent that the presence of a hydroxyl group in the modifier molecules is important.

The solubilization of long-chain alcohols generally lowers the critical micelle concentration (CMC) [11,12]. The CMC value of SDS in the present buffer without the modifiers was determined to be $4.6 \cdot 10^{-3}$ $M$ by the electrical conductivity method. Therefore, in this study where the total concentration of SDS was 0.075 $M$, the increment of the surfactant forming micelles with the decrease in CMC should be negligibly small (less than 6%). According to a study employing fluorescence quenching measurements [13], the aggregation number of SDS is decreased by the addition of alcohols but the micelle size is nearly invariant. On the other hand, recent work using dynamic light scattering [14] showed that the size of SDS micelles increases with increasing concentration of 1-octanol. Anyway, it is difficult to explain the selective effect of the modifiers in MEKC in terms of the changes in CMC, aggregation number and micelle size.

In general, solutes that possess hydrophilic functional groups are mainly solubilized in the surface palisade layer of the micelle, as shown in Fig. 3a, whereas solutes that possess no hydrophilic groups are incorporated in the hydrocarbon core of the micelle [15]. As shown in Fig. 3b, the organic hydroxy compounds as modifiers should be solubilized in the surface palisade layer, and it is probable that the saturation of the palisade layer with the modifiers causes a decrease in the partition coefficient of analytes that possess hydrophilic groups. On the other hand, if there is an attractive interaction between the modifier and analyte molecules in the micelle as shown in Fig. 3c, the partition coefficient of the analyte should be increased. In the less polar field inside of the micelle, stronger interaction via hydrogen bonding is expected between acidic (phenols) and basic (alcohols, acetophenone and nitrobenzene [16,17]) solutes than in the aqueous

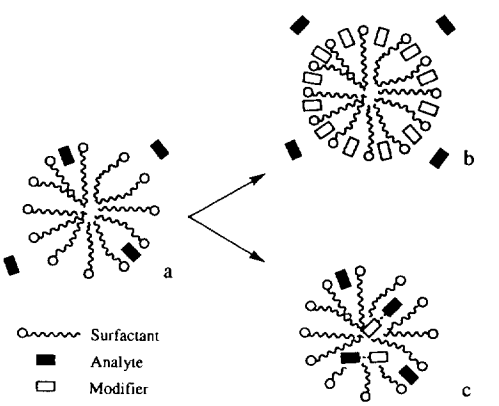


Fig. 3. Schematic illustrations explaining the effect of organic hydroxy modifiers on the capacity factors of analytes that possess hydrophilic functional groups. (a) Partitioning of the analyte between micellar palisade layer and bulk water; (b) decrease in the partition coefficient of the analyte caused by saturation of the palisade layer with the modifier; (c) increase in the partition coefficient of the analyte caused by interaction between the analyte and the modifier in the micelle.

phase. When the combination of modifier and analyte is such an acid–base pair, the effect shown in Fig. 3c should compensate for the effect in Fig. 3b, and apparently the effect of the modifers should become small. Consequently, the effects of the modifiers on the capacity factors of analytes that possess hydrophilic groups, shown in Table 1 and Fig. 2, are explained in terms of the saturation of the micellar palisade layer and the intermolecular hydrogen bonding in the micelles.

The effect of increasing the capacity factors of solutes that possess no hydrophilic groups can be attributed to the expansion of the micellar core with the hydrocarbon part of the solubilized modifiers. The greatest effect of 1-hexanol in increasing the capacity factor corresponds to the deeper permeation of its straight hydrocarbon chain into the micellar core than the cyclic chains of cyclohexanol and phenol.

### 3.2. Applicability to improvement of MEKC separation

In Fig. 4, the micellar electrokinetic chromatograms of *o*-cresol, *p*-cresol and nitrobenzene with 0.075 *M* SDS solution in the absence and presence of 0.10 *M* 1-hexanol are shown. The partition coefficients of these analytes between the micellar and aqueous phases are similar and complete separation with the pure micelles is difficult. However, in the presence of 1-hexanol, baseline separation is achieved accompanying a change in retention order. This improvement in separation is primarily due to the selective decrease in the capacity factor of nitrobenzene.

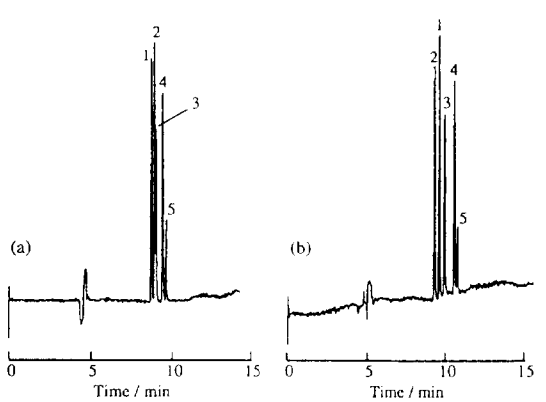


Fig. 4. Improvement in the MEKC separation of (1) *o*-cresol, (2) nitrobenzene, (3) *p*-cresol, and (4) 2-chlorophenol by the addition of 1-hexanol. Micellar solution: (a) 0.075 *M* SDS in phosphate–borate buffer (pH 6.8); (b) 0.075 *M* SDS and 0.10 *M* 1-hexanol in phosphate–borate buffer (pH 6.8).

Generally, resolution in MEKC becomes higher when the value of $t_{mc}/t_0$ and the number of theoretical plates ($N$) increase [1]. The addition of 1-hexanol, cyclohexanol and phenol did not influence the $t_0$ value but increased the $t_{mc}$ value: $t_{mc}/t_0$ was 3.63 with 0.075 *M* SDS and rose to 4.47, 4.23 and 3.99 in the presence of 0.1 *M* 1-hexanol, cyclohexanol and phenol, respectively. Further, the $N$ value was increased by the addition of the alcohols, e.g., $N$ for acetophenone rose from $1.7 \cdot 10^5$ to $2.2 \cdot 10^5$ and that for 2-naphthol from $2.6 \cdot 10^5$ to $3.2 \cdot 10^5$ when 0.10 *M* cyclohexanol was added. Although the reasons for the increases in $t_{mc}/t_0$ and $N$ are still unclear, the micellar modification with the hydroxy compounds is obviously effective for the improvement of resolution in MEKC.

## 4. Conclusions

This work clearly shows that the solubilization selectivity of SDS micelles is modified by the addition of a small amount of organic hydroxy compounds to the micellar solution. Such micellar modification with organic solutes offers a simple and useful method for controlling the MEKC separation and makes it possible to separate analytes that are hardly separated with pure micelles. In addition, this method improves the resolution by increasing the migration time of micelles and the number of theoretical plates.

When using UV adsorption detectors in MEKC, aliphatic alcohols are favourable as modifiers because they show little absorbance in the UV range. Of course, various hydrophobic compounds other than hydroxy compounds can be used, and further investigations are in progress.

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