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Bioelectrochemistry 52 (2000) 91–101

Bioelectrochemistry

www.elsevier.com/locate/bioelechem

Testing of nonlinear electrofrictiophoresis in agarose gel

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Received 12 August 1999; received in revised form 26 May 2000; accepted 24 July 2000

Abstract

It was theoretically predicted earlier that if a periodic force without constant component is applied to a particle, then the particle can produce a directed drift in some direction. The effect is named nonlinear electrofrictiophoresis, because it is crucial for its appearance that the friction force depends on the particle's velocity in a nonlinear manner. We test a possibility to observe this effect when a mixture of fragments of DNA (the DNA ladder) moves in the agarose gel. For this purpose, we study the nonlinear characteristics of a DNA ladder movement in the gel. The gels with the ladder were run under various electric field strengths. It was found that the friction coefficient for each DNA fragment in the ladder depends on the migration velocity, suggesting that energy dissipation during migration is a nonlinear function of velocity. This nonlinearity makes the system under consideration suitable for observing nonlinear electrofrictiophoresis. A possible velocity of directed drift under periodic electric drive without constant component was estimated numerically for experimentally observed dependencies. The velocity appeared to be comparable with that of migration under a constant field of moderate strength. A possible mechanism of energy dissipation during movement of DNA through the gel is discussed.

Keywords: Nonlinear friction; Electrofrictiophoresis; DNA; Agarose gel

1. Introduction

Directed drift of charged dispersed particles in fluids is observed in electrophoresis [1] and its various modifications [2]. Several variations of electrophoresis are known and used in practice: (i) ordinary electrophoresis — directed movement of colloidal particles in a medium under the action of a non-varying uniform electric field; (ii) dielectrophoresis — directed movement of dielectric particles in a spatially non-uniform electric field [3–5]; (iii) dipolophoresis — directed drift of colloidal particle in a spatially non-uniform AC electric field [6].

In the literature on electrokinetic effects, it is assumed (e.g., [6], p. 118 and 119) that it is impossible to obtain directed drift by means of a spatially uniform periodic field (the field has no zeroth harmonic, thus its time-averaged value is exactly zero). This point of view will be valid provided that nonlinear effects can be ignored. On the other hand, the possibility of ignoring nonlinear effects depends on the experimental conditions, which include the property of the substance used for medium and of the dispersed material, as well as the range of velocities of the particles involved. One source of nonlinearity is deviation of the rheological properties of the dispersion medium from Newtonian behavior [7]. Due to this kind of nonlinearity, the friction–velocity dependence in the medium becomes nonlinear, and this fact has intriguing consequences for the movement of colloidal particles under periodic field without a constant component [8]. Indeed, a directed drift can be produced by a periodic field, which has no constant component.

There are many fluids displaying nonlinear properties under moderate shearing strain rates, among which are weak gels, such as cytoplasmic media [9], and polymer solutions [10].

Gels which are used for DNA separation do not fall into these categories, but they have similar structure and could, therefore, display some nonlinear features. Information

Abbreviations: DNA — deoxyribonucleic acid; EtBr — ethidium bromide; AC — alternating current; DC — direct current

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0302-4598/00/$ - see front matter
PII: S0302-4598(00)00088-X

usually supplied with commercially available gels is concerned with pore size and contains no knowledge about friction–velocity dependence. Therefore, the only way to decide whether a given medium is nonlinear, and is therefore suitable for nonlinear electrofrictiophoresis, is to determine that dependence experimentally.

The purposes of this study have been to determine experimentally the friction–velocity dependence and to estimate theoretically the possible drift velocity caused by a periodic electric field of moderate strength without a constant component.

2. Materials and methods

2.1. Materials

2.1.1. Gel preparation

Experiments were performed in two series. A sufficient amount of agarose (Sigma, A-9539) was weighed by analytical balance and dissolved in 0.5 × TBE (0.045 M Tris–borate, 0.001 M EDTA, pH 7.95–8.10) buffer at the final concentration of 1.50% (w/v) agarose. For each experimental series, the gel preparation conditions and the equipment used were kept the same to prevent possible concentration variations between individual experimental trials, although the way of gel preparation for each series was slightly different.

For the first series, microwave oven was used to heat the agarose buffer mixture. Briefly, the agarose buffer mixture was heated with 70% of full power for 1 min, immediately followed by heating for an additional minute with full power. The combination of heating duration and the power applied was just enough to obtain a clear solution, free of visible swollen agarose particles. Prior to gel pouring, the beaker was cooled under running tap water. For the second series of experiments, to prevent the loss water due to evaporation, the agarose-buffer mixture was heated in a tightly closed bottle. The bottle was immersed in a boiling water bath for 15 min and the solution was let to stand at room temperature for 20 min to cool down prior to gel pouring.

2.1.2. Gel casting

After the agarose solution was cooled to 50–60°C, it was poured into the casting tray with the comb in its place which was located on a level surface. The tray was allowed to wait for 30 min at room temperature for solidification the gel. Hoefer (Model: HE 100 Supersub) horizontal unit was used as an electrophoretic chamber.

2.1.3. DNA ladder and loading

One hundred base pairs DNA ladder (Promega, G210) which consists of 11 fragments with sizes of 100, 200, 300, 400, 500, 600, 700, 800, 900, 1,000 and 1,500 bp

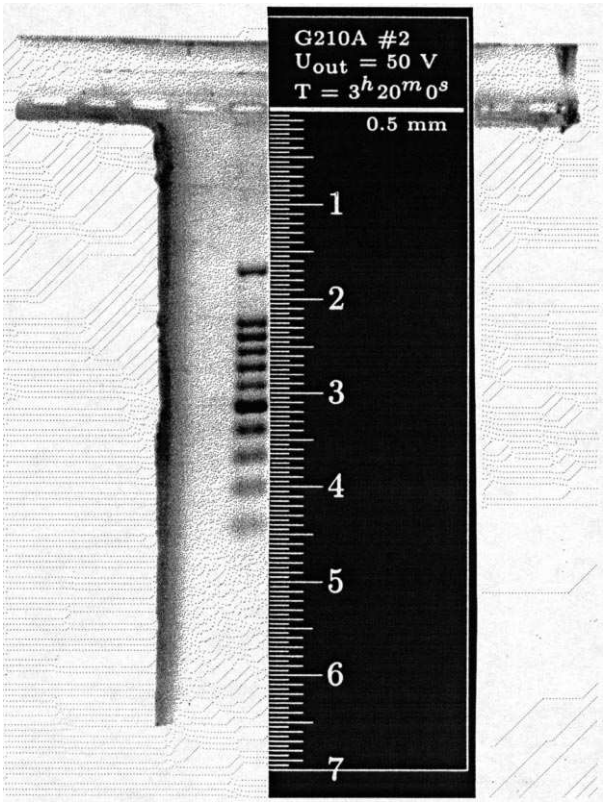

Fig. 1. Photographed gel with the ruler positioned at one side, as seen on the Multi-Analyst/PC program screen (negative colors are used here). Note that the ruler was attached to the photograph, not to the gel. Therefore, the positions read from this image must be multiplied by 1.178 (scaling factor of the camera) in order to obtain real displacements. Running conditions are given on top of the ruler. The Multi-Analyst/PC program allows various transformations of the image, such as changing scale, brightness, contrast, and colors. It also allows to draw auxiliary lines and rectangulars overlapping with the gel and and the ruler simultaneously. By using all this possibilities, and taking into account that the ruler is precise with the tics width equal exactly to 0.1 mm, and the intertics space — 0.4 mm, we were able to measure displacements with 0.1 mm precision.

(Fig. 1) was run in 0.5 × TBE buffer in all experiments. Seven microliter of DNA ladder (70 ng/band/lane except the 500 bp fragment which contains 210 ng/band/lane DNA) in storage buffer (10 mM Tris–HCl, pH 7.4 and 1 mM EDTA) was mixed with 1.2 μl of 30% glycerol in distilled water and loaded into the well without using any tracking dye. The same lane was loaded with DNA sample for all runs.

2.1.4. Staining and photography of DNA in agarose gel

DNA in agarose gel was stained with ethidium bromide (EtBr). The gels were immersed in distilled water containing EtBr (0.5 ml/ml) for 30 min. and then photographed on Polaroid films (Polaroid 667 ASA 3,000) using a transilluminator. To obtain the same scale for all prints, all photographs were taken at the same fixed distance with the same aperture and exposure parameters.

Table 1
Gel running conditions

U (V)	50	90	130	170	210	250
T	3 h, 20 min, 0 s	1 h, 51min, 6 s	1 h, 16 min, 55 s	58 min, 48 s	47 min, 36 s	40 min, 0 s
t (°C)	30.3	30.1	29.6	28.7	27.2	24.8
$E(1)$ (V m^{-1})	185	335	490	653	803	928
$E(2)$ (V m^{-1})	184	331	478	629	783	930

U is the power supply output voltage; T is the running time; t is the heat bath water temperature, which is different for different U for the reasons explained in the text; $E(1)$ and $E(2)$ are the electric field strength in the gel slab for the first and second gel, respectively. The field strength is highly sensitive to the chamber buffer level due to redistribution of potential between the slab and anode and catode buffer chambers, and due to electrodes polarization. Therefore, $E(1)$ and $E(2)$ are not equal. For each column, $T \times U = 600\,000$ V · s.

2.2. Methods

2.2.1. Thermal calibration of electrophoretic chamber

During electrophoresis, electric current produces heat in the gel slab. The heat can affect the characteristics of DNA movement in the gel. As we intend to compare experimental data for different voltages, the temperature should be kept the same for all voltages, even if current through the gel varies.

The electrophoresis chamber that we used allowed connection to a constant temperature bath for stabilizing the temperature. The power supply used was Atta, Model: Constapower 3500. In a preliminary set of experiments, run with the buffer only, the ability of the constant temper-

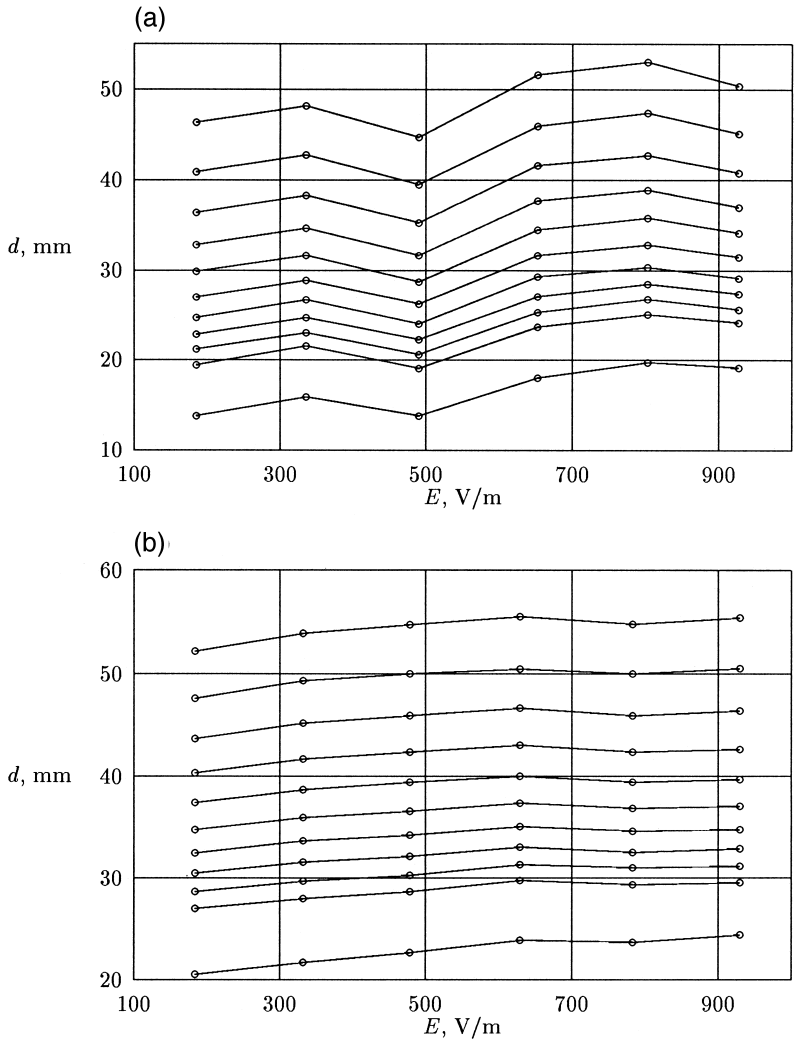

Fig. 2. Displacement versus electric field strength. First (a) and second (b) gel preparation procedure. Curves from top down correspond to DNA fragment lengths from 100 bp at 100 bp increments up to 1000 and 1500 bp. The experimental points were measured once; therefore, error bars are absent here and further. Note the essential influence of the gel preparation procedure.

ature bath to keep the temperature constant when the power supply output voltage changed from 50–250 V was estimated. Since the specific resistance of the buffer decreases as its temperature increases, temperature variations in the buffer can be monitored by measuring buffer resistance. For this purpose, an additional pair of electrodes was introduced to measure the voltage directly across the gel slab. The gel slab was placed over a narrow (2.5-cm-wide) channel restricted by thick glass blocks. The intensity of current through the channel was read from the power supply indicator. It appeared that heat exchange between the bath water and buffer was not sufficient to obtain a constant buffer temperature and therefore, a constant buffer resistance in the range of supply voltages used if the water bath temperature was adjusted to one and the same temperature in each case. This was because the two compartments were separated from each other by the Plexiglass material of the electrophoresis chamber. Therefore, for each of the six voltages used, a specific water bath temperature was determined by trial and error which would result in the same gel temperature when the supply voltage was changed. Temperatures used are listed in Table 1.

2.2.2. Choosing voltages and running times

When a charged particle moves in the gel with constant velocity driven by a constant electric field of strength E, the friction force $F(v)$ is exactly balanced by the electric force applied to the particle:

$$F(v) + QE = 0, \quad (1)$$

where Q is the net charge on the particle. For linear dissipation, the function $F(v)$ is linear:

$$F(v) = kv, \quad (2)$$

where k is the friction coefficient. Thus, in the linear paradigm, the velocity will be linearly proportional to E. In this case, if we choose running times inversely proportional to E, for a given particle (or DNA fragment), migration distance should be the same for all driving

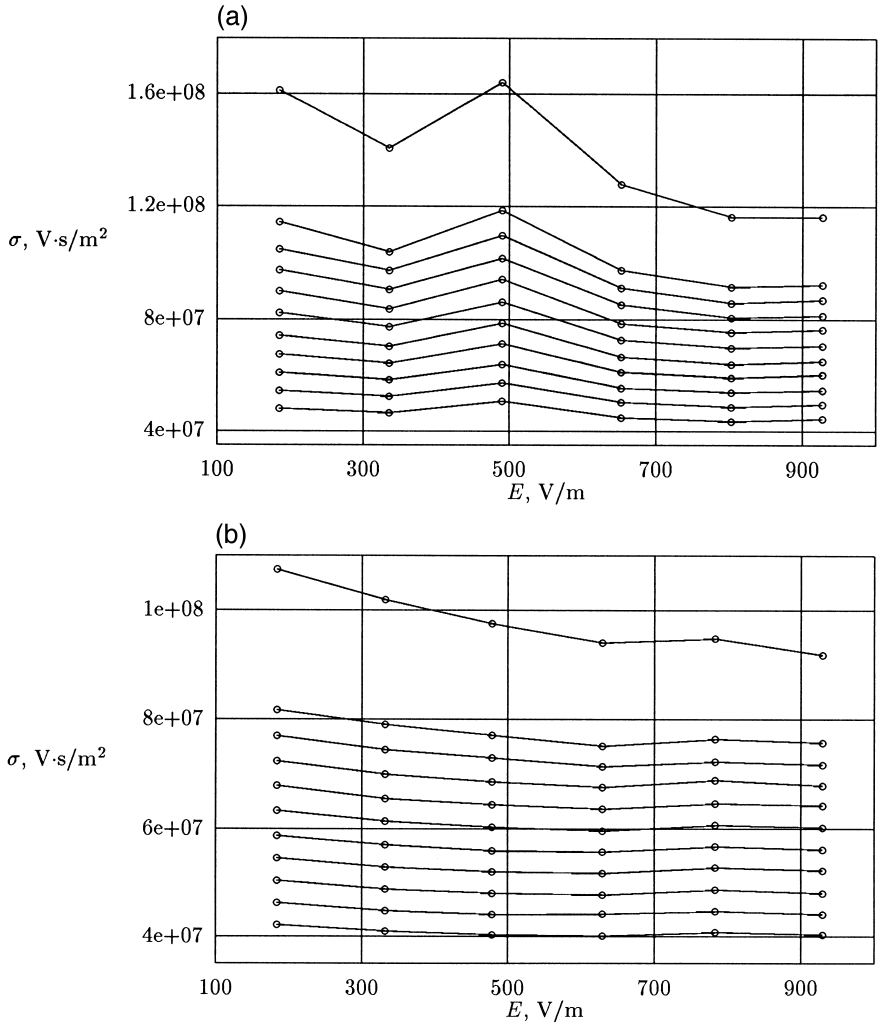

Fig. 3. Inverse mobility (σ) vs. electric field strength (E). First (a) and second (b) gel preparation procedure. Curves from bottom up correspond to DNA fragment lengths from 100 bp at 100 bp increments up to 1000 and 1500 bp.

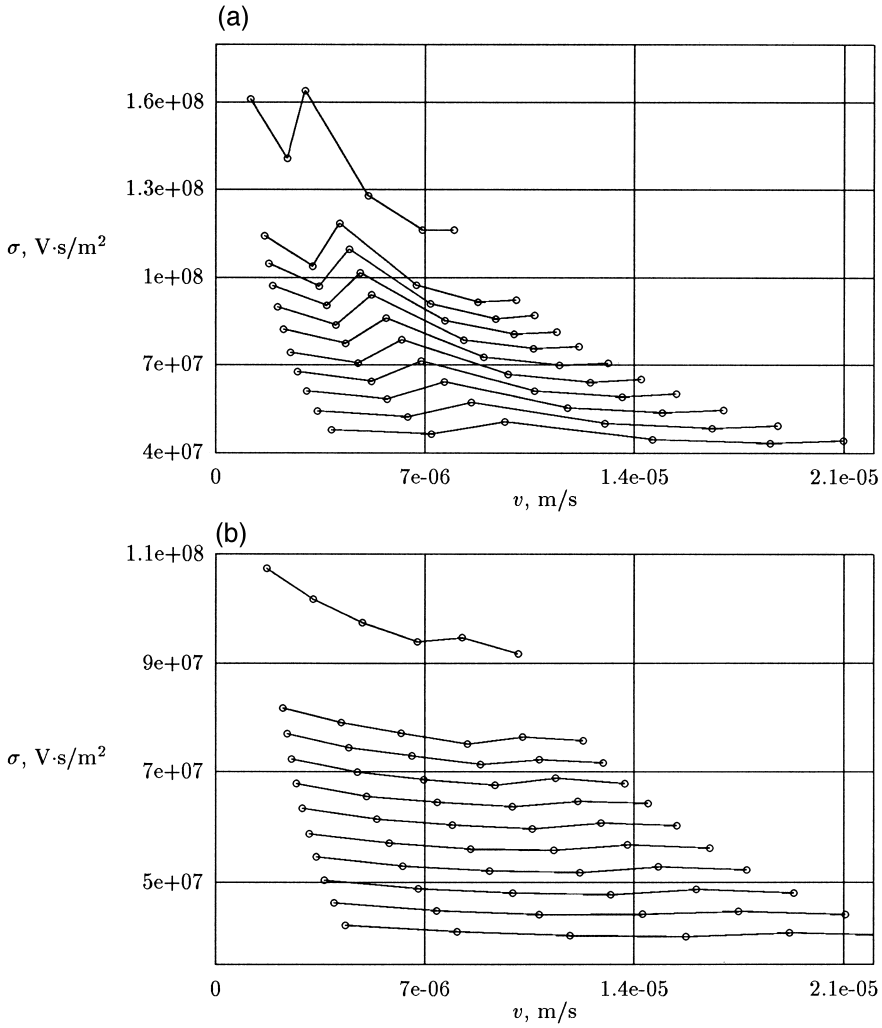

Fig. 4. Inverse mobility (σ) versus migration velocity (v). First, (a) and second (b) gel preparation procedure. Curves from bottom up correspond to DNA fragment lengths from 100 bp at 100 bp increments up to 1000 and 1500 bp.

voltages. Different migrating distances in different experiments will suggest that friction is nonlinear. Therefore, one must choose the running times and voltages in such a way that their product, $U \times T$ is the same, where U is the output voltage of the power supply. Here, we must have in mind that E should be proportional to U, provided the buffer volume and electrode polarization is exactly the same for all voltages. Otherwise, the redistribution of voltage between the channel and anode and cathode buffer chambers will be different for different experiments, which abolishes the linearity between U and E. Therefore, for data processing, we use the voltage measured directly across the gel slab by an additional pair of electrodes (see Table 1).

If the left-hand side of Eq. (2) is nonlinear, the friction coefficient k on its right-hand side becomes velocity-dependent. In order to express this dependence in terms of measured values, let us substitute Eq. (2) into Eq. (1).

$$kv + QE = 0. \tag{3}$$

For a DNA fragment, the net charge $Q = n \times 2e$, where n is the number of base pairs in the fragment; $e = 1.6 \cdot 10^{-19}$ C is the elementary charge. Substituting this into Eq. (3) we get the following.

$$kv + 2neE = 0,$$

or

$$\frac{k}{2ne} = -\frac{E}{v}. \tag{4}$$

Here, the left-hand side is the friction coefficient per single base pair divided by $2e$, and the right-hand side consists of experimentally measured quantities. Let us define the inverse mobility, σ as

$$\sigma = \frac{E}{v}. \tag{5}$$

It follows from Eq. (4), that in the linear case, σ will be velocity-independent; otherwise, its values will be different for different voltages. Investigating those possibili-

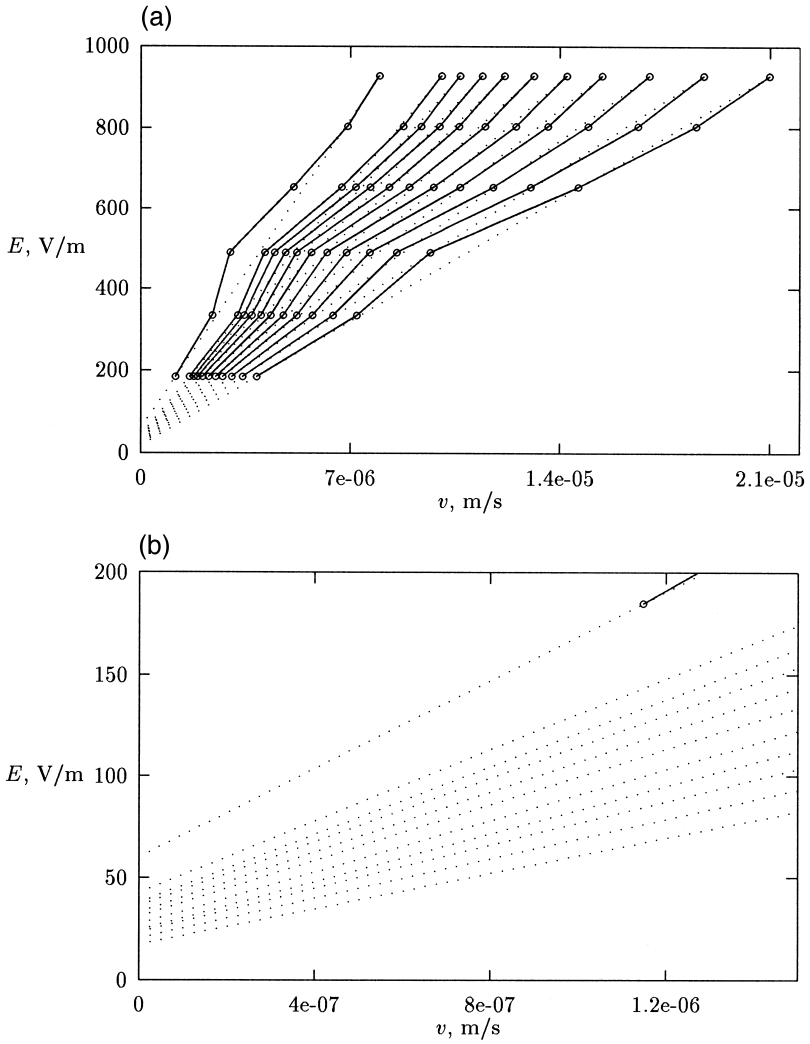

Fig. 5. (a) Dependence of driving electric field on velocity for the first gel preparation procedure. Solid lines — experimental data; dotted lines — linear approximation of the experimental curves. Curves from bottom up correspond to DNA fragment lengths from 100 bp at 100 bp increments up to 1000 and 1500 bp. (b) Same as (a) but in fine scale.

ties, or more precisely, finding the dependence $\sigma(v)$, and/or $\sigma(E)$ has been the main purpose of the experiments conducted in this study.

Note. The effective charge of a DNA base pair in a buffer is smaller than $2e$ due to screening by counterions. This does not change our conclusions concerning the nonlinear friction. Also, the effective charge depends on the field strength. This latter effect might take place for electric field strength greater then 10 000 V/m. For the fields used here (Table 1), the effective charge is strength independent with high precision.

2.2.3. Measurement of migration distances

After each run, the gels were stained by EtBr and photographed under UV light by a polaroid camera. Precise ruler bars with 0.5-mm divisions with 0.1-mm line thickness were prepared by PC and printed by 600 dpi resolution printer. The photographs[1] with glued rulers were scanned on the imaging densitometer (BIO-RAD, Model GS-700) gel scanner, and positions of lines were measured by means of the BioRad software (imaging software: Multi-Analyst/PC). The magnification of polaroid camera was determined by photographing the ruler itself. During measurements, a graphic rectangular having the same width as the thickness of the loading well (0.5 mm) was put at the center of a line, and the position of its front edge was used to measure the migration distance. This procedure allows to measure the migration (displacement) with a precision of 0.1 mm or better. An illustrative example is given in Fig. 1. Note that Fig. 1 is for illustration only. In real measurements, the image contrast and brightness were chosen for each line separately in order to

[1] All photographs in the pcx format are available from the following web site: nonlin.bitp.kiev.ua.

obtain the line image with sharp boundary and width exactly equal to that of loading well.

2.2.4. Calculating drift in a time-periodic field

As has been mentioned before (see Section 1), if the friction force dependence on velocity is nonlinear, directed drift can be obtained by applying a periodic field without a constant component. The conditions necessary to have a drift are discussed in Refs. [8,11,12]. The main requirement is that the electric force $E(t)$, has more than one harmonic. For example, a waveform represented by the following expression would be suitable.

$$E(t) = A\sin(\omega t) + B\sin(2\omega t + \varphi). \quad (6)$$

In this case, as it is proven in Refs. [8,11,12], the drift direction and magnitude depends on the phase shift, φ, and relative magnitudes of the harmonics, A, and B, provided that the maximal value of $E(t)$ remains the same and A, B, φ vary. For A and B fixed, there are two values of phase, φ_1, and $\varphi_2 = \varphi_1 + \pi$, for which the drift is absent. For all other phasxce shifts, the particle which is subjected to the action of the time dependent electric field (Eq. (6)) will drift in one or the other direction.

The magnitude of the drift can be found by integrating over one period of the driving force the steady-state (periodical) solution, $v^*(t)$, of the equation of motion:

$$m\dot{v} + F(v) = QE(t), \quad (7)$$

where m is the particle mass; Q is its net charge; $F(v)$ is the function describing dependence of the friction force on velocity.

In Refs. [8,11,12], several methods are offered for the estimation of drift in a periodical solution to Eq. (7) type. All of them require that $F(v)$ is a smooth function of v. Unfortunately, the experimentally found dependence is not smooth due to the finite jump for $v = 0$ (see Figs. 5 and 6). Another possible method is to find the periodical solution by direct numerical integration of Eq. (7) with

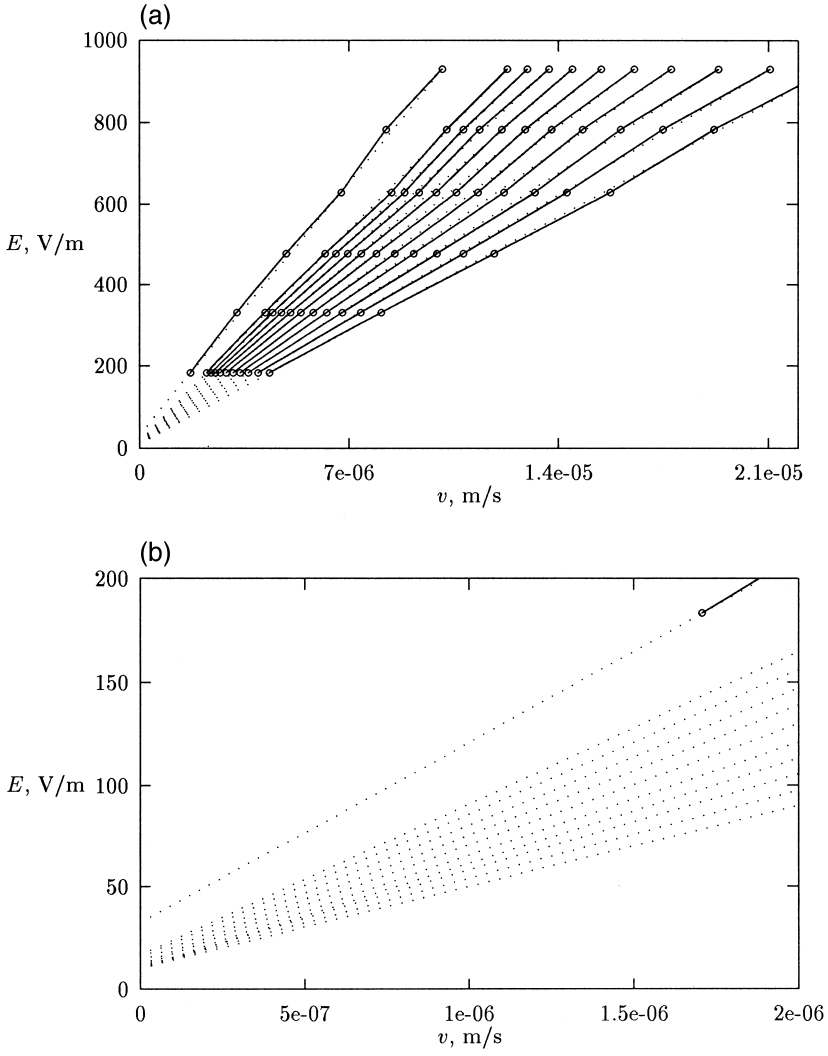

Fig. 6. (a) Dependence of driving electric field on velocity for the second gel preparation procedure. Solid lines — experimental data; dotted lines — linear approximation of the experimental curves. Curves from bottom up correspond to DNA fragment lengths from 100 bp at 100 bp increments up to 1000 bp and 1500 bp. (b) Same as (a) but in fine scale.

experimentally determined $F(v)$. Namely, using the definition from Eq. (5) of σ, we can rewrite Eq. (7) in the following form:

$$\frac{m}{Q}\dot{v} = -\sigma(v)v + E(t), \tag{8}$$

where $\sigma(v)$ is the experimentally found dependence. For DNA $m/Q = 3.44 \cdot 10^{-6}$ kg/C. (See also Note in Section 2.2.2). Keeping in mind that $E(t)$ and $\sigma(v)$ have relatively large values, we can ignore the left-hand side in Eq. (8).

$$0 = -\sigma(v)v + E(t). \tag{9}$$

Let us introduce the function $f(v)$ by the following equation.

$$f(v) = \sigma(v)v.$$

For positive v values, the graph of the $f(v)$ is represented in Fig. 5 (curve for the 1500 bp), due to relationship (Eq. (9)). For negative values of v, the $f(v)$ is defined as $f(v) = -f(-v)$ ($-v$ is positive). Since $f(v)$ grows monotonously, it has the inverse function denoted as $f^{-1}(E)$. The table of function $f^{-1}(E)$ can be obtained by swapping two columns in the table of function $f(v)$. This last table is exactly the table used for drawing graph for 1500 bp in the Fig. 5. Now we can represent solution of the Eq. (9) based on definition of inverse function.

$$v^*(t) = f^{-1}(E(t)), \tag{10}$$

where the $f^{-1}(E)$ is defined by the above mentioned table in the experimental points and calculated by means of linear interpolation in intermediate points.

3. Results

The results obtained are presented in the figures in this section.

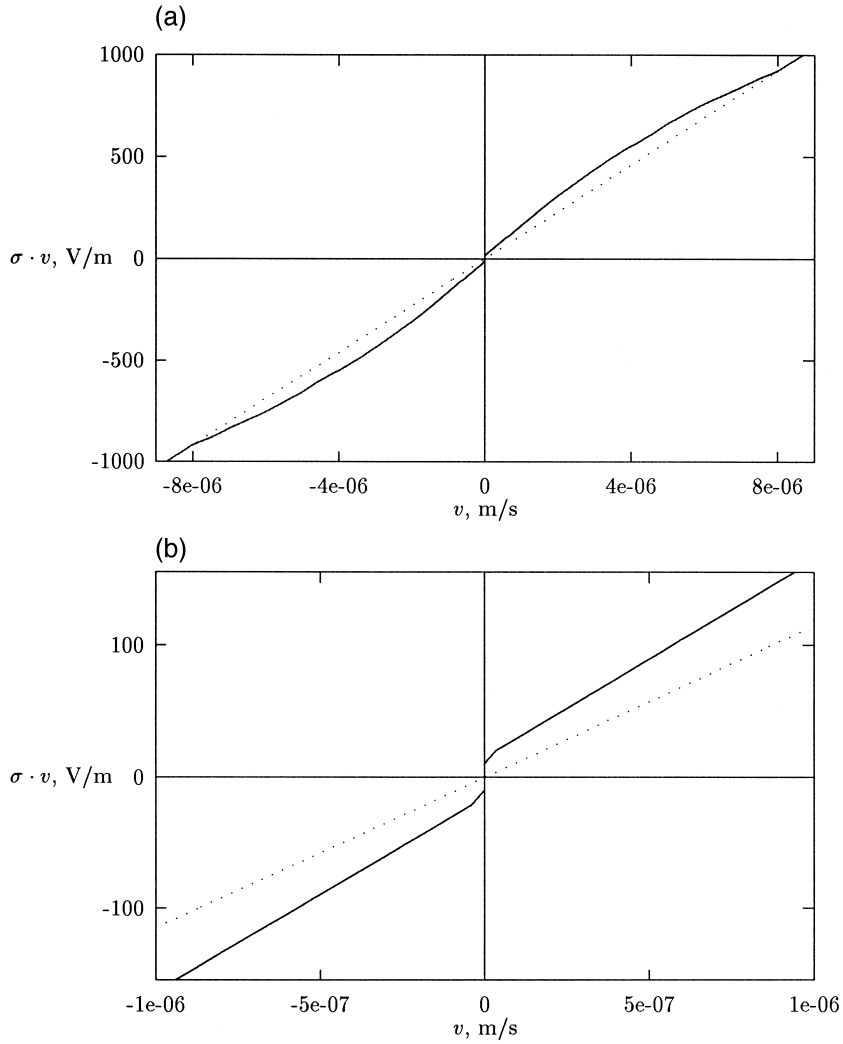

Fig. 7. (a) Solid line — the relationship $f(v) = \sigma(v)v$ vs. v used for numerical estimates; dotted line — linear approximation of $f(v)$. (b) Same as (a) but in fine scale.

Table 2
Parameters of the time periodic electric field, $E(t)$ found by the optimization procedure

No	1	2	3	4	5
Amplitudes (V m^{-1})	627.2	431.6	31.3	126.9	97.1
Phases (rad.)	4.00	1.53	2.42	6.20	4.35
Frequencies (Hz)	1	2	3	4	5

3.1. Displacements obtained

After running the gels under conditions specified in Table 1, they were stained and photographed and displacements for each line were measured, and scaled, as described in Section 2.2. The displacements obtained are shown in Fig. 2. Experimental point for $E = 630$ V/m ($U = 170$ V) was measured twice for the second gel preparation procedure, and the average value of the two measurements ($d_1 = 49.8$, 45.6, 42.2, 39.0, 36.4, 34.0, 32.0, 30.0, 28.6, 27.2, 22.0 mm, $d_2 = 44.5$, 40.1, 37.1, 34.1, 31.6, 29.6, 27.6, 26.1, 24.6, 23.4, 18.4 mm) was plotted. As can be seen from the graphs, the displacement for each line has a tendency to be higher for higher field strength, which suggests that friction velocity dependence may be nonlinear (see Section 2.2.2).

3.2. Dependence of inverse mobility on field strength and velocity

Based on the data Presented in Fig. 2 the inverse mobility was calculated as a function of the field strength, and velocity. The results obtained are presented in Figs 3 and 4.

For linear friction–velocity dependence, all plots must be horizontal straight lines. But the obtained curves suggest that the friction–velocity relationship is nonlinear for DNA moving in agarose gel.

3.3. Dependence of friction force on field strength and velocity

We can calculate the dependence of friction force, $F(v)$ on velocity for every DNA fragment based on measured displacements (Section 3.1) and the data in Table 1. But in Eq. (8), we have another function, namely, $f(v) = \sigma(v)v$, which is proportional to $F(v)$.

$$F(v) = -Qf(v).$$

Both functions will have similar graphical appearance, with different scales on the vertical axes. Furthermore, as the experimental data of Section 3.1 are obtained under constant electric fields, Eq. (9) becomes exact (not approximate) for these data. Therefore, displaying the E dependence on v, we obtain graphs of $f(v)$ for every DNA fragment. These graphs are presented in Figs. 5 and 6.

3.4. Theoretical estimation of possible electrofrictiophoresis effect

Estimation of possible drift in the periodic solution to a nonlinear equation of type Eq. (7) or Eq. (9) is not a well defined mathematical task, because the drift depends in a very complicated manner on the exact time course of the applied electric field $E(t)$. Nevertheless, it is certain that the drift velocity cannot exceed a velocity, v_{max}, caused by a constant field with magnitude equal to the maximum value of $|E(t)|$, even though it can be quite close to the v_{max} (see Refs. [8,11,12]). Having a concrete dependence for $f(v)$ as displayed in Figs. 5 and 6, we can choose a periodic field with amplitude

$$E_{max} \equiv \sup_{0 \le t \le T} |E(t)| \le 927.7\,\text{V/m}, \qquad (11)$$

which corresponds in our case to the power supply output voltage of 250 V, and can then calculate the periodic solution $v^*(t)$ by means of Eq. (10) and the drift v_{dr} by:

$$v_{dr} = \int_0^T v^*(t)\,dt.$$

By means of varying the parameters of $E(t)$ with condition in Eq. 11 preserved, we can try to find the time dependence, $E(t)$ which causes maximum drift.

The above program has been realized for the relationship $f(v) = \sigma(v)v$ displayed in Fig. 5 for the 1500 bp DNA fragment. The experimental curve was smoothed and the final function, $f(v)$ used for numerical estimates is displayed in Fig. 7.

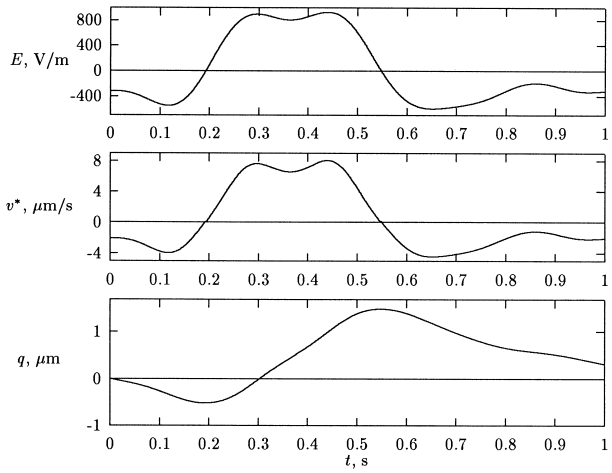

Fig. 8. Time courses of electric field, $E(t)$, steady-state velocity, $v^*(t)$, and displacement, $q(t)$ determined by the optimization procedure. The drift value $v_{dr} = 0.31$ μm/s.

The time course of $E(t)$ was realized as the sum of five sinusoidal harmonics with different amplitudes and phase shifts.

$$E(t) = \sum_{1 \le n \le 5} A_n \sin(2\pi nt + \varphi_n). \quad (12)$$

A simple optimization procedure was used to find a combination of 10 parameters in Eq. (12), which provides maximum drift with condition in Eq. (11) preserved. Here, one phase of the five can be chosen deliberately and fixed, therefore we have here only nine independent parameters. The parameters found are listed in Table 2, and time courses of $E(t)$, $v^*(t)$, and particle displacement, $q(t)$ during steady-state movement are displayed in Fig. 8.

4. Discussion

In this study, we have tried to estimate the suitability of agarose gel/DNA electrophoresis for an experimental observation of the nonlinear electrofrictiophoresis. This effect has been predicted theoretically [8,11,12]. This is a preliminary report dealing with the possible nonlinear properties of the agarose gel. Experimental data obtained here (see Section 3.2) support the conjecture that the agarose gel–DNA fragment system has nonlinear frictional properties and therefore is suitable for observing nonlinear electrofrictiophoresis. Theoretical estimates obtained with an experimentally observed friction–force relationship (see Section 3.4) gave a drift (v_{dr}) of 0.31 μm/s for the 1500 bp DNA fragment under a periodic electric field without a constant component (Figs. 4–6). This is comparable to the drift obtainable under a 50-V driving potential. (see Figs. 4–6).

4.1. Energy dissipation during movement of a molecular chain in a gel

Experimental data obtained in the present study allow us to discuss briefly the possible mechanism of energy dissipation when a macromolecular chain moves through a gel. First of all, even if a gel with a buffer can be modelled as composed of two interlacing fluids with different viscosities [13], our data do not support the idea that the friction force is produced by movement of a chain in a viscous media. Indeed, the inverse mobility, σ defined by Eqs. (4) and (5), has the physical meaning of a friction coefficient per single base pair divided by $2e$. This quantity should not depend on the length of the chain in the case of pure viscous friction. Therefore, in the viscous fluid paradigm, the net friction coefficient for a molecular chain should be linearly proportional to its length, n. Taking into account the fact that the electric force applied to the fragment is linearly proportional to n, we may also conclude that electrophoretic separation based on chain length would be impossible in the viscous friction paradigm.

The data presented in Figs. 3 and 4 suggest that it is σ which is linearly proportional to n. Therefore, the net friction coefficient for a fragment is proportional to n^2. This fact alone (dependence of σ on n) makes the separation possible (note that ratio Q/m in the motion of Eq. (8) does not depend on n). What are the reasons for this n^2-dependence? A possible explanation can be found if we consider the movement of a molecular chain in a gel as dispersion over obstructions [13]. The movement should involve considerably long periods of time when the chain (or its part) moves without touching any obstruction. The probability to hit an obstruction per unit length of the trajectory is linearly proportional to n. The intensity of energy exchange during the hit is linearly proportional to the mass being accelerated before the hit, and therefore again to n. (Additional possibility: the "hit" has a prolonged duration. The moving DNA, after touching the flexible gel fragment, deforms it (see Ref. [13]). In this case, the dissipated energy is proportional to the force, QE, which is linearly proportional to n as well.) These two n-dependent effects could give rise to the n^2-dependence in the net friction coefficient. The necessary condition for this mechanism to be effective is that the pore size should be much larger than an elementary fragment (base pair, for DNA).

4.2. Possible source of nonlinearity of energy dissipation

The linear friction paradigm originates from Stokes's formula which is valid for a friction force exerted on a sphere moving in a Newtonian fluid. But the concept of Newtonian fluid is an ideal one, and any real material including pure water deviates under proper conditions from the ideal [14,15]. If the mechanism of the previous section or any other complicated mechanism (e.g., Ref. [13]) takes place, then it is natural to expect a deviation from Newtonian behavior. Another mechanism which introduces nonlinearity can be offered on the basis of the data presented in Figs. 5 and 6. From these data, it follows that there should be a threshold force (electric field strength), which must be reached before the movement starts. This introduces nonlinearity to the friction–velocity relationship, because for the linear case, one should obtain a straight line passing through the origin.

4.3. Perspective for separation by means of nonlinear electrofrictiophoresis

It follows from the nature of the effect observed in this study that it could be a basis for a new separation technique, provided that the friction–velocity dependencies of the separating substances are different. In principle, a small difference in friction–velocity relationship allows separation in a constant field, but it could require a very

long gel length. By applying a suitable periodic field without a constant component, displacements due to the constant field could be excluded and the migrating particles could be confined to a short gel.

Acknowledgements

All authors thank anonymous referees for their critical remarks. A.K. Vidybida wishes to express his gratitude to the Scientific and Technical Research Council of Turkey (TÜBİTAK) for supporting this research project, and to Ege University for the excellent working and living conditions that have been provided to him during September–November 1998.

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