Dynamical Light Scattering for DNA-CTMA:DR1 chains: Wormlike Semi-Flexible Model, Coil Size and Persistence Length

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ABSTRACT

Recent experimental Dynamic Light Scattering (DLS) studies of the coil sizes of DNA-CTMA:Rh solutions have lead to numerical discrepancies with theoretical predictions amounting to one-two orders of magnitude.[1] In this paper, which has partially character of a tutorial, we present the basic theoretical concepts underlying an analysis of the polymer coil sizes from DLS experiments. In particular, we discuss the limitations of those methods. We present a wormlike model of a polymer chain which is a promising candidate for inferring information about the spatial structure of the DNA chain from experimental data.

Keywords: DNA, Dynamic Light Scattering, coil size, persistence length, wormlike model

1. OUTLINE

Quite recently a solution of DNA-CTMA:Rh was studied using DLS technique in order to characterize the morpho-structural control of functionalized DNA based systems.[1] An attempt to interpret the experimental data - coil sizes - using a gaussian model has lead to large numerical discrepancies, amounting to one-two orders of magnitude. The origin of the discrepancies was unknown.

The aim of this paper is twofold: (i) to discuss critically the basic theoretical concepts related to the interpretation of DLS data and (ii) to present a wormlike model as a candidate for the theoretical description of the DNA coil sizes.

The paper is organized as follows. Section 2 introduces basic parameters used for the characterization of spatial organization of a polymer chain. Section 3 is devoted to Zimm dynamics of polymer chains in good solvents. Wormlike model is described in Section 4. In Section 5 we discuss an origin of limitations of the theoretical approach introduced in previous sections.

2. POLYMER CHAIN: SCALES AND DEFINITIONS

For theoretical modelling we treat the polymer chain as a continuous elastic beam. Below, we present the most important geometric scales - sizes - used in further analysis.

The position of an element of the chain is given by the radius vector $\vec{r}(s)$, where $s = 0 \dots l_c$ is an arc length along the polymer contour (Fig. 1, left). l_c denotes the length of the polymer contour. Vector $\frac{d\vec{r}(s)}{ds}$ is tangent to the contour at point $\vec{r}(s)$.

The end-to end distance l_e reads:

$$l_e = |\vec{r}(s = l_c) - \vec{r}(s = 0)|. \tag{1}$$

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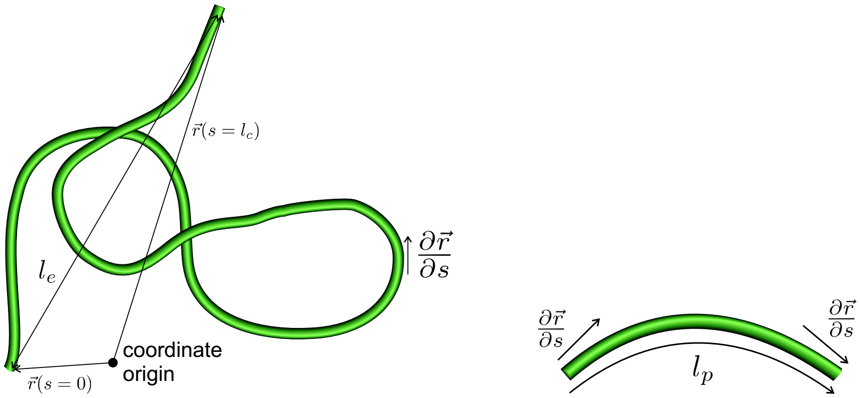

Figure 1. Polymer chain - scales and definitions

The radius of giration r_g is an average square distance between the elements of the chain:

$$(r_g)^2 = \frac{1}{l_c^2} \int_{s_2=0}^{l_c} \int_{s_1=0}^{s_2} \langle |\vec{r}(s_1) - \vec{r}(s_2)|^2 \rangle ds_1 ds_2. \tag{2}$$

Both l_e and r_g offer estimations for the linear size of the spatial organization of the polymer chain.

The persistence length l_p (Fig. 1, right) is the distance over which the orientations of tangent vectors become statistically independent. Mathematically, the averaged scalar product of two such vectors decays exponentially with their arc distance along the contour; the emerging spatial scale is the persistence length:

$$\left\langle \left. \frac{\partial \vec{r}}{\partial s} \right|_{s_1} \cdot \left. \frac{\partial \vec{r}}{\partial s} \right|_{s_2} \right\rangle = \exp\left(-\frac{|s_2 - s_2|}{l_p} \right). \tag{3}$$

The above formulas correspond to the case when the chain is treated as a continuous medium. In some theoretical approaches (see below) the chain is represented by a system of beads connected by bonds (discrete bead-bond model); the positions of beads are given by a set of discrete vectors $\vec{r}_i, i = 1 \dots N$. In this case in the above formulas the integration is replaced by the summation and the continuous variable s by a discrete index. In the case when the bonds are harmonic springs one obtains one of the most important discrete models of a polymer chain, namely the bead-spring model.[2]

3. ZIMM DYNAMICS OF DNA CHAINS IN GOOD SOLVENTS AND DYNAMIC LIGHT SCATTERING

In this Section we explain, step by step, the main concepts and approaches which are used to interpret the results of Dynamic Light Scattering in terms of the radius of giration of a polymer chain in a solvent or of a diluted system of chains with the same giration radii.

3.1 Einstein-Stokes relation

Brownian motion of a particle of mass m is described by the Newton's equation

$$m \frac{dv(t)}{dt} = -\frac{1}{\mu} v(t) + g\, \xi(t), \tag{4}$$

where $\xi(t)$ denotes the white noise: $<\xi(t)>= 0, <\xi(t)\xi(t')> \propto \delta(t - t')$ and the brackets $< \ldots >$ denote the average over the realizations of $\xi(t)$.[3,4] μ is the mobility, its inverse is called a friction constant ζ. The solution of Eq.(4) is the Ornstein-Uhlenbeck process.[3] In equilibrium the Einstein relation holds:

$$D = \frac{k_B T}{\zeta}, \tag{5}$$

where the diffusion constant reads (via Fokker-Planck equation[3]) $D = (g/m)^2$. This formula relates the thermal fluctuations (D) to the response of the system (ζ) and is a specific version of the fluctuation-dissipation theorem.[5]

In an important case of a spherical particle of radius a immersed in a solvent of viscosity η Stokes formula holds: $\mu = \frac{1}{6\pi\eta a}$. The Einstein formula takes the form of Einstein-Stokes formula:

$$D = \frac{k_B T}{6\pi\eta a}. \tag{6}$$

3.2 Zimm dynamics

Zimm model describes the dynamics of a single chain in a solvent. It is a non-draining model which assumes that the motion of the solvent close to the chain is strongly suppressed. The hydrodynamic effects are accounted for by the Navier-Stokes equation and lead to the long-range hydrodynamic interactions characterized by the Oseen tensor.[2] Each of the beads undergoes a Brownian motion in a harmonic potential due to the harmonic forces between the neighbouring beads. In the equilibrium the dynamics of the beads is described by the following equation,[6] where $\vec{v}_i$ describes the flow of the solvent close to the bead $\vec{r}_i$, and k_0 stands for the spring constant:

$$\eta \ \ \frac{d\vec{r}_i(t)}{dt} - \vec{v}_i(t) \ \ = k_0 \left[\vec{r}_{i+1}(t) + \vec{r}_{i-1}(t) - 2\vec{r}_i(t)\right] + \vec{\xi}_i(t). \tag{7}$$

The excluded volume interaction resulting from the fact that two elements of a chain cannot occupy the same volume element in the space at the same time can be also taken into the account. Calculations[2] show that the diffusion coefficient $D^{(Zimm)}$ of the center of the mass of the chain is proportional to $(l_c)^{-1/2}$. This is in a fair agreement with experimental result:[7]

$$D^{(exp)} \approx 3 \cdot 10^{-12} \left(\frac{l_c}{1\mu\mathrm{m}}\right)^{-0.57}. \tag{8}$$

Another seminal model of the dynamics of polymer chains - the Rouse model - predicts that $D^{(Rouse)} \propto (l_c)^{-1}$. Because of this (and other, see Ref[2]) failures, the Rouse model is regarded as inappropriate for the description of the hydrodynamic-driven dynamics of diluted polymer systems.

The final expression for diffusion coefficient in Zimm dynamics reads[2]

$$D \approx \frac{k_B T}{\eta r_g}. \tag{9}$$

Equation (9) has the form of Einstein-Stokes equation (6) and thus sets one of the milestones for an experimental analysis of the sizes of polymer chains. Namely, it states that the polymer diffusion in the dilute solution is similar to that of a dilute system of spheres with radius $R = r_g$. In other words, as far as the hydrodynamic diffusion is taken into account, polymer chains can be treated as spheres. Once the diffusion coefficient is measured (see Section (3.3)), Eq. (9) yields the giration radius of the chain. Comparison with an experiment shows that Zimm model is accurate enough to approximate the diffusion of DNA when $R \approx r_g/3$.[7] Then, the above equation reads

$$D = \frac{k_B T}{2\pi\eta\, r_g}. \tag{10}$$

3.3 Dynamic Light Scattering

The Brownian motion of polymers in the solvent can be studied using Dynamic Light Scattering (DLS) technique which yields the time correlations of the intensity of the scattered light. The dynamical structure factor (the correlation function) reads:

$$g(\vec{k},t) = \frac{1}{N} {}_{n,m} \exp\left(i\vec{k}\cdot(\vec{r}_n(t) - \vec{r}_m(0))\right. \quad . \tag{11}$$

In the regime $kr_g \ll 1$ only the overall translatory motion of the polymer can be observed, represented by the translatory movement of the center of mass of the chain. In the long time limit[2]

$$g(\vec{k},t) \propto \exp(-\Gamma_k t), \tag{12}$$

where

$$\Gamma_k = Dk^2 = \frac{k_B T k^2}{2\pi\eta}\frac{1}{r_g}. \tag{13}$$

Formulas (12) and (13) illustrate the principle of DLS measurements of the characteristic size of a polymer chain - radius of giration - from DLS measurements: it is inferred from the decay constant of the correlation function $g(\vec{k},t)$. Let us point again that this result is valid only for a single chain or for a strongly diluted system.

4. WORMLIKE CHAIN MODEL

To analyze theoretically the experimental data one needs a model of a polymer chain which depends on two experimentally measurable parameters: l_c and r_g. Such a model should set a relation between l_c and r_g such that relation (8) holds at least approximately. In the literature there are a few such models:[2] wormlike chain, freely jointed chain, freely rotating chain, bead-spring chain and others.

In what follows we present the main features of the wormlike chain model which treats the polymer as a circularly symmetric elastic beam element with a flexural rigidity YI, where Y and I denote, respectively, the Young modulus and the second moment of the area of the beam.[7] The energy of bending is then

$$U_{bend} = \frac{1}{2}YI\int_{s=0}^{l_c}\left|\frac{d^2\vec{r}}{ds^2}\right| ds. \tag{14}$$

The description of statistical properties of the chain is obtained in the framework of statistical mechanics, where the probability of a specific configuration of the chain is given by the Gibbs factor $\exp\left(-\frac{U_{bend}}{k_BT}\right.$. Let us summarize the main results of such an analysis. The average end-to-end distance $\langle l_e\rangle$ reads

$$\langle l_e\rangle = \overline{\quad 2l_pl_c - 2(l_p)^2\ \ 1-\exp\left(-\frac{l_c}{l_p}\right)\Big]}. \tag{15}$$

In the limit $l_p \approx l_c$ the chain is rod-like and $\langle l_e\rangle \approx l_c$. In another important limit $l_p \ll l_c$ $\langle l_e\rangle$ becomes

$$\langle l_e\rangle = \sqrt{2l_cl_p} = \sqrt{6}\langle r_g\rangle. \tag{16}$$

This equation can be used to infer the persistence length of long DNA chains. The persistence length is proportional to the flexural rigidity of the polymer: $l_p = \frac{YI}{k_BT}$. This formula sets a relation between l_c and r_g:

$$YI = 3k_BT(r_g)^2(l_c)^{-1}. \tag{17}$$

At room temperature the model DNA polymer backbone has a flexural rigidity $YI \approx 2\cdot 10^{-28}\,\mathrm{Nm}^2$. This implies that $r_g \propto (l_c)^{1/2}$. Upon inserting this result into Eq. (9) one finds that $D \propto (l_c)^{-1/2}$, in fair agreement with experimental result Eq. (8).

5. CONCENTRATION OF THE SOLUTION: LIMITATIONS OF THE THEORY

5.1 Dilute and semidilute solutions

Let us discuss the spatial organization of the solution. To start with, let us divide polymer solutions in good solvents into three groups, following Ref.:[2] dilute, semidilute and concentrated. In a dilute solution the DNA chains are away from each other and their interaction can be neglected. In this regime the coil size is independent on the concentration of the solution. For the dilute solution the interactions play a minor role and various physical quantities attain the dependence on the density through virial corrections which, as a rule, are small. As the density increases, the polymer chains come closer and start to overlap. The concentration ρ^* at which the overlap starts is estimated as follows[2]

$$\frac{\rho^* \, N_A}{M} \, \frac{4\,\pi}{3} \, R_g^3 \approx 1, \tag{18}$$

with M the molecular mass and N_A the Avogadro constant. This condition simply states that in the volume with radius equal to the giration radius there is, on the average, a single coil. Since $\rho^* \propto M^{-4/5}$, for large molecular weight ρ^* can become quite small. For example, for a polystyrene of $M \approx 10^6$, ρ^* is about 5 g/L (about 0.5% in weight). For $M = 8 \cdot 10^6$ an extra factor 8 in M decreases ρ^8 by $8^{4/5} \approx 5$ and yields ρ^* about 1 g/L. Assuming that this argumentation is weakly dependent on the nature of the polymer chain we tentatively estimate the concentration for which the $M = 8 \cdot 10^6$ DNA coils starts to overlap as

$$\rho^*_{DNA} \approx 1\,\mathrm{g/L}. \tag{19}$$

This analysis shows that for large molecular weight one can have a solution where the molecules are overlapped but occupy a small volume fraction - by the definition, it is a semidilute solution.

5.2 Mean chain to chain distance: an example

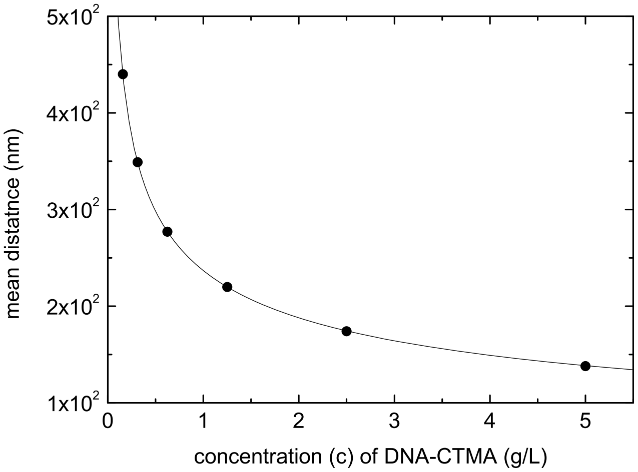

Figure 2. Distance between centers of mass of DNA-CTMA chains against concentration

To illustrate the transition form dilute to semidilute solutions let us consider the case of $M = 8 \cdot 10^6$ Da double stranded DNA-CTMA-DR1 (dsDNA) solution in butanol, studied recently using DLS technique; the concentrations varied form 0.01 g/L to 10 g/L.[8] The mass of DNA backbone is

$$M = 8 \cdot 10^6\,\mathrm{Da} = 8 \cdot 10^6 \cdot 1.66 \cdot 10^{-27}\,\mathrm{kg} = 1.328 \cdot 10^{-20}\,\mathrm{kg}.$$

Consider the case of the concentration $c = 10\,\frac{g}{L}$. The number N of DNA chains in $V = 1$ L of the solvent is equal to

$$N = \frac{0.01}{M} = 7.53 \cdot 10^{17}.$$

Thus, the average volume V_{ch} per chain is:

$$V_{ch} = \frac{V}{N} = 1.328 \cdot 10^{-21}\,\frac{\mathrm{m}^3}{\mathrm{chain}}.$$

If every chain occupies a box, then its side is equal to

$$a = (V_{ch})^{\frac{1}{3}} = 1.1 \cdot 10^{-7}\,\mathrm{m} = 0.11\,\mu\mathrm{m}.$$

This result means that mean distance between adjacent DNA chains is approximately equal to $0.11\,\mu$m. On the other hand, experimental DLS data[8] show that the typical coil size of DNA-CTMA biopolymer at this concentration is larger than 0.5 μm. Those estimations clearly show that the DNA chains overlap and the system is not dilute.

For an arbitrary concentration c the box side a reads:

$$a = \left(\frac{M}{c}\right)^{\frac{1}{3}}. \tag{20}$$

The plot of function $a(c)$ is shown in Fig. 2. We conclude that even for the lowest concentrations used in experiment (0.01 g/L), the distance between centers of chains is comparable with the characteristic coil size. This result is supported by the Monte Carlo simulations using bond fluctuating method.[9] Fig. 3 shows an instantaneous configuration of polymer chains consisting of 20 beads for the concentration of chains comparable to the concentration 0.01 g/L in experiment. An eye inspection shows that some of the chains overlap.

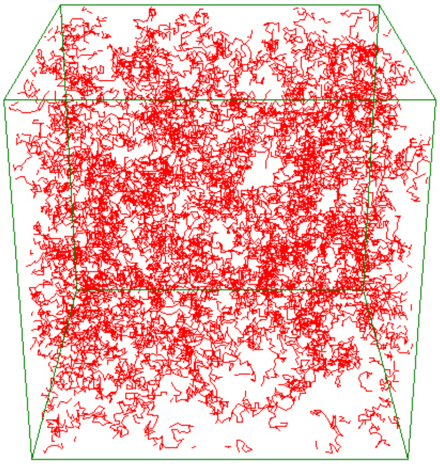

Figure 3. Instantaneous configuration of a model polymer system with low concentration (see text for the details).

6. CONCLUSIONS

We have presented methodological aspects of an experimental and theoretical analysis of the results of DNA coils size measurements via Dynamic Light Scattering method. The main conclusion is that the theoretical analysis relies heavily on the assumption that the system is dilute and the chains do no overlap. This sets an upper limit for the concentrations of the DNA solutions. For higher concentrations a very careful treatment of the DLS results is required and some modifications of the approach presented in this paper is necessary. The results of such an analysis for recent experiment[8] will be published elsewhere.

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REFERENCES

1. A. Tane, I. Rau, A. Meghea, and A.D. Nistor, U.P.B. Sci. Bull., Series B, Vol. **76**, Iss. 3, 99 (2014).
2. M. Doi M., and S.F Edwards, *The Theory of Polymer Dynamics*, Clarendon Press, Oxford (2013).
3. K. Jacobs, *Stochastic Processes for Physicists*, Cambridge University Press, Cambridge (2010).
4. D.S. Lemons, *An Introduction to Stochastic Processes in Physics*, The John Hopkins Iniversity Press, Baltimore (2002).
5. L.D. Landau and E. M. Lifshitz, *Statistical Physics. Course in Theoretical Physics*, Vol. 5, Pergamon, Oxford, (1961).
6. T. Kawakatsu, *Statistical Physics of Polymers*, Springer (2004).
7. B.J. Kirby, *Micro- and Nanoscale Fluid Mechanics*, Cambridge University Press (2010).
8. C.A. Lazar, F. Kajzar, and I. Rau, unpublished.
9. H.P. Deutsch, K. Binder, J. Chem. Phys. **94**, 2294 (1991).
10. C.A. Lazar, A.C. Mitus, W. Radosz, G. Pawlik, F. Kajzar, and I. Rau, in preparation.