Theoretical aspects on the regulation of electroosmotic flow in capillary electrophoresis by adding charged amphiphiles[1]

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Abstract

The regulation of electroosmotic flow (EOF) in capillary electrophoresis is of great importance in many analytical applications. In this paper, the variation of EOF with the concentration of amphiphile is studied by adding a charged amphiphile to the background electrolyte (BGE) when using a C_8-coated capillary. A theory relating the change in concentration of amphiphile in the BGE to the variation in observed EOF is presented. The basis of the theory is that the adsorbed amphiphile creates an electrostatic surface potential that is calculated from the linearised Poisson-Boltzmann equation. The adsorption isotherm of the amphiphile to the capillary surface is described by an electrostatic surface potential modified linear isotherm. By assuming that the ζ-potential in the Smoluchowski equation is proportional to the electrostatic surface potential, an equation describing the EOF as a function of amphiphile concentration is obtained. The proposed theory is experimentally tested by adding octane sulfonate, tetrabutylammonium or tetrapentylammonium ion to the BGE. It is shown that, in these cases, the proposed theory describes the EOF as a function of amphiphile concentration well and that, as expected, the proportionality constant between the electrostatic surface potential and the ζ-potential is less than one. Some practical aspects and problems that occur with this type of measurement are also discussed. © 1997 Elsevier Science B.V.

Keywords: Electroosmotic flow; Background electrolyte composition; Coated capillaries; Amphiphiles

1. Introduction

The first observation of electroosmosis was made in 1807 by Reuss [1], but a physico-chemical explanation of the process was not presented until almost a hundred years later by Smoluchowski [2] and this was further developed into its present form [3,4]. The phenomenon of electroosmosis occurs when an electric field is applied parallel to a charged surface in the presence of an electrolyte. In electrophoretic applications, electroosmotic flow (EOF) has usually been considered as an unwanted process that should be repressed or controlled [5]; this may be achieved with different coating techniques or by applying an external field over the capillary wall.

Many papers that deal with the control of electroosmosis and the minimization of adsorption have been published since Jorgensson and Lukacs [6] improved and popularised capillary electrophoresis (CE). The papers discuss external field applications [7–9], dynamic coating of fused-silica surfaces [10–

18], the use of background electrolytes (BGEs) containing organic solvents [19], covalent modification of the capillary surface [20–27] and combinations of the two last-mentioned techniques [28–33]. Theoretical models have been developed that describe different approaches for controlling electroosmosis and which take into account the adsorption of analytes to the capillary surface [34], the application of an external field over the capillary wall in order to control electroosmosis [35,36] and the use of polymer-coated capillaries with covalently bonded sulfonate groups to create an EOF that is pH independent [37]. Corradini et al. [38,39] have presented a Langmuirian type of model for the adsorption of cationic amphiphiles to the silica surface of capillaries, where the basicity and size of the amphiphile is taken into account.

Adsorption of analytes is a common problem in CE and the effect is pronounced when the analyte and the capillary wall are of opposite charge. Fused-silica capillaries have a negative charge when pH>2 and the coulombic force that is the main contributor to the surface affinity of the analytes can be reduced by using low pH values or a high ionic strength. The most common technique used to minimize the electrostatic interaction between cationic analytes and the capillary wall is to add cationic amphiphiles to the BGE. Several types of amines have been used to dynamically coat the silica surface and the results from coating with cetyltrimethylammonium chloride indicate that adsorption of the amphiphile involves a mechanism that comprises both electrostatic and hydrophobic forces [12].

Determination of adsorption isotherms of tetraalkylammonium salts has been thoroughly investigated in reversed-phase liquid chromatography and the physico-chemical model presented in [40,41] can also be applied to a CE system.

In this study, the capillary surface consisted of silica that had been made hydrophobic using covalently bound C_8-alkyl chains. A charged amphiphile is added to the BGE. The amphiphiles adsorb on the covalently bound hydrophobic chains at the surface and create a charged surface, which, in turn, regulates the EOF in the capillary. The hydrophobicity, net charge and bulk concentration of the added amphiphile will determine the magnitude of the electrostatic potential created at the hydrophobic

capillary surface. The surface potential modified linear adsorption isotherm is used to describe the relation between surface and bulk concentration for charged amphiphiles. However, the zeta-potential in the Smoluchowski equation and the electrostatic surface potential in the adsorption isotherm are not necessarily equal. The adsorption isotherm is then used, in combination with the Smoluchowski equation, to estimate the EOF and any possible difference between the two is taken into account in the presented theory.

The adsorption of charged amphiphiles to a hydrophobically coated capillary surface provides a possible means of controlling the magnitude and the direction of the EOF. Giddings [42] derived a general relation for resolution in electrophoresis, and it has been shown that optimal resolution can be achieved if the EOF is tuned to a certain direction and magnitude. The regulation of EOF can be used to improve the resolution in critical separations [33] or to reduce the run-times in the determination of e.g. anions with indirect detection [43]. It has been shown that the addition of an amphiphile to the BGE can also be used to minimize electromigration dispersion and to regulate the peak shape of overloaded peaks [44].

2. Experimental

2.1. Chemicals

Tetrabutylammonium (TBA) hydroxide (0.4 M) and octanesulfonic acid were obtained from Eastman Kodak (Rochester, NY, USA) and the tetrapentylammonium (TPeA) chloride was purchased from Aldrich-Chemie (Steinheim, Germany). Benzylalcohol was obtained from Merck (Darmstadt, Germany).

The BGE was made up of amphiphile, NaOH and H_3PO_4, adjusted to a pH value of 3.0, with a constant ionic strength during the measurement series. The ionic strength was 0.05 for the tetraalkylammonium-containing BGEs and 0.02 for octanesulfonic acid solutions.

The water used throughout this study was purified using a Modulab Analytical Research Grade RO/Polishing System from Continental Water Systems (San Antonio, TX, USA).

2.2. Capillary electrophoresis system

A Beckman P/ACE System 2100 and a P/ACE 5000 (Beckman Instruments, Palo Alto, CA, USA) were used with UV detection at 214 nm. The temperature was set at 25°C. CElect C_8 bonded capillaries were obtained from Supelco (Bellefonte, PA, USA). The columns used for the tetraalkylammonium measurements had an inner diameter of 50 μm and a length of 40/47 cm (effective/total length) and those used for the octanesulfonic acid measurements had an I.D. of 50 μm and a length of 50/57 cm.

2.3. Procedures

EOF was measured by injection of 0.01% (w/w) benzyl alcohol dissolved in water, and the ionic strength was $I=0.05$ M during the measurement of the EOF for tetraalkylammonium ions and was $I=0.02$ M during the determination of the EOF for octanesulfonic acid.

First, the C_8 capillary was rinsed with acetonitrile for 5 min and then equilibrated with amphiphile ions by flushing for 2 min with phosphate buffer containing a given concentration of amphiphile. The capillary column was flushed with running buffer between injections and rinsed with purified water at the end of the day.

The EOF marker was injected at a pressure of 0.5 p.s.i. (1 p.s.i.=6894.76 Pa) for 5 s. The voltage was 20 kV using octanesulfonic acid as the modifier of EOF, generating a current of 30–35 μA, and was 15 kV during the tetraalkylammonium measurements, generating a current of 55–60 μA.

2.4. Theory

Electroosmosis occurs when an electric field is applied parallel to the axis of a capillary tube and it refers to the motion of liquid induced by the field. The flow results from the presence of an electric double layer at the tube wall, since the applied electric field causes the ions in the double layer to move towards one of the electrodes. The motion of these ions gives rise to a body force on the liquid in the double layer and this body force sets the liquid in motion. When charged amphiphilic molecules are

dissolved in the BGE, they adsorb on the capillary surface and thereby change the concentration of ions in the double layer. A consequence of this is that the electroosmotic flow changes when the concentration of amphiphiles in the BGE varies. It is the purpose of this work to study and present a simple model for the variation of the EOF with amphiphile concentration. The theoretical analysis consists of two steps; in the first, an adsorption isotherm for the amphiphile on the capillary surface is described. In the second step, the change in the electric double layer induced by the adsorbed ions is combined with the Smoluchowski equation to give the relationship between amphiphile concentration and EOF.

2.5. Adsorption isotherm of the amphiphile

A simple adsorption isotherm that considers the electrostatic surface potential as well as a maximum possible concentration of the capillary surface may be derived in the following way.

Consider the equilibrium:

$$A^-(l) + S \rightleftharpoons A^-S \tag{1}$$

Where $A^-(l)$ and A^-S represent a negatively charged amphiphile in the liquid phase and adsorbed to the capillary surface, respectively and S is the part of the capillary surface that is not occupied by the amphiphile. The thermodynamic condition for equilibrium is:

$$\mu_A + \mu_S = \mu_{AS} \tag{2}$$

where μ_i represents the electrochemical potential for species i ($i=A^-$, A^-S and S). In solution, the electrochemical potential for these species is:

$$\mu_A = \mu_A^0 + RT \ln\left(\frac{c_A}{c_{0A}}\right) \tag{3}$$

$$\mu_S = \mu_S^0 + RT \ln X_S \tag{4}$$

$$\mu_{AS} = \mu_{AS}^0 + RT \ln X_{AS} + Z_A F \Delta\psi_0 \tag{5}$$

Here c_A is the amphiphile concentration in the liquid phase and c_{0A} is the standard state for the amphiphile in the solution i.e. 1 mol/l. The term $(\frac{c_A}{c_{0A}})$ is simplified to c_A in the following expressions. The charge of the amphiphile is expressed as Z_A, F is the Faraday constant, μ_i^0 is the electrochemical potential

of the standard state and $\Delta\psi_0$ is the change in electrostatic potential of the capillary surface caused by the adsorbed amphiphile. X_{AS} is the fraction of the capillary surface occupied by the amphiphile, i.e.

$$X_{AS} = \frac{n_A}{n_0} \quad \text{and} \quad X_S = 1 - X_{AS} \tag{6}$$

Here n_A is the surface concentration of amphiphile and n_0 is the monolayer capacity of the surface. The adsorption isotherm is obtained by combining Eqs. (2)–(6):

$$n_A = \frac{n_0 K_{AS} C_A e^{\frac{-Z_A F \Delta\psi_0}{RT}}}{1 + K_{AS} C_A e^{\frac{-Z_A F \Delta\psi_0}{RT}}} \tag{7}$$

where

$$K_{AS} = \exp\left(\frac{-(\mu_{AS}^0 - \mu_A^0 - \mu_S^0)}{RT}\right) \tag{8}$$

For low surface concentrations of amphiphile, the term $K_{AS} C_A e^{\frac{-Z_A F \Delta\psi_0}{RT}}$ is much smaller than unity. In this case, Eq. (7) is simplified to the surface potential modified linear adsorption isotherm.

$$n_A = n_0 K_{AS} C_A e^{\frac{-Z_A F \Delta\psi_0}{RT}} \tag{9}$$

Eq. (9) will be used in the ensuing calculations do determine the dependence of EOF on amphiphile concentration in the BGE.

When the charged amphiphile adsorbs onto the capillary's surface, a change in the electrostatic surface potential is obtained. From the Debye–Hückel approximation of the Poisson–Bolzmann equation, the relationship between the surface concentration of amphiphile and the induced change in the electrostatic surface potential is:

$$\Delta\psi_0 = \frac{Z_A F n_A}{\kappa \varepsilon_0 \varepsilon_r} \tag{10}$$

where ε_0 is the permittivity of a vacuum, ε_r the dielectric constant of the fluid medium and $1/\kappa$ is the Debye length, which is a measure of the thickness of the electric double layer, where κ is defined as

$$\kappa = F \left[\frac{2I}{\varepsilon_0 \varepsilon_r RT} \right]^{1/2} \tag{11}$$

where I is the ionic strength of the BGE.

2.6. Electroosmotic flow

When the radius of the capillary is large compared to the Debye length, the velocity of the fluid beyond the double layer is given by the Smoluchowski equation:

$$v_{e0} = -\frac{\varepsilon_0 \varepsilon_r \zeta E}{\eta} \tag{12}$$

where v_{e0} is the fluid velocity, E is the applied electric field parallel to the tube axis and η is the viscosity of the fluid medium in the capillary. ζ is the zeta-potential, which represents the electrostatic potential at the plane of shear. The value of ζ is therefore not a priori equal to Ψ_0, which represents the electrostatic potential at the plane of adsorption at the capillary surface. For reasons that are discussed below, it is appropriate to set

$$\Delta\zeta = k\Delta\Psi_0 \tag{13}$$

where k is a proportionality constant with a numerical value of less than one and $\Delta\zeta$ is the change in the zeta-potential caused by the adsorption of the amphiphile to the capillary surface.

The difference in velocity of the EOF with and without amphiphile in the liquid is obtained by combining Eqs. (12) and (13):

$$v_{e0} - v'_{e0} = -\frac{\varepsilon_0 \varepsilon_r k \Delta\Psi_0 E}{\eta} \tag{14}$$

where v'_{e0} is the velocity of the liquid in the absence of amphiphile. By combining Eqs. (9) and (10) with Eq. (14), the adsorption isotherm and electrostatic surface potential are expressed in terms of $(v_{e0} - v'_{e0})$ as

$$-\frac{\eta(v_{e0} - v'_{e0})}{k\varepsilon_0 \varepsilon_r E}$$

$$= \frac{Z_A F n_0 K_{AS} c_A \exp\left(FZ_A \dfrac{\eta(v_{e0} - v'_{e0})}{k\varepsilon_0 \varepsilon_r ERT}\right)}{\kappa\varepsilon_0 \varepsilon_r} \tag{15}$$

The EOF is usually measured as the time it takes a marker molecule to migrate a distance, L, from the point of injection to the point of detection, t_{e0}, so that:

$$t_{e0} = \frac{L}{\nu_{e0}} \tag{16}$$

Taking the logarithm of Eq. (15), inserting Eq. (16) and rearranging the final equation for the dependence of migration time on the amphiphile concentration in the liquid phase, one gets

$$\frac{1}{t_{e0}} = \frac{1}{t'_{e0}} + \frac{k\varepsilon_0\varepsilon_r RTE}{|Z_A|F\eta L}\left(\ln\left(\frac{\kappa\eta L}{|Z_A|kEFn_0 K_{AS}}\right)\right.$$
$$\left. + \ln\left(\frac{c_A}{\dfrac{1}{t_{e0}} - \dfrac{1}{t'_{e0}}}\right)\right) \tag{17}$$

where t'_{e0} corresponds to ν'_{e0}. The experimental data are therefore plotted as $1/t_{e0}$ as a function of $\ln(c_A/(1/t_{e0} - 1/t'_{e0}))$, giving a linear relation with a slope containing physical constants and the parameter k, which corrects for the difference between the surface potential and the zeta-potential. To make the final equation independent of the sign of the amphiphile, the direction of the EOF and the direction of the field, the absolute of the numerical value for the charge of the amphiphile is introduced in Eq. (17).

3. Results and discussion

In this paper a description of the regulation of EOF by varying the ζ-potential via dynamic coating of C_8 capillaries is presented. The magnitude of the EOFs is described as a function of the amphiphile concentration in the BGE. The use of hydrophobic capillaries for the adsorption of amphiphiles to the surface opens up the possibility of regulating the EOF in both directions. This can be accomplished by adding anionic or cationic amphiphiles, such as octanesulfonic acid and tetrabutylammonium ions, the structures of which are shown in Fig. 1a. As can be seen in Fig. 1a, the EOF is regulated by varying the concentration of both amphiphiles over a 0–10 mM concentration range. This can be used to control the resolution of two adjacent peaks. In Fig. 1b, the EOF is shown at different tetrapentylammonium (TPeA$^+$) concentrations. Coating the surface using the more hydrophobic TPeA$^+$ compared with the TBA$^+$ should result in a steeper increment in the EOF, however, as shown in Fig. 1b, the increment
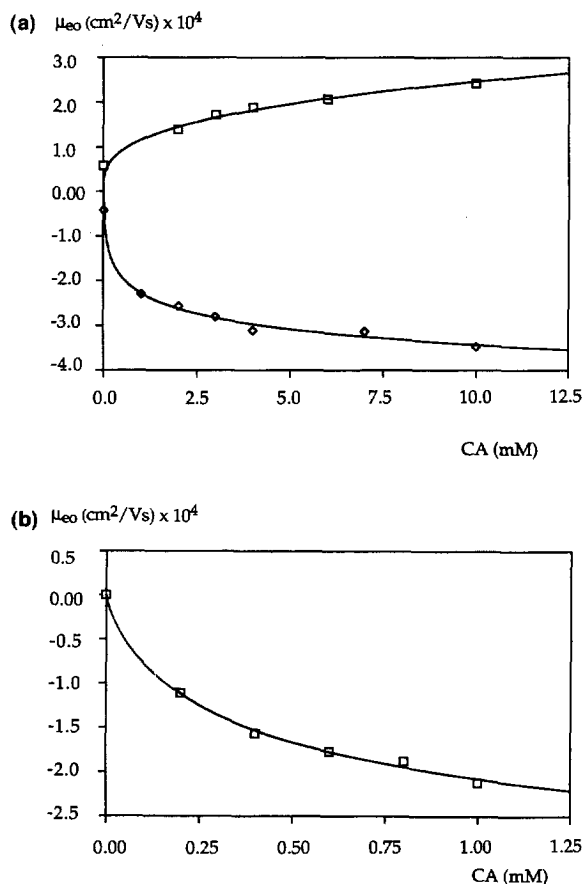

Fig. 1. (a) Electroosmotic mobility as a function of the concentration of octanesulfonic acid (□) and TBA$^+$ (◇), respectively, at pH 3.0 using a constant ionic strength of 0.05 M, for tetrabutylammonium ions, and of 0.02 M for the octanesulfonate acid experiment. (b) EOF is plotted versus the concentration of TPeA$^+$ in the BGE. The electroosmotic charge was chosen according to Hunter [4]. The solid lines in (a) and (b) were obtained using a logarithmic curve fit function and the EOF measurements were repeated at each amphiphile concentration ($n > 5$). For further information, see Section 2.

for TPeA$^+$ is of the same magnitude as that for TBA$^+$. A reasonable explanation of this unexpected result may be due to differences in the hydrophobicity of the C_8-coated capillaries used. If the capillary surface is less hydrophobic in the TPeA$^+$ experiment, the obtained EOFs could be explained. Calculation of the maximum ζ-potential in the concentration range studied, using Eq. (12), gives a ζ-potential of $+44.9$ mV, at a TBA$^+$ concentration of 10 mM. For 1 mM TPea$^+$, the calculated ζ-potential

was +27.5 mV, using tabulated constants for water at 25°C. The data obtained when 10 mM octanesulfonic ions were used as the dynamic coating gave a ζ-potential of −31.4 mV. Deviations from linearity are expected for surface potentials higher than 25 mV using the Debye–Huckel approximation of the Poisson–Bolzmann equation (Eq. (10)).

Several capillaries and amphiphiles with varying degrees of hydrophobicity have been tested during our investigation of the adsorption of amphiphiles to hydrophobic surfaces [33]. The use of more hydrophobic tetraalkylammonium ions, such as cetyltrimethylammonium chloride, as surface modifiers affected the EOF strongly at concentrations below 10 μM and when less hydrophobic amphiphiles, such as tetramethylammonium ions, were added to the BGE, the effect on EOF was low in the concentration range of 1–10 mM. The amphiphiles used in this study were chosen because they had suitable affinity for the hydrophobic surface and they were also used as co-ions with a matching mobility [44]. As can be seen in Fig. 1a,b, low EOFs were obtained when no amphiphiles were added to the BGE. One explanation for these results may be varying degrees of the covalently bonded C_8 chains attached to the silica surface, where residual silanol groups can cause an EOF in the direction of the cathode. Additionally, trace amounts of positively charged surface active agents originating from the glass material may explain the negative EOF that is shown in Fig. 1a. The measured EOFs in the absence of amphiphiles were $5.1 \cdot 10^{-5}$ (cm^2/V s) for octanesulfonic acid, $-4.2 \cdot 10^{-5}$ (cm^2/V s) for TBA$^+$ and less than $\pm 8.7 \cdot 10^{-6}$ (cm^2/V s) for TPeA$^+$. The presented theory can be applied without distortions, since differences in the EOF are used in the calculations.

The presented theory is tested using Eq. (17), describing the EOF as a function of the amphiphile concentration in the BGE, by plotting the calculated values of the EOF represented by $1/t_{e0}$ versus the amphiphile concentration in the BGE, calculated as ln $[c_A/(1/t_{e0} - 1/t'_{e0})]$. A visual evaluation of the slopes in Figs. 2–4 indicates that the data points were equally distributed along the regression lines and that the lines not were curved. A statistical evaluation of the experimental slopes was performed to estimate the standard deviation for the regression line of y on x [45]. Normal conditions using this kind
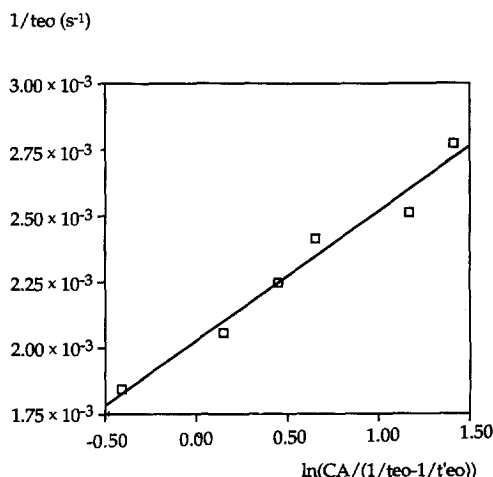

Fig. 2. Experimental data obtained for TBA$^+$, plotted according to Eq. (17) ($r = 0.984$).

of calculation of the standard deviation for the slopes were not fulfilled since there is an uncertainty in both the x and y values. Moreover, an error in the x value is correlated to an error in the corresponding y value, hence, it is not possible to conclude that the error is equal along the regression line. Despite these shortcomings, the method described in reference [45] was used to give an estimate of the uncertainty of the calculated slopes using Eq. (17). The following values were obtained for the slopes with their
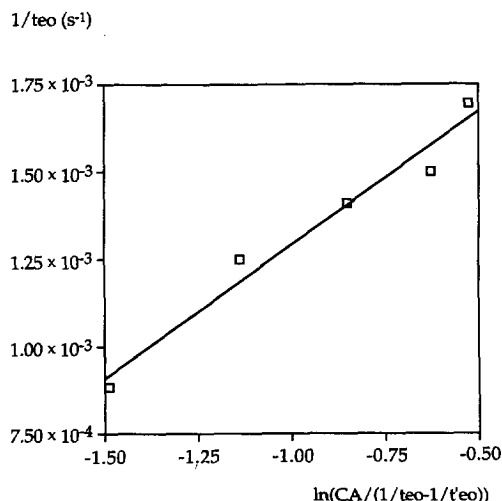

Fig. 3. Experimental data obtained for TPeA$^+$, plotted according to Eq. (17) ($r = 0.983$).
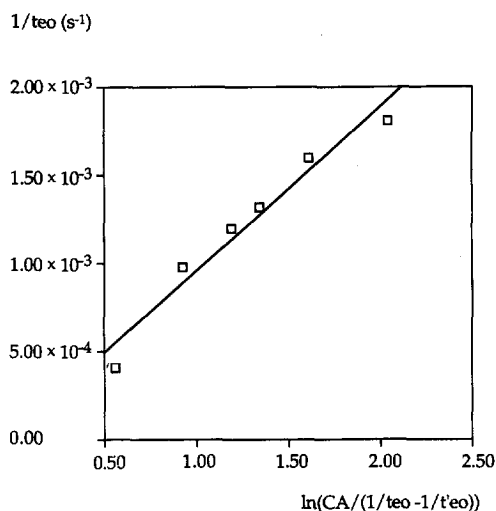
1/teo (s⁻¹)

Fig. 4. Experimental data obtained for octanesulfonic acid, plotted according to Eq. (17) ($r = 0.978$).

individual standard deviation; $7.6 \pm 0.8 \cdot 10^{-4}$ for $TPeA^+$, $4.9 \pm 0.40 \cdot 10^{-4}$ for TBA^+ and $9.3 \pm 1.0 \cdot 10^{-4}$ for octanesulfonic acid. It can be seen that the relative standard deviation for the slopes was approximately 10% in all three cases. A comparison of the experimental slope values, calculated from the EOF measurements with the theoretical value, gives a numerical value for k, i.e. the relation between surface potential (ψ_0) and the ζ-potential. The theoretical values of the slopes were obtained from Eq. (17) by inserting numerical values for the physical constants and the experimental conditions. The theoretically calculated values, using $k = 1$, were $15.7 \cdot 10^{-4}$ for the experiments with tetraalkylammonium ions and $13.8 \cdot 10^{-4}$ for the experiments with octane sulfonate ions. By dividing the experimental slope by the theoretical slope, the k values in Eqs. (13) and (17) are obtained. Provided that the factors relating k to the slope are known with negligible error, a relative standard deviation of 10% also holds for the k value. The standard deviation was then multiplied by a factor of three, giving the following intervals of the k values, TBA^+ 0.21–0.39, $TPeA^+$ 0.35–0.56 and 0.49–0.91 for octanesulfonic acid. The factor of three is a coverage factor, taking into account the approximations made in estimating the uncertainty of the slopes.

The highest k value for the slopes was achieved

with octanesulfonate, which was run with a lower ionic strength (0.02) compared to the cationic amphiphiles, where an ionic strength of 0.05 was used. The slopes of TBA^+ and $TPeA^+$ were expected to have equal values according to the theory, but the lines should be shifted since the ions have different affinities for the surface (K_{AS} value in Eq. (17)), which changes their intercepts. A comparison of the TBA^+ and $TPeA^+$ slopes, taking into account the propagation of random errors from the slopes, gives a relative standard deviation of 14% for the quotient slope TBA^+/slope $TpeA^+$, which gives an approximate value for the upper limit of $0.64 \cdot (1 + 3 \cdot 0.14) = 0.92$. This statistical evaluation indicates that there is a significant difference between the two slopes.

The k value is defined as the ratio between the electrostatic potential at the plane of adsorption of the amphiphilic molecules and the plane of shear between the stationary fluid at the surface and the moving fluid. This interpretation of the k value is in accordance with the colloid chemistry view that there is a difference in location between these planes. The exact position of the plane of shear has been a matter of debate for many years [4]. From the Gouy-Chapman theory and the obtained k values, it is possible to estimate that in the investigated systems, the plane of shear is located 7–10 Å from the plane of adsorption. This is somewhat higher than the expected 5–7 Å and one possible reason for this may be that, in the applied electric field, the adsorbed amphiphilic ions migrate in the opposite direction to the EOF and, therefore, the plane at which the fluid velocity is zero moves further out from the surface. These approximate calculations are based on the assumption that the bulk values for ε and η for water holds close to the surface. For low surface potentials (<200 mV), there is no need to assign special properties for water close to the surface [46].

4. Conclusions

Regulation of adsorption effects and the possibility of controlling the EOF is of major importance in CE and the developed theory takes into account the important physico-chemical relationships that alter the magnitude of the EOF in CE capillaries. The proposed theory was tested by adding anionic and

cationic amphiphiles to the BGE and it was shown that the theory is good for describing the EOF as a function of amphiphile concentration. The proportionally constant (k) between the surface potential (ψ_0) and the ζ-potential was less than one, as expected. Determination of the EOF as a function of the amphiphile concentration in the BGE is difficult due to the many possible disturbances that may appear at the surface of the capillary. There is a need for further investigations to verify the presented theory e.g. varying the ionic strength, using different amphiphiles and types of capillaries.

Acknowledgments

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