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Separation of alkylbenzyl quaternary ammonium compounds by capillary zone electrophoresis Effect of organic solvent in sample solution

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Abstract

The important role played by the organic solvent in the sample solution in the capillary electrophoretic separation of alkylbenzyldimethyl ammonium compounds (ABDACs) is demonstrated. An organic solvent must be added to disrupt the micelles in the sample solution for effective separation of ABDACs. An organic solvent must also be added to the background electrolyte to disrupt micelles. Typically, methanol at a minimal content of 60% (v/v) or acetonitrile at 30% (v/v) is necessary for complete micelle disruption of a sample at a concentration 0.01 m*M* when using a phosphate buffer (20 m*M*) containing 30–40% (v/v) acetonitrile at pH 5.0. The effect of organic solvent in the sample solution is interpreted in terms of the absorption of organic solvents by micelles and the disruption of micelles into individual surfactant ions. The extent of disruption of micelles depends on the concentration of the sample. For complete disruption of micelles of ABDACs in a polar non-aqueous solvent, acetonitrile (40%, v/v) must be added to the phosphate buffer.

Keywords: Capillary electrophoresis; Sample solution composition; Quaternary ammonium compounds, alkylbenzyl; Surfactants

1. Introduction

Capillary electrophoresis (CE) is used to separate diverse analytical samples [1–4]. This technique provides high resolution, extremely high efficiency, rapid analysis and low consumption of solvent in comparison with high-performance liquid chromatography. However, difficulties are encountered in the determination of alkylbenzyldimethyl ammonium compounds (ABDACs) because these compounds, having long alkyl chains ($\geq C_{12}$), form micelles and because these surfactants probably adsorb on the capillary wall. CE is successfully applied to separate ABDACs [5,6]. Weiss et al. [5] reported that this analytical method required organic modifiers to disrupt the formation of micelles by surfactants with a long alkyl chain. When a high tetrahydrofuran concentration (i.e. 57.5%, v/v) was used as an organic modifier, the sensitivity was sufficient to enable separation of a mixture of ABDACs with a sample concentration of 1

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m*M*; the addition of methanol or acetonitrile instead of tetrahydrofuran did not improve peak shape [5]. Acetonitrile (30%, v/v) or tetrahydrofuran (40%, v/v) or acetone (50%, v/v) in a phosphate buffer solution was proved necessary to disrupt micelles of these surfactants and to effectively separate the ABDACs when samples with a concentration of ≤0.01 m*M* were dissolved in methanol–water (60:40, v/v) [6]. As micelles of 18-ABDAC were not completely disrupted even on addition of a large amount of methanol (e.g., 80%, v/v) to the buffer, these results clearly demonstrate that the effectiveness of the organic modifier to disrupt micelles decreased in the order acetonitrile > tetrahydrofuran > acetone > methanol. Apparently, a discrepancy exists between our results and those obtained by Weiss et al. [5]; we suspect that varied sample treatment may be primarily responsible for this discrepancy. Thus, the presence of an organic solvent in a sample solution plays an important role in the determination of ABDACs.

The effects of the organic modifier in the sample solution on the shape of the analyte signal and the resolution were reported by other researchers [7–9]. The effect of methanol on the separation of alkyl-substituted phenols was remarkable because sharp and distinct sample signals became broadened and diffuse with increasing proportions of methanol in the sample [7]. The influence of the concentration of organic solvent in a sample on the resolution was demonstrated in an analysis of drugs containing flavonoids [8]. Peak broadening or even peak splitting was observed when acetone-2,4-dinitrophenylhydrazone was dissolved in pure acetonitrile or in solvents containing a large amount of acetonitrile [9]. All those reports illustrate that the presence of an organic solvent in the sample solution affects the separation of analytes to a large extent.

In the present paper we studied the effect of the presence of an organic solvent in a sample solution on the effectiveness of the separation of alkylbenzyldimethyl ammonium compounds by capillary zone electrophoresis.

2. Experimental

2.1. Chemicals

Alkylbenzyldimethyl ammonium compounds (ABDACs) were benzyldimethyldodecyl ammonium bromide (12-ABDAB), benzyldimethyltetradecylammonium chloride dihydrate (14-ABDAC), benzyldimethylhexadecylammonium chloride monohydrate (16-ABDAC), and benzyldimethyloctadecylammonium chloride monohydrate (18-ABADC) (Aldrich). Sodium dihydrogenphosphate monohydrate and tetrahydrofuran (UV grade) were purchased from E. Merck. Methanol, acetonitrile, and acetone were HPLC grade (Fisher). Stock standard solutions (10 m*M*) of the quaternary ammonium compounds were prepared with either organic solvent or doubly deionized water. Working standard solutions were obtained by diluting the stock standard solution with either organic solvent or doubly deionized water to appropriate concentrations. Before use, all phosphate electrolyte solutions were filtered through a membrane filter (0.45-μm) and degassed. Stock sample solutions were filtered through a membrane filter (0.22-μm) before use.

2.2. Apparatus and procedures

Electrophoretic experiments were carried out on a Model 500 capillary electrophoresis instrument (Spectra-Physics, Fremont, CA, USA) fitted with a fused-silica capillary of 44 cm total length (75 μm I.D.; Polymicro Technologies, Phoenix, AZ, USA). The capillary window was made by scraping off the polyimide coating (ca. 3 mm) before mounting in the cassette; the distance from the anodic end of the capillary to the detection window was 37 cm. The UV detector was operated at 210 nm. Electrophoresis was performed at a constant applied voltage of 15 kV and the temperature was 25°C. The mode of injection was hydrodynamic and the duration was typically 2 s. A new capillary was washed with 1.0 *M* NaOH for 20 min at 60°C, followed

by 0.1 *M* NaOH for 20 min at 60°C and doubly deionized water for 30 min at 60°C, and finally with doubly deionized water for 5 min at 25°C.

Before use, the capillary was washed with 1.0 *M* NaOH for 5 min at 60°C, followed by doubly deionized water for 5 min at 60°C, and with doubly deionized water for 5 min at 25°C. Between runs, the capillary was prewashed with 0.1 *M* NaOH for 2 min and equilibrated with buffer solution for 2 min before each run. Samples dissolved in water–organic solvent mixtures with various compositions were equilibrated at least overnight before injection. Establishment of equilibrium between micelles and free surfactant ions seems to be rapid as judged from the insignificant variations in conductivity measured only a few minutes after preparation of the sample solutions. The data were collected on a microcomputer. A Model SP-701 pH meter (Suntex, Taipei, Taiwan) was used to adjust the pH of the buffer, with an accuracy ±0.01 unit. A Model SC-170 conductivity meter (Suntex) served to measure the conductivity of sample solutions, with an accuracy better than ±0.04 μS/cm.

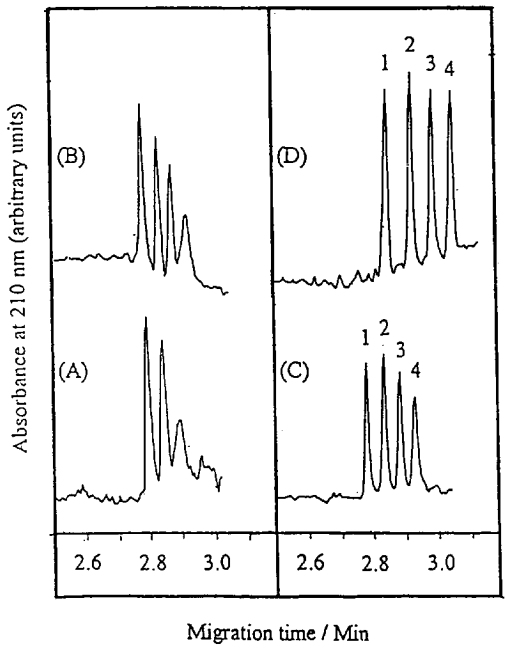

Fig. 1. Effect of methanol added in varied proportions to a sample solution on the separation of ABDACs (0.01 m*M*) with various amounts of acetonitrile [A–C, 30% (v/v); D, 40% (v/v)] in phosphate buffer: (A) 0%, (B) 40%, (C) 60%, and (D) 60%.; carrier electrolyte, NaH_2PO_4 (20 m*M*); applied voltage, 15 kV; detection wavelength, 210 nm; injection mode, hydrodynamic for 2 s. Peaks: 1 = 12-ABDAB; 2 = 14-ABDAC; 3 = 16-ABDAC; 4 = 18-ABDAC.

3. Results and discussion

3.1. Influence of organic solvent in a sample solution

As illustrated in previous work [6], effective separation of alkylbenzyldimethyl ammonium compounds (ABDACs) was achieved upon addition of 30% (v/v) acetonitrile to a phosphate buffer (20 m*M*) at pH 5.0 using a fused-silica capillary at 15 kV for a sample (0.01 m*M*) in a solution containing 60% (v/v) methanol. Methanol must be added to the sample solution to effectively separate the ABDACs.

Fig. 1 shows the effect of methanol concentration of the sample solution on the effectiveness of the separation of ABDACs with a sample concentration of 0.01 m*M* and with a phosphate buffer containing 30% (v/v) acetonitrile at pH 5.0. Without methanol in the sample solution, micelles of 12-ABDAC and 14-ABDAC are completely disrupted and micelles of 16-ABDAC are only partially disrupted, whereas little or no disruption was observed for micelles of 18-ABDAC. As the electropherogram of ABDACs dissolved in a solution containing 20% (v/v) methanol resembles that of ABDACs in a solution containing no methanol, few, if any, micelles of 16-ABDAC and 18-ABDAC are disrupted when 20% (v/v) methanol is added to the sample solution. The disruption of micelles of 16-ABDAC and 18-ABDAC increased considerably upon addition of 40% (v/v) methanol to the sample solution (Fig. 1B). On increasing the proportion of methanol in the sample solution from 40 to 60% (v/v), the degrees of disruption of micelles of 16-ABDAC and 18-ABDAC increased continually, but the disrup-

tion of micelles of 18-ABDAC reached only about 80% at most (Fig. 1C). No further disruption of micelles of 18-ABDAC was observed upon increasing the methanol content in the sample solution up to 80% (v/v), when the phosphate buffer solution containing 30% (v/v) acetonitrile was used. However, micelles of 18-ABDAC were completely disrupted when the content of acetonitrile in the separation buffer solution was increased from 30 to 40% (v/v), with a constant concentration of methanol in the sample solution (Fig. 1D). Thus, the results confirm previous findings that 60% (v/v) methanol must be added to the sample solution for an effective separation of ABDACs, apart from the necessity to add acetonitrile (30–40%, v/v) to an acetonitrile–phosphate buffer. The results also reveal that, although 60% (v/v) methanol must be added to the sample solution, acetonitrile must also be added (40%, v/v) to the phosphate buffer to completely disrupt the micelles of the ABDACs.

An organic solvent in a sample solution of surfactants acts through the combination of two processes: (1) the micelles absorb the methanol, and (2) the micelles are disrupted into individual surfactant ions when the concentration of the organic solvent in the sample solution is high. As methanol possesses only a weakly hydrophobic character due to its methyl group, it is not expected to penetrate the interior of the micelle [10]. Methanol thus becomes absorbed in the micelle palisade layer that contains ionic head groups, water, and the first methylene groups of the alkyl chain of the surfactant [11]. In the presence of methanol, the solvophobic (or hydrophobic) interactions between the hydrocarbon chains in the micelles are enhanced, resulting in a decreased charge density on the micellar surface and in an increased degree of ionization of the micelles [11]. This partial absorption of methanol stabilizes the micelles proportional to the amount of methanol in the micelles [10]. Methanol decreases the relative permittivity of the medium. As the water in the palisade layer becomes partly replaced by methanol, the decreased relative permittivity tends to increase the critical micelle concentration (CMC) and the decreased polarity increases the repulsions between the ionic head groups [11]. Hence, the micelles become less stable and surfactant ions dissociate to a certain extent to decrease the repulsions. These effects may lead to a decreased micellar aggregation number and eventually to disruption of the micelles when enough methanol is added [11,12].

The solvophobic effects and the relative permittivity of the palisade layer, operating against each other, depend on concentration. With only a little methanol added to the sample solution (or absorbed in the palisade layer), these effects are small. With increasing methanol concentration, the effects increase and the micellar composition substantially changes, resulting in an increased CMC and a decreased aggregation number. The micelles may even become completely disrupted at a still higher concentration of methanol.

As no absorption was detected in the UV range for ABDACs in their micellar form but sharp and distinct absorption characteristics were detected for ABDACs in their monomeric form, the degree of disruption of micelles of a particular ABDAC species was estimated on the basis of the ratio of the peak area for a particular species to its maximum peak area obtained on complete disruption of the micelles. For instance, an estimate of the degree of disruption of micelles of 18-ABDAC is based on the ratio of the peak area due to 18-ABDAC to its maximum peak area, which is equivalent to the ratio of peak areas of 18-ABDAC and 12-ABDAC. The maximum peak area due to 18-ABDAC measured under conditions of completely disrupted micelles is assumed equal to that of 12-ABDAC because the molar absorptivity of 18-ABDAC is similar to that of 12-ABDAC. Hence, we make two assumptions: (1) that micelles of 12-ABDAC are completely disrupted under the operating conditions used, and (2) that the absorption of a particular monomeric species at the detection wavelength has no contribution from the micellar species.

The influence of acetonitrile in the sample

solution on the separation of ABDACs is shown in Fig. 2. Micelles of 16-ABDAC and 18-ABDAC were only partially disrupted on addition of acetonitrile (20%, v/v) to the sample solution when a phosphate buffer solution containing 30% (v/v) acetonitrile was used (Fig. 2A). As acetonitrile disrupts micelles better than methanol [6], less acetonitrile in the sample solution is required to disrupt micelles effectively. Micelles of 16-ABDAC and 18-ABDAC are nearly completely disrupted on addition of 30% (v/v) acetonitrile to the sample solution (Fig. 2B), and micelles of all ABDACs are completely disrupted on addition of 40% (v/v) acetonitrile to the sample solution. Hence, the results further support previous findings obtained for methanolic solutions.

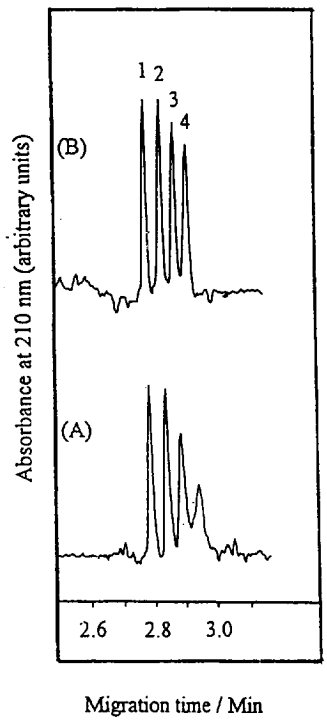

Fig. 2. Effect of acetonitrile concentration in the sample solution on the separation of ABDACs (0.01 m*M*) with acetonitrile (30%, v/v) in the phosphate buffer: (A) 20%; (B) 30%. Peak numbering and other electrophoretic conditions as for Fig. 1.

3.2. Disruption of micelles in a polar non-aqueous solvent

Solvophobic interactions of the hydrocarbon tails of surfactants are assumed to be the driving force for aggregation in polar non-aqueous solvents; thus the micelles are considered to have a "normal" structure, but with a smaller aggregation number that depends on the concentrations of the surfactant and the organic modifier [12,13]. Data on the proper aggregation model and on solvent effects on the stability of micelles are unavailable or few [12]. As aggregation of dodecyltrimethylammonium bromide does not occur in methanol [10], it is of interest to study how effectively micelles of ABDACs, especially 18-ABDAC, are disrupted in a polar non-aqueous solvent, such as methanol or acetonitrile.

To investigate the aggregation of these surfactants, we determined the CMC of 18-ABDAC in pure methanol or acetonitrile and in aqueous solutions containing methanol or acetonitrile. The CMC determined from conductivity measurements for 18-ABDAC in pure methanol and in 60% (v/v) methanol solution are $1.5 \cdot 10^{-5}$ *M* and $1.3 \cdot 10^{-5}$ *M*, respectively, i.e. about twice as large as the CMC in water. The CMCs of 18-ABDAC in pure acetonitrile and in 30% (v/v) acetonitrile solution are ca. $(1.5 \pm 0.2) \cdot 10^{-5}$ *M*. The CMCs in pure organic solvents and in 30% (v/v) acetonitrile are determined less precisely because the variation in conductivity of 18-ABDAC is gradual.

Fig. 3 presents electropherograms of ABDACs dissolved in pure methanol and acetonitrile with a separation buffer containing acetonitrile (30%, v/v). Comparison of the electropherograms in Fig. 3A and Fig. 1C indicates that micelles of ABDACs in pure methanol seem less effectively disrupted than micelles in aqueous methanol (60%, v/v) solution. Micelles of 18-ABDAC in pure methanol are only disrupted for ca. 50%. As the CMC of 18-ABDAC measured in pure methanol is about the same as that measured in aqueous methanol (60%, v/v), we suspect that micelles of 18-ABDAC are slightly more stable

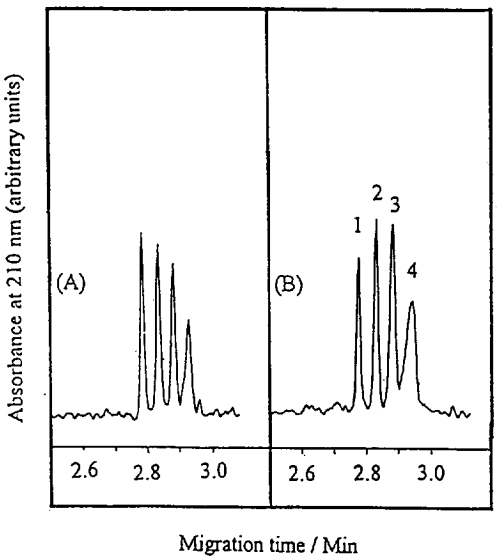

Fig. 3. Electropherograms of ABDACs (0.01 m*M*) dissolved in pure organic solvent with acetonitrile (30%, v/v) added to the phosphate buffer: (A) methanol; (B) acetonitrile. Peak numbering and other electrophoretic conditions as for Fig. 1.

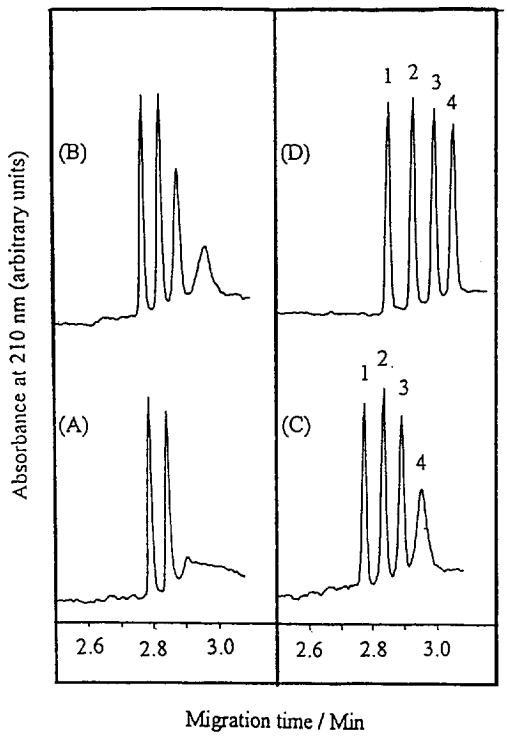

Fig. 4. Effect of methanol added to a sample solution on the separation of ABDACs (0.05 m*M*) with various amounts of acetonitrile [A–C, 30% (v/v); D, 40% (v/v)] in phosphate buffer at the proportions: (A) 0%, (B) 40%, (C) 80%, and (D) 60%. Peak numbering and other electrophoretic conditions as for Fig. 1.

in pure methanol than in 60% (v/v) methanol because of increased solvophobic interactions.

A similar phenomenon was observed for ABDACs dissolved in pure acetonitrile which has a strongly dipolar character. Comparison of the electropherograms in Fig. 3B and Fig. 2B indicates that micelles of 18-ABDAC are less effectively disrupted in pure acetonitrile than in the sample solution containing 30% (v/v) acetonitrile. These results can be rationalized according to an argument similar to that for pure methanol. For completely disrupted micelles of 18-ABDAC in pure acetonitrile, acetonitrile at 40% (v/v) in the electrolyte solution is sufficient.

Peak broadening was observed for surfactant ions when micelles were disrupted incompletely (Fig. 3B and Fig. 4A–C). Perhaps this phenomenon resulted from the presence of surfactant ions with a slightly varied aggregation number.

3.3. *Effect of sample concentration*

As demonstrated previously [6], the effect of sample concentration on the effectiveness of separation must be taken into consideration. The disruption of micelles is less complete at a sample concentration of 0.05 m*M* than at 0.01 m*M*. Hence, the larger the concentration of the sample, the larger the concentration of organic modifier necessary to completely disrupt the micelles. Figs. 4A–C present electropherograms obtained for samples at 0.05 m*M* with various proportions of methanol in the sample solution. In the absence of methanol, only the micelles of 12-ABDAC and 14-ABDAC are completely disrupted, whereas micelles of 16-ABDAC are slightly disrupted (Fig. 4A). With increasing proportions of methanol, up to 80% (v/v), the disruption of micelles of 18-ABDAC is only about 75% complete with a phosphate buffer containing 30% (v/v) acetonitrile as organic modifier (Fig. 4C). However, micelles were completely disrupted in this sample dissolved in

60% (v/v) methanol with a phosphate buffer containing 40% (v/v) acetonitrile.

Conversely, micelles of ABDACs were completely disrupted in samples dissolved in 60% (v/v) methanol or 30% (v/v) acetonitrile at 0.0025 m*M* when 30% (v/v) acetonitrile was added to the phosphate buffer. All micelles of ABDACs were completely disrupted in pure acetonitrile, and nearly completely disrupted in pure methanol when 30% (v/v) acetonitrile was added to the phosphate buffer. Again, micelles of ABDACs in a sample (0.0025 m*M*) were completely disrupted in pure methanol when 40% (v/v) acetonitrile was used in the phosphate buffer. All these results indicate that the concentration of the sample affects the extent of disruption of the micelles.

4. Conclusion

The results of the present investigation clearly demonstrate that addition of an organic solvent to a sample solution markedly affects the capillary zone electrophoretic separation of alkylbenzyldimethyl ammonium compounds with a long alkyl group. For effective separation, micelles in the sample solution must be disrupted by means of an organic solvent at an appropriate concentration, while at the same time an organic modifier is needed to disrupt the micelles in the background electrolyte solution. The concentration of the sample also affects the extent of disruption of the micelles.

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