Modified electroosmotic flow by cationic surfactant additives in capillary electrophoresis
Evaluation of electrolyte systems for anion analysis

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Abstract

A detailed experimental study of the variables affecting flow reversal was conducted in electrolyte systems commonly used for the determination of small anions. The electroosmotic flow mobilities of chromate (5 mM, pH 8) and 3,5-dinitrobenzoate solutions (concentration range 1.25–10 mM and pH 4–9) were determined by monitoring the migration time of pure water with indirect UV detection at 254 nm. The addition of the cationic surfactants cetyltrimethylammonium bromide, tetradecyltrimethylammonium bromide and lauryltrimethylammonium bromide, computed as fractions of the standard critical micelle concentration (std CMC), was investigated. The results suggested that flow reversal follows a single transition adsorption mechanism, in which the capillary inner surface is occupied by a layer of hemi-micelles, i.e. two dimensional aggregates formed by lateral interaction of surfactant tails. Once flow reversal is achieved, with surfactant concentration approximately above 10% std CMC, the nature of the electrolyte anion as well as the pH of the solution does not exert any significant effect on the magnitude of the electroosmotic flow. However, at this condition, the electroosmotic flow mobility varies linearly with the logarithm of the anion concentration.

Keywords: Electroosmotic flow; Buffer composition; Surfactants; Anions

1. Introduction

The practice of electrophoresis in fused-silica capillaries is associated with a distinct phenomenon known as electroosmosis, the flow of solution induced by an electric field [1]. The electroosmotic flow (EOF) has a significant impact in the design of electrophoretic separations, which is supported by the large volume of publications in the past years concerning flow description and control [2]. Perhaps, the most interesting aspect of flow manipulation is the dynamic deactivation of the capillary inner surface by adsorption of cationic surfactants [2,3].

The proper choice of the surfactant characteristics and concentration allows a direct control of the flow magnitude, including flow reversal.

The reversed electroosmotic flow is used advantageously in the electrophoretic separation of small inorganic and organic anions [3]. Due to the fact that the anion mobility is usually quite large when compared to the electroosmotic mobility, under normal flow conditions, small anions migrate contrarily to the flow and therefore, their motion towards the detector is retarded. Even when the flow is suppressed, with the use of permanent surface-coated capillaries, anion analysis is unfavorably long and potentially more costly and troublesome. The reversion of the capillary surface charge allows the anions to migrate in the same direction of the electroosmotic

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flow, which imparts a substantial shortening of the analysis time and consequent gain in resolution.

Despite the interest that normal electroosmotic flow has conveyed in the last years, our knowledge of the physicochemical aspects of flow reversal has been restricted to a few reports. The study of cationic surfactant additives recalls the pioneering work of Fuerstenau et al., who first postulated a concentration-dependent surface adsorption of hemi-micelles [4,5]. With the implementation and initial developments of the capillary electrophoresis for the analysis of small anions [6–9], the use of alkyl ammonium salts soon became widespread [10–20]. However, very few articles attempted to offer a critical explanation of the mechanism by which flow reversal is achieved. In the literature to date, many studies are tied to specific conditions [10–13], others examine few variables at a time [14–16]. Another particularly frustrating aspect of the literature is the number of application articles employing proprietary additives of unknown identity [3,17,18]. It was only recently, in the work of Kaneta et al. [21] and Lucy and Underhill [22], that the role of the surfactant in the development of reversed flow was rationalized. Especially in this last article [22], a clear description of the mechanism of adsorption and the morphology of the resultant hemi-micelle is presented. Furthermore, the variables influencing the bilayer assembly at the capillary surface are investigated.

The present work examines the premises of the hemi-micelle adsorption model for electrolytes commonly employed in the analysis of small anions. The electroosmotic flow mobilities of the electrolytes chromate and 3,5-dinitrobenzoate are characterized in the presence of the cationic surfactants cetyltrimethylammonium bromide (CTAB), tetradecyltrimethylammonium bromide (TTAB) and lauryltrimethylammonium bromide (LTAB). The reversal of the electroosmotic flow is investigated as a function of surfactant chain length and concentration, as well as electrolyte type, concentration and pH.

2. Experimental

2.1. Instrumentation

The experiments were conducted in a capillary electrophoresis system (model 270A-HT, Perkin-Elmer, Applied Biosystems Division, Foster City, CA, USA) equipped with a variable UV–Vis detector, set at 254 nm, a temperature control device maintained at 30 °C and a data acquisition station (TURBOCHROM software). A fused-silica capillary (Polymicro Technologies, Phoenix, AZ, USA) with dimensions 70 cm total length×75 µm I.D., 365 µm O.D. was installed. A detection window of approximately 0.2 cm was created at 50 cm from the capillary inlet, by removing the polyimide coating. Samples were injected hydrodynamically (5 p.s.i. for 0.5 s; 1 p.s.i.=6894.76 Pa). The electrophoresis system was operated under constant voltage conditions, either at +30 or −30 kV, depending on the surfactant concentration evaluated.

2.2. Reagents and solutions

All standards were prepared from reagent-grade chemicals and deionized water. Stock aqueous solutions of the cationic surfactants CTAB, TTAB and LTAB were prepared at 9.2, 30.2 and 150.0 mM, respectively, which correspond to ten times the standard critical micelle concentrations (std CMC) [23]. Stock solutions of the electrolytes potassium chromate and 3,5-dinitrobenzoic acid were prepared at 125 mM (pH 8) and 62.5 mM (pH 5), respectively.

2.3. Procedure

Working solutions containing appropriate amounts of surfactant and electrolyte were prepared fresh daily by dilution of the stocks and filtered through a 0.2 µm membrane filter just prior to use. The pH adjustments, when necessary, were made by addition of 0.1 M NaOH to the working solution. The capillary was conditioned by a flush with 1 M NaOH (5 min), followed by a flush with deionized water (10 min) and a flush with the working solution (15 min), every time a new set of conditions was established. In between runs, the capillary was just replenished with fresh electrolyte solution (2 min flush).

2.4. Calculations

The magnitude of the electroosmotic flow mobility

was determined by the measurement of the migration time of pure water (t_{osm}) and the knowledge of the capillary dimensions and instrumental parameters, according to the expression:

$$\mu_{osm} = \frac{L_{tot}L_{det}}{Vt_{osm}} \qquad (1)$$

where L_{tot} is the capillary total length, L_{det} is the distance from the injection inlet to the detector position and V is the applied voltage.

3. Results and discussion

3.1. Effect of the surfactant concentration and chain length on EOF

To investigate the influence of the surfactant chain length on the development of the electroosmotic flow, the mobility of chromate solutions was measured in the presence of CTAB, TTAB, and LTAB, at various concentrations, as depicted in linear scale (Fig. 1A). Within the range of concentration evaluated, all three surfactants can cause flow reversal in a single step, which is in accordance with a single transition adsorption isotherm [22]. However, there is a different transition interval from normal to reversed flow for each surfactant in particular. This transition is related to the assembly of the bilayer at the capillary inner surface, which is characterized by the presence of adsorbed hemi-micelles.

The formation of hemi-micelles and stability of the bilayer assembly are directly related to the surfactant structural features, giving that the ionic strength and solvation characteristics of the medium are kept constant. The strength of the dispersion interaction between the surfactant tails is dependent on the chain length, the longer the chain, the stronger the interaction. Therefore, it is expected that CTAB favors the assembly of the bilayer more effectively than TTAB and LTAB, in this order, causing inversion of the flow at smaller concentrations. This behavior is clearly observed in Fig. 1A. The practical outcome of this observation within the anion analysis context is that by working with smaller concentrations of surfactant, an undesirable increase of the medium viscosity is avoided, leading to faster separations. Moreover, peak distortions due to discrepan-

cies between the conductivity of the solute zone and the electrolyte solution are minimized. And finally, a lower current is generated, causing less Joule heating to dissipate.

If the comparison of the surfactant efficacy to accomplish flow reversal is based on a fraction of CMC, structural differences are somehow compensated. Fig. 1B shows the mobility of chromate solutions as a fraction of the standard critical micelle concentration. The CMC values were obtained from the literature, at standard conditions, aqueous solutions at 25 °C, (CTAB = 0.92, TTAB = 3.02 and LTAB = 15 mM) [23]. It is expected that ionic strength, dictated mostly by the electrolyte concentration, will change the std CMC values into conditional ones and this effect will be slightly different for different chained surfactants. Nevertheless, the percentage std CMC scale is used here simply as a reference to help choosing an appropriate concentration of a surfactant to cause stable inverted flow. Therefore, as observed in Fig. 1B, the electroosmotic flow reaches a common constant value for the three surfactants at a concentration approximately above 10% std CMC. Different surfactants have different abilities to assembly the bilayer, but once the charged surface sites are saturated by hemi-micelles and the flow has been inverted, an increase in surfactant concentration in the bulk has no effect on the extent of adsorption at the surface and the flow magnitude remains practically constant. It is worthwhile to mention that in the studies of flow reversal in phosphate buffered solutions by Lucy and Underhill [22], a conditional value for the critical micelle concentration of CTAB was found to be approximately 0.15 mM. The interesting outcome of this observation is that anion analysis, as widely practised in 0.5 mM CTAB–5 mM chromate solutions, has been performed in fact under micellar electrokinetic capillary chromatography and not in free solution capillary electrophoresis as widely claimed [3]. The practical implication of this finding is that anion–micelle association may occur for a number of anions and should be taken into consideration in studies aiming at selectivity improvement.

3.2. Effect of electrolyte type and concentration

Fig. 2 shows the electroosmotic mobility of chromate and 3,5-dinitrobenzoate solutions as a
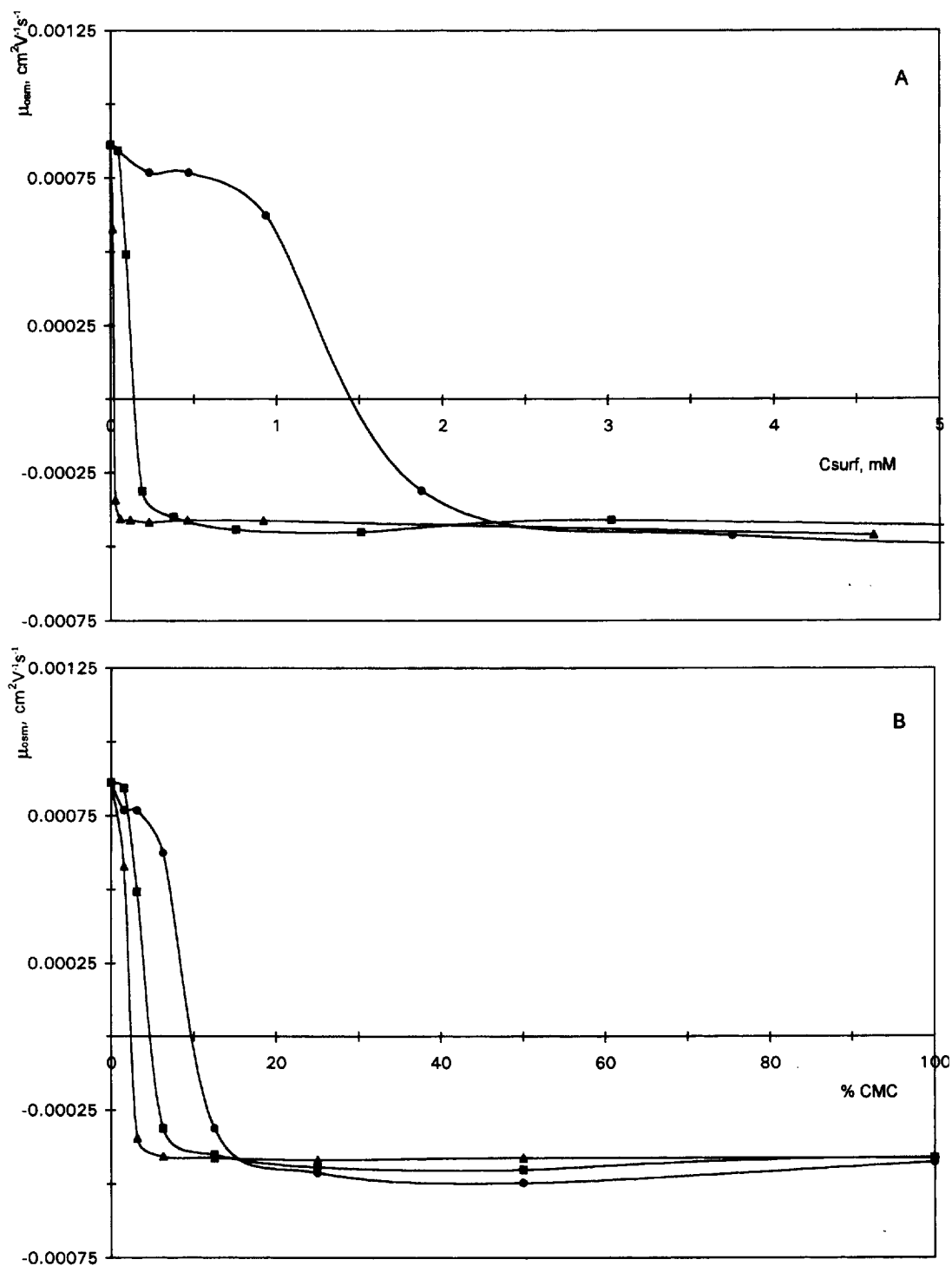
Fig. 1. Effect of the surfactants CTAB (▲), TTAB (■) and LTAB (●) on the magnitude of the electroosmotic flow of 5 m*M* chromate solutions at pH 8, shown in a linear scale (A) and as a fraction of the standard critical micelle concentration (B).
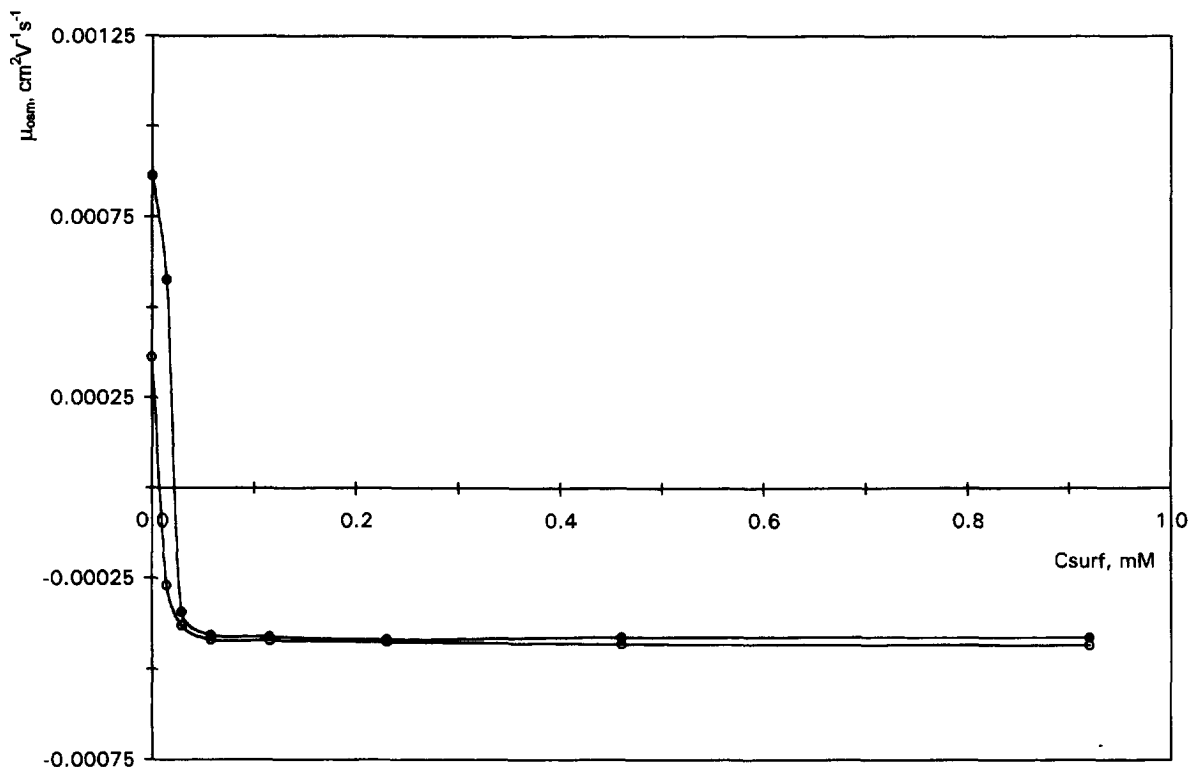
Fig. 2. Effect of the CTAB concentration on the magnitude of the electroosmotic flow of the electrolyte systems 5 mM chromate at pH 8 (●) and 5 mM 3,5-dinitrobenzoate at pH 5 (○).

function of CTAB concentration. It is noticeable that approximately at 0.1 mM, which corresponds to 10% std CMC, the flow becomes independent not only of the surfactant concentration but also of the electrolyte type. In normal electroosmotic flow, the magnitude of the flow is directly related to the distribution of charges in the double layer at the interface solution–surface, and is by all means affected by the electrolyte anion type [24]. In reversed flow, the surface is no longer the solid backbone of silica, but a dynamic structure represented by the bilayer assembly.

A similar mechanism for cation adsorption in the development of normal electroosmotic flow [25] can be used here to explain the effect of the anion type on the reversed flow. An ion-exchange reaction can be written as:

$$(SiO^- \ {}^+SURF^+)A^- + X^- \overset{K_{ie}}{\leftrightarrow}$$

$$(SiO^- \ {}^+SURF^+)X^- + A^- \qquad (2)$$
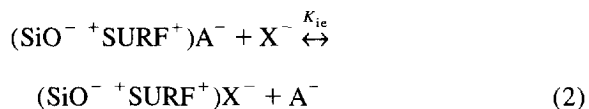
where X^- is the electrolyte anion in solution, ${}^+SURF^+$ is the hemi-micelle adsorbed onto the capillary wall and A^- is the anionic portion of the double layer.

If it is assumed that differences in flow magnitude arise from distinct ion-exchange behavior, which is reflected by the magnitude of the constant K_{ie}, the curves of Fig. 2 suggest that the adsorption of both chromate and 3,5-dinitrobenzoate follows a similar mechanism, regardless of their chemical diversity. However, an increase in the electrolyte concentration would have the same effect that it does in normal electroosmotic flow: compression of the double layer thickness, followed by a decrease in the absolute value of zeta potential, and a resulting decrease in the flow magnitude [24]. It is interesting to observe that like normal flow [24], the reversed flow also varies linearly with the logarithm of the electrolyte concentration (Fig. 3), suggesting that the bilayer assembly at the capillary surface behaves similarly to an ion-selective electrode.
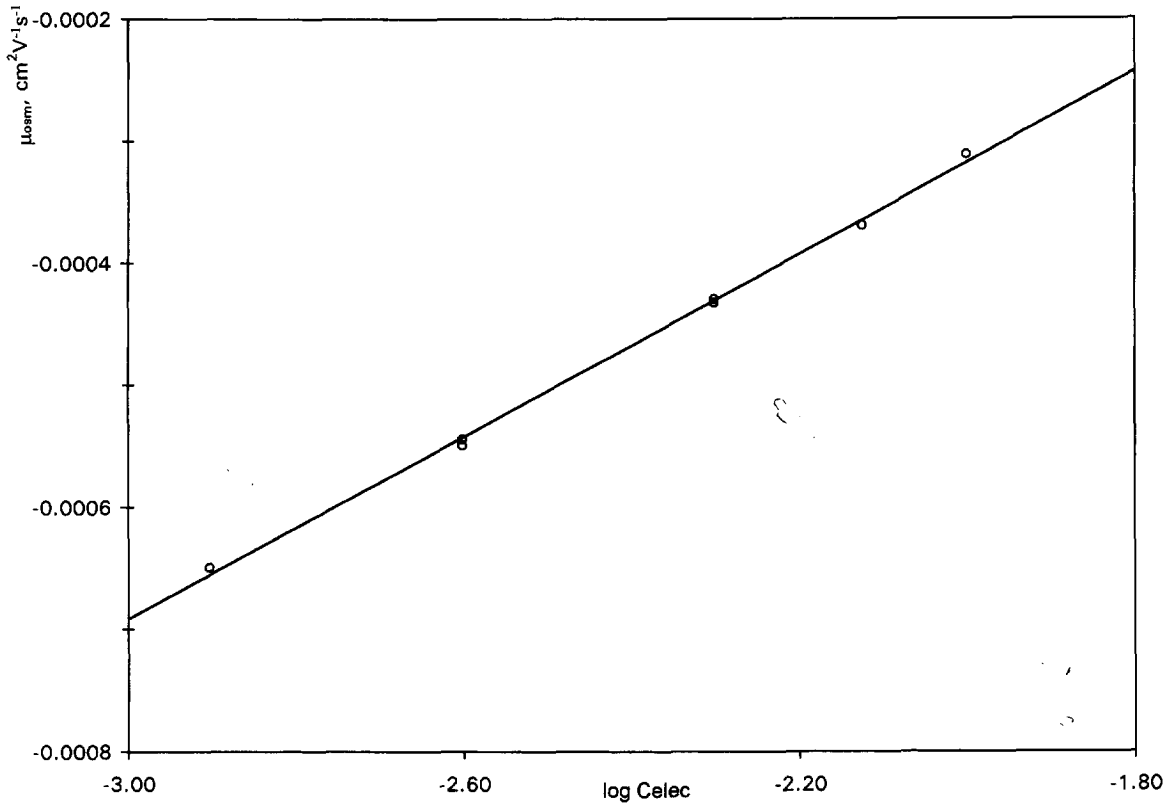
Fig. 3. Effect of the electrolyte concentration on the magnitude of the electroosmotic flow of 3,5-dinitrobenzoate solutions, at pH 5 and 0.115 mM CTAB (10% std CMC).

3.3. Effect of pH

To understand the effect of pH on the development of reversed electroosmotic flow, the mobility of 3,5-dinitrobenzoate solutions, with and without CTAB, in the pH range 5–9 was compared. Without the surfactant, the influence of pH is pronounced (Fig. 4A). The titration of the surface silanol groups with the increase of pH exposes a large number of charged sites, and the flow rises considerably. This behavior is not observable in the presence of CTAB (Fig. 4B). In fact, the flow remains practically constant. As the pH is lowered, there is an increased competition between protons and surfactant monomers for the charged sites at the surface. Therefore, a pH effect would only be expected at conditions where the formation of a monolayer is feasible. However, since the concentration of CTAB used in this study (10% std CMC) was large enough to

promote a complete assembly of the bilayer, the surface charged sites were already saturated by the surfactant and no longer accessible to protons.

4. Conclusions

The results revealed that CTAB, in a concentration approximately above 10% std CMC, is the most suitable flow modifier. Both chromate and 3,5-dinitrobenzoate can be used indistinctly to produce the same flow magnitude. The reversed flow mobility varies with the electrolyte concentration in a logarithmic fashion and is independent of pH. By establishing the relative importance of the variables capable of altering the flow characteristics, these studies have helped us to improve our overall understanding of reversed electroosmotic flow in capillary electrophoresis.
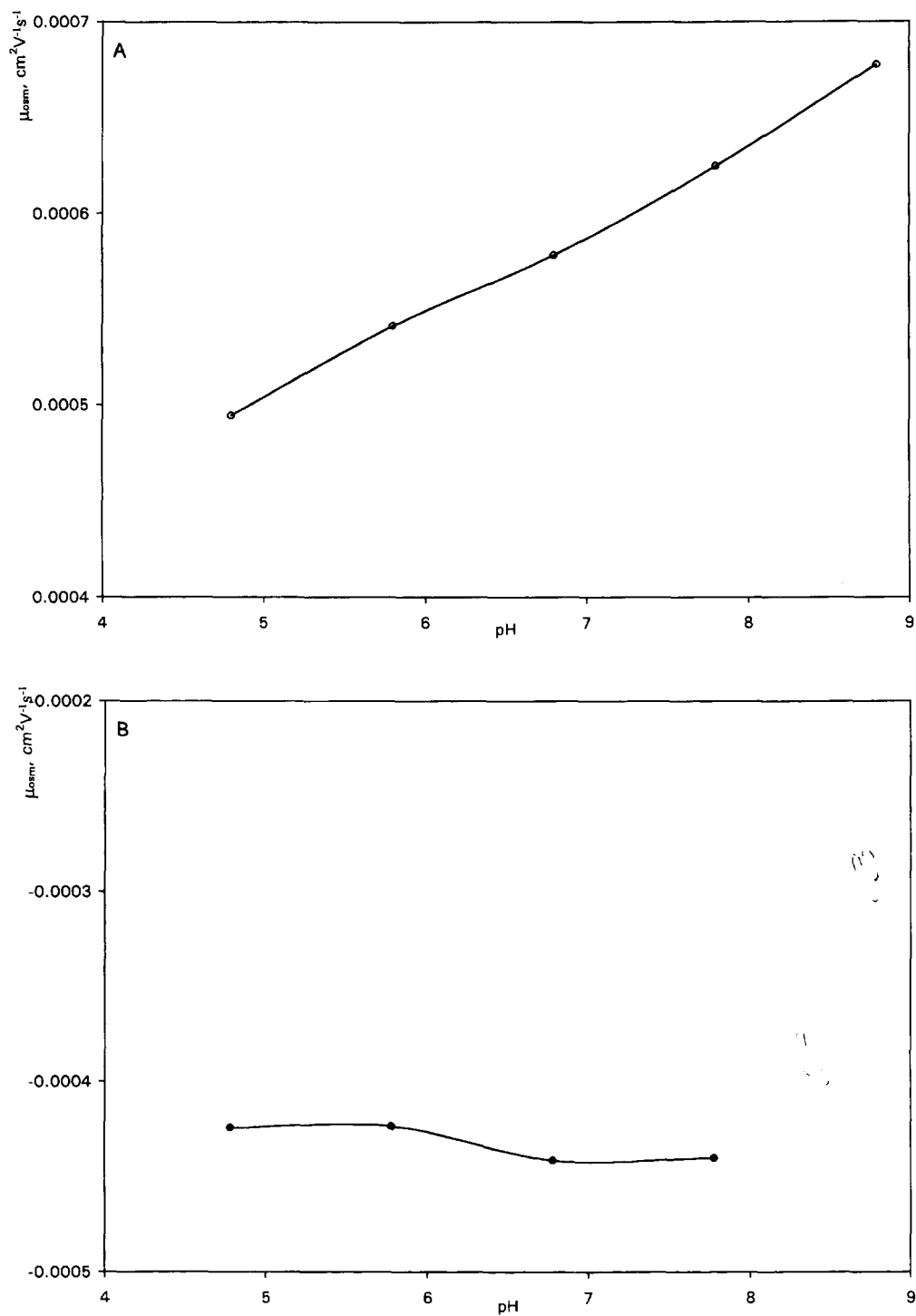
Fig. 4. Effect of pH on the magnitude of the electroosmotic flow of 3,5-dinitrobenzoate solutions with no surfactant (A) and with 0.115 mM CTAB (10% std CMC) (B).

5. Applications

The above conclusions can be applied advantageously in the design and optimization of anion separations. For instance, since the flow is not influenced by the electrolyte type, the choice of the electrolyte system would be related only to peak symmetry considerations [3,19,26]. Additionally, since the flow is not affected by pH, the choice of the electrolyte pH would depend exclusively on the assessment of the effective mobility versus pH curves of the components in the mixture under investigation [26]. Moreover, since the electrokinetic injection depends on the reproducibility of the flow, and the flow was found to be constant and quite stable within a large surfactant concentration range (at CTAB>10% std CMC), the precision of electrokinetic injections should improve considerably. Such conditions are very desirable for the determination of anions at trace level.

Acknowledgments

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