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Electromigration behaviour of DNA molecules at the free electrolyte–polymer solution interface

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Abstract

The velocity at which DNA molecules migrate across an interface between a free solution of an electrolyte and a sieving medium was investigated in capillaries. A model was proposed where the DNA molecules are supposed to be stacked at the interface and then, due to consecutive conformational changes, their velocities increase and reach the value of their effective electrophoretic mobilities in the polymer solution filling the rest of a capillary. This behaviour was shown by measuring the electromigration injection bias and by extrapolation of the migration times in capillaries of different lengths to the zero migration path. The experiments showed an extraordinarily high size-based separation selectivity of the electromigration across the interface, which seems to offer a potential for successful high resolution separation of DNA fragments in capillaries as short as several centimetres.

Keywords: Electrophoretic mobility; Interfaces, electrolyte–polymer solution; DNA

1. Introduction

The conformation of the chain, together with the net total charge and size of a molecule, are the parameters that control the electromigration of polyelectrolytes in sieving media [1]. While the charge and molecular size remain constant, chain conformation varies during electrophoresis. Conformational changes are induced by drag forces exerted on a moving charged molecule by a sieving medium [2,3]. Thus, the mean conformational entropy of a molecule migrating in free solution is higher than its entropy when passing through meshes of a polymer network. If the frequency of interactions with such barriers is high enough, the polymer coil will be elongated permanently and effective electrophoretic mobility increases, while the separation selectivity decreases. It has been shown both theoretically and experimentally that the entropic changes that occur in a DNA polymer coil are responsible for the size-selective hindering of molecules migrating through a sieving medium [3,4]. Consequently, a molecule at the interface between a free electrolyte and a sieving medium can be expected to be slowed down more and with a higher size-selectivity than in the rest of the migration path. Not only classical chemical or physical gels, but also solutions of linear hydrophilic polymers can be used as the sieving medium [5–10]. Even in polymer solutions, long molecules of polyelectrolytes can be stretched at electric field strengths higher than 250 V/cm [11]. Under optimum conditions, the separation selectivity is expected to be as high as that found in chemical gels [7]. In this paper, the behaviour of DNA fragments at the interface between the free solution of an electrolyte and an agarose solution has been studied.

The behaviour of polyelectrolytes across this

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interface, situated at the start of the capillary, determines their injected amount by electromigration. Thus, quantitative evaluation of the electromigration injection provides a very sensitive tool for investigation of the behaviour of DNA fragments at the interface. In our previous paper [12], we have shown that the rates at which DNA molecules of different sizes are introduced into the separation column are not equal. We have evaluated the effect of the electrophoretic mobilities of DNA fragments in the sample free solution and in polymer sieving media on this electromigrational injection bias. If electroosmosis is negligible, the injection bias can be induced only by differences in the electrophoretic mobilities of various substances inside the sample vial and is independent of the mobilities inside the capillary. This conclusion holds true for an assumption of the existence of a quasi-equilibrium on both sides of the interface. In other words, the amount of a sample that reaches the outer side of the interface is conserved and migrates at an adjusted concentration into the capillary. Nevertheless, we have observed an injection bias of DNA fragments, even if their mobility in free electrolyte is size-independent. In fact, due to significant electroosmotic flow, which acts against electrophoretic migration, some of the sample is repulsed. Under such conditions, the actual amount injected is controlled by the effective electrophoretic mobility of a DNA fragment at the polymer solution side of the interface. In this paper, we have evaluated this effective electrophoretic mobility by comparing the real injected amount with the theoretical value of our model. The importance of this investigation also has a practical aspect. The conformationally driven migration across the interface seems to be the main process providing very fast and high resolution DNA separations in capillaries as short as several centimetres. The feasibility of such separations has already been published [9,13]. The objective of this paper is to describe the migration of DNA fragments across the interface and to explain its possible effect on an electromigration injection bias and the resolution of ultrafast separations.

2. Theory

The electrophoretic mobilities of DNA fragments in gels and polymer solutions have been regarded as constant quantities during an analysis in capillaries. We have proved experimentally that the fragments are slowed down more at the capillary injection point than during migration through a sieving medium in the rest of the capillary. Such a phenomenon can be revealed by comparing the injected amount of DNA fragments with theoretical expectations and/or by an extrapolation of migration times to the zero migration path. The following theoretical models explain our experimental strategy.

2.1. The effect of an interface on the injected amount of polyelectrolyte

Some considerations of the quantitative aspects of electromigration injection have been introduced in our previous paper [12]. In our theoretical model, an injection size bias is shown to be dependent on the mobilities of fragments in a sieving medium and on electroosmosis. The predicted bias of our previous model is, however, smaller than the experimental one. Comparison of the theoretical prediction of our model (Eq. 24 of reference [12]) with experimental results is shown in Fig. 1. Here, the quantitative composition, expressed as the peak height percentage, is plotted against the size of the DNA fragments (ΦX 174 DNA, Hae III digest). The differences between the theoretical and experimental results

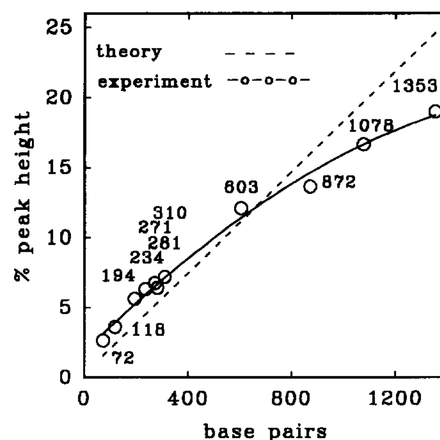

Fig. 1. Comparison of the theoretically evaluated electromigration injection bias (Eq. 24 of Reference [12]), - - -, with experimental values – ◦ – ◦ – ◦ –. Separation conditions: 2% agarose SeaPrep in 1×TBE; temperature, 30°C; injection, 8 s at E=120 V/cm; electrophoresis, at E=182 V/cm; capillary, 50 (54.6) cm.

provide evidence of the more complex behaviour of DNA fragments across the interface.

To overcome the discrepancy, we consider the region of the interface as another phase 2. The scheme is depicted in Fig. 2. The electromigration of DNA molecules at the interface is described by a mobility, $u_{j,1}$, in the free solution of a sample (phase 1) and by mobilities, $u_{j,3}$, inside the capillary (phase 3). In region 2, the electrophoretic mobilities, $u_{j,2}$ (t), of fragments, j, vary with time. DNA molecules are supposed to be stacked at the outer side of the interface $[u_{j,2}$ $(t)=0]$ and, as a result of conformational deformations, consecutively enter the sieving medium inside the capillary. Since the width of phase 2 is very small, the penetration of molecules, j, into the capillary is described by the mean effective mobilities, $u_{j,2}$. Behind phase 2, the fragments migrate inside the sieving medium (phase 3) with effective electrophoretic mobilities, $u_{j,3}$. The migration through phases 2 and 3 is affected by bulk electroosmotic flow, described by electroosmotic mobility, u_{eo}, which acts against electromigration.

It follows from Eqs. (4,7) in Ref. [12] that we cannot expect any injection bias, if the mobilities, $u_{j,1}$, of all fragments, j, are the same and if their mass fluxes through all three phases are equal $J_{j,1}=J_{j,2}=J_{j,3}$. In this case, the concentrations of individual constituents vary with electrophoretic mobility and electric conductivity in the respective phases (Eq. (5) in [12]), but the composition of the mixture does not change. Any change in the composition of a mixture can be brought about only by a loss of injected fragments. This is possible during the injection procedure when a part of the injected sample is pushed out from the capillary due to the effect of electroosmosis. Thus, the concentration changes at the outer side of the interface, expressed by molar concentrations, $c_{j,2}$, are controlled by the following continuity equation. Mass flux to the interface, $J_{j,1}$, is split up into the bulk electroosmotic flow, J_{eo}, out of the capillary and the electromigration, $J_{j,2}$, into the capillary.

$$J_{j,1} = s_1 E_{i,1} u_{j,1} c_{j,1} = J_{eo} + J_{j,2} = s_2 E_{i,2}[u_{eo} + (u_{j,2} - u_{eo})]c_{j,2} \quad (1)$$

where $s_{1(2)}$ are cross-sections and $E_{i,1(2)}$ are electric field strengths during injection at the respective phases. It is convenient to express these quantities in Eq. (1), in a manner similar to that of Eq. 5 in Reference [12], with the help of specific conductivity, $\kappa=I/(sE)$. Electric current, I, is the same through the whole system. Thus, we obtain the equation of a concentration adjustment in the form of

$$c_{j,1}\frac{u_{j,1}}{\kappa_1} = c_{j,2}\frac{u_{j,2}}{\kappa_2} \quad (2)$$

The mobilities, $u_{j,1}$, of DNA fragments in a free solution are known to be size-independent, because of the constant total charge-to-length ratio of these polyelectrolytes [2]. The net injected amount, m, of a fragment, j, is then given by the electromigration flux into the capillary $J_{j,2}$ (Eq. (1)) for the injection time, t_i

$$m_j = c_{j,1}\frac{u_{j,1}(u_{j,2} - u_{eo})}{u_{j,2}}\frac{I_i}{\kappa_1}t_i \quad (3)$$

where I_i is the electric current during an injection.

If we want to compare quantitative predictions of our model with experimental results, we must be able to evaluate the injected amounts of sample components from the respective peak areas or peak heights in an electropherogram. We will follow the concept introduced in Reference [12] and derive the basic relationships for quantitative analysis, based on our new model. The instantaneous detector response of a DNA fragment in absorbance units R_j[AU] is given by the product of the absorbance coefficient of one base pair, ϵ_j, a number of base pairs (bp) of a fragment p_j and its concentration in the detection

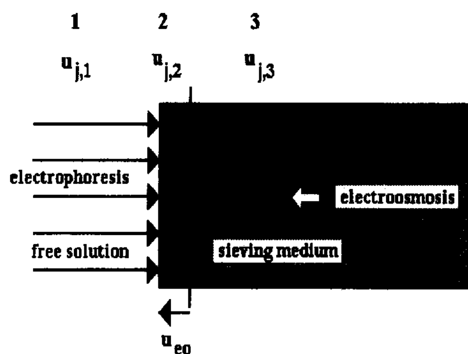

Fig. 2. Schematic representation of injection by electromigration. The electrophoretic mobilities, $u_{j,1}$ of the DNA fragments in sample free solution (phase 1) are independent of size. The size-selective mobilities at the inner side of the interface (phase 2), $u_{j,2}$, and in the rest of the capillary (phase 3), $u_{j,3}$ are affected by electroosmosis, u_{eo}.

window $c_{j,3}$. Then the peak area A_j[AU·s] can be expressed as the time integral of the detector response $R_j(t)$

$$A_j = \epsilon_j p_j \int_{t_1}^{t_2} c_{j,3}(t)\mathrm{d}t \tag{4}$$

The integration limits are given by the passage times of the beginning and the end of the peak. The integral of the concentration distribution $c_{j,3}(t)$ in a detection window can be evaluated with the help of the total injection amount, m_j (Eq. (3)). This injected amount gives a spatial concentration distribution, $c_{j,3}(x)$, during passage through the capillary and can be expressed as an integral, $m_j = s_3 \int c_{j,3}(x)\mathrm{d}x$. Fragments, j, migrate through the detection window at a velocity of $E(u_{j,3} - u_{eo})$ and, thus, the axial co-ordinate, x, can be transformed into the time co-ordinate by the relationship $x = E(u_{j,3} - u_{eo})t$. Then, we obtain the equation of total injected amount in the form of

$$m_j = s_3 E(u_{j,3} - u_{eo}) \int_{t_1}^{t_2} c_{j,3}(t)\mathrm{d}t \tag{5}$$

The cross-section of the capillary, s_3, is equal to the cross-section of the interface, s_2, and E is the electric field strength inside the capillary during the electrophoretic run. The product, s_3E can be substituted in manner similar to that in Eq. (2) by I/κ_3. As the time integrals in Eqs. (4,5) are identical, we can express the integral in Eq. (5) with the help of Eq. (3) and insert it into Eq. (4). Then, for the peak area, we have

$$A_j = \epsilon_j p_j c_{j,1} \frac{u_{j,1}(u_{j,2} - u_{eo})}{u_{j,2}(u_{j,3} - u_{eo})} \cdot \frac{I_i}{\kappa_1} \cdot \frac{\kappa_3}{I} \cdot t_i \tag{6}$$

For an investigation of the injection bias, the relative amounts of individual components are important. The relative peak area of fragment, j, with respect to the total peak area of an electropherogram of a sample mixture is

$$\phi_{j,A} = \frac{p_j \dfrac{u_{j,2} - u_{eo}}{u_{j,2}(u_{j,3} - u_{eo})}}{\sum_j p_j \dfrac{u_{j,2} - u_{eo}}{u_{j,2}(u_{j,3} - u_{eo})}} \tag{7}$$

The equation holds true for electropherograms of equimolar mixtures of restriction fragments, where $c_{j,1}$ values are the same for fragments of all sizes.

The equation for prediction of the peak height can easily be evaluated if the concentration profile of a peak is approximated by a Gaussian curve. As follows from the theory, the maximum height of a Gaussian curve is directly proportional to the area under the curve [12]. If we introduce a proportionality coefficient, k, into the equation of the total injected amount, m_j (Eq. (3)), we get, for the maximum concentration of a zone

$$c^*_{j,3} = kc_{j,1} \frac{u_{j,1}(u_{j,2} - u_{eo})}{u_{j,2}} \cdot \frac{I_i}{\kappa_1} \cdot t_i \tag{8}$$

and for the related peak height (in absorbance units)

$$h_j = k\epsilon_j p_j c_{j,1} \frac{u_{j,1}(u_{j,2} - u_{eo})}{u_{j,2}} \cdot \frac{I_i}{\kappa_1} \cdot t_i \tag{9}$$

Obviously, the peak height is not dependent on the migration velocity but it does strongly depend on zone spreading through the coefficient, k. The relative peak height of fragment j, with respect to the total peak heights of an electropherogram of a sample mixture is

$$\phi_{j,h} = \frac{p_j \left(1 - \dfrac{u_{eo}}{u_{j,2}}\right)}{\sum_j p_j \left(1 - \dfrac{u_{eo}}{u_{j,2}}\right)} \tag{10}$$

Here, the equation is valid only for electropherograms of equimolar mixtures of restriction fragments. The theoretical predictions of Eqs. (7,10) will be compared with experimental results.

2.2. Selectivity of a size-based separation at the interface

In Section 2.1, we have introduced a two-stage migration process and have shown its role for the electromigration injection. It is now interesting to evaluate the contribution of both unsteady and steady stages of migration on the overall separation selectivity. If our idea of DNA conformational changes during the process of penetration into a sieving medium across the interface is correct, we can expect a higher separation selectivity at the injecting end

than in the rest of the capillary. Thus, the migration delay should contributes to the overall resolution of separated components and a reasonable separation can be achieved even at the entrance to the capillary. The resolution of two components can be given by $R_s = \Delta t/(4\sigma_t)$, where Δt is the difference in their migration times to a detector fixed at a position and σ_t is the standard deviation of concentration distributions of their zones, in time units [1], and this can be expressed for a two-stage process as

$$R_s = \frac{\Delta t_2 + \Delta t_3}{4\sigma_t} = \frac{S_2\bar{t}_2 + S_3\bar{t}_3}{4\sigma_t} \tag{11}$$

where $S = \Delta t/\bar{t}$ is the separation selectivity of two components and $\bar{t}$ is the mean time of their passage to the detector [1]. The subscripts, 2 and 3, denote the separations at the interface and in the rest of the capillary, respectively. Eq. (11) shows the effect of two independent processes with different selectivities on the overall resolution, if the zone spreading, expressed by σ_t, is independent of the migration time. This is the case when separations occur in short capillaries. Here, spreading due to the injection will be the predominant contribution to the total width of a zone, independent of the separation process in the rest of the capillary. It follows from Eq. (11) that the resolution cannot be optimised by the length of a migration path. The resolution does not decrease with migration length. Reasonable resolution, however, can be attained even if $\bar{t}_3$ is minimised. In this case, values of S_2 and σ_t play the crucial role.

Eq. (11) can also be written in the form

$$R_s = \frac{S'_2\bar{t}_2 + S'_3\bar{t}_3}{4\sigma_t} \cdot \frac{\Delta p}{\bar{p}} \tag{12}$$

where S' is the selectivity of a size-based separation, $S' = |\mathrm{d} \ln t/\mathrm{d} \ln p|$ and Δp is the size difference of two fragments with a mean size, $\bar{p}$, in number of bp [3,13,14]. In contrast to the selectivity, S, which characterises the separation of two particular components only, the selectivity, S', is a universal continuous function that quantifies the ability of a sieving medium to separate molecules according to their size. Then the ratio $\Delta p/p$ quantifies the separation problem in question. We use this selectivity for the interpretation of our experiments.

3. Experimental

3.1. Chemicals

Fragments of ϕX-174 DNA–Hae III digest (New England BioLabs) in the range of 72–1353 bps was used as the model mixture. The agarose used as a sieving medium was SeaPrep (catalogue No. 50302; FMC Bioproducts, Rockland, MD, USA) and agarose BRE (No. 1503; FMC Bioproducts). Solutions (2%) were prepared gravimetrically in 89 m*M* Tris, 89 m*M* boric acid, 2.5 m*M* Na_2EDTA (1×TBE) or 0.1 *M* Tris, 0.1 *M* N-tris(hydroxymethyl)methyl-3-aminopropanesulfonic acid (TAPS) at boiling temperature.

3.2. Capillary

A fused-silica capillary (100 μm I.D.×367 μm O.D.) was obtained from Polymicro Technologies (Phoenix, AZ, USA) and installed in a laboratory-assembled cartridge (Bio-Rad, catalogue No. 148-3050). The inner wall of the capillary was coated with linear polyacrylamide using Hjertén's procedure [15]. The total lengths of the capillaries were from 11 to 54.6 cm and the effective lengths were from 4.5 to 50 cm. A window was cut out (with a blade) under a microscope. The electroosmotic mobility was determined by measuring the migration time of a diluted sample of neutral mesityl oxide placed in the anodic electrode vial. The electroosmotic mobility, u_{eo}, was found to be $1.83 \cdot 10^{-9}$ m^2 V^{-1} s^{-1}.

3.3. Apparatus

The experiments were performed using a BioFocus 3000 system (Bio-Rad, Hercules, CA, USA) at 30°C and at an electric field strength of 146–438 V/cm. The capillary was kept at a constant temperature using distilled water as a thermostating medium. The separations were monitored at 260 nm by an absorbance detector. After each analysis run, the capillary was rinsed with fresh agarose solution, under pressure. Both the cathodic and anodic chambers, with a volume of 0.6 ml, were filled with agarose solution. Samples were injected by electromigration. In the case where the effective length was 4.6 cm, the sample was injected into the original

outlet end of the capillary and the whole procedure was performed under the opposite polarity.

A laboratory-assembled system with laser-induced fluorescence (LIF) detection was used in this work to demonstrate the ultrafast separation (at an electric field strength of 1182 V/cm) in a capillary with a length of 5.5 (11) cm. DNA fragments were intercalated using ethidium bromide at a concentration of 5 μg/ml in both the background electrolyte and the sample. Ethidium bromide was excited using an argon ion laser (488 nm, LGR 7801 M5, Zeiss, Germany). The fluorescence was observed using the objective lens of a microscope (40×0.65, Oriel) and the image was focused onto an iris. The iris was adjusted to transmit fluorescence and block scattered laser light. A system of two filters, a blocking filter, 488 nm (Oriel), and a band-pass interference filter, 580 nm (Oriel), was used to separate either the 488 or 514 nm lines of the laser beam. Fluorescence was detected using a photomultiplier tube (R 647-01, Hamamatsu). An improved version of the data acquisition and evaluation system CSW 1.6 (Data Apex Dobřichovice, The Czech Republic) was used to record the detector signal at a sampling rate of 100 Hz.

4. Results and discussion

In the first series of experiments, we investigated the time that it takes a DNA molecule to penetrate into a capillary across the interface between free solution in a sample vial and polymer solution in the capillary. This time is independent of the length of the capillary and contributes to the overall migration time to a detector. Hence, extrapolation of migration times of DNA fragments in capillaries of different lengths to the zero migration path should reveal any delay at the injection point. We have consecutively cut the capillary and injected a model mixture of ϕX-174 DNA–Hae III digest under the same separation conditions, i.e., electric field strength, temperature and the composition of the separation medium. The results are presented in Fig. 3, where the migration times of the DNA fragments are plotted against the effective length of the capillary. The low-molecular-mass compounds, picric acid (PA) and sulphosalicylic acid (SSA), were added as reference molecules as they are not retarded by sieving. The result of the extrapolation is shown in the insert. The lines corresponding to fragments of DNA do not pass through the origin. The longer the DNA molecule, the longer is the intercept. This fact confirms our idea that consecutive conformational changes occur in DNA fragments during passage across the free electrolyte–polymer solution interface. It is known that DNA molecules adopt a lower conformational entropy when migrating through a sieving medium in an electric field [3,4]. Thus, the migration velocity is very low at the beginning of the separation and attains its steady state value after an initial period of time. It is interesting to note that this period of time is longer than the injection time in our case. Fig. 3 shows that fragments of about 1000 bp spend more than 1 min in this unsteady migration regime. The lines for the low-molecular-mass reference compounds intersect the origin of co-ordinates, within experimental error.

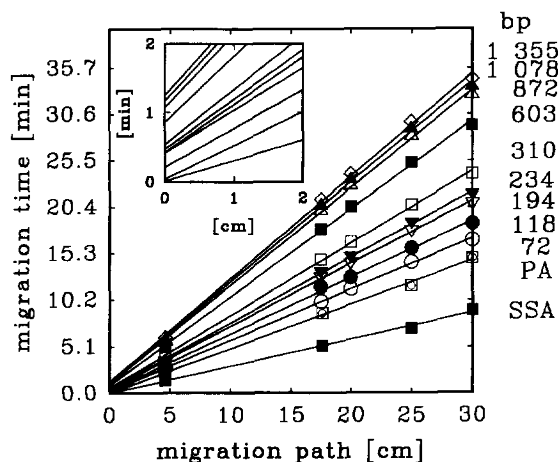

Fig. 3. Extrapolation of migration time to the zero path. Separation conditions: 2% agarose SeaPrep in 0.1 M Tris–TAPS; temperature, 30°C; injection, for 5 s at $E=146$ V/cm; electrophoresis, at $E=146$ V/cm; capillaries, 4.6 (34.6), 17.2 (21.8), 20 (24.6), 25 (29.6) and 30 (34.6) cm. PA=picric acid and SSA= sulphosalicylic acid.

As stated above, high selectivity of the separation at the interface can also be expected. As an example, the separation selectivity, $S=\Delta t/\bar{t}$, of two fragments (1078 and 1353 bps) is 0.012 in the longest capillary, while a value of 0.069 is obtained as the extrapolation to the zero migration path. Thus, the selectivity

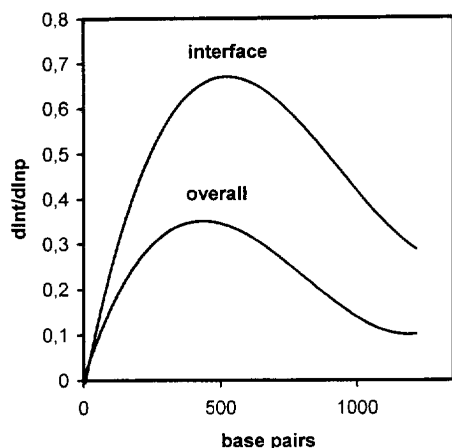

Fig. 4. Comparison of the selectivities of size-based separations, d ln t/d ln p, at the interface and in the capillary as a whole.

at the start of the capillary is more than five times higher than the overall selectivity. In Fig. 4, the selectivity values for the size-based separation, $S' = |\mathrm{d}\ln t/\mathrm{d}\ln p|$, are plotted. Also, we can conclude that the ability to separate fragments of different sizes is better at the interface than in the capillary as a whole. We have a simple explanation for this. Conformational changes that accompany the migration of polyelectrolytes in sieving media improve the selective effect of polymer length on the migration velocity. Thus, molecular stretching, if followed by relaxation, acts positively on the separation selectivity: What must be avoided in electrophoretic systems with sieving media is permanent stretching.

Our conclusions on the behaviour of DNA molecules at the interface also support the experimental results obtained by quantification of the electromigration injection bias. The consistency with theoretical predictions is shown in Fig. 5A and B, where the experimental results are compared to the theoretical evaluations of the relative composition of a model mixture (Eqs. (7,10)). Here, the percentages for peak area (Fig. 5A) and peak height (Fig. 5B) are plotted against the molecular sizes of the model mixture. The following theoretical suppositions lead to the best fit (squares) of the experimental data (circles): (1) as has been discussed in Section 2, the relative composition of an injected sample reflects changes in the effective electrophoretic mobilities of DNA fragments at the interface $u_{j,2}$; (2) the mo-

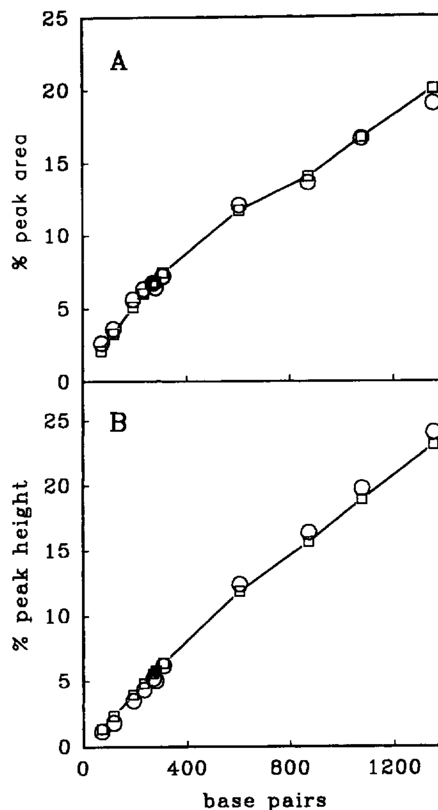

Fig. 5. Comparison of the theoretically evaluated electromigration injection bias (Eqs. (7,10)) -□-□-□-, with experimental values, ○ ○ ○. Separation conditions: 2% agarose SeaPrep in 1×TBE; temperature, 30°C; injection, 8 s at $E=120$ V/cm; electrophoresis, at $E=182$ V/cm; capillary, 50 (54.6) cm.

bilities of fragments at the interface, $u_{j,2}$, are in a simple relationship to the related effective mobilities evaluated from the migration time through the whole capillary, $u_{j,3}$. In fact, $u_{j,3}$ represents the steady state values of $u_{j,2}$ after some conformational changes of migrating molecules occurs, and both quantities are determined by conformation. Since the selectivity at the interface (based on $u_{j,2}$) is approximately twice as high as the overall selectivity (based on $u_{j,3}$) (Fig. 4), the mobility, $u_{j,2}$, must be a quadratic function of $u_{j,3}$, following from the definition of S'. This is in accordance with the idea that the factor, f, in the relationship between both mobilities

$$u_{j,2} = u_{j,3}/f \tag{13}$$

is inversely proportional to $u_{j,3}$ and

$$f = f^*/u_{j,3} \quad (14)$$

showing that selective retardation of a molecule occurs at the interface. Thus, we obtained the relationship

$$u_{j,2} = u_{j,3}^2/f^* \quad (15)$$

for evaluation of the effective electrophoretic mobility that controls an electromigration injection. The best fit of the experimental data in Fig. 5A and B was attained for $f^* = 52$. The fit is reasonable for the peak areas and heights as well. The values of the coefficient, f, which characterises molecular retardation at the interface, together with the mobilities $u_{j,2}$ and $u_{j,3}$, are shown in Table 1. While fragments of about 100 bp in length migrate into the capillary during injection at a mean velocity that is 2.5 times smaller than its steady state value, the injection velocity of 1000 bp fragments is four times smaller.

The period of time of unsteady migration is dependent on the electric field strength, since polyelectrolytes are deformed faster and penetrate into a sieving medium easily at high field strengths. As a result, a steady state migration velocity should be attained faster. Because of this, we can expect the electromigration injection bias to be strongly dependent on the electric field strength, if the injection time is shorter than the period of time of the unsteady migration. Fig. 6 shows the peak area percentage vs. the electric field strength during a very short injection (3 s). Under this condition, the longest fragments migrate into the capillary at the steady state velocity only at relatively high field strengths. The pronounced bias is evident for electric field strengths of lower than 146 V/cm. The appearance of the electropherograms shown in Fig. 7 clearly confirms our conclusions on the bias. The

Table 1
Values of $u_{j,2}$, $u_{j,3}$ and f

Base pairs	$u_{j,3}\cdot10^9$ ($m^2\,V^{-1}\,s^{-1}$)	$u_{j,2}\cdot10^9$ ($m^2\,V^{-1}\,s^{-1}$)	Retardation f
72	20.73	8.26	2.51
118	19.07	6.99	2.73
194	17.78	6.08	2.92
234	17.30	5.76	3.00
271	16.54	5.26	3.14
281	16.32	5.12	3.19
310	16.17	5.03	3.22
603	14.01	3.77	3.71
872	12.88	3.19	4.04
1078	12.70	3.10	4.09
1353	12.51	3.02	4.16

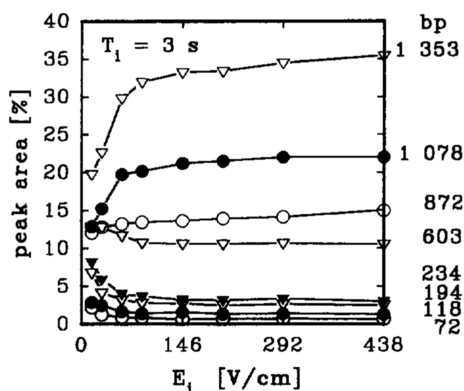

Fig. 6. Effect of the electric field strength, E_i on electromigration injection bias. Separation conditions: 2% agarose SeaPrep in 0.1 *M* Tris–TAPS; temperature, 30°C; injection time, 3 s; electrophoresis, at $E = 146$ V/cm; capillary, 4.6 (34.6) cm.

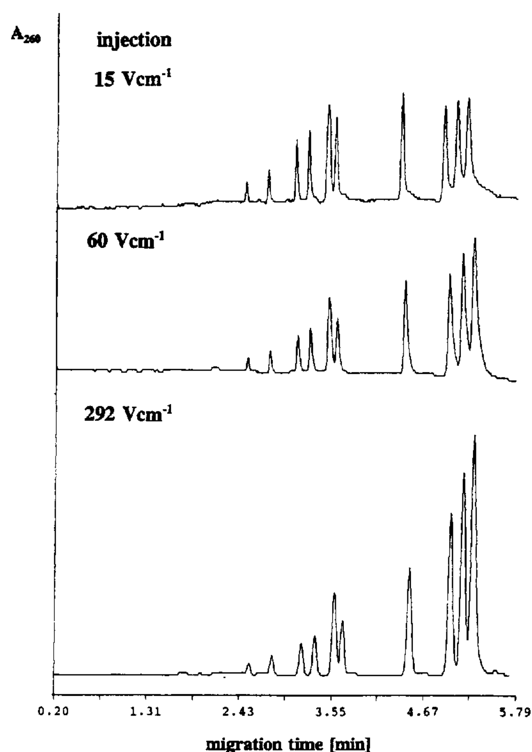

Fig. 7. Electropherograms of ϕX-174 DNA–Hae III digest at three values of E_i. Separation conditions are as in Fig. 6.

peaks of the three longest fragments are smaller than that of the peak for a 603-bp fragment at an injection field strength of 15 V/cm.

It is normally accepted in an analytical praxis that the amount of sample injected is linearly proportional to the injection time. As follows from our model, the mobility that controls an injection varies with time and, thus, the condition of linearity is not fulfilled. The non-linear dependence of the peak area on the injection time is shown in Fig. 8. There are two reasons for the application of a very low electric field strength of the injection in these experiments: (1) At 28 V/cm, the unsteady migration regime affects the injection over a broad range of injection times; (2) The system is not overloaded and good resolution of peaks is attained, even for long injection times. The non-linear dependence of the injected amount on the injection time provides more evidence of the unsteady migration character of DNA fragments during an electromigration injection. The respective electropherograms are shown in Fig. 9. At an injection time of 12 s, the peaks for the longest fragments are lost in the noise, while the peaks for the smaller ones are visible. The sensitivity of the injection bias to injection conditions is demonstrated in Fig. 10. While a moderate bias is the result of either a relatively long injection (25 s) at a low field (of 28 V/cm) or a short injection (3 s) at a high field (of 292 V/cm), the longer fragments are injected in a very small amount at 28 V/cm for a period of 15 s.

An example of a fast separation in a short capillary at extremely high electric field strength (over 1 kV/cm) is shown in Fig. 11. Here, the separation of the model sample was completed in 29 s, while the period of time for the separation of eleven fragments from 72 to 1353 bps was only 6 s. The remarkably high separation selectivity at the beginning of the capillary is supposed to be a reason for this high resolution analysis at an extremely high electric field strength. To find direct evidence for this conclusion is the subject of further investigations. A crucial point for the successful use of short capillaries is a reasonably small contribution of the injection and detection to the overall spreading of a zone. Thus, the highly sensitive LIF detection of DNA fragments, intercalated by ethidium bromide, was used. The effective length of the detection

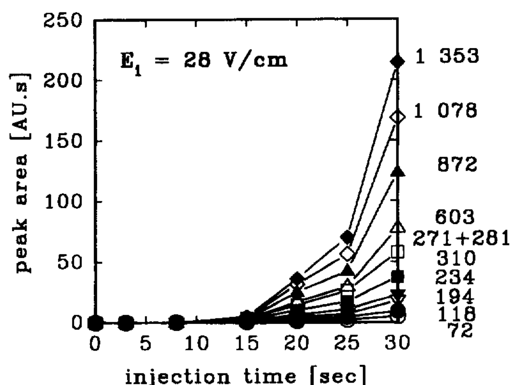

Fig. 8. Non-linear dependence of peak area on injection time. Separation conditions: 2% agarose SeaPrep in 0.1 *M* Tris–TAPS; temperature, 30°C; injection, at $E=28$ V/cm; electrophoresis, at $E=146$ V/cm; capillary, 4.6 (34.6) cm.

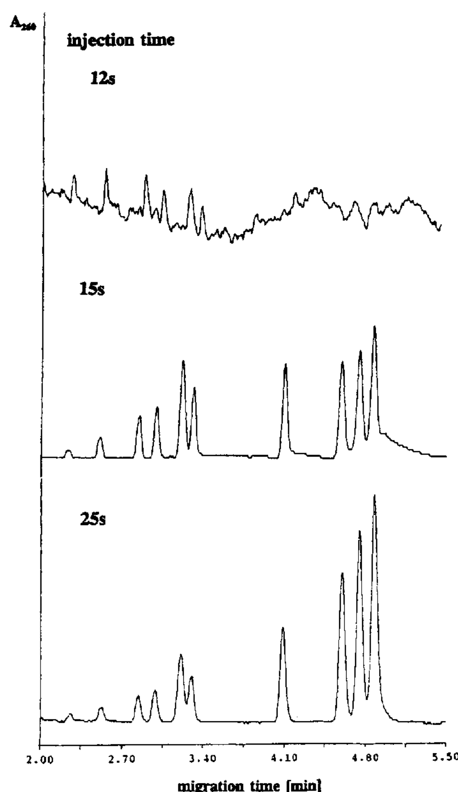

Fig. 9. Electropherograms of ϕX-174 DNA–Hae III digest at three injection times. Separation conditions as in Fig. 8.

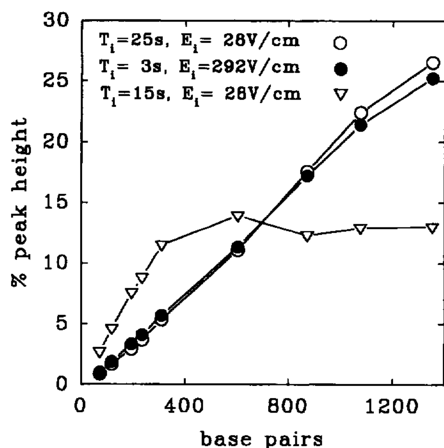

Fig. 10. Electromigration injection bias under different injection conditions. Separation conditions: 2% agarose SeaPrep in 0.1 *M* Tris–TAPS; temperature, 30°C; electrophoresis, at $E=146$ V/cm; capillary, 4.6 (34.6) cm.

window was about 70 μm. Moreover, this sensitive detection method allowed a very narrow zone of sample to be injected ($E_i=91$ V/cm, $t_i=7$ s), which would not be detectable by an absorbance detector.

5. Conclusions

The electromigration of DNA fragments at the interface between a free solution of a sample and a sieving medium inside a capillary is controlled by an electrophoretic mobility that is substantially smaller than the effective electrophoretic mobility in the rest of the capillary. It is known that the conformational entropy of DNA fragments in an electric field is lower in an environment of mechanical obstacles than in a free solution [3]. Thus, the retardation of molecules at the interface is supposed to be caused by conformational changes of polyelectrolytes during electromigration from a free solution into a capillary. These changes are more pronounced than those during migration through a sieving medium inside the capillary. As a result, the overall migration time of a fragment is given by a delay at the injection point and the time that the fragment spends in travelling from the injection point to the detector. The delay is independent of the migration path to the detector, but it is a function of the molecular mass, the electric field strength and the concentration of a sieving polymer. Extrapolation of the migration times of DNA fragments through capillaries of different lengths to the zero migration path provides intercepts that represent values of the delays of individual fragments (Fig. 3). The separation selectivity at the interface is higher than the overall selectivity of the separation in the capillary as a whole (Fig. 4, Eq. (15)).

The electrophoretic mobility of a sample component at the interface controls its injection by an electromigration (Eq. (3)). Therefore, differences in these mobilities result in an electromigration injection bias, which has to be considered if results of a quantitative analysis are to be evaluated. On the other hand, the changes in the composition of the originally equimolar mixture of restriction fragments provide us with valuable data on the behaviour of DNA fragments at the interface. Based on the composition of an analysed mixture, the effective mobility at the interface was evaluated (Eqs. (7,10)) and the selective retardation was proven (Fig. 5). The selective retardation of DNA molecules, expressed by the factor f (Eq. (13)), is dependent on the molecular size (Eq. (14)), the electric field strength and the concentration of the sieving medium. The f values of individual fragments (Table 1) indicate a conformation-driven process, where a polymer coil has to undergo stretching to be able to enter the solution. The longer a DNA molecule is, the longer it takes to penetrate into a polymer solution.

The electromigration injection bias is dependent on the electric field strength (Figs. 6 and 7) and on the fraction of the injection time that is occupied by the unsteady migration regime (Figs. 8–10). Consequently, the injection bias is reduced at a high injection electric field strength and/or by a long injection time. The amount injected by electromigration is not a linear function of the injection time, if the injection is performed under unsteady migration conditions (Fig. 8).

The migration character of DNA fragments across the interface can theoretically be the main separation process, providing reasonable resolution in very short capillaries (Eqs. (12,15)). The ultrafast separation introduced in Fig. 11 is supposed to be an example confirming this hypothesis. Nevertheless,

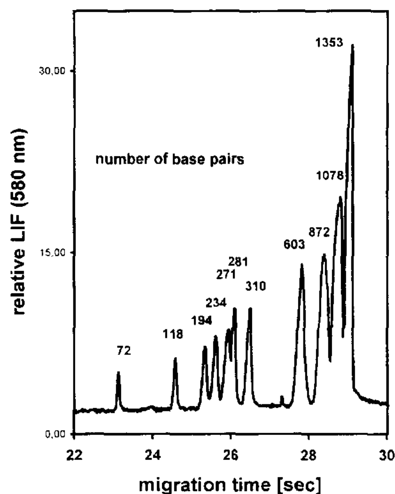

Fig. 11. Ultrafast capillary electrophoresis of ϕX 174 DNA–Hae III digest with LIF detection. Separation conditions: 2% agarose BRE in 20 m*M* Tris–TAPS; temperature, 25°C; injection, for 7 s at 91 V/cm; electrophoresis, at $E=1182$ V/cm; capillary, 5.5 (11) cm.

the exact contribution of the high separation selectivity at the interface to the overall resolution in short capillaries must be proved directly. Successful separations in very short capillaries suggest that the internal potential of capillary electrophoresis in solutions of polymers for ultrafast separations has not yet been fully exploited. The enormous effect of conformational changes on the separation selectivity has lead us to investigate separation columns with concentration-modulated medium or separations on membranes.

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