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Sample matrix effects in micellar electrokinetic capillary electrophoresis

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Abstract

Several factors related to sample matrix which can influence peak height in micellar electrokinetic capillary chromatography were studied. The ionic strength of the sample did not affect greatly the peak height. High concentration of surfactants or organic solvents in the sample decreased the peak height. On the other hand, using a surfactant in the sample different from the one in the electrophoresis buffer or the addition of polyethylene glycol to the sample enhanced slightly the peak height. A high surfactant concentration in the buffer increased the migration time as well as the plate number and the peak height. Matrix effects are more profound with large than with small sample injections. In general, the effect of sample matrix in MECC is much less than that observed in capillary zone electrophoresis. It is recommended to prepare the standards in the same matrix as that of the sample or to add the analytes directly to the sample to avoid any bias in the results.

1. Introduction

Samples from biological and industrial sources often have a complex matrix such as high proteins or salts which can play an important role in the separation in capillary zone electrophoresis CZE [1]. Sample matrix has practical implications on both resolution and quantitation. It can improve or worsen the separation depending on the CE conditions selected [1]. Also, it affects the accuracy of the quantification if the sample and the standard do not share a similar composition [1,2]. In general, in CZE, high salts and proteins present in biological samples deteriorate the separation [3,4], while a low ionic strength in the sample improves the separation through stacking effects [5,6].

Micellar electrokinetic capillary chromatography (MECC) is a very powerful tool for separating neutral and hydrophobic molecules [7,8]. Hydrophobic compounds are not very soluble in water. They necessitate the addition of organic solvents to the sample for solubilization which can interfere later on with the separation. The surfactants in MECC have the added advantage of solubilizing proteins which can eliminate sample extraction or deproteinization in many analyses [9]. Little information is known about sample matrix effects in MECC. Here we investigate several aspects of sample preparation including the addition of organic solvents which can influence peak height in MECC.

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2. Experimental

2.1. Equipment

A Model 2000 capillary electrophoresis instrument (Beckman Instruments, Fullerton, CA, USA) was set at 214 nm and 35°C. The capillary was 500 mm × 50 μm I.D., and the running buffer was boric acid 100 mmol/l adjusted to pH 8.4 ± 0.1 with 2 mmol/l NaOH and contained 55.4 mmol/l sodium dodecyl sulfate (SDS). The SDS was dissolved in the buffer by sonication for 5 min. Between runs, the capillary was washed for 1 min with phosphoric acid 100 mmol/l and for 1 min with the running buffer. The containers were filled daily with fresh buffer and the capillary was washed for 3 min with each of the following: NaOH (2 mol/l), phosphoric acid (100 mmol/l) and the running buffer. The instrument was set at a fixed current of 38 μA. The voltage reading was approximately 16 kV. The sample was introduced by pressure injection for 20 s (about 3% of the capillary volume) or as specified. The capillary, under these conditions, is overloaded with sample in order to study the factors which can improve the peak height and separation.

2.2. Chemicals

Polyethylene glycol 8000 (PEG) was purchased from Fisher Scientific (Fairlawn, NJ, USA). Acetaminophen and SDS were obtained from Sigma Chemicals (St. Louis, MO, USA). Caffeine and deoxycholic acid sodium salt were obtained from Matheson Coleman and Bell (Norwood, OH, USA). Octyl sodium sulfate and acetoacetanilide were bought from Eastman Kodak (Rochester, NY, USA). Felbamate (2-phenyl-1,3-propanediol dicarbamate) was obtained from Carter-Wallace (Cranbury, NJ, USA).

2.3. Standard

A stock solution of 250 mg/l of felbamate was prepared in 10% methanol in water. Acetoacetanilide (200 mg/l) was dissolved in 2% methanol, while caffeine and acetaminophen (100 mg/l) were dissolved directly in water. The stock standard solutions were diluted five-fold in water or in the different buffers as specified later.

3. Results and discussion

Initially, we noticed a difference in the peak height and shape between serum and water samples spiked with the new antiepileptic drug felbamate using MECC, especially with large injection volumes (Fig. 1) [9]. This difference can lead to erroneous calculations based on peak heights which prompted us to investigate here a few variables in the sample which might influence peak height. Felbamate and acetoacetanilide are used as examples of non-water-soluble, and acetaminophen and caffeine as examples of water-soluble compounds. Peak height is used rather than area because it reflects better here the changes in plate number and consequently the resolution.

3.1. Sample size

Since the light path is very limited in CE, a large sample volume would be desirable in order to increase the sensitivity of detection. However, in practice peak height does not increase appreciably with increase in sample size. Plate number and the separation in general deteriorate rapidly with increase in sample size due to the increase in band broadening or sample overloading [1].

In CZE, the sample volume which can be injected into the capillary without overloading is dependent on the running buffer concentration, or more accurately, on the ratio of the running buffer to the sample buffer [2,3]. A high ratio allows a larger sample volume to be loaded and consequently a higher peak height to be obtained. This effect is related to the stacking force [10]. As a rule of thumb, the sample length should be less than 0.5% of the capillary length.

Since in MECC, the separation, in general, is dependent on the partition between the pseudo-

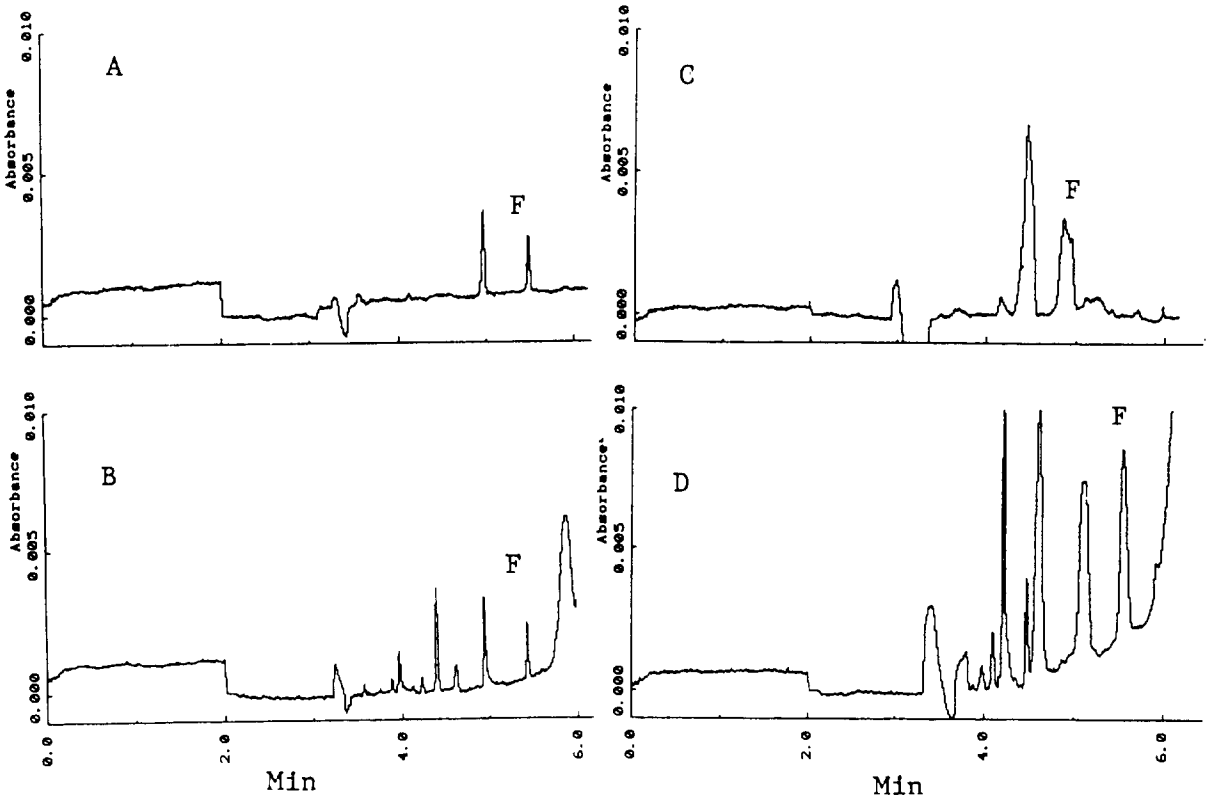

Fig. 1. Felbamate F (50 mg/l) diluted: (A) in water, 3-s injection; (B) in serum, 3-s injection; (C) in water, 20-s injection; (D) in serum, 20-s injection.

phase of the micelles and the aqueous buffer or the capacity factor (k'), the ionic strength of the running buffer has little or no effect on sample size or loading for felbamate (Fig. 2a). Increasing the sample size did not cause proportional increase in peak height, especially at low buffer or SDS concentration. On the other hand, a high SDS concentration in the running buffer caused an increase in peak height (Fig. 2b). The increase in peak height reflects an increase in plate number (N) as can be seen in Fig. 3. The N for felbamate increased from ca. 12 000, to 35 000, to 96 000 as the SDS concentration is increased from 0.8, 1.6 to 3.2%, respectively. In addition to an increase in N, the migration time increased and the separation improved when SDS concentration increased (Fig. 3). According to Terabe et al. [11], the plate height H due to longitudinal diffusion (at $t_o/t_{mc} > 0.1$) =

$$[2(D_a + k'D_m)/(1 + t_o)/t_{mc}]1/v_{eo}$$

where D_a is diffusion coefficient of the aqueous, D_m is the diffusion coefficient of the micelle, t_{mc} is migration time of the micelle, t_o is the migration time of the bulk solution, and v is the EOF velocity.

The increase in SDS leads to an increase in k' (Fig. 3) and a decrease in the diffusion coefficient. Consequently a smaller H is achieved [12].

SDS concentration does not affect the peak height of felbamate only. The peak heights of all the compounds are improved by the increase in SDS concentration (Fig. 3), especially when the sample volume is relatively large (Fig. 2b). However, at the same time, a high SDS may also produce undesirable effects on excessive current generation, long migration time (Fig. 3) or on the optimum resolution. From a practical point

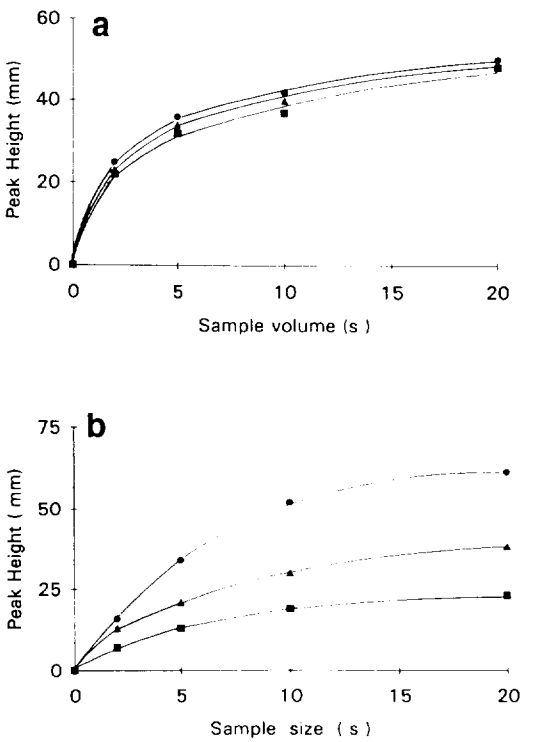

Fig. 2. Effect of sample size on felbamate peak height as related to: (a) the running buffer concentration in mmol/l (■ = 50 m*M*, ▲ = 100 m*M*, ● = 200 m*M*), and (b) SDS percentage in the buffer (■ = 0.8%, ▲ = 1.6%, ● = 3.2%).

of view, SDS concentration in the buffer has to be optimized for sensitivity (peak height) as well as resolution.

The subsequent studies were performed with large sample volumes with 10 or 20 s injection (as specified), i.e. overloading, in order to investigate the conditions which can enhance the peak height.

3.2. Sample pH

Since felbamate is a neutral compound, the pH of the sample should not affect its migration in the sample zone (or its stacking properties) and consequently it should not affect peak height. Using a phosphate buffer of 50 mmol/l for the sample the pH between 6.1 and 11.3 did not affect the peak height (results are not shown).

3.3. Sample ionic strength

A high ionic strength in the sample in CZE decreases the plate number and deteriorates the separation [1,5]. Here, in MECC, the buffer concentration in the sample (5–200 mmol/l) did not have appreciable effect on peak height (results are not shown). There was a slight increase in peak height (ca. 20%) when NaCl was added at a concentration of about 0.2%–0.5%. Probably the NaCl causes a slight decrease in solubility of the felbamate in the aqueous phase.

3.4. Organic solvents

MECC is often employed to separate non-polar compounds which are non-water-soluble. Thus, these compounds have to be prepared or solubilized in organic solvents. Unfortunately, the addition of organic solvent to the sample decreases peak height as well as the resolution (Fig. 4). The effect is more pronounced with large sample injections (Fig. 5). This effect is probably due to altering the distribution of the analyte between the micelle and aqueous phase. It can be seen in Fig. 5D that the absorbance at t_0 is much higher when methanol is added to the sample indicating that a large portion of the analytes remained in the aqueous phase unsolubilized by the micelle. It is interesting that water-miscible organic solvents such as methanol and acetonitrile do not degrade the separation in CZE. In fact they can improve the separation by a special stacking mechanism [1,4]. Thus, from a practical point, the minimum amount of organic solvent in the sample should be used in MECC.

3.5. The effect of surfactant in the sample

In general, a high concentration in the sample of the same surfactant used in the electrophoresis buffer decreases the peak height (Fig. 6). However, a surfactant in the sample different from that in the running buffer is preferred (Figs. 6 and 7). The peaks with higher k' are more affected by the surfactants in the buffer (Fig. 7). It is difficult to explain the effects of the

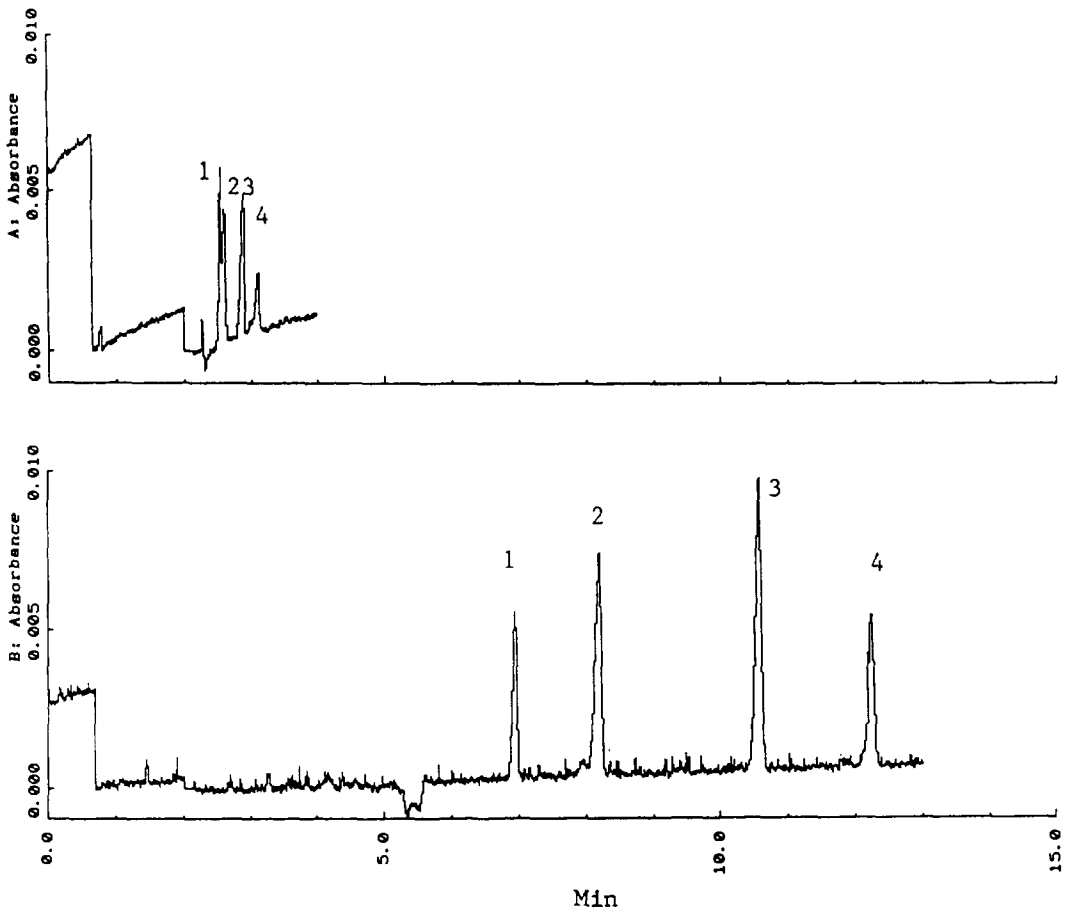

Fig. 3. Effect of SDS concentration on peak height, migration time and separation: SDS 0.8% (top) and SDS 3.2% (bottom); 1 = acetaminophen; 2 = caffeine; 3 = acetoacetanilide; 4 = felbamate. The sample was injected for 10 s.

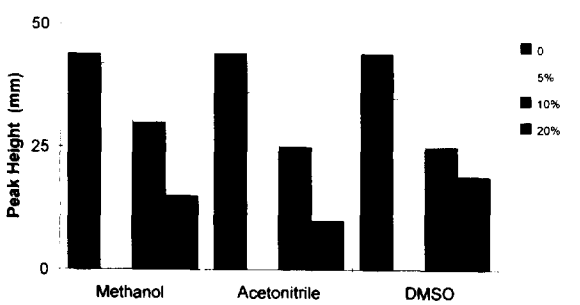

Fig. 4. Effect of different organic solvents in the sample on felbamate peak height. The stock standard was diluted five-fold either in water (0%), methanol, acetonitrile, or dimethylsulfoxide (DMSO) at the specified concentrations.

different surfactants. It seems that some surfactants tend to exert less band spread on some analytes in the sample.

3.6. *Effect of proteins*

Specific serum proteins such as albumin (20 mg/ml) increased the peak height slightly (ca. 25%) compared to water. However, when both albumin (20 mg) and sodium chloride (5 mg/ml) are present in the sample the peak height increases by ca. 50%. γ-Globulins did not have any effect.

Surfactants solubilize proteins and thus, in MECC the sample does not require deproteinization or extraction provided the proteins do not

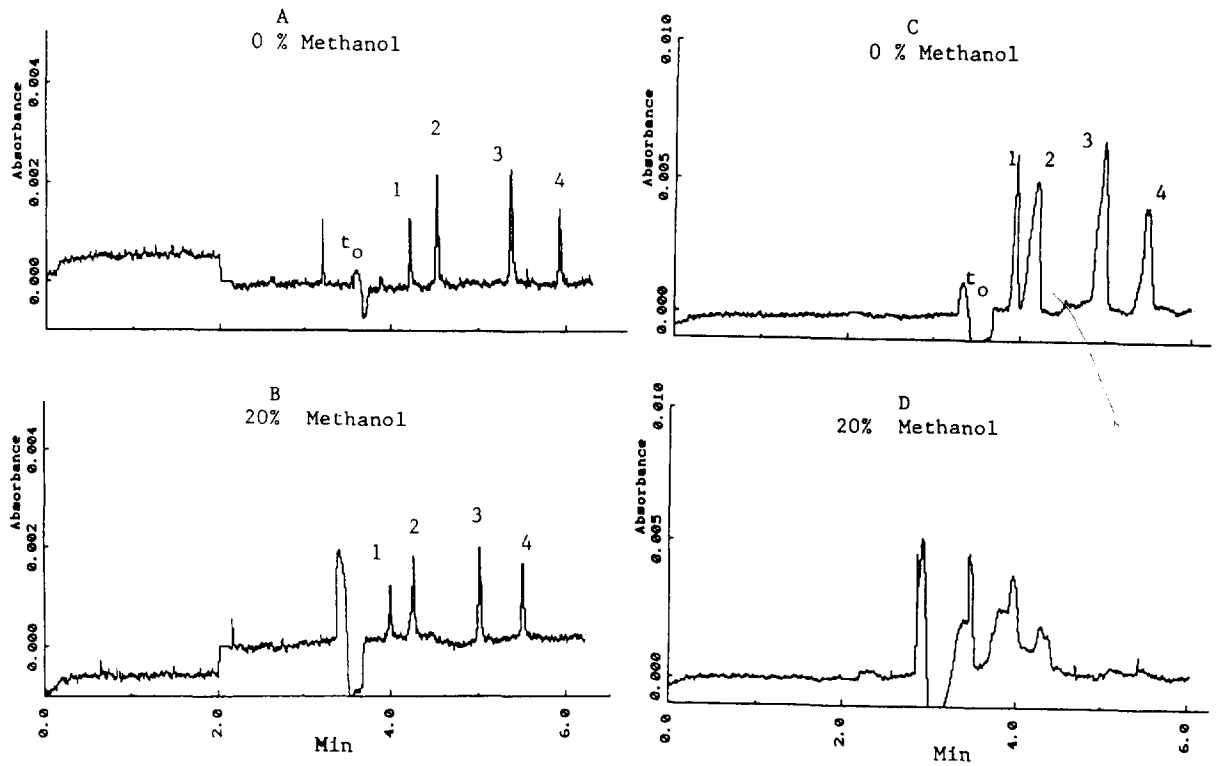

Fig. 5. Effect of addition of 20% methanol on the peak height and separation using 2- and 20-s injections: (A) injection for 2 s in absence of methanol, (B) 2 s with methanol. (C) 20 s in absence of methanol, and (D) 20 s in presence of methanol. Compounds as in Fig. 3.

co-migrate with the analyte of interest. In CZE proteins affect the reproducibility of the assay more than the separation [1]. On the other hand, in MECC, proteins have little effect on reproducibility because they are solubilized by the surfactants.

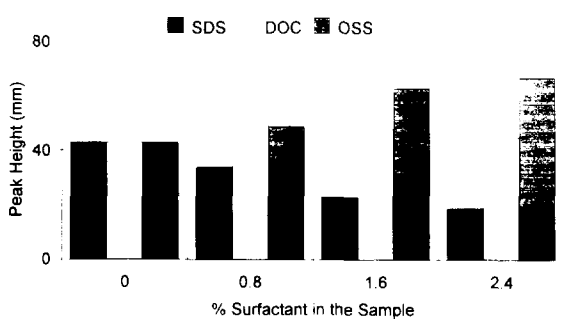

Fig. 6. Effect of different concentrations of SDS, sodium deoxycholate (DOC) and octyl sodium sulfate (OSS) in the sample on the peak height of felbamate.

3.7. Polyethylene glycol

The addition of PEG in the sample, which can also decrease the solubility of many small molecules, increased the peak height slightly (Fig. 8). The effect of PEG is more evident when the sample size is large, especially for felbamate and acetoacetanilide, both non-water-soluble compounds (Fig. 9), while the peak height for acetaminophen, a water-soluble compound with low k', deteriorated with addition of PEG. It is difficult to explain these effects without further experiments; however, it seems that PEG increases the absorbance at t_o similar to that for the organic solvents in the sample.

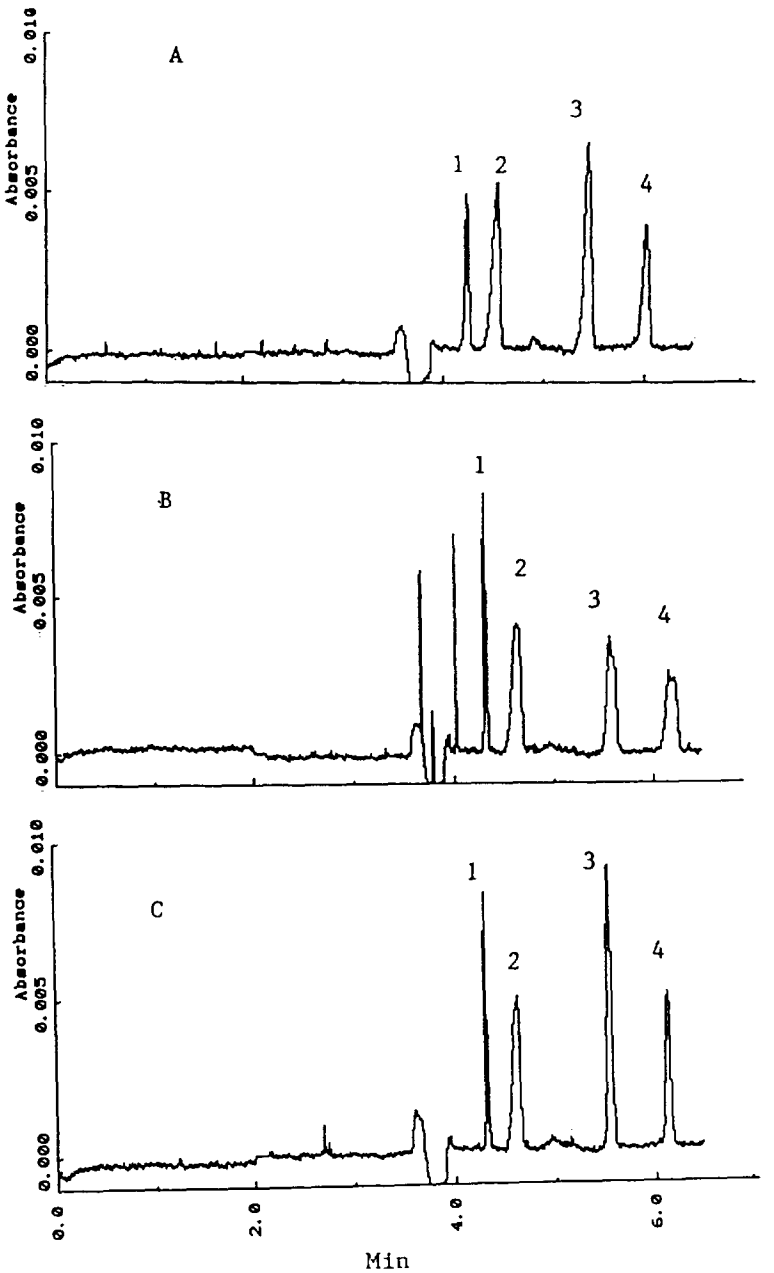

Fig. 7. Effect of addition of surfactants in the sample on peak height. Sample injection 10 s: (A) no surfactant, (B) SDS 0.8%, and (C) octyl sodium sulfate 1.6%.

4. Conclusions

Whole serum contains a mixture of proteins, salts, and buffers which all affect the peak height of the analytes (Fig. 1) as discussed earlier. It seems that sample matrix in MECC as in CZE affects both resolution and quantification. The effect of sample matrix in MECC is less dramatic

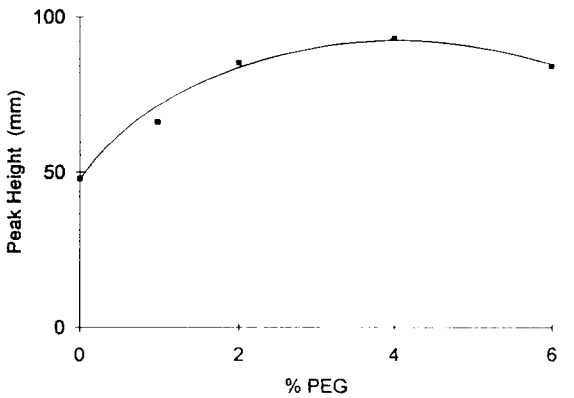

Fig. 8. Effect of the addition of PEG at different concentrations to the sample on peak height for felbamate.

than that in CZE. For example, peak height in CZE can be increased 5- to 10-fold by manipulating the sample [1], while in MECC only 20–100% increase is obtained. These effects, on the one hand, can be beneficial in terms of sample matrix and can be more tolerated in MECC; on the other hand, this effect can not be used to enhance the peak height too much as in CZE. However, erroneous calculations or poor separation can occur if the standard and the sample do not share the same matrix. The effects of sample matrix in MECC are more obvious at large rather than small volumes. To avoid any bias in the analysis, it is recommended to dissolve the standard in the same matrix as the sample, or to add the standard directly to the sample. These studies should be helpful in the analysis of neutral molecules by MECC.

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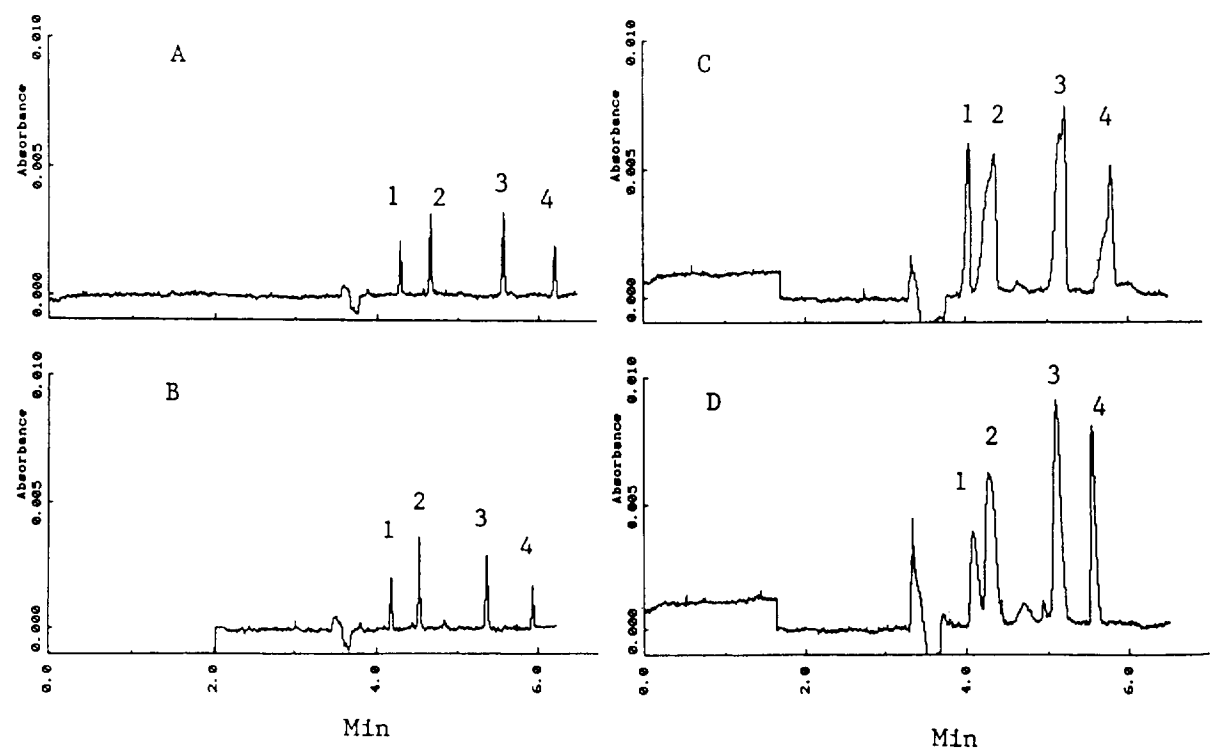

Fig. 9. Effect of the addition of 3% PEG to the sample on peak height: (A) injection for 2 s in absence of PEG, (B) 2 s with PEG, (C) 20 s in absence of PEG, and (D) 20 s in presence of PEG. Compounds as in Fig. 3.

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