



ELSEVIER

Journal of Chromatography A, 684 (1994) 311–322

JOURNAL OF
CHROMATOGRAPHY A

Dependence of the electroosmotic mobility on the applied electric field and its reproducibility in capillary electrophoresis

Michael S. Bello¹, Laura Capelli, Pier Giorgio Righetti*

Faculty of Pharmacy and Department of Biomedical Sciences and Technologies, University of Milan, Via G. Celoria 2, 20133 Milan, Italy

First received 5 April 1994; revised manuscript received 8 June 1994

Abstract

Experimental results on the electroosmotic mobility in fused-silica capillaries are presented for different applied voltages and solutions of different pH. The electroosmotic mobility is shown to be dependent on the applied voltage and this dependence cannot be attributed to the temperature effects. Results of the electroosmotic mobility measurements are found to be dependent also on the electrophoresis unit they have been performed in. The explanation given and the relevant theory presented are based on the hypothesis that these effects are produced by a radial electric field inevitably existing in any electrophoresis unit. The concept of the limiting electrophoretic mobility, i.e. extrapolated to the zero applied voltage, is introduced in order to characterize the properties of the solution–wall interface. The slope of the electroosmotic mobility dependence on the applied voltage depends on the solution pH and the surroundings of the capillary. Theoretical estimations agree well with both experimentally found limiting mobilities and slopes. Long-term variations of the electroosmotic mobility are supposed to be related with the cation penetration into the capillary wall.

1. Introduction

The electroosmotic flow in a capillary column is produced by an electric field and transmitted by the drag of ions acting in a thin sheath of charged fluid adjacent to the silica wall column. The origin of charge in this sheath is an unbalance between positive and negative ions in the bulk solution which have to balance the fixed negative charge on the silica wall. Electroosmosis (EO) in capillary electrophoresis has at-

tracted much attention because of its importance for understanding results of separation and run reproducibility. EO flow is superimposed on the electrophoretic motion of the analyte and, thus, effects its migration time. If the direction of the EO flow is the same as that of the electrophoretic motion, it reduces the migration time and decreases resolution. For micellar electrokinetic chromatography with neutral micelles, electroosmosis is the only source of motion and, therefore, determines the reproducibility of the analysis.

A traditional explanation of electroosmosis [1–3], based on the concept of the electric double layer at the boundary of the solid-phase–electrolyte solution, assumes that the mobile part of

* Corresponding author.

¹ Permanent address: Institute of Macromolecular Compounds, Russian Academy of Sciences, Bolshoi 31. St. Petersburg 199004, Russian Federation.

the double layer moves parallel to the boundary with a certain velocity U under the influence of the applied tangential electric field E . The mobile part of the double layer has an opposite charge to the immobilized surface charge originating from the dissociation of surface molecules or ion adsorption to the surface.

Electroosmotic mobility (EM) μ is defined as the ratio of the electroosmotic velocity to the intensity of the electric field which generates the electroosmotic flow. The well-known formula of Von Smoluchowski relates μ with the properties of the liquid (dielectric constant and viscosity) and ζ potential.

$$\mu = \frac{\epsilon \epsilon_0 \zeta}{\eta} \quad (1)$$

where ϵ is the dielectric constant of the electrolyte solution, ϵ_0 is the dielectric permittivity of vacuum, η is the solution viscosity and ζ is the zeta potential, which is, as a first approximation, equal to the electric potential drop between the wall and the bulk of the electrolyte solution.

The surface charge determining ζ potential is formed by the dissociated charged groups and is known to be controlled by the solution pH (as shown by Lukacs and Jorgenson [4], Schutzner and Kenndler [5], Schwer and Kenndler [6], Kohr and Engelhardt [7]), by organic modifiers [6], and its ionic strength and composition, see Van Ormann [8]. The important feature of the EO mobility generated by the immobilized surface charge is that it does not directly depend on the applied axial electric field. Indirectly, EO mobility depends on the electric field through the dependence of the solution viscosity on temperature which, in case of inadequate cooling, depends on the square of the electric field strength.

A way to control EO directly by applying a radial electrical field was proposed recently by Lee and co-workers [9,10] and by Ghowsi and Gale [11]. This radial electric field is produced by applying a potential difference between the electrolyte solution within the capillary and the outer surface of the capillary [12–17]. The effect of the radial electric field on the EO is explained in the literature as it induces an additional surface charge density on the inner fused-silica

surface which effects the total ζ potential and, thus, according to Eq. 1, the EO mobility.

The dependence of the EO mobility on the electric field measured in conventional CE units was experimentally observed and reported by Rasmussen and McNair [18] and by Issaq et al. [19] and was attributed to the viscosity dependence on the temperature mentioned above. Another explanation for the dependence of the EO mobility on the applied electric field, based on new findings [9–17], was recently proposed by Van De Goor et al. [20] and by the present authors [21]. The idea was that the electric potential difference between the part of the capillary maintained at high voltage and the grounded parts of the equipment creates a radial electric field in the capillary wall. Therefore, application of the electric field to the capillary leads to effects similar to those reported for capillaries with a voltage offset at the outer surface.

In this paper we present a theoretical model for explaining the existence of the radial electric field in conventional CE units and experimental data showing direct dependence of the EO mobility on the applied voltage in two types of commercially available CE units. In the theoretical treatment the capillary surroundings in conventional CE units are modelled by an outer grounded cylinder containing the capillary. The theory derives a formula for the EO mobility in this system from the basic equations of the electric field. Reproducibility of the EO flow is studied and discussed for various experimental conditions.

2. Theory

A capillary in conventional CE units is contained in a grounded box or a cartridge and has nearby other parts of the unit, such as a carousel or a detector having the electric potential of the ground. The high positive voltage applied to the anode creates not only a uniform electric field within the capillary bore, but also an electric field of a very complex configuration outside the capillary, and, in particular, a radial electric field

in the capillary wall. Obviously, if two points in space have different electric potential, an electric field exists between them. In the case of a capillary these points are the high voltage part of the capillary and grounded parts of the unit. Additionally, for a coiled capillary, parts of the capillary with different electric potential are close to each other and, thus, produce a radial electric field [22].

The radial electric field directed outward from the capillary can only be created by a positive electric charge. As the buffer solution is electrically conductive, the only possible location of the positive charge is in the vicinity of the capillary wall. This positively charged layer, having the thickness of the Debye–Hückel radius, generates the EO flow under the influence of the axial electric field. Note that in the case of an induced positive charge, the existence of the neutralizing negative immobilized charge is not necessary.

In order to calculate the electric field outside and inside the capillary one has to solve the three-dimensional Poisson equation taking into account the geometry of the capillary surroundings and their electrical properties. Although this problem can be solved in principle, it is extremely laborious. As an approximation, we propose to model the surroundings of the capillary by an outer grounded metal cylinder of the large radius r_c .

Consider a cross-section of the capillary at a distance z from the high voltage end of the capillary and having the inner wall radius r_w and the outer capillary radius r_o (Fig. 1). The capillary is contained within an outer metal cylinder of the radius r_c large enough to be assumed as a model of a container, a box or a cartridge surrounding the capillary and having the electrical potential of the ground. The potential difference between the metal cylinder and the bulk of the solution is $V(z)$.

Assume the capillary to be long in comparison with the radius of the outer cylinder. Then, when calculating the radial electric field for the high voltage part of the capillary the axial electric field may be neglected in comparison to the radial one. This approximation should be valid for the part of the capillary distant enough from

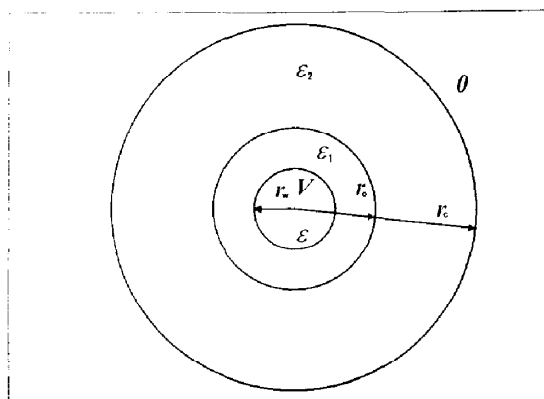


Fig. 1. Scheme of the capillary cross section and its surroundings. r_w is the inner capillary radius, r_o is the capillary outer radius, r_c is the radius of the imaginary grounded outer cylinder, modeling the capillary surroundings, ϵ is the dielectric constant of the solution, ϵ_1 and ϵ_2 are the dielectric constants of the capillary wall and the substance filling the space between the capillary and the grounded parts of the CE unit.

both ends of the capillary. Therefore, we consider a simplified one-dimensional model for the electric field distribution within the capillary instead of a complex three-dimensional one. The errors caused by this simplification do not allow us to suppose a quantitative agreement with experiments, although a qualitative agreement is expected.

2.1. ζ Potential

At this point it is possible to derive the electric potential drop between the bulk of the electrolyte solution in the capillary and its surface, i.e. the ζ potential, and, hence, according to Eq. 1, the expression for the EO mobility. The equation governing electric field in the electrolyte solution is the well-known Poisson–Boltzmann equation

$$\nabla^2 \varphi = -\frac{1}{\epsilon \epsilon_0} F \sum_i z_i C_{i0} \exp\left(-\frac{z_i F(\varphi - V)}{RT}\right) \quad (2)$$

where ∇^2 is the Laplace differential operator, φ is the electric potential in the capillary bore, ϵ is the solution dielectric constant, ϵ_0 is the dielectric permittivity of vacuum, F is the Faraday

constant, i is the counting index of ions in the solution, z_i the valence of the ion i , C_{i0} is its concentration in the bulk, R is the gas constant and T is the absolute temperature in Kelvin.

The first boundary condition at the interface electrolyte–capillary wall follows from the Gauss electrostatic theorem and is given by

$$\varepsilon \frac{\partial \varphi(r_w^-)}{\partial r} = \varepsilon_1 \frac{\partial \varphi_1(r_w^+)}{\partial r} + \frac{\sigma_w}{\varepsilon_0} \quad (3a)$$

where r is the radial coordinate, ε_1 is the dielectric constant of the capillary wall, σ_w is the surface density of the immobilized electric charge, the arguments r_w^- and r_w^+ mean that the derivatives are calculated at the inner and outer side of the boundary $r = r_w$, respectively.

The surface charge density σ_w includes all charges bound to the wall: ionized groups (silanols in case of fused silica) and adsorbed cations. We do not distinguish here the Stern layer but assume it to be included in the immobilized surface charge and to belong to the solid phase.

The second boundary condition at $r = r_w$ expresses the continuity of the electric potential

$$\varphi(r_w^-) = \varphi_1(r_w^+) \quad (3b)$$

In the bulk of the solution the radial electric field should be equal to zero as the radial electric current does not exist and, thus, the electric potential should be equal to V . The term “bulk” means here a domain of the capillary bore distant several Debye–Hückel radii inward from the capillary wall. We denote a length equal to several Debye–Hückel radii by δ_x and set the third boundary condition for Eq. 2 as

$$\varphi(r_w - \delta_x) = V \quad (3c)$$

The ion concentrations in the bulk are subject to the electroneutrality condition

$$\sum_i z_i C_{i0} = 0. \quad (4)$$

The electric potentials φ_1 in the capillary wall and φ_2 in the space between the capillary and the outer cylinder are governed by the Poisson equation.

$$\nabla^2 \varphi_1 = 0, \quad \nabla^2 \varphi_2 = 0. \quad (5)$$

The boundary conditions at the surfaces $r = r_o$ and $r = r_c$ are the continuity of the electric displacement, continuity of the electric potential, and the equality of the potential φ_2 to zero at the grounded surface of the outer cylinder.

$$\varepsilon_1 \frac{\partial \varphi_1(r_o^-)}{\partial r} = \varepsilon_2 \frac{\partial \varphi_2(r_o^+)}{\partial r}, \quad (6)$$

$$\varphi_1(r_o^-) = \varphi_2(r_o^+), \quad \varphi_2(r_c) = 0.$$

Linearizing the Poisson–Boltzmann Eq. 2² for relatively small $F(\varphi - V)/RT$ and ignoring the curvature of the capillary inner surface on the space scale of δ_x one obtains for the electric potential in the solution

$$\varphi = V - \varphi_s \exp\left(-\frac{r - r_w}{\delta}\right), \quad (7)$$

$$\delta = \frac{1}{F} \sqrt{\frac{\varepsilon_0 \varepsilon R T}{\sum_i C_{i0} z_i^2}}$$

where φ_s is an integration constant and δ is the Debye–Hückel radius.

Solutions to Eqs. 5 have the form

$$\varphi_1 = A_1 + A_2 \ln(r), \quad \varphi_2 = B_1 + B_2 \ln(r) \quad (8)$$

where A_1 , A_2 , B_1 and B_2 are constants.

All five unknown constants in Eqs. 7 and 8 can easily be found from the five boundary conditions in Eqs. 3a, 3b, and 6. Eq. 3b can be substituted by a simpler one: $\varphi_1(r_w^+) = V$ because the electric potential difference between the bulk of the electrolyte and the capillary inner surface is not expected to be greater than a hundred millivolts which is negligible in comparison with V (kilovolts).

The solution for the intensity of the electric field in the capillary wall at the interface with the capillary bore is

$$E_1(r_w^+) = \frac{V}{r_w} \left(\ln \frac{r_o}{r_w} + \frac{\varepsilon_1}{\varepsilon_2} \ln \frac{r_c}{r_o} \right)^{-1} \quad (9)$$

² Linearization of the Poisson–Boltzmann equation does not lead to any significant errors for the results presented below.

where E_1 is the radial intensity of the electric field within the capillary wall.

For the ζ potential defined as the potential drop between the bulk of the solution and a capillary inner surface it follows from Eqs. 3a, 7 and 9

$$\zeta = \varphi_s = GV\delta - \frac{\sigma_w \delta}{\epsilon \epsilon_0}, \quad (10)$$

$$G = \frac{\epsilon_1}{\epsilon r_w} \left(\ln \frac{r_o}{r_2} + \frac{\epsilon_1}{\epsilon_2} \ln \frac{r_c}{r_o} \right)^{-1}$$

where G is the geometrical factor. It can be seen from Eq. 10 that the ζ potential comprises two terms. The first term in the right-hand side of Eq. 10 represents an induced ζ potential, vanishing if the applied voltage reduces to zero. The second term is associated with the immobilized surface charge. In the general case, Eq. 10 is not linear but an implicit non-linear equation for ζ , since, as will be discussed below, the surface charge density depends on ζ .

2.2. Induced electroosmotic mobility

According to Eq. 1, EO mobility is directly proportional to the ζ potential. Since the first term in the right hand side of Eq. 10 is not zero if a voltage is applied, the EO mobility is not zero even at very low pH values when silanols are not ionized, and it is proportional to the electric field. We shall call the EO mobility at zero wall charge the "induced EO mobility"

In the case of zero surface charge, one obtains for the EO mobility from Eqs. 1 and 10

$$\mu_i = \beta V, \quad \beta = \frac{\epsilon \epsilon_0 G \delta}{\eta} \quad (11)$$

where μ_i is the induced electroosmotic mobility and β is the slope of the induced EO mobility dependence on the applied voltage. This slope has a dimension of $\text{m}^2 \text{V}^{-2} \text{s}^{-1}$ and is proportional to the capacity of two cylindrical capacitors in series. The expression for the induced electroosmotic mobility can formally be obtained from a capacitor model in a similar way to that presented in [14]. However, the interpretation is different. In a number of papers the induced EO

mobility is explained by an additional polarization charge induced at the capillary surface by the radial electric field. Here, in contrast, we have shown that EO can exist even in the absence of both immobilized surface charge (set $\sigma_w = 0$ in Eq. 10) and the induced polarization charge (set $\epsilon_1 = 1$ and $\epsilon_2 = 1$, no polarization). Even if there is no polarization or immobilized surface charges, the mobile charged layer, which is responsible for electroosmosis, exists due to the existence of the radial potential gradient.

It follows from Eq. 11 that the factor β and, thus, the induced EO mobility depends both on the dielectric constant of the material (possibly coolant) filling the space between the capillary and the distance between the capillary and the grounded cylinder or grounded part of the unit. In general, if the surface charge is not zero the slope β depends on the surface charge due to the non-linearity of Eq. 10. An expression for the slope can be derived from Eqs. 1 and 10.

$$\beta = \frac{\epsilon \epsilon_0 G \delta}{\eta} \left(1 + \frac{d\sigma_w}{d\zeta} \frac{\delta}{\epsilon \epsilon_0} \right)^{-1} \quad (12)$$

Evidently, Eq. 12 generalizes Eq. 11. It is seen from Eq. 12 that the slope of the EO mobility dependence on the applied voltage decreases when the surface charge density depends on the ζ potential.

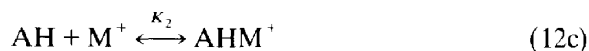
The magnitude of the induced electroosmotic mobility can be estimated as follows. Assume the capillary I.D. to be equal to $75 \mu\text{m}$, $r_o = 176 \mu\text{m}$, $r_c = 3 \text{ cm}$, $\epsilon_1 = 3$ (fused silica), $\epsilon_2 = 1$ (air), $V = 10 \text{ kV}$, $\delta = 5 \text{ nm}$. Eq. 12 gives a mobility of $\mu_i \approx 0.21 \cdot 10^{-8} \text{ (m}^2/\text{Vs)}$ which is not negligible. This value of the electroosmotic mobility corresponds to the induced ζ potential $\zeta_i \approx 3 \text{ mV}$. This value lies within the limit ($\approx 25 \text{ mV}$) of the applicability of the linearized Poisson-Boltzmann equation. If the space between the capillary and the outer cylinder is filled with a fluorocarbonic refrigerant having a dielectric constant $\epsilon_2 \approx 2$ then $\mu_i \approx 0.38 \cdot 10^{-8} \text{ (m}^2/\text{Vs)}$, which is twice as high as in air. One can imagine a situation in which the space is filled with water. For this case $\epsilon_2 = 80$ and Eq. 12 gives $\mu_i \approx 2.1 \cdot 10^{-8} \text{ (m}^2/\text{Vs)}$, which approaches the maximum

value of the induced EO mobility of $2.3 \cdot 10^{-8}$ (m^2/Vs). The latter value is derived from Eq. 12 by setting $r_c = r_o$ and neglecting the second term in the parentheses in the right hand side of Eq. 12. This situation corresponds to a conductive sheath installed at the surface of the capillary [15].

2.3. Limiting electroosmotic mobility and properties of the surface

It can be seen from Eqs. 10 and 11 that at the limit of zero applied voltage the ζ potential and, therefore, the EO mobility, approaches a certain value determined by the immobilized surface charge. We shall denote this limiting value of the ζ potential as ζ_0 and the corresponding EO mobility as μ_0 . In order to calculate the surface charge density and the ζ potential one has to adopt a model of the surface reactions. Several models have been proposed in literature and all of them include the dissociation of the surface silanols. Additionally, Ghowsi and Gale [11] assumed formation of complexes SiOH_2^+ , SiOH_2Cl and SiOK at the surface; Salomon et al. [23] considered complexation of the ionized silanols with the metal ions SiOM ; Huang et al. [13] included in their model only interaction between metal ions and non-ionized silanols. Almost all models supposed the existence of the Stern layer the width of which was used as an additional fitting parameter.

The model used in this paper assumes the following reactions taking place on the surface of the capillary wall, involving dissociation of the surface groups (silanols in the case of fused silica)



where A is the ionized surface group, H^+ is the proton and M^+ is the counter ion. Constants K_a , K_1 and K_2 are the equilibrium constants used for the algebraic relations between the concentrations.

The total surface density of the surface sites is assumed to be fixed and the following equation relates it with the surface concentrations of the ionized and non-ionized groups and positive and neutral complexes.

$$[\text{A}^-] + [\text{AH}] + [\text{AHM}^+] + [\text{AM}] = N \quad (13)$$

where quantities in brackets denote surface concentrations of the ionized groups, non-ionized groups, positive complexes and neutral complexes. N is the total surface concentration of sites.

The surface charge density can be found as

$$\sigma_w = - \frac{eNK_1(K_aK_2 - [\text{M}_s^+][\text{H}_s^+])}{K_1K_2K_a + [\text{M}_s^+]K_2K_a + [\text{H}_s^+][\text{M}_s^+]K_1 + [\text{H}_s^+]K_1K_2} \quad (14)$$

where e is the electron charge.

Concentrations $[\text{M}_s^+]$ and $[\text{H}_s^+]$ of the counter ions and protons are related to their bulk values by the Boltzmann factor [11,13]:

$$\begin{aligned} [\text{M}_s^+] &= [\text{M}^+] \exp\left(\frac{F\zeta}{RT}\right), \\ [\text{H}_s^+] &= [\text{H}^+] \exp\left(\frac{F\zeta}{RT}\right) \end{aligned} \quad (15)$$

It's worth noting that the last relations, Eq. 15, are the source of non-linearity of Eq. 10 as they contain the ζ potential.

Constants of the surface group dissociation K_a and of the counter ion binding K_1 and K_2 were found from the fitting of the experimental data on the dependence of the limiting EO mobility on the solution pH. The geometrical factor G was found from the dependence of the induced EO mobility on the applied voltage and used the same in all calculations.

3. Experimental

Measurements of the EO mobility were performed for solutions in the pH range from 2.3 to 8 using a Beckman P/ACE System 2100 and at pH 4 using a Waters Quanta 4000. In order to exclude all effects of the initial treatment of the capillaries with strong base (a sharp increase in the EO mobility [6,14]), in most experiments the

capillaries were initially rinsed with water for 30 min and with the solution for another 30 min. Between runs the capillaries were stored in the solution. The EO mobility was assessed by measuring the migration time of a neutral marker (phenol or acrylamide). The electric current dependence on the applied voltage was always linear for runs made by using the Beckman system. This indicated no increase in the temperature of the solution. When working with the Waters system, the electric current dependence of the applied voltage was used to find mobilities recalculated to 25°C. The software package described in [24] was utilized for the recalculation. Fused-silica capillaries were from Polymicro (Phoenix, AZ, USA), 375 μm O.D., 75 μm I.D. In series of experiments with a given buffer, the capillary was rinsed with water and then with that particular buffer, as opposed to treatment with KOH (see Fig. 2). The ionic strength in the majority of experiments was kept constant and equal to 5 mM. The solutions used were acetic acid and Tris-acetate solutions. Acetic acid, Tris (hydroxymethylaminomethane) and phenol used as a neutral marker were obtained from Merck (Darmstadt, Germany). Acrylamide used as a neutral marker for measurements at high pH values was from Bio-Rad (Hercules, CA, USA).

4. Results and discussion

4.1. Experimental results

EO mobilities measured in two fused-silica capillaries in the solution of the acetic acid pH = 2.3 are shown in Fig. 2. The ionic strength of the solution is 5 mM and corresponds to the Debye-Hückel radius $\delta = 4.34$ nm; the concentration of the acetic acid is 1.41 M. The neutral marker is 1 mM phenol solution. The upper black boxes represent the mobilities obtained in the capillary treated with KOH before the EO measurements. The group of the experimental points clustering near the lowest line shows the mobilities obtained in different days in the capillary rinsed only with the solution and water. Different

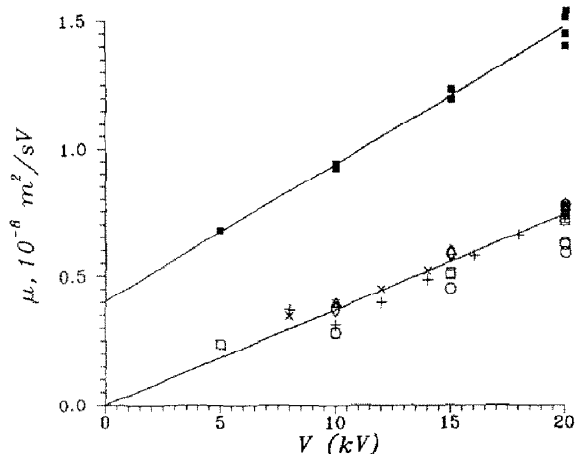


Fig. 2. Dependence of the electroosmotic mobility on the applied voltage in a 1.41 M solution of acetic acid, pH = 2.3, ionic strength 5 mM. Black boxes represent measurements made in a capillary treated before with KOH. Other symbols show results obtained in a capillary rinsed only with water and the solution. Different symbols represent series of measurements performed on different days. Beckman P/ACE System 2100.

symbols correspond to the different days when the measurements were performed. Both groups of experimental points show the dependence of the electroosmotic mobility on the electric field. The significant difference in the mobilities obtained in the capillary treated with KOH and that treated only with the solution and water is in accord with previous findings [6,14]. However, in contrast to [14] the EO mobility shows a voltage dependence in the capillary pretreated with KOH. The limiting mobility in the capillary rinsed with KOH has a non-zero value even at this very low pH indicating a negative charge on the fused-silica surface, whereas in the capillary rinsed only with water and the solution the limiting mobility and the surface charge are negligible.

The best-fit solid line in Fig. 2 is given by the equation

$$\mu = 0.37 \cdot 10^{-12} V$$

where V is the applied voltage in volts. The slope $\beta = 0.37 \cdot 10^{-12}$ was used to calculate the geometrical factor G according to Eq. 11.

EO mobility as a function of the applied

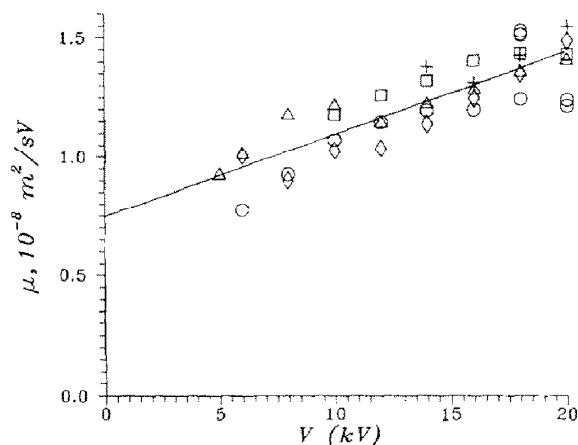


Fig. 3. Dependence of the electroosmotic mobility on the applied voltage in a 6 mM solution of acetic acid, pH = 3.5, ionic strength 0.32 mM. Different symbols represent series of measurements performed on different days. Beckman P/ACE System 2100.

voltage for the 6 mM acetic acid solution pH = 3.5 is shown in Fig. 3. The ionic strength of the solution is 0.32 mM which corresponds to $\delta = 17.2$ nm, i.e. about four times as the Debye-Hückel radius corresponding to the previous case. Different symbols again represent different days when measurements were performed. The linear best fit of the experimental points gives $\mu_0 = 0.75 \cdot 10^{-8} \text{ m}^2 \text{ V}^{-1} \text{ s}^{-1}$ and $\beta = 0.35 \cdot 10^{-12} \text{ m}^2 \text{ V}^{-2} \text{ s}^{-1}$, indicating approximately the same slope as the best-fit line in Fig. 2. However, the limiting mobility μ_0 is rather high. It suggests that at this pH there exists not only induced ζ -potential but also the ζ -potential created by ionized silanol groups at the inner capillary wall.

Fig. 4 shows EO mobility as a function of the applied electric field for the 93.9 mM acetic acid solution and 5 mM Tris which corresponds to pH = 3.5 and ionic strength equal to 5 mM (the same as in Fig. 2). The best-fit line is described by $\mu = 0.16 \cdot 10^{-8} \text{ m}^2 \text{ V}^{-1} \text{ s}^{-1}$ and $\beta = 0.21 \cdot 10^{-12} \text{ m}^2 \text{ V}^{-2} \text{ s}^{-1}$ showing a gentler slope than the previous best-fit lines. The limiting mobility has a value lower than that for the solution of a low ionic strength. It is of interest to note that the slope β here is less than the slope in Fig. 2 corresponding to the solution of the same ionic strength but lower pH.

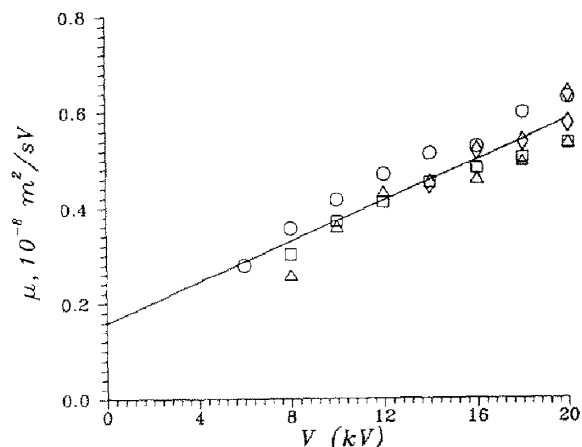


Fig. 4. Dependence of the electroosmotic mobility on the applied voltage in a solution of 93.9 mM acetic acid and 5 mM Tris, pH = 3.5, ionic strength 5 mM. Different symbols represent series of measurements performed on different days. Beckman P/ACE System 2100.

Fig. 5 shows the EO mobility as a function of the applied electric field in the Tris-acetate buffer, pH = 4.75; concentration of acetic acid is 10 mM, concentration of Tris is 5 mM, making the ionic strength equal to 5 mM. Black boxes in the figure show the first series of experiments with this buffer. The dashed line fitting open squares corresponds to the series of measurements obtained after a 40-hour pause (after a

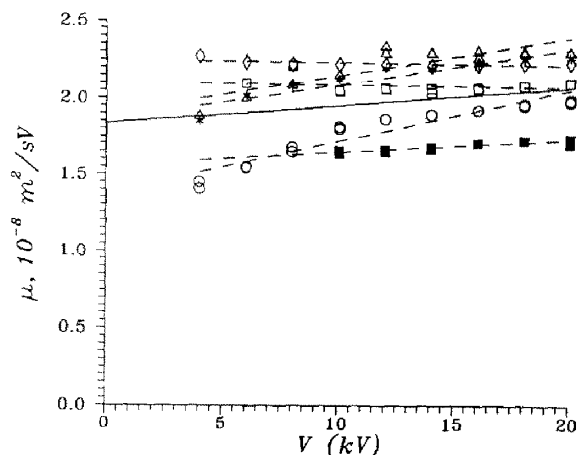


Fig. 5. Dependence of the electroosmotic mobility on the applied voltage in a solution of 10 mM acetic acid and 5 mM Tris, pH = 4.75, ionic strength 5 mM. Different symbols represent series of measurements performed on different days. Beckman P/ACE System 2100.

weekend) and is almost horizontal. The next line with a slope fits experimental points obtained in successive days. The upper horizontal dashed line fits points measured after the second 40-hour pause and the upper line with a slope fits data measured in successive days. It can be seen that the reproducibility of the EO flow improves with time suggesting a saturation of a slow transport process. The line fitting all experimental points gives $\mu_0 = 1.83 \cdot 10^{-8} \text{ m}^2 \text{ V}^{-1} \text{ s}^{-1}$ and $\beta = 0.12 \cdot 10^{-12} \text{ m}^2 \text{ V}^{-2} \text{ s}^{-1}$.

The first series of measurements for a solution of 5 mM acetic acid and 5.25 mM Tris making pH=7 and ionic strength 5 mM are shown by open circles in Fig. 6. Then, unexpectedly, the next day (open squares) the EO mobility dropped. The measurements performed on following days showed less dispersion and gave values grouped between the highest and lowest series. The values for μ_0 and β are $2.18 \cdot 10^{-8} \text{ m}^2 \text{ V}^{-1} \text{ s}^{-1}$ and $0.6 \cdot 10^{-12} \text{ m}^2 \text{ V}^{-2} \text{ s}^{-1}$, respectively.

Experimental data for a solution of 5 mM acetic acid and 7.5 mM Tris making pH=8 and ionic strength 5 mM are shown by open circles in Fig. 7. In contrast to the previous case but similar to the case of pH=4.75, the lowest EO mobilities were found on the first day of measurements (open circles). The second day gave slightly higher values and then, after a 40-hour

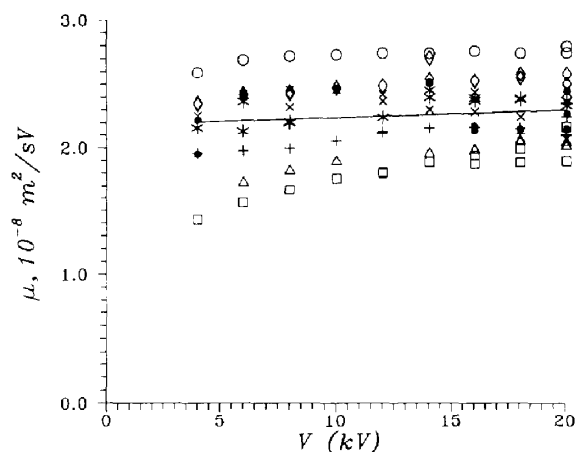


Fig. 6. Dependence of the electroosmotic mobility on the applied voltage in a solution of 5 mM acetic acid and 5.25 mM Tris, pH=7, ionic strength 5 mM. Beckman P/ACE System 2100.

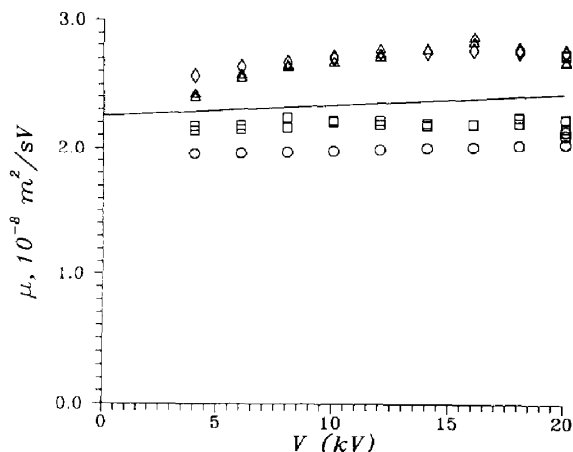


Fig. 7. Dependence of the electroosmotic mobility on the applied voltage in a solution of 5 mM acetic acid and 7.5 mM Tris, pH=8, ionic strength 5 mM. Beckman P/ACE System 2100.

pause, the EO mobility increased significantly (triangles) and then remained approximately the same (diamonds). μ_0 and β are $2.25 \cdot 10^{-8} \text{ m}^2 \text{ V}^{-1} \text{ s}^{-1}$ and $0.9 \cdot 10^{-12} \text{ m}^2 \text{ V}^{-2} \text{ s}^{-1}$, respectively.

Fig. 8 shows the EO mobility measured in a solution of 33.1 mM acetic acid and 5 mM KOH having pH=4 and ionic strength 5 mM. The measurements were performed on the Waters Quanta 4000 having a geometry of the capillary environment different from that of the Beckman P/ACE 2100 system. Three groups of experimen-

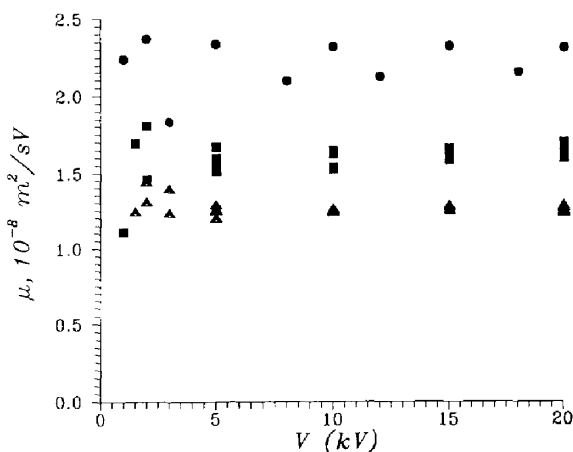


Fig. 8. Dependence of the electroosmotic mobility on the applied voltage in a solution of 33.1 mM acetic acid and 5 mM KOH, pH=4, ionic strength 5 mM. Waters Quanta 4000.

tal points correspond to the measurements performed with the time interval of three weeks. During this time the capillary contained the buffer solution inside. The highest mobility corresponds to the latest series of measurements. The main characteristic for all the series of measurements shown in this figure is the absence of a measurable slope, i.e. $\beta = 0$.

4.2. Comparison of the experimental results with the theory

Limiting mobilities obtained as the results described in the previous section together with their confidence intervals are shown by the circles in Fig. 9. The solid line in this figure is found by numerically solving Eq. 10 with $V = 0$ and with the surface charge density given by Eqs. 14 and 15. The total surface density of silanol sites N was taken from literature as $N = 8.31 \cdot 10^{-6} \text{ mol/m}^2$ (5 nm^{-2}). The $\text{p}K_a$ of silanols was found by fitting the experimental points and happened to be equal to 6.3 which is exactly the same value as found by Huang [13]. However, this could represent a mean $\text{p}K$ value and does not per se exclude the existence of a spectrum of $\text{p}K$ values. A $\text{p}K_a$ less than 6.3 would contradict that the limiting mobility at $\text{pH} = 2.3$ is as close to zero as shown in Fig. 9. The binding constants

K_1 and K_2 were also determined from the fitting. Their $\text{p}K$'s were found to be equal to $\text{p}K_1 = 4.92$ and $\text{p}K_2 = 0.25$, respectively. Neither scaling nor introduction of additional fitting parameters like Stern's layer capacity or immobile layer capacity was necessary to fit the experimental data.

It is of interest to note that the steep transition in Fig. 9 has the inflection point between $\text{pH} 4$ and 5 whereas $\text{p}K_a$ of silanols was found to be 6.3. Therefore the transition should be attributed to the combined action of the ion complexation and silanol ionization at the capillary surface. The EO mobility at high pH is determined to a great extent by the value of the constant K_1 , controlling complexation of the cations with the ionized silanols. If this mechanism were not taken into account in our model for the surface charge, Eq. 12b, we would obtain EO mobility values 6–7 times higher than those experimentally found. It was necessary to include in the model additional complexation of the neutral silanols with the buffer cations described by the constant k_2 , Eq. 12c, to fit intermediate values of the limiting EO mobility at $\text{pH} = 3.5$ and 4.75.

A relatively high value for the experimentally obtained limiting mobility at $\text{pH} = 4.75$ suggests the possibility of an additional mechanism affecting the surface charge. It might be additional binding of protons by neutral silanols, a change of binding constants and silanol $\text{p}K$ with the increase of the surface charge, or, finally, existence of a spectrum of the silanol $\text{p}K$'s in stead of a single value.

Experimentally measured (circles) and theoretically found (asterisks) slopes β as a function of pH are plotted in Fig. 10. It can be seen from this figure that the theory explaining the EO voltage dependence based on the concept of the radial electric field is in agreement with experiments. Fig. 10 shows a decrease of the slope β , i.e. a decrease of the voltage control of the EO flow with the pH increase. This fact agrees with experiments performed in the capillaries with the radially applied electric field [13].

The results presented in Fig. 3 for the low ionic strength solution of acetic acid are in relative agreement for the limiting mobility [$0.75 \cdot 10^{-8} \text{ m}^2 \text{ V}^{-1} \text{ s}^{-1}$ (experimental) and $1.15 \cdot$

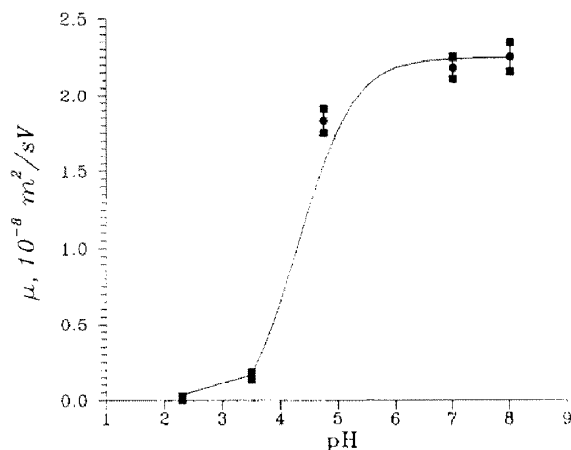


Fig. 9. Limiting EO mobilities corresponding to the measurements shown in Figs. 2, 4, 5, 6 and 7. Solid line represents simulation.

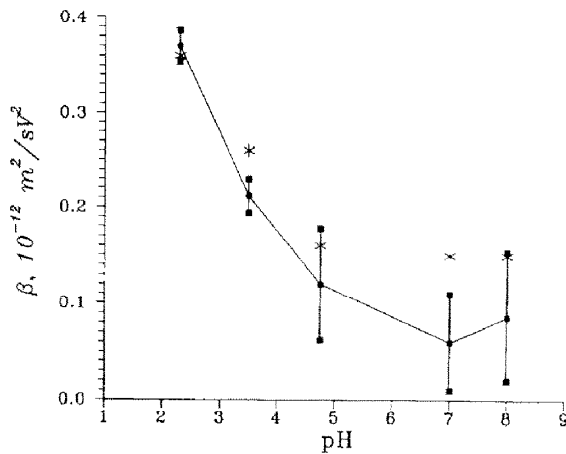


Fig. 10. Slopes of the dependencies of the EO mobilities against applied voltage corresponding to the measurements shown in Figs. 2, 4, 5, 6 and 7. Asterisks represent simulation.

$10^{-8} \text{ m}^2 \text{ V}^{-1} \text{ s}^{-1}$, (theoretically calculated)] and show a discrepancy for the slope [$0.35 \cdot 10^{-12} \text{ m}^2 \text{ V}^{-2} \text{ s}^{-1}$ (experimental) and $0.94 \cdot 10^{-12} \text{ m}^2 \text{ V}^{-2} \text{ s}^{-1}$ (theoretical)]. Therefore, the model for the surface interaction with the electrolyte solution still needs improvement.

The short-term (within hours) and long-term (days and weeks) variations of the EO mobility found in this study were attributed to the ion-exchange properties of the silica wall [25] and, also, the influence of the radial electric field. This radial electric field keeps embedding cations into the silica wall (physically driving them to penetrate the wall) to such an extent as to continuously alter the EOF (which keeps increasing as the voltage is augmented). Only after several hours (or even days) of continuous operation does the EOF approach a plateau, probably as a compromise between electrical forces driving cations into the wall and diffusive forces pushing them out. It should also be noted that different geometries of the metal box surrounding the capillary can have a profound effect on the EOF. Thus in the Beckman unit, where the metal frame is very close to the capillary, there is a stronger dependence of EOF on voltage since the radial voltage is quite high. Conversely in the Waters Quanta, where the metal box is quite a

distance from the capillary, such voltage dependence of the EOF is much reduced.

Therefore, we suppose that cations are not only attracted by the negatively charged surface, but that they can also enter the capillary wall. This continuous penetration of ions (strongly enhanced by the radial electric field) might result in an additional ionization of silanols (which would normally be protonated). An experimental evidence for the ion penetration into the capillary wall at high pH was given recently by Wu et al. [26].

As a final remark, our data could shed some light on the work of Lambert and Middleton [27], who reported a pH hysteresis effect with silica capillaries. They observed that the EOF would depend on the preconditioning of the fused-silica column. EOF values obtained from a column previously exposed to acidic conditions for varying periods of time were consistently lower than the values from columns previously exposed to alkaline conditions, particularly in the pH 4–6 region. The equilibration of surface charges on the capillary thus preconditioned could take as long as two weeks. These data can now be interpreted on the basis of our hypothesis of penetration of cations inside the wall: clearly, when preconditioning at pH 2 such penetration would be negligible, whereas a preconditioning step at pH 12 would considerably enhance this phenomenon.

5. Conclusions

1. The EO flow is observed in fused-silica capillaries even in solutions of very low pH as 2.3. However, the EO flow shows linear dependence on the applied voltage and the EO mobility extrapolated to zero voltage approaches zero.

2. The experimentally found dependence of the EO mobility on the intensity of the applied electric field is explained by the radial electric field caused by the surroundings of the capillary. The latter indicates that EO mobility measurements depend on the instrument used for measurements and that the characteristic of the

capillary wall should be evaluated from the EO mobility extrapolated to zero voltage.

3. The reproducibility of the EO flow decreases with the increase of the solution pH. A probable explanation is the interaction of cations (adsorption and diffusion enhanced by the electric field) with the fused-silica wall.

Acknowledgements

Supported in part by a grant from the European Community (Human Genome Analysis, N. GEN-CT93-0018) and by Progetto Strategico Comitato Chimica. (CNR, Roma).

Note added

After this manuscript was accepted, we heard of a paper from the group of E. Gruska [28] also describing the unreproducibility of EOF flow and ways to control it. According to them, additives such as amines added to the background electrolyte completely stabilize the EOF flow. Although they do not mention the radial electric field as a possible source for EOF unreproducibility, they mention a mechanism for such phenomenon which is indeed complementary to our observations. According to them (verbatim): the amines (added to the standard buffer) "should shield the wall from impurities in the buffer that otherwise might be adsorbed onto it. The adsorption of impurities will change the nature of the double layer on the wall and therefore change EOF". What we are saying is (verbatim): "this radial electric field keeps embedding cations into the silica wall (physically driving them to penetrate the wall) to such an extent as to continuously alter the EOF (which keeps increasing as the voltage is augmented)." Clearly, while hypothesizing different causes, we are talking about the same mechanism for EOF unreproducibility!

References

- [1] D.J. Shaw, *Electrophoresis 1969*. Academic Press, London.
- [2] P.D. Grossman, in P.D. Grossman and J.C. Colburn (Editors), *Capillary Electrophoresis 1992*, Academic Press, San Diego, 3–43.
- [3] R.J. Hunter, in J.O'M. Bockris, B.E. Conway and E. Yeager (Editors), *Comprehensive Treatise of Electrochemistry, Vol. 1: The Double Layer*, Plenum Press, New York, 1980, pp. 397–437.
- [4] K.D. Lukacs and J.W. Jorgenson, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 8 (1985) 407–411.
- [5] W. Schutznier and E. Kenndler, *Anal. Chem.*, 64 (1992) 1991–1995.
- [6] C. Schwer and E. Kenndler, *Anal. Chem.*, 63 (1991) 1801–1807.
- [7] J. Kohr and H. Engelhardt, *J. Microcol. Sep.*, 3 (1991) 491–495.
- [8] B.B. Van Ormann, G.G. Liversidge and G.L. McIntire, *J. Microcol. Sep.*, 2 (1991) 176–180.
- [9] C.S. Lee, W.C. Blanchard and C.-T. Wu, *Anal. Chem.*, 62 (1990) 1550–1552.
- [10] C.S. Lee, D. McManigill, C.-T. Wu and B. Patel, *Anal. Chem.*, 63 (1991) 1519–1523.
- [11] K. Ghowsi and R.J. Gale, *J. Chromatogr.*, 559 (1991) 95–101.
- [12] C.S. Lee, C.-T. Wu, T. Lopes and B. Patel, *J. Chromatogr.*, 559 (1991) 133–140.
- [13] T.-L. Huang, P. Tsai, C.-T. Wu and C.S. Lee, *Anal. Chem.*, 65 (1993) 2887–2893.
- [14] M.A. Hayes and A.G. Ewing, *Anal. Chem.*, 64 (1992) 512–516.
- [15] M.A. Hayes, I. Kheterpal and A.G. Ewing, *Anal. Chem.*, 65 (1993) 27–31.
- [16] M.A. Hayes, I. Kheterpal and A.G. Ewing, *Anal. Chem.*, 65 (1993) 2010–2013.
- [17] C. A. Keeley, R.H. Holloway, T.A.A.M. van de Goor and D. McManigill, *J. Chromatogr. A*, 652 (1993) 283–289.
- [18] H.T. Rasmussen and H.M. McNair, *J. Chromatogr.*, 516 (1990) 223–231.
- [19] H.J. Issaq, I.Z. Atamna, G.M. Muschik and G.M. Janini, *Chromatographia*, 32, (1991) 155–161.
- [20] T.A. van de Goor, C.A. Keely, R.R. Holloway and D. McManigill, presented at the *Sixth International Symposium on HPCE, Jan 31–Feb 3, 1994, San Diego, CA*.
- [21] M.S. Bello, L. Capelli and P.G. Righetti, *Sixth International Symposium on HPCE, Jan 31–Feb 3, 1994, San Diego, CA*, poster p-335.
- [22] W.D. Cole and D. McManigill, *Sixth International Symposium on HPCE, Jan 31–Feb 3, 1994, San Diego, CA*, poster p-326.
- [23] K. Salomon, D.S. Burgi and J.C. Helmer, *J. Chromatogr.*, 559 (1991) 69–80.
- [24] M.S. Bello, E.I. Levin and P.G. Righetti, *J. Chromatogr. A*, 652 (1993) 329–336.
- [25] R. Virtanen, *Acta Polytechnica Scand.*, 123 (1974) 1–67.
- [26] C.T. Wu, T.L. Huang and C.S. Lee, *J. Chromatogr. A*, 652 (1993) 277–281.
- [27] W.J. Lambert and D.L. Middleton, *Anal. Chem.*, 62 (1991) 1585–1587.
- [28] N. Cohen and E. Grushka, *J. Chromatogr. A*, 678 (1994) 167.