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GEL ELECTROPHORESIS OF DNA — NEW MEASUREMENTS AND THE REPTON MODEL AT HIGH FIELDS*

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The paper is dedicated to Prof. Andrzej Fuliński for his 70th birthday

New experimental data are presented on the gel electrophoresis of DNA. Experiment was made for molecules of length 173 kbp, in 1 percent agarose gel, in TAE 1× buffer and the field intensity between 5 and 9 V/cm. The results are compared with our computer simulations, performed within the repton model of Duke and Rubinstein. The ranges of field and molecule length are determined, where the geometration effect appears. We investigate also the field dependence of the velocity and the diffusion coefficient at the border of the geometration regime.

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1. Introduction

Separation of molecules of DNA is of current interest in biophysics, because of existing and new applications in genetic technology. For this purpose, the gel electrophoresis (GE) is a standard technique [1]. However, our understanding of the underlying physical processes remains incomplete. Then GE is a fertile area for theory and computational science [2–4]. Indeed, even with much model simplifications, the problem is quite complex.

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Most briefly, the technique of GE is to apply an electric field to a sample of molecules of DNA dropped at a given point of gel, embedded in the buffer. In these conditions, molecules of DNA are charged and they move in the electric field. The key point is that the friction coefficient is a nonlinear function of the molecule length. As a result, molecules of different length move with different velocities, what enables their separation.

This kind of movements of the DNA chains in gel was described within various theoretical and computational models, valid at different ranges of the chain length $S = Na$, where a is the persistence length, and of the applied electric field E. The latter is usually expressed by the ratio ε of electric energy to thermal energy. For the case of short molecules (shorter than $\approx 5\,\mathrm{kbp}$), Ogston model is expected to be valid [5]. For intermediate sizes of the molecules and moderate field, the repton model [6, 7] has been used. In this case the mobility $\mu = v/E$ changes as

$$\mu = \left[\frac{1}{9N^2} + \frac{4\varepsilon^2}{25}\right]^{1/2}, \tag{1}$$

and does not change with the field for $N\varepsilon < 5/6$ [8]. At zero field, the diffusion coefficient is [8]

$$D = \frac{1}{3N^2}\left[1 + 5N^{-2/3}\right]. \tag{2}$$

In the presence of field, there is no definite expression for D. However, basing of arguments of Ref. [2] we can expect that the evaluations obtained within the biased reptation model and the biased reptation model with fluctuations [9] remain valid also for the repton model discussed here. These evaluations are: $D \propto N^{-2}$ for $N < \varepsilon^{-2/3}$, $D \propto N^{-1/2}\varepsilon$ for $\varepsilon^{-2/3} < N < \varepsilon^{-2}$ and $D \propto \varepsilon^2$ for $N > \varepsilon^{-2}$ [9]. We note that up to our knowledge, the diffusion coefficient at high fields and long molecules has not been evaluated within the repton model. In the case of very long chains and high electric fields, analytical geometration model has been used. According to this approach, the mobility is approximately constant with N and the diffusion coefficient D increases with N [10–12].

For the sake of simulations, molecules are represented as chains of the so-called reptons, *i.e.* rigid fragments of the chain, connected with flexible bonds. The gel is reduced to a periodic lattice of cells. Within this simple picture, a bond can be in three states: parallel or antiparallel to the field, or squeezed. These bond states are represented by ±1 or zero. Then, the spatial conformation of a linear molecule can be written as a string of numbers. Each repton moves in the field according to the Monte Carlo rules, with the condition of chain continuity. This latter condition means that the distance between neighboring reptons cannot exceed the distance between neighboring cells of the gel. This picture is known as the repton or particle model [8].

In experiment, diffusion in the range of long molecules and high electric fields (above $E = 5\,\mathrm{V/cm}$) was investigated only recently by the present authors [12]. Our measurements show that the velocity v of long molecules does not depend on their length S, as is already known [2]. A new effect is that the diffusion coefficient D increases with the molecule length. This behaviour is opposite to what is observed at low fields, where D is known to decrease with S [2]. The latter effect is well reproduced by the computer simulations for zero field [8]. Both effects occurring at high fields coincide with the so-called geometration, what means that the molecule length oscillates during its motion [10,13].

The aim of this work is *(i)* to report new experimental data on the diffusion coefficient, *(ii)* to verify the repton model, when confronted with the above mentioned experimental facts. This is the content of two subsequent sections. In Section 4 we compare quantitatively experiment and theory. Section 5 is devoted to final conclusions.

2. Experiment

For all experiments the agarose gel was made in a standard way. 1 percent gel (250 mg of agarose powder with 25 mL TAE 1× buffer) was obtained by microwave-heating. Water evaporating from the boiled gel was supplemented to preserve appropriate concentration of the gel. After boiling 25μL of ethidium bromide (EtBR, Sigma $500\mu\,$g/mL) was added to the gel and the agarose was cooled down at room temperature for 5 min. Then the agarose was gelified for 30 min in the electrophoresis chamber at room temperature. During electrophoresis the gel was submerged in 265 mL TAE 1× buffer and electrophoresis tank was kept in ice to keep approximately constant temperature. To each line 100 ng of phage T4 (173 kbp of length $\approx 80\mu$m) or λ (48.5 kbp of length $\approx 16.5\mu$m) or λ cut with HindIII was added. Experiment was made for fields intensity between 5 and 9 V/cm.

The results presented in Figs. 1 and 2 confirm that the applied range of parameters, the molecule length and the electric field, are appropriate for the high-field geometration regime. As we see, for $N > 150$–200 the velocity does not depend on the molecule length S, and the diffusion coefficient increases with S. In these figures, the molecule length is expressed by $S = N/b$, where b is a persistence length of the molecule. Evaluating $b = 5\times10^{-8}$ m after [14], we obtain the number of reptons $N = 150$–200, above which $v = \mathrm{const}(N)$, roughly equivalent to 22–29 kbp. The diffusion coefficient D increases with N for $\varepsilon = 0.72$ and higher.

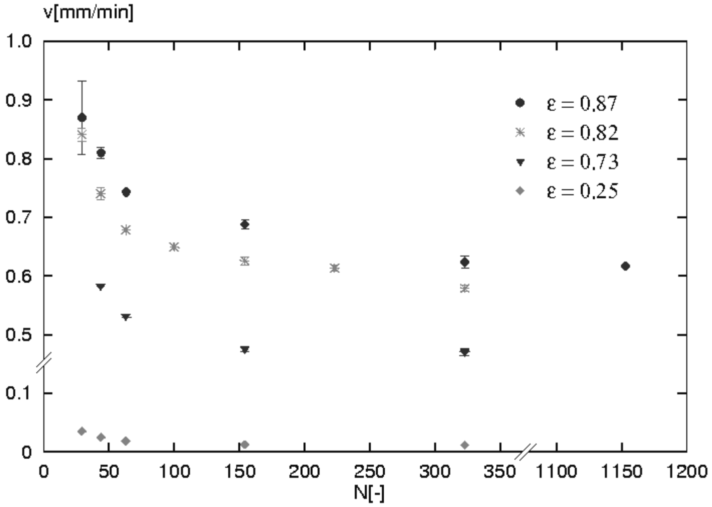

Fig. 1. Experimental dependence of the velocity on the molecule length.

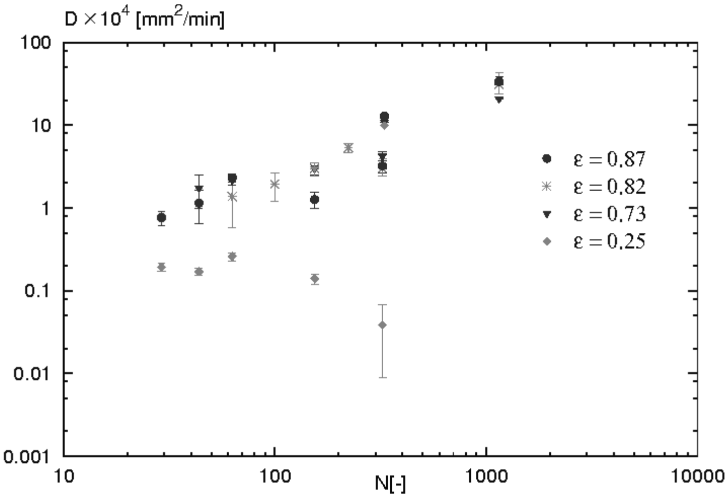

Fig. 2. Experimental dependence of the diffusion coefficient on the molecule length.

The data presented in Fig. 3 and 4 show that the limit value of field for the nonlinear regime is not smaller than $\varepsilon = 0.5$. Above this value, the mobility $\mu = v/E$ increases with field. In this region, also the diffusion coefficient increases with field.

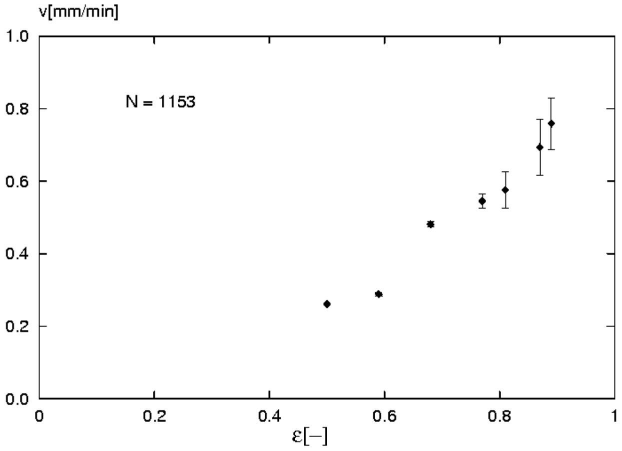

Fig. 3. Experimental dependence of the velocity on the applied electric field.

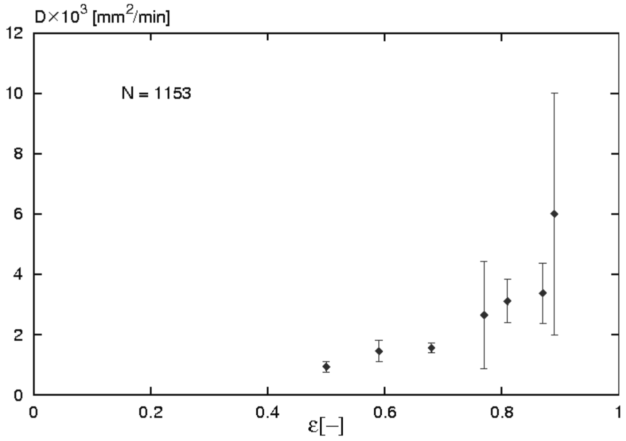

Fig. 4. Experimental dependence of the diffusion coefficient on the applied electric field.

3. Simulation

We use standard Monte Carlo one-dimensional version of Duke–Rubinstein repton model [6, 7] with some numerical tricks proposed by Barkema and Newman [8]. These tricks were introduced in order to increase the efficiency of the algorithm, which still has, for the high fields case, complexity of the order of N^3. Also, we estimate that the time of calculations increases with field as ε^2. A course of a simulation for a chain is as follows. After randomizing of numbers' sequence which represents the initial shape of the chain in gel, we apply the rules of motion, allowing the molecule to reach a stationary state with a constant time average of its velocity v. If the simulated molecule is immobile for a very long (arbitrarily chosen) time, it is excluded from the ensemble. (We note that after sufficiently long time every molecule starts to move with a constant velocity.) In this way, transient effects last shorter. Initial size of our ensemble is 2000 molecules. With the above trick, we have still not less than 1000 molecules.

Figs. 5–8 are to be compared with Figs. 1–4. The results can be concluded as follows. We observe the same effects, as in the experiment: length

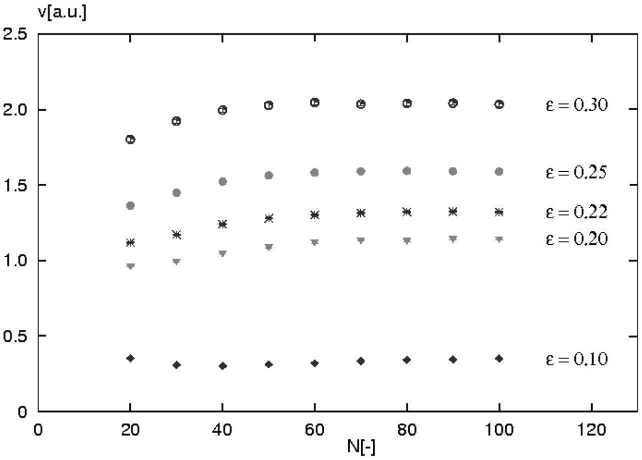

Fig. 5. Calculated dependence of the velocity on the molecule length.

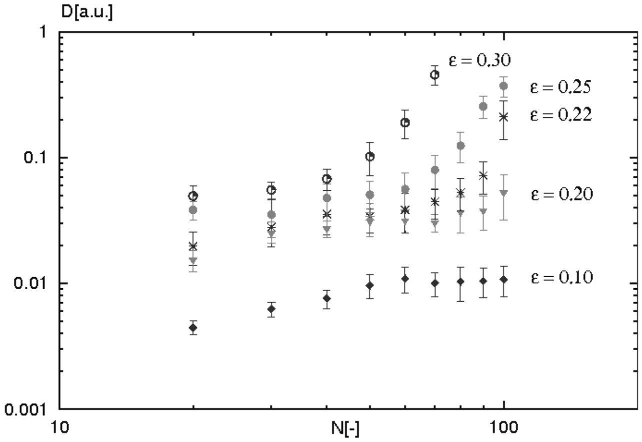

Fig. 6. Calculated dependence of the diffusion coefficient on the molecule length.

dependence of the velocity reaches a plateau above some length value, and the diffusion coefficient increases with the molecule length. Also, above some field value the mobility increases with field as does the diffusion coefficient.

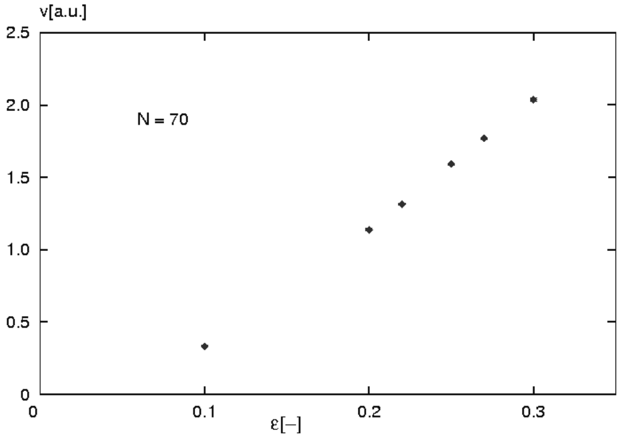

Fig. 7. Calculated dependence of the velocity on the applied electric field.

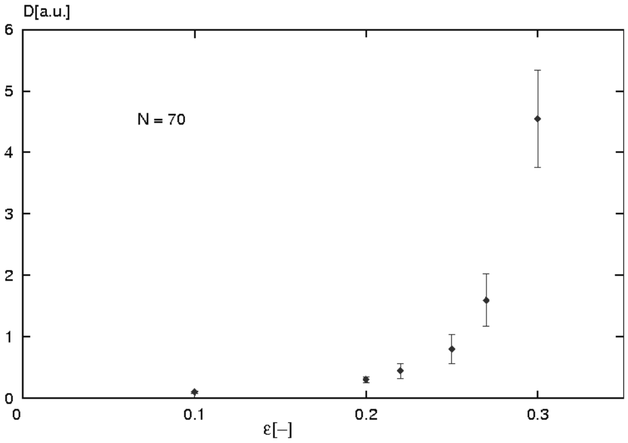

Fig. 8. Calculated dependence of the diffusion coefficient on the applied electric field.

However, the range of parameters (N, ε), where these effects appear, are different than in the experiment. In particular, the number of reptons where v starts to be a constant on N, is about 40, what is markedly smaller than the above reported $N = 150$. On the other hand, the respective field range is quite narrow, and it falls between 0.1 and 0.25. This is a remarkable difference when compared to the experimental data, where in principle no upper limit of the field was observed for the observed dependences. For $\varepsilon = 0.3$, the variance of the position of molecules seems to increase with time in a nonlinear way, as in the case of anomalous diffusion. With our statistics we cannot state, whether this is the case, or whether the reason is that the transient time to get a stationary state becomes extremely long.

4. A quantitative comparison

For a quantitative evaluation of the results, a dimensionless quantity $Y = D/(Sv)$ has been proposed in Ref. [12]. In the numerical calculations, the length is expressed in units of the cell size and simultaneously in the repton size. Then, the version of the algorithm applied here demands $a = b$. With this condition, numerical equivalent of Y is equal $D/(vN)$.

The experimental and calculated values of Y are shown in Fig. 9. In the range of $N < 100$, where the data can be compared, we observe an agreement between the experimental data for $\varepsilon = 0.87$ and the calculated results for ε between 0.1 and 0.2. For higher values of ε, the results of the simulation show a sharp increase of Y. As it was commented in the previous section, this behaviour can be due to some transient effect. As we are interested in the geometration, we concentrate on the range of the parameters where Y can be approximated as const(N). This particular behaviour was suggested by the thermal theory of geometration [10, 11]. The result of this theory is added in Fig. 9 as a dashed line. As we see, the obtained value of Y is higher than both calculated and experimental values.

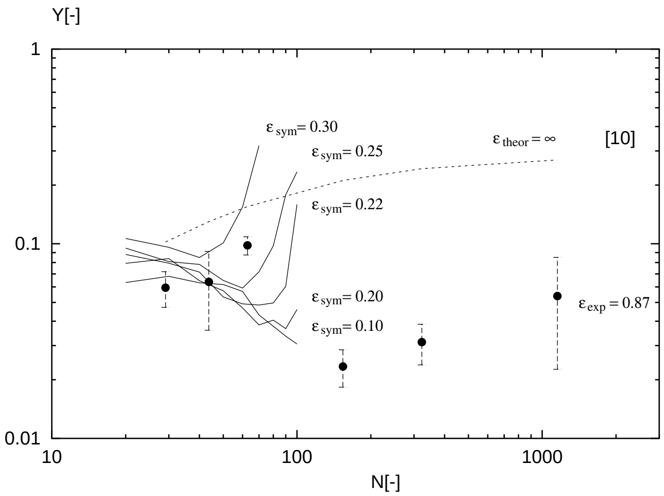

Fig. 9. The dimensionless constant Y, as dependent on the molecule length.

5. Discussion

The obtained difference between the range of parameters N and ε, where the experimental behaviour is reproduced by the calculations within the repton model, could indicate that the evaluation of these parameters is false. The values of ε are influenced by temperature. In the simulation, temperature is introduced via the Monte Carlo probabilities of the movements of reptons. It would be straightforward to argue, that these probabilities should be renormalized by some factors, and even to introduce a phenomenological temperature, which could be reduced to the real temperature rescaled by an appropriate factor. We believe that the reason for the above discrepancy is rather the crude simplifications closed in the assumptions of the repton model. The allowed set of statistical events is reduced in the simulation to shifts of one repton from one cell to another. This is certainly a strong limitation with respect to the actual movement of a molecule of DNA in disordered gel. The discrepancy between the respective ranges of the numbers of reptons is, in our opinion, of the same origin.

With these qualifications, we admit that there are physical, really existing effects, which are reproduced by the repton model. The argument is the qualitative accordance between the data obtained from experiment and model simulations. This accordance is seen between Fig. 1 and Fig. 5, Fig. 2 and Fig. 6, Fig. 3 and Fig. 7, Fig. 4 and Fig. 8. The model of geometration [10, 12] provides a qualitative explanation of the observed effects: the plateau of the velocity with the molecule length and the increase of the diffusion coefficient with the molecule length. We conclude that the effect of geometration is present in the simulation and in the experiment.

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