



ELSEVIER

Journal of Chromatography A, 709 (1995) 63–68

JOURNAL OF
CHROMATOGRAPHY A

Contribution of the electroosmotic flow to peak broadening in capillary zone electrophoresis with uniform zeta potential

Bohuslav Gaš^{a,*}, Milan Štědrý^a, Ernst Kenndler^b

^aFaculty of Science, Charles University, Albertov 2030, CZ-128 40 Prague 2, Czech Republic

^bUniversity of Vienna, Institute of Analytical Chemistry, Währinger Strasse 38, A-1090 Vienna, Austria

Abstract

An expression is derived which gives the plate height contribution caused by an electroosmotic flow (EOF) in a cylindrical capillary with longitudinally uniform zeta potential. The derivation is made in terms of effective thickness of the electric double layer (an analogue to the Debye length). Typical values of the effective thickness calculated for common situations are given. The resulting expression for the plate height, $H_{\text{eof}} = \beta^2 v_{\text{eof}} / D$, enables one to calculate the plate height simply as a function of the diffusion coefficient, D , of the solute, the electroosmotic velocity, v_{eof} , and the thickness of the electric double layer, β . The impact of peak broadening by the EOF is compared with that from longitudinal diffusion and extra-column effects for solutes with widely varying diffusion coefficients.

1. Introduction

On the border between a liquid and a solid phase, the intensity of the electric field is not necessarily zero owing to the presence of an electric double layer. The structure of this double-layer is described by various models, of which the Stern–Gouy–Chapman model [1] is generally accepted. According to this model, it consists of a compact or Stern layer that adheres on the solid surface and the diffuse layer in the liquid, where the volume charge density is significantly greater than in the rest of liquid. For an aqueous solution of small ions in contact with, e.g., fused silica, glass or even an organic polymer such as PTFE, the Stern layer mostly possesses an excess

of negative charge. This is a result of adsorption of negative ionic species and/or protolysis of dissociable groups on the surface.

The excess of negative charge on the surface must be compensated for by an excess of positive ions in the diffuse layer. Consequently, the electroneutrality condition in the diffuse layer is no longer valid.

The plane formed by the centres of the ions from the diffuse layer, which are in closest proximity to the compact layer, is named the outer Helmholtz plane. The diffuse layer in principle ranges from the outer Helmholtz plane to infinite distance and it is of primary importance for electrokinetic effects. The excess of net charge in the diffuse layer is responsible for the electroosmotic flow (EOF) that can occur in electrophoretic separation columns. In this case, the longitudinal driving electric field acting in the

* Corresponding author.

direction of the column axis generates the driving electroosmotic force in the diffuse layer. The net charge in the layer, however, drops to zero very quickly when the distance from the wall exceeds several nanometers under circumstances usual in capillary zone electrophoresis (CZE).

The potential profile in the diffuse layer for a double layer with planar geometry can be attained by solving the Poisson–Boltzmann equation [2,3]. Rice and Whitehead [4] calculated stationary velocity profiles of the EOF in cylindrical capillaries employing a linear approximation of the exponential terms in the Poisson–Boltzmann equation. They pointed out, however, that this approximation fails when the potential in the diffuse layer becomes higher than ca. 50 mV.

Martin and co-workers [5,6] determined the contribution of the radial electroosmotic velocity profile in a capillary tube to the sample peak dispersion. They approximated the original velocity profile either by a power function or by a partly flat and a partly quadratic profile. It should be noted that they also employed the linear approximation of the Poisson–Boltzmann equation [5,6].

Andreev and Lisin [7] made a detailed study of potential and velocity radial profiles in a capillary tube numerically without any linearization. The temperature effects acting significantly in CZE were also taken into account.

It is the main aim of this paper to give a derivation of the contribution of the EOF to the peak dispersion in open CZE columns assuming a longitudinally uniform driving electroosmotic force along the capillary column. This is assessed in terms of the fraction of the solute in the diffuse layer which lags behind the travelling peak. The profiles of the electric potential in the diffuse layer are calculated for cylindrical symmetry based on the Poisson–Boltzmann equation without any approximation in accordance with Andreev and Lisin [7]. This approach is not limited to either the magnitude of the potential or any linearization. This approach enables us to express the peak dispersion contribution of the EOF by a simple closed equation.

2. Assumptions of the model

An open cylindrical capillary tube of radius a with homogeneous properties across the length is assumed to be filled with a homogeneous liquid solution of a background electrolyte consisting of n species of ions, the i th of which has a concentration c_i^0 and charge number z_i . As a consequence of the longitudinal homogeneity, the properties of the electric double layer are also homogeneous across the length. Further, the setting of the problem exhibits cylindrical symmetry. As the studied process is homogeneous with respect to the coordinate which can be identified with the axis of the capillary, there is only one spatial coordinate, the radial coordinate r , on which all quantities depend. If the net charge density at a distance r from the axis of the capillary is denoted by $\rho(r)$, the outer driving electric field of constant strength E exerts a driving force $E\rho(r)$ on a unit volume of the electrolyte.

The radial distribution of the electric potential ϕ is given by the Poisson–Boltzmann equation in the cylindrically symmetrical setting

$$\begin{aligned} \frac{1}{r} \cdot \frac{d}{dr} \left(r \cdot \frac{d\phi}{dr} (r) \right) \\ = - \frac{1}{\varepsilon} \sum_{i=1}^n c_i^0 z_i F \exp \left[- \frac{z_i F \phi(r)}{RT} \right] \end{aligned} \quad (1)$$

where ε is the permittivity of the electrolyte solution, R is the gas constant, T is the absolute temperature and F is the Faraday constant. With the two boundary conditions

$$\frac{d\phi}{dr} (0) = 0, \phi(a) = \zeta \quad (2)$$

Eq. 1 determines the potential ϕ on $r \in \langle 0, a \rangle$. The value of the potential ϕ on the outer Helmholtz plane, for simplicity identified here with the wall of the capillary, is denoted by ζ and is called the electrokinetic or zeta potential.

Only the axial component, v_x , of the velocity of the EOF depends on the r coordinate, i.e., $v_x = v_x(r)$. It can be derived that the stationary radial velocity profile of v_x is

$$v_x(r) = \frac{\varepsilon E}{\eta} [\phi(r) - \zeta] \quad (3)$$

where η is the dynamic viscosity of the liquid. Since $\phi(0)$ is nearly zero in electrophoretic columns with diameters above 10 μm , the stationary velocity of the flow at the centre of the capillary is

$$v_x(0) \equiv v_{eo} = -\varepsilon E \zeta / \eta \quad (4)$$

This is identical with the well known Helmholtz–Smoluchowski relationship.

3. Derivation and discussion

3.1. Effective thickness of the electric double layer

The total axial flux J_x of an analyte with concentration c_a moving in a capillary tube is given by

$$J_x = c_a 2\pi \int_0^a r v_x(r) dr \quad (5)$$

In the derivation of the lagged part, it is assumed that the same flux J_x is transported by the velocity v_{eo} but in a cylinder with radius $a - \beta$, so

$$J_x = c_a \pi (a - \beta)^2 v_{eo} \quad (6)$$

where β is the effective thickness of the diffuse layer and can be interpreted as the thickness of the cylindrical sheath of liquid sticking on the column wall without being moved. Equating Eqs. 5 and 6, β is then

$$\beta = a - \sqrt{\frac{2}{v_{eo}} \int_0^a r v_x(r) dr} \quad (7)$$

The effective thickness β is an analogue of the Debye length (or thickness) κ^{-1} , which is the effective thickness of the ionic atmosphere but obtained from the linearized Poisson–Boltzmann equation and is defined for, e.g., uni–univalent electrolytes as

$$\kappa^{-1} = \frac{1}{F} \sqrt{\frac{\varepsilon RT}{2c}} \quad (8)$$

For the calculation of β , the following two steps are to be followed:

- (i) Calculation of the potential $\phi(r)$ by solving the differential Eqs. 1 and 2. The equations were solved numerically by the Hamming predictor–corrector method [8] after substituting $d\phi/dr$. This enables one to solve the set of two differential equations of the first order. Integration starts from the point $r = a$ with $\phi(a) = \zeta$. The boundary condition in $r = 0$, $d\phi/dr(0) = 0$, is attained by iterative shooting.
- (ii) Determining β by numerical integration of Eq. 7 using Eq. 3. The numerical integration was performed using the Simpson formula.

Table 1 shows the effective thickness β calculated for various ζ potentials (or corresponding electroosmotic mobilities u_{eo} , defined as $u_{eo} = v_{eo}/E$) and various concentrations c^0 of an aqueous solution of a uni–univalent electrolyte ($i = 1, 2$; $c_1^0 = c_2^0 \equiv c^0$; $z_1 = 1$; $z_2 = -1$) acting as the background electrolyte. For comparison, the corresponding Debye lengths are also given in Table 1. The difference between β and κ^{-1} is caused by linearization of the exponential terms when deriving the Debye length κ^{-1} . Although the linearization may be well founded in deriving the ionic atmosphere of a single ion [9], it fails in the case of an electric double layer when the zeta potential exceeds about 50 mV [4] (see also Table 1), which corresponds to an electroosmotic mobility of about $35 \cdot 10^{-9} \text{ m}^2 \text{ V}^{-1} \text{ s}^{-1}$. However, such a situation is commonly reached in CZE when using untreated silica capillaries. It can be seen that the deviation is significant for high zeta potentials where β is found to be nearly a factor of two smaller than κ^{-1} .

3.2. Peak dispersion due to longitudinally uniform EOF

The total peak dispersion is the result of the individual contributions. For a linear case the

Table 1
Dependence of the effective thickness β on concentration of a uni-univalent electrolyte and zeta potential ζ

ζ (V)	u_{eo} ($10^{-8} \text{ m}^2 \text{ V}^{-1} \text{ s}^{-1}$)	Concentration c^0 (mmol l $^{-1}$)				
		0.01	0.1	1	10	100
β (nm)						
0.02	1.38	95.1	30.1	9.51	3.01	0.951
0.05	3.45	91.1	28.8	9.11	2.88	0.911
0.1	6.91	79.8	25.2	7.98	2.52	0.798
0.2	13.81	55.8	17.7	5.58	1.77	0.558
κ^{-1} (nm)						
		95.9	30.3	9.59	3.03	0.959

u_{eo} = Electroosmotic mobility; κ^{-1} = Debye length.

final variance of the peak is the sum of the individual peak variances. In the case of EOF the lagging sheath of liquid in the diffuse layer near the column wall causes a radial diffusion flux of the lagged part of the analyte from the wall to the centre. Consequently, the axial profile of the sample moving in a capillary column will be dispersed. This will be the contribution of the uniform EOF to the total peak dispersion.

From the effective thickness β , the fraction F of liquid sticking on the column wall and virtually not moving can be found. Assuming $\beta \ll a$ in common electrophoretic columns, this fraction is given by

$$F = \frac{2\pi a\beta}{\pi a^2} = \frac{2\beta}{a} \quad (9)$$

Knowing the fraction F , an approximate derivation of the influence of the radial velocity profile $v_x(r)$ on peak dispersion in CZE can be performed. Aris [10] and Giddings [11] derived that the variance σ^2 of a solute peak caused by a non-movable fraction F at the capillary wall but undergoing radial diffusional transport in the case of plug-like flow with velocity \bar{v} is

$$\sigma^2 = \frac{F^2(1-F)a^2}{4D} \cdot \bar{v}^2 t \quad (10)$$

where D is the diffusion coefficient of the ana-

lyte. This model can be adopted for derivation of the peak dispersion due to a uniform EOF when assuming that $\beta \ll a$. In the case under discussion, the fraction F is very small compared with 1 and the assumptions that $\bar{v} \approx v_{\text{eo}}$ and $1 - F \approx 1$ are acceptable. Eq. 10 can further be simplified by substituting F and introducing β , leading to

$$\sigma_{\text{eo}}^2 = \frac{\beta^2}{D} \cdot v_{\text{eo}}^2 t \quad (11)$$

where σ_{eo}^2 is the variance caused by the uniform EOF. Transformation of this expression using the relationship between the length-based variance and the theoretical plate height H_{eo} leads to the simple equation

$$H_{\text{eo}} = \frac{\beta^2}{D} \cdot v_{\text{eo}} \quad (12)$$

This expression enables one to determine easily the dispersion caused by the EOF for a given electroosmotic velocity. Naturally, the validity of Eq. 12 is restricted to cases where the dimension of molecules or particles of the analyte is considerably smaller than the effective thickness β .

It should be realized that the described derivation is in fact a simplification of the approach introduced by Martin and co-workers [5,6] for conditions that are typical in CZE. Further, it is worth noting that the plate height is not depen-

dent on the radius of the column, at least if $\beta \ll a$.

It is useful to compare the contribution of the uniform EOF to the plate height with that originating from the inevitable longitudinal diffusion, H_{dif} , which is expressed by $H_{\text{dif}} = 2D/v_{\text{eo}}$. The relationship between these two contributions can clearly be seen from the ratio $H_{\text{eo}}/H_{\text{dif}}$, which reflects the impact of the EOF on the efficiency relative to diffusion and is expressed by

$$\frac{H_{\text{eo}}}{H_{\text{dif}}} = \frac{\beta^2 v_{\text{eo}}^2}{2D^2} \quad (13)$$

For given experimental conditions, this ratio depends strongly on the nature of the solute because the diffusion coefficient of the particular compound is squared in the denominator. In Fig. 1, the ratio $H_{\text{eo}}/H_{\text{dif}}$ is depicted as a function of the diffusion coefficient for various velocities. For the usual conditions, e.g., a concentration of

0.01 mol l⁻¹ of a uni-univalent background electrolyte (β is about 3 nm, see Table 1), the effect of peak broadening due to the EOF is in fact negligible for small solutes for all realistic velocities. It increases with decreasing diffusion coefficient (and increasing mass). For a velocity of 1–2 mm s⁻¹ (which is common in CZE) it reaches the same value as the longitudinal diffusional dispersion for compounds with a diffusion coefficient in the range $2 \cdot 10^{-12}$ – $5 \cdot 10^{-12}$ m² s⁻¹; here the ratio is unity.

It is obvious that even under these conditions both diffusional and electroosmotic contributions will probably not play a significant role in peak broadening because other effects not taken into account here, such as extra-column or adsorption effects, will dominate. Therefore, not only the relationship between diffusional and electroosmotic dispersion must be considered, but also the magnitude of the individual peak broadening contributions. For the above-mentioned usual conditions in CZE (background concentration 0.01 mol l⁻¹, $\beta \approx 3$ nm, electroosmotic velocity 1–2 mm s⁻¹), the contribution of the EOF will be negligible for almost all solutes.

The situation can change when the concentration of the background electrolyte is decreased. Some examples of these contributions are given in Table 2 for a concentration of 1 mmol l⁻¹ and not too large ζ potentials ($\beta \approx 10$ nm) for six compounds with various diffusion coefficients. For a small molecule such as an aromatic acid, H_{dif} is about 10^{-6} m, in contrast to the minute value of $6 \cdot 10^{-11}$ m for H_{eo} . For large solutes, however, both plate height contributions are in the same range of 10^{-8} – 10^{-7} m.

Such a plate height may be of the same order of magnitude as that caused by extra-column peak broadening, e.g., due to the finite length of the injection zone. Taking a length of 1 mm in a capillary with a 250-mm distance between the injector and detector, the corresponding contribution of the initial sample plug length to peak broadening (expressed again by the plate height) is $3.3 \cdot 10^{-7}$ m.

For lower concentrations of the background electrolyte, e.g. 10^{-4} mol l⁻¹ (leading to $\beta \approx 25$ nm), the electroosmotic dispersion might in fact

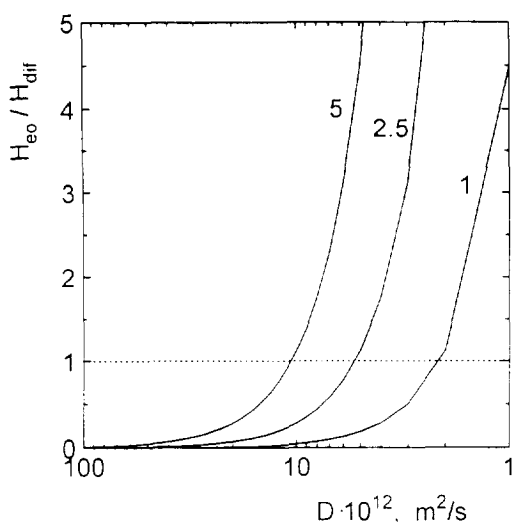


Fig. 1. Contribution of the EOF to the plate height, H_{eo} , related to that caused by longitudinal diffusion, H_{dif} , as a function of the diffusion coefficient, D , of the solute. Curves for electroosmotic velocities of 1, 2.5 and 5 mm s⁻¹ are shown. The effective thickness, β , is 3 nm, which corresponds to a concentration of the background electrolyte of about 10 mmol l⁻¹.

Table 2
Contributions of EOF and longitudinal diffusion to peak broadening for five compounds with different diffusion coefficients

Compound	Molecular mass ^a	Diffusion coefficient (m ² s ⁻¹) ^a	H _{eo} (m)	H _{diff} (m)
Naphthalenesulphonic acid	200	5 · 10 ⁻⁹	6 · 10 ⁻¹¹	3.3 · 10 ⁻⁶
Myoglobin	18 000	1 · 10 ⁻¹⁰	3 · 10 ⁻⁹	6.6 · 10 ⁻⁸
Albumin	69 000	6 · 10 ⁻¹¹	5 · 10 ⁻⁹	4 · 10 ⁻⁸
Glutamate dehydrogenase	313 000	4 · 10 ⁻¹¹	7.5 · 10 ⁻⁹	2.7 · 10 ⁻⁸
α ₂ -Macroglobulin	820 000	2.4 · 10 ⁻¹¹	1.3 · 10 ⁻⁸	1.6 · 10 ⁻⁸

Conditions: electroosmotic velocity $v_{eo} = 3 \text{ mm s}^{-1}$; concentration of the background electrolyte $c^0 = 1 \text{ mmol l}^{-1}$ ($\beta = 10 \text{ nm}$).

^a Molecular masses and diffusion coefficients of proteins were taken from Ref. [12].

dominate both of the other effects (diffusion and extra-column) for large solutes.

Hence one can conclude that a significant contribution to peak dispersion due to the EOF can arise in such rare, but not unrealistic, cases.

Acknowledgement

This work was supported by the Grant Agency of the Czech Republic, grant No. 203/93/0718.

References

- [1] P. Delahay, Double Layer and Electrode Kinetics, Interscience, New York, 1965.
- [2] W.J. Moore, Physical Chemistry, Prentice-Hall, Englewood Cliffs, NJ, 1972.
- [3] O.F. Devereux and P.L. De Bruyn, Interaction of Plane Parallel Double Layers, MIT Press, Cambridge, MA, 1963.
- [4] C.L. Rice and R. Whitehead, J. Phys. Chem., 69 (1965) 4017–4024.
- [5] M. Martin and G. Guiochon, Anal. Chem., 56 (1984) 614–620.
- [6] M. Martin, G. Guiochon, Y. Walbroehl and J.V. Jorgenson, Anal. Chem., 57 (1985) 559–561.
- [7] V.P. Andreev and E.E. Lisin, Electrophoresis, 13 (1992) 832–837.
- [8] A. Ralston, A First Course in Numerical Methods, McGraw-Hill, New York, 1965.
- [9] J.C. Poirier, in B.G. Conway and R.G. Barradas (Editors), Chemical Physics of Ionic Solutions, Wiley, New York, 1934.
- [10] R. Aris, Proc. R. Soc. London, Ser. A, 252 (1959) 538.
- [11] J.C. Giddings, Dynamics of Chromatography, Marcel Dekker, New York, 1965.
- [12] H. Ch. Curtius and M. Roth (Editors), Clinical Biochemistry, Principles and Methods, Vol. I, Walter de Gruyter, Berlin, 1974.