ELSEVIER

Journal of Chromatography A, 781 (1997) 277–286

JOURNAL OF CHROMATOGRAPHY A

Protein band spreading in capillary zone electrophoresis Effects of sample zone length and presence of polymer

Sergey P. Radko[a,*], George H. Weiss[b], Andreas Chrambach[a]

[a]*Section on Macromolecular Analysis, Laboratory of Cellular and Molecular Biophysics, National Institute of Child Health and Human Development, National Institutes of Health, Building 10, Room 6C215, Bethesda, MD 20892, USA*

[b]*Physical Sciences Laboratory, Division of Computer Research and Technology, National Institutes of Health, Building. 12A, Room 2002, Bethesda, MD 20892, USA*

Abstract

It is commonly accepted that intra-column zone dispersion in CZE rests on multiple mechanisms, viz. diffusion, interaction of analyte with the capillary walls, Joule heat and conductivity differences between sample zone and the surrounding buffer. The most important extra-column contributor to bandwidth is thought to be the starting zone width. The present study shows that the length of the starting zone above 10 mm is linearly related to the bandwidth of R-phycoerythrin (M_r $290 \cdot 10^3$). Below that length, bandwidth demonstrates a plateau preceded by a slight rise. Within the 'plateau range', the ratio of bandwidth to effective capillary length is close to constant while it is independent of electric field strength in the range of 37 to 370 V cm^{-1} and of protein concentration in the range of 0.1 to 1000 μg ml^{-1}. The experimental observations support the notion that the analyte–wall interaction is the determining source of intra-column zone dispersion. A slight rise observed at initial zone lengths of less than 2 mm was accounted for by a diffusion model taking into account a non-local initial concentration of analyte. The presence of polyethyleneglycol in the buffer within a concentration range up to 6% does not affect bandwidth. Above that concentration, the level of constant bandwidth is raised. © 1997 Elsevier Science B.V.

Keywords: Band profiles; Buffer composition; Proteins

1. Introduction

Capillary zone electrophoresis (CZE) is widely considered to be a highly effective separation method with an efficiency, expressed in terms of a theoretical plate number of several hundred thousand, assuming that axial diffusion be the sole source of peak dispersion. Yet, that theoretical efficiency was rarely achieved in practical separations. It has been realized, from early on, that the peak width in CZE can have a multiplicity of causes [1,2]. To date, diffusion (i), Joule heating (ii), the difference in conductivity between the sample zone and surrounding buffer (iii) and interactions between analyte and capillary walls (iv) can be thought of as being the most important intra-column causes of peak broadening (e.g. [2,3]). In the particular case of electrophoresis in sieving media, the interaction of analyte molecules with the polymeric matrix (v) has also been noted as an additional possible source of peak broadening [4]. All of these five factors, together with the width of starting and detection zones (vi), as long as on-column injection and detection are employed, determine the total (final) peak width constituting the plate number and, ultimately, resolution.

In the application of CZE to proteins it has recently been shown that size-dependent retardation ('molecular sieving') exerted by polymers contained

*Corresponding author.

0021-9673/97/$17.00
PII S0021-9673(97)00730-9

in an electrophoretic buffer at concentrations above the entanglement threshold is governed by the ratio of protein Stokes' radius to the screening length [5,6], conceived as the average size of 'mesh' of the polymer network formed in the solution. However, a prediction of resolving capacity of a polymeric sieving medium requires in addition an understanding of how the medium affects peak broadening. To answer that question an insight into the mechanisms responsible for the broadening is required. Thus, it is necessary to quantitatively evaluate contributions of each potential source of peak broadening. Knowing what these factors are and to what extent they contribute to the final band width might both facilitate optimizing separation efficiency and allow one to assess the limits of optimization. Recently, such an approach has been utilized in capillary gel electrophoresis of DNA fragments [7,8] as well as CZE of amino acids [9] and peptides [10].

The present experimental study aimed at determining which factor or factors dominantly affect peak width in CZE of proteins under conventional conditions both in the presence and absence of polymeric media. Those conditions include a commercial apparatus with an effective cooling system, a low conductivity electrophoretic buffer and capillaries with an internal polymer coating to sufficiently suppress electroosmotic flow.

Since the viscosity of polymer solutions depends on polymer concentration and molecular mass as well as the rate of flow of the injection, it is difficult to adjust the size of starting zones for different solutions. It seems to be more appropriate to analyze the effects of the polymeric media on bandwidth by comparing bandwidth as a function of starting zone length obtained in a buffer alone with that in buffer containing the polymer. Thus, the emphasis has been placed on studying the contribution to bandwidth arising from a variation of starting zone length.

A native fluorescent protein, R-phycoerythrin (M_r $290 \cdot 10^3$), was used as a model analyte in view of a number of its advantageous properties. R-Phycoerythrin is a highly homogeneous protein whose shape might be approximated by that of a rigid sphere of 4 nm radius considering its sedimentation constant [5]. Its native fluorescence allows one to vary its concentration over a wide range without a corresponding loss in detection sensitivity.

2. Experimental

2.1. Materials

(2-[N-cyclohexylamino]-ethanesulfonic acid) (CHES) was obtained from Sigma (St. Louis, MO, USA, Cat. No. C-2885). The native fluorescent protein, R-phycoerythrin (M_r $290 \cdot 10^3$) was a product of Polysciences (Warrington PA, Cat. No. 18188). Polyethyleneglycol (PEG) with M_r $20 \cdot 10^3$, $40 \cdot 10^3$, $100 \cdot 10^3$, $200 \cdot 10^3$, $300 \cdot 10^3$, $400 \cdot 10^3$, $600 \cdot 10^3$, $1000 \cdot 10^3$, $4000 \cdot 10^3$ and $8000 \cdot 10^3$ obtained from Sigma and mesityl oxide from Aldrich (Milwaukee, WI, USA, Cat. No. 141-79-7) were used.

2.2. Capillary zone electrophoresis (CZE)

CZE was carried out in 0.025 *M* CHES, 0.05 *M* Tris, pH 9.0, in the presence or absence of PEG, using the HPCE-2100 apparatus (Beckman Instruments, Fullerton, CA, USA) equipped with fluorescence and ultraviolet detectors. In our preparation of buffer containing PEG, the polymer was dissolved for 0.5 to 3 days, depending on its M_r, in deionized water with gentle agitation. The resulting aqueous solution was mixed with a 10-fold concentrate of the buffer. Capillaries of 27 to 67 cm length and 100 μm diameter were used. Capillaries were internally coated with 3% polyacrylamide [11]. The detection window was formed at 7 cm from the anodic end of the capillary prior to internal coating. Other conditions were those previously described [12]. Electroendosmosis (EEO) was measured prior to CZE as the time of displacement to the detector of buffer containing 0.05% mesityl oxide from the anodic reservoir. Capillaries exhibiting EEO in excess of 1×10^{-5} $cm^2\ V^{-1}\ s^{-1}$ (4% of the measured mobility of phycoerythrin) were discarded.

2.3. Data processing

2.3.1. Length of starting zone

The length of starting zone, L_o, can be calculated on the basis of the Poiseuille law (e.g. [13]) as:

$$L_o = \Delta p r^2 t / 8 \eta L_t \quad (1)$$

where Δp is the pressure difference across the

capillary, r is the internal radius of capillary, t is the injection time, η is the solution viscosity, and L_t is the total length of the capillary. Since, $r=50$ μ, $\eta \simeq 0.9$ cPs, and according the manufacturer, $\Delta p=$ 0.5 p.s.i. (1 p.s.i. = 6894.76 Pa), the length of the starting zone for the minimal injection time provided by the HPCE-2100 apparatus ($t=1$ s) may be written as:

$$L_o(\text{cm}) = 11.9/[L_t(\text{cm})] \quad (2)$$

The length of starting zone can also be calculated by using the time required to replace the buffer in the capillary with the same buffer containing a detectable agent. That time was experimentally measured as the passage time of the buffer containing 0.05% of mesityl oxide to the detector at a given injection pressure for capillaries of different lengths (27 to 67 cm). For a 1 s injection it gives the length of the starting zone as:

$$L_o(\text{cm}) = (16.7 \pm 1.4)/[L_t(\text{cm})] \quad (3)$$

which is greater than the expected value by a factor of 1.4. That fact is thought to be due to a real value of injection pressure higher than 0.5 psi. Eq. (3) was used to calculate values of L_o shown in the figures. Gravity flow injection was used (e.g. 2) to obtain a starting zone less than the minimal one provided by the injection system of the HPCE-2100 apparatus. In that case, the length, L_o, was calculated on the basis of the proportionality between phycoerythrin peak area and that length according to:

$$L_o(g)/L_o(p) = F(g)/F(p) \quad (4)$$

where $L_o(g)$ and $F(g)$ are the length of starting zone and peak area obtained at a gravity flow injection, respectively, and $L_o(p)$ and $F(p)$ are the length of starting zone and peak area at the given injection pressure, respectively.

2.3.2. Spatial width of the peak at half-height (bandwidth), $\Delta X_{1/2}$

The migration times corresponding to the half-heights of the ascending and descending limbs of the detected peak, t_1 and t_2, as well as the mode of the peak, t_m, were measured. The conversion from the 'temporal' width of a zone, $\Delta t_{1/2}=(t_1-t_2)$, with units of time, to the spatial width of the zone passing through the detector position, $\Delta X_{1/2}$, with units of length, was made as follows [13]:

$$\Delta X_{1/2} = (\Delta t_{1/2}/t_m)L - d \quad (5)$$

In this relation L is the effective capillary length (capillary length up to the detector) and d is the spatial width of the detector window ($d=0.5$ mm in our case). For convenience, the spatial width of the peak will be referred to as bandwidth analogous to the terminology used in gel electrophoresis.

3. Results

3.1. Non-monotonic dependence of bandwidth on the starting zone length

The bandwidth of R-phycoerythrin in CZE increases as a linear function of the starting zone length, L_o, when $L_o>10$ mm and the effective capillary length is in the range of 200–600 mm (Fig. 1). The parameters of that linear function are listed in Table 1.

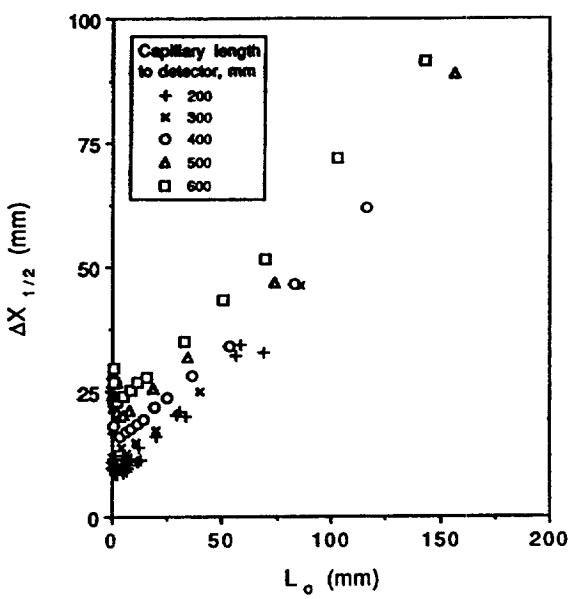

Fig. 1. Bandwidth as a function of starting zone length: CZE of R-phycoerythrin (10 μg ml^{-1}), 0.025 M CHES, 0.05 M Tris, pH 9.0, 25°C, 370 V cm^{-1}. Bandwidth, $\Delta X_{1/2}$, and starting zone length, L_o, as defined under Section 2.

Table 1
Parameters of the linear function relating bandwidth and length of the starting zone: $\Delta X_{1/2} = AL_o + B$

L (mm)	A	B (mm)	R^2
200	0.42	7.4	0.956
300	0.43	9.1	0.996
400	0.41	13.5	0.998
500	0.46	15.7	0.995
600	0.50	19.5	0.996

Where L=effective length of capillary.

3.2. Proportionality between parameter B and the effective capillary length

The parameter B of Table 1 represents an expression of the joint contribution of all factors other than the starting zone length to bandwidth. It increases linearly as a function of the effective length of the capillary, with slope A of 0.5 when $L_o \geq 10$ mm. The parameter B can be evaluated as contributing 70% or more to the total bandwidth when $L_o < 10$ mm. On a semilogarithmic scale the same data exhibit a plateau of bandwidth in the range below $L_o = 10$ mm (Fig. 2)

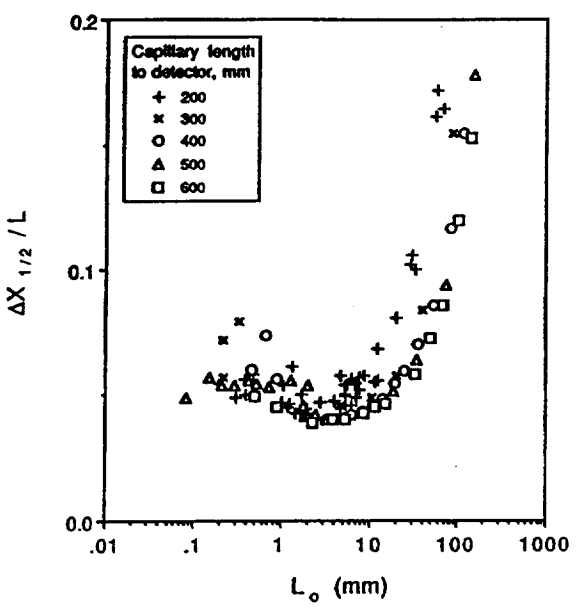

Fig. 2. Non-monotonic dependence of bandwidth on starting zone length: Semi-logarithmic plot of the data in Fig. 1. Bandwidth is expressed relative to effective capillary length.

as expected from a diffusion analysis discussed in the Appendix.

3.3. Independence of bandwidth of electric field strength and sample concentration

Under the conditions of CZE used and within the 'plateau range' (1 mm$< L_o <$10 mm), the bandwidth of R-phycoerythrin is unaffected by variations both in field strength over a range of 37 V cm^{-1} to 370 V cm^{-1} (Fig. 3A) and in sample concentration from 0.1 to 1000 μg ml^{-1} (Fig. 3B).

3.3.1. Independence of bandwidth on PEG molecular mass at $\Delta L_o > 2$ mm

When the length of the starting zone exceeds 2 mm, the bandwidth is independent of the molecular weight of PEG present in the electrophoretic buffer (Fig. 4A and B). Below $\Delta L_o = 2$ mm the bandwidth increases. The increase is most pronounced at a low molecular mass of PEG (ibid.)

3.4. Independence of bandwidth on PEG in the moderate concentration range and increase of bandwidth at high PEG concentrations

In the concentration range of 1 to 6% PEG, the bandwidth is independent of PEG concentration; and above 6% PEG, the bandwidth of phycoerythrin is augmented (Fig. 5).

4. Discussion

Band spreading is of central concern for CZE insofar as it affects separation efficiency (theoretical plate height, H). As a working hypothesis for a theoretical treatment of zone dispersion, the contributions of all of various sources of band spreading in terms of variance, σ^2, have been defined and treated as if they were independent of one another and consequently additive [14]. It is implied in the notion of variance, σ^2, that the distribution function is normalized for the amount of analyte, i.e. it is given a value of 1. Neglecting the width of the detection zone, one might write (e.g. [2]):

$$\sigma^2_{tot} = \sigma^2_l + \sigma^2_d + \sigma^2_{\Delta T} + \sigma^2_{\Delta k} + \sigma^2_w \quad (6)$$

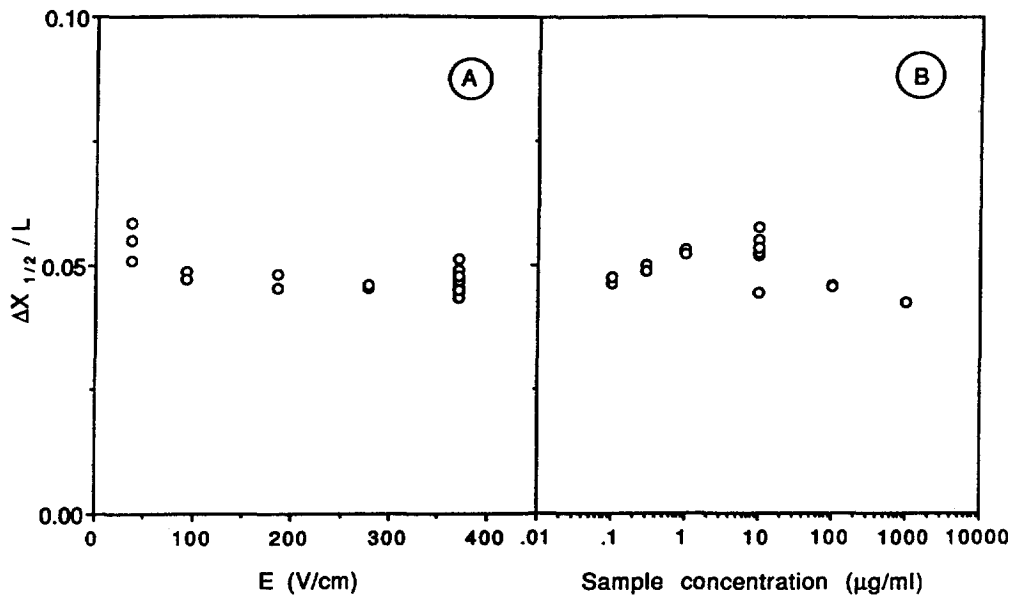

Fig. 3. Relative bandwidth of R-phycoerythrin as a function of field strength (panel A) and sample concentration (panel B). Other conditions as in Fig. 1. Effective capillary length = 200 mm.

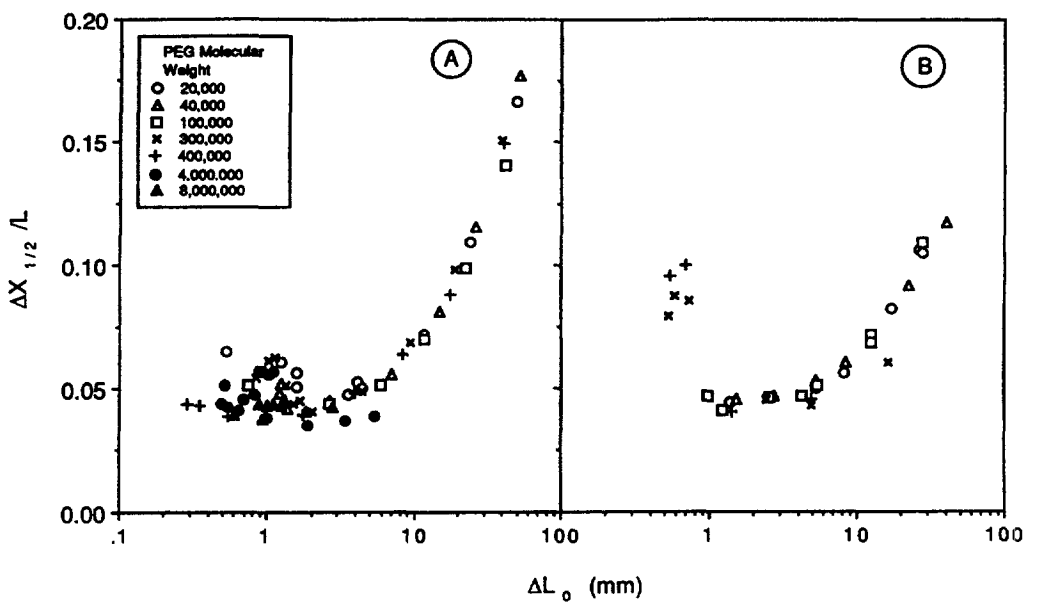

Fig. 4. Relative bandwidth of R-phycoerythrin as a function of the starting zone length at various M_r of PEG. PEG concentrations of 1% (panel A) and 4% (panel B). Other conditions as in Fig. 3.

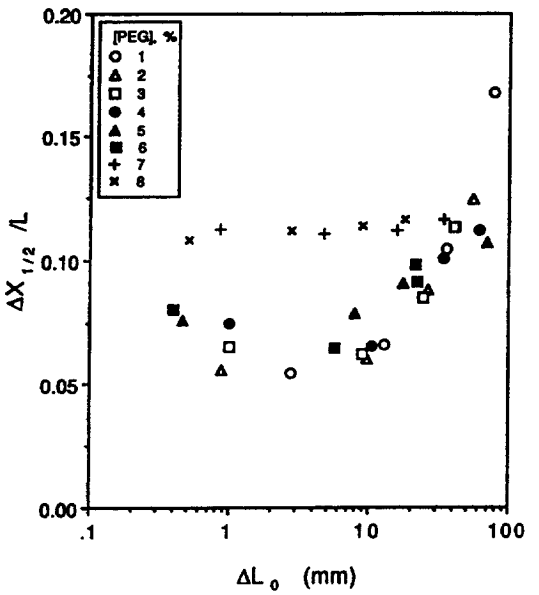

Fig. 5. Relative bandwidth of R-phycoerythrin as a function of the starting zone length, using various PEG ($M_r = 2 \cdot 10^5$) concentrations. Other conditions as in Fig. 3.

where σ^2_{tot} is the total variance and σ^2_l, σ^2_d, $\sigma^2_{\Delta T}$, $\sigma^2_{\Delta k}$ and σ^2_w are the variances due to a finite length of starting zone, axial diffusion, a temperature gradient, conductivity differences and an analyte–wall interaction, respectively.

4.1. Diffusion

For the variance due to axial diffusion, σ^2_d, one might write [2]:

$$\sigma^2_d = 2DL/\mu E \quad (7)$$

where D and μ are the diffusion coefficient and electrophoretic mobility of the analyte, respectively. For phycoerythrin, $D = 4 \cdot 10^{-7}$ cm^2 s^{-1} [3,15]. Its mobility, μ, was measured as $24 \cdot 10^{-5}$ cm^2 V^{-1} s^{-1}. The lowest field strength, E, used was 37 V cm^{-1}. Since the total variance of peak, σ^2_{tot}, is related to the peak width at half height (bandwidth) as $\sigma^2 = (\Delta X_{1/2})^2/5.54$, it may be evaluated on the basis of experimental values of $\Delta X_{1/2}$. Taking the lowest value of $\Delta X_{1/2}$ observed experimentally for the capillaries of each length used (Figs. 1 and 2), one may show that the relative contribution of the diffusional dispersion to bandwidth cannot exceed 1% under the conditions of the study.

4.2. Joule heat

In the absence of convection, Joule heating can still give rise to band spreading by generating a temperature gradient inside of the capillary. The gradient is a result of heat being dissipated along the capillary wall while induced uniformly in the bulk of the capillary. Its profile is close to parabolic [16]. Since electrophoretic mobility is a function of temperature, the temperature gradient necessarily induces a velocity gradient across the capillary cross-section which results in band spreading.

The mathematical treatment of the contribution of the parabolic temperature profile to band spreading applied an analogy with the Taylor dispersion in chromatography [16,2] and provides the following expression for $\sigma^2_{\Delta T}$:

$$\sigma^2_{\Delta T} = r^6 E^5 k^2 \theta^2_T \mu L / 1536 D k^2_T \quad (8)$$

where k is a specific electroconductance of the buffer, θ_T is a temperature coefficient of the electrophoretic mobility ($\theta_T = 0.02$ K^{-1} [2]) and k_T is a specific thermoconductivity of the buffer. The parameter k can be calculated from $k = I/\pi r^2 E = 0.0004$ W^{-1} cm^{-1} (since the current, I, was 12 μA at $E =$ 370 V cm^{-1}). The thermoconductivity of the buffer can be taken as that of water, $k_T = 0.006$ W cm^{-1} K-1 (e.g. [2]). Thus, even for the longest capillary used ($L = 60$ cm) and $E = 370$ V cm^{-1}, the variance due to the temperature gradient will be of the order of 10^{-6} cm^2, i.e. far below the experimentally observed values of total variance [$(\Delta X_{1/2})^2/5.54$] (Figs. 1–3). This agrees with the observation that bandwidth is nearly independent of the field strength (Fig. 3A). If the temperature profile would contribute sufficiently to band spreading under the imposed conditions, in the absence of an appreciable contribution due to diffusion, a sharp increase of bandwidth with the field strength ($\propto E^{2.5}$) would have to be expected. This is not observed experimentally.

4.3. Conductivity difference

The difference in conductivity between the sample

zone and surrounding buffer is known to be responsible for both band spreading and peak asymmetry in electrophoresis [1–3]. The phenomenon was first theoretically treated by Mikkers et al. [17] for univalent ions. To describe conductivity-induced band spreading quantitatively in a general case, Hjerten [3] has proposed a simple approximate expression:

$$\sigma_k^2 = (L/16)^2 \times (\Delta k/k)^2 \quad (9)$$

where Δk is the conductivity difference between the buffer and the analyte zone, and k is the conductivity of the buffer. Since Δk has to be expected to be proportional to analyte concentration, the contribution of the conductivity difference to band spreading can easily be tested by varying the protein concentration of the sample. As is seen in Fig. 3B, both increasing and decreasing the sample concentration by a factor of 100 (compared to the concentration 10 $\mu g\ ml^{-1}$ at which the rest of the experiments have been carried out) had no appreciable effect on the measured bandwidth of phycoerythrin. Thus, the 'electrophoretic' contribution to band spreading can be ruled out under the conditions used.

4.4. Starting zone length and analyte–wall interaction

Since the axial diffusion, Joule heating and conductivity difference can be neglected as contributors to the observed band spreading, such spreading must be due to the finite length of the starting zone and an interaction of analyte with the capillary walls. To what extent each of those factors contributes to bandwidth can be understood by studying the dependence of bandwidth on the starting zone length.

If the sample is introduced into the capillary as a rectangular pulse, the variance due to injection, σ_i^2, is proposed to enter into Eq. (6) as $L^2/12$ (e.g. [2]). However, that approach appears correct only for small injection lengths when diffusional dispersion can convert an initial analyte distribution into a near-Gaussian. For relatively large injection plugs, the bandwidth has to be thought to approach the length of the starting zone [13], resulting in their proportional increase with L_o. The experimental findings are in reasonable agreement with such expectation. The increase of bandwidth with starting zone length can be approximated by a linear function (Fig. 1 and Table 1) beginning with L_o above 10 mm. The coefficient A of the observed linear dependence is near-constant for all effective lengths of capillaries used while the term B is nearly linearly dependent on those lengths (Table 1). Thus, one may consider that the term which depends on L_o in the equation stated in Table 1 is a contribution to bandwidth due to the length of starting zone. The term B is likely to reflect analyte–wall interactions during electrophoresis.

A somewhat unintuitive result in Fig. 2 is the occurrence of a minimum in the bandwidth considered as a function of starting zone length. However, this can be shown to follow from an analysis based on diffusion theory, as indicated in the Appendix.

As shown in Section 4.1, the ordinary diffusion along the capillary axis does not contribute to any significant extent to final bandwidth and thus cannot be responsible for the observed anomaly. The physical source of the anomaly is believed to be the analyte–wall interaction. Rigorously, a non-Markovian generalization of the Eq. (A.1) of the Appendix has to be used [18]. Yet, the main feature of the model is that it takes into account an impact of the non-local initial condition, viz. one of the finite starting zone length, on the zone kinetics in CZE. Hence, the simplification of the process by use of a linear diffusion model seems appropriate under the condition that the diffusion constant in Eq. (A.1) is an apparent 'diffusion' constant defined by the actual band spreading.

4.5. Presence of polymer in the buffer

To evaluate the effect of polymer on bandwidth, two essential parameters, the entanglement threshold, c^*, and the screening length, ξ, are needed and require definition. The entanglement threshold, c^*, denotes the transition from a dilute regime to a network (semi-dilute regime). The relation between c^* and M_r is rewritten [19] as:

$$c^* = 1.5/(KM_r^a) \quad (10)$$

where K and a are constants for a specific polymer and solvent (for PEG in aqueous solution, $a = 0.76$

and $K=0.019\ cm^3\ g^{-1}$ [20]). The average size of the mesh of the network formed in the concentration range above c^* can be considered to equal the screening length, ξ, which is independent of polymer M_r but depends on polymer concentration (e.g. [19]). For PEG, the values can be calculated as:

$$\xi \simeq 0.6c^{-0.75} \quad (11)$$

with dimensions of ξ and c in nm and $g\ cm^{-3}$, respectively. A derivation of Eq. (11) rests on Eq. 1, 2, 3 of [12]. When the particle diameter, $2R$, is less than ξ, the particle interacts with the polymer network through the occupancy of spaces in the network; if it is more than ξ, the interaction follows a different mechanism, viz. one of displacement of the network by the particle [12].

A polymer network in solution can be generated either by increasing the concentration of a polymer of defined M_r, or by increasing the M_r of the polymer at a defined concentration. Both approaches were taken in the present study to evaluate band spreading.

First, c^* was varied at constant PEG concentration of 1 and 4% by varying the value of M_r of PEG from $20\cdot10^3$ to $8000\cdot10^3$ (at constant ξ). The relative bandwidth of phycoerythrin was shown to be independent of c^* (Fig. 4), i.e. independent of the existence of a polymer network. This means that the contribution of the Giddings–Weiss mechanism [18] of an interaction with the polymer to form stationary and mobile states is not appreciable under the conditions used.

The second approach was to study dispersion in a network formed by a polymer solution at various concentrations and constant M_r. Concentrations were chosen to cross the transition between values of ξ larger than $2R$ (0.01 to 0.04 g PEG per cm^3) or smaller than $2R$ (0.05 to 0.08 g PEG per cm^3).

One sees (Fig. 5) that in the two regimes, relative bandwidth varies with the length of sample zone in similar fashion. However, the values of bandwidth are shifted upwards upon the transition from an occupancy mechanism ($2R<\xi$) to a network displacement mechanism ($2R>\xi$). The transition between the two regimes appears at PEG concentrations above 0.04 g cm^3, corresponding to values of ξ smaller than 6.7 nm. Considering that the diameter of phycoerythrin is 8 nm, the close approximation between the two values supports the notion that the upward shift of relative bandwidth is indeed related to a change of the mechanism of retardation.

Irrespective of the mechanism of retardation in polymer solutions, relative bandwidth is independent of polymer concentration, presence or absence of polymer in the solution, and remains constant in the range of small lengths of injected zone (Fig. 5). Thus, a contribution of the polymer to band spreading can be neglected under the conditions used.

An anomaly observed in the present study which deserves comment is the sharp rise of bandwidth at very low values of the starting zone length which occurs only in a relatively concentrated solution (4%) of PEG (Fig. 5B). That rise in bandwidth appears to be coincident with a lowering of mobility values by about 20%. This suggests that possibly the phenomenon which is unexplained at present may be susceptible to future analysis upon the availability of sufficient bandwidth, band shape and mobility data in the range of interest.

5. Conclusions

CZE of R-phycoerythrin shows that bandwidth under the conditions used cannot be accounted for by diffusion, Joule heating or the conductivity difference between solute and solvent. It is therefore assumed to be due to the interaction of the analyte with the inner wall of the capillary. The finding of increased bandwidth with decreased length of the starting zone is shown by mathematical modeling to support that assumption. The presence of polymer (polyethyleneglycol) in the buffer does not affect bandwidth unless the size of the analyte exceeds the average mesh size of the polymer network.

6. Appendix: Kinetics of CZE with a non-local initial concentration

Our analysis is based on the assumption that the protein concentration satisfies a linear diffusion model for motion in a field. Let $c(x,t)$ be the concentration at x at time t, which accordingly satisfies:

$$\frac{\partial c}{\partial t} = D\frac{\partial^2 c}{\partial x^2} - \mu E\frac{\partial c}{\partial x} \tag{A.1}$$

with D the diffusion constant, μ the mobility and E the electric field. The end of the channel is at $x=L$ from which point the protein is eluted. Therefore Eq. (A.1) is to be supplemented by the boundary condition $c(L,t)=0$. In the analysis, it is convenient to work in dimensionless units by defining a dimensionless time τ and a dimensionless distance y by:

$$\tau = \mu E t/L, \quad y = x/L \tag{A.2}$$

which transforms Eq. (A.1) to:

$$\frac{\partial c}{\partial \tau} = \epsilon\frac{\partial^2 c}{\partial y^2} - \frac{\partial c}{\partial y} \tag{A.3}$$

in which $\epsilon = D/(\mu EL)$ is a dimensionless diffusion constant. In the set of units in Eq. (A.2) the elution point is $y=1$ so that $c(1,\tau)=0$.

To study the effects of the non-local initial condition we assume that it is uniform over an interval, therefore having the form:

$$c(y,0) = \begin{cases} 1, & 0 \le y \le \theta \\ 0, & \theta \le y \le 1 \end{cases} \tag{A.4}$$

where θ is the fraction of the channel initially occupied. Consider a protein molecule initially at $y=\xi \le 0$. The probability that it is eluted at a time between τ and $\tau+d\tau$ will be denoted by $j(\tau|\xi)d\tau$ where $j(\tau|\xi)$ is related to the concentration by:

$$j(\tau|\xi) = -\epsilon\frac{\partial c}{\partial y}\bigg|_{y=1} \tag{A.5}$$

it being understood that $c(y,\tau)$ is to be found as the solution to Eq. (A.3) subject to the initial condition $c(y,0)=\delta(y-\xi)$ where $\delta(z)$ is a delta function. The amount of protein leaving the channel per unit time is equal to the flux, a quantity to be denoted by $J(\tau|\theta)$. This, in turn, is related to the concentration by:

$$J(\tau|\theta) = \int_0^{\theta} j(\tau|\xi)\mathrm{d}\xi \tag{A.6}$$

The solution to Eq. (A.3) subject to the indicated initial condition and the absorbing boundary point at $y=1$ is found using standard techniques in the theory of partial differential equations. One finds:

$$c(y,\tau) = \frac{\exp\left(\frac{y-\xi}{2\epsilon} - \frac{\tau}{\epsilon}\right)}{\sqrt{4\pi\epsilon\tau}}\left\{\exp\left[-\frac{(y-\xi)^2}{4\epsilon\tau}\right] - \exp\left[-\frac{(2-y-\xi)^2}{4\epsilon\tau}\right]\right\} \tag{A.7}$$

The combination of Eqs. (A.5) and (A.7) then yields an expression for $j(\tau|\xi)$ of the form:

$$j(\tau|\xi) = \frac{1-\xi}{\sqrt{4\pi\epsilon\tau^3}}\exp\left[-\frac{(1-\xi-\tau)^2}{4\epsilon\tau}\right] \tag{A.8}$$

An integration of this expression according to Eq. (A.6) leads finally to:

$$J(\tau|\theta) = \sqrt{\frac{\epsilon}{\pi\tau}}\left\{\exp\left[-\frac{(1-\theta-\tau)^2}{4\epsilon\tau}\right] - \exp\left[-\frac{(1-\tau)^2}{4\epsilon\tau}\right]\right\} + \frac{1}{2}\left[\mathrm{erf}\left(\frac{1-\tau}{\sqrt{4\epsilon\tau}}\right) - \mathrm{erf}\left(\frac{1-\theta-\tau}{\sqrt{4\epsilon\tau}}\right)\right] \tag{A.9}$$

where

$$\mathrm{erf}(z) = \frac{2}{\sqrt{\pi}}\int_0^z e^{-u^2}\,\mathrm{d}u \tag{A.10}$$

The detected bandwidth must he calculated numerically from Eq. (A.9).

Fig. 6 illustrates the non-monotonic variation of

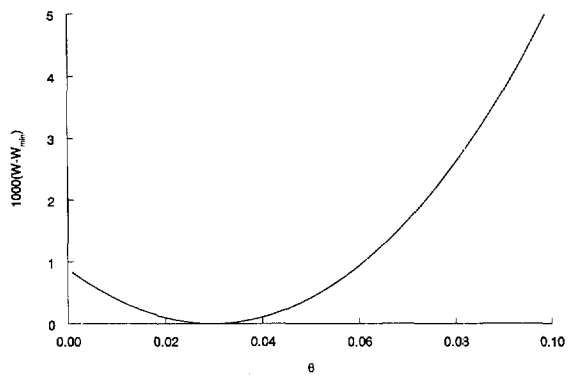

Fig. 6. A curve of bandwidth as a function of starting zone length generated from a model based on diffusion theory.

the bandwidth at the detector (W) plotted as a function of θ. The ordinate is arbitrary since, in physical units, it will be proportional to μE which can take any value. Therefore the curve, as displayed, is plotted as a function of $1000(W-W_{min})$ where W_{min} is the minimum bandwidth. In calculating the curve we have used the physically realistic value $\epsilon = 0.005$.

References

[1] G.O. Roberts, P.C. Rhodes, R.S. Snyder, J. Chromatogr. 480 (1989) 35.
[2] P.D. Grossman, J.C. Colburn (Eds.), Capillary Electrophoresis: Theory and Practice, Academic Press, San Diego, CA, 1992.
[3] S. Hjerten, Electrophoresis 11 (1990) 665.
[4] E. Yarmola, H. Sokoloff, A. Chrambach, Electrophoresis 17 (1996) 1416.
[5] S.P. Radko, A. Chrambach, J. Phys. Chem. 100 (1996) 19461.
[6] S.P. Radko, A. Chrambach, Biopolymers 42 (1997) 183.
[7] J.A. Luckey, T.B. Norris, L.M. Smith, J. Phys. Chem. 97 (1993) 3067.
[8] J.A. Luckey, L.M. Smith, Anal. Chem. 65 (1993) 2841.
[9] J. Liu, V. Dolnik, Y.-Z. Hsieh, M. Novotny, Anal. Chem. 64 (1992) 1328.
[10] H.G. Lee, D.M. Desiderio, J. Chromatogr. 665 (1994) 9.
[11] S. Hjerten, J. Chromatogr. 347 (1985) 191.
[12] S.P. Radko, A. Chrambach, Electrophoresis 17 (1996) 1094.
[13] X. Huang, W.F. Coleman, R.N. Zare, J. Chromatogr. 480 (1989) 95.
[14] J.C. Giddings, Dynamics of Chromatography, Marcel Dekker, New York, 1965.
[15] H.A. Sober, Handbook of Biochemistry, CRC Press, Cleveland, OH, 1973, p. C-28.
[16] R. Virtanen, Acta Polytech. Scand. 123 (1974) 7.
[17] F.E.P. Mikkers, F.M. Everaerts, Th.P.E.M. Verheggen, J. Chromatogr. 169 (1979) 1.
[18] G.H. Weiss, H. Sokoloff, S.F. Zakharov, A. Chrambach, Electrophoresis 17 (1996) 1325.
[19] J.L. Viovy, C. Heller, in: P.G. Righetti (Ed.) Capillary Electrophoresis in Analytical Biotechnology, CRC Press, Boca Raton, FL, 1996, p. 478.
[20] P. Molyneux, Water-Soluble Synthetic Polymers: Properties and Behavior, Vol. 1, CRC Press, Boca Raton, FL, 1983, p. 24.