Dynamic light-scattering studies of hydroxyethyl cellulose solutions used as sieving media for electrophoretic separations

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ABSTRACT

Dynamic light-scattering experiments are performed on semi-dilute solutions of hydroxyethyl cellulose which have been shown to be suitable as molecular sieving media for electrophoretic separations in a capillary format. The multi-exponential data analysis program CONTIN is used to determine the various relaxation processes present. The relationship between polymer concentration, C (g/ml), and the mesh-size of the entangled network, ξ, is found to be $\xi(\text{Å}) = 6.0\ C^{-0.68}$, which is in good agreement with predictions based on the scaling theory of De Gennes and intrinsic viscosity measurements.

INTRODUCTION

Recently it has been demonstrated that low-viscosity (< 2 cP) semi-dilute polymer solutions can be used as molecular sieving media for electrophoretic separations performed in micro-capillary tubes [1–7]. These systems promise significant practical advantages over traditional rigid-gel electrophoresis media in that no gel preparation step is required. Furthermore, because of their low viscosity, these solutions are well suited for use in automated capillary electrophoresis instrumentation. In essence, the use of semi-dilute polymer solutions in a capillary electrophoresis format decouples the two roles of a traditional electrophoresis gel: that of an anti-convective support and of a molecular sieve.

In our previous report [3] the scaling theory of De Gennes [8] and traditional theories of electrophoretic migration were used to relate the properties of the mesh-forming polymer to the mesh size of the polymer network and to the resulting electrophoretic migration behavior of a series of DNA restriction fragments. It was shown that the mesh size of the entangled polymer solution was similar to that of traditional agarose gels and that the migration mechanism of the DNA fragments was the same as that found in traditional rigid-gel matrices. Furthermore, a relationship was proposed which could be used to design optimal entangled polymer systems for different electrophoresis applications.

Since the first successful application of scaling theory to polymer solutions by De Gennes [8], dynamic light scattering (also known as photon correlation spectroscopy or quasielastic light scattering) has played an important role in the study of the dynamics of semi-dilute polymer solutions. Here we apply the technique of dynamic light scattering to measure the mesh size of the hydroxyethyl cellulose (HEC) solutions which were shown to be useful as sieving media in electrophoretic separations of DNA restriction fragments. In particular, we confirm the predictions for the mesh size based on scaling theory and intrinsic viscosity measurements for aqueous HEC solutions used in our previous electrophoresis study.

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THEORY

Physical basis

Dynamic light-scattering measurements yield directly the mutual diffusion coefficient of a scattering species undergoing Brownian motion. The time dependence of the intensity fluctuations of the scattered light, $I(t)$, can be related to the transport properties of the scattering species.

A simple picture of a light-scattering photometer is given in Fig. 1. A monochromatic laser light is used to illuminate a region of the sample solution. The sample scatters light in all directions and a record of the intensity fluctuations of the light scattered through a small range of angles is collected using a photodetector. These intensity fluctuations are then related to the Brownian motion of the scattering species.

In the case of semi-dilute polymer solutions, light scattering is caused by fluctuations in the concentration of the sample polymer induced by its thermal motion within the illuminated volume. The relaxation rate of these concentration fluctuations can be determined using the scattered intensity autocorrelation function, $G(2)(\tau)$, where

$$G(2)(\tau) = \langle I(t)I(t+\tau) \rangle = \lim_{T \to \infty} \frac{1}{T} \int_0^T I(t)\,I(t+\tau)\mathrm{d}\tau$$

(1)

where $I(t)$ is the time dependent intensity of the scattered light, τ is the delay time of the fluctuations, T is total time for the measurements and the angle brackets denote a statistical average. The relaxation rate of the intensity fluctuations, Γ, is calculated by fitting experimental data to a theoretical formulation of $G(2)(\tau)$.

The diffusive nature of the relaxation process responsible for the decay in $G(2)(\tau)$ indicates that the scattered intensity autocorrelation function is characterized by an exponentially decaying function, with the time constant given by Γ^{-1}, where

$$\Gamma = D|q|^2$$

(2)

where Γ is the relaxation rate of the process, D is the diffusion constant and q is the scattering vector. The scattering vector is a measure of the characteristic length associated with the diffusive fluctuations
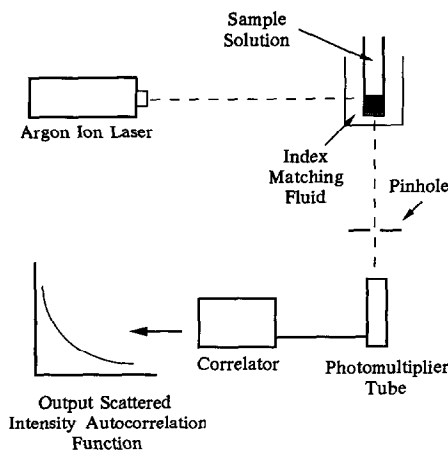

Fig. 1. Schematic diagram of light scattering apparatus.

which can be measured at a given scattering angle with a given incident radiation. The magnitude of q is given by the relation

$$q \cong \frac{4\pi n}{\lambda} \sin\left(\frac{\theta}{2}\right)$$

(3)

where θ is the angle between the incident and scattered light, n is the refractive index of the scattering medium, and λ is the wavelength of the incident light in vacuo. Thus, knowing Γ, one can determine the value for D.

In the limit of infinite dilution, the self diffusion coefficient, D_0, of a spherical particle is given by the Stokes-Einstein relation as

$$D_0 = \frac{kT}{6\pi\eta R}$$

(4)

where k is Boltzmann's constant, T is the absolute temperature, η is the viscosity of the pure solvent and R is the particle radius. According to the scaling theory of De Gennes [8], in semi-dilute polymer solutions, the cooperative diffusion coefficient of the polymer, D_c, is given by

$$D_c = \frac{kT}{6\pi\eta\xi_d}$$

(5)

where ξ_d is the dynamic correlation length of the polymer, which can be interpreted as a measure of the mesh size of the polymer network. De Gennes
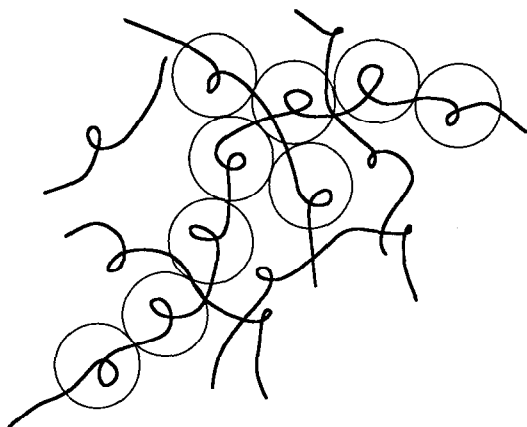
Fig. 2. Schematic illustration of a semi-dilute polymer solution according to the scaling theory of De Gennes [8].

theory assumes that semi-dilute polymer solutions can be modeled as a group of spherical polymer "blobs" having a characteristic size ξ_d whose motion is uncorrelated (see Fig. 2). Thus, given an experimentally measured value of Γ, in a semi-dilute solution, using eqns. 2 and 5, one can obtain an estimate of the value ξ_d. The value of Γ is calculated from the measured autocorrelation function of $I(t)$. This procedure will be discussed in the following section.

The autocorrelation function

In general, correlation functions provide a method for expressing the degree to which dynamic properties are correlated over time. As mentioned earlier, dynamic light-scattering experiments measure the intensity autocorrelation function through the recorded time dependent intensity of the scattered light, $I(t)$. However, because the dielectric constant fluctuations which are responsible for light scattering are proportional to the *electric field* of the scattered light rather than the *intensity*, it is necessary to know the relationship between the *scattered intensity* and the *scattered electric field* autocorrelation functions. (The structural information is contained in the behavior of the scattered electric field, but all we can measure directly is the scattered intensity.) The scattered electric field autocorrelation function, $g(1)(\tau)$ is defined as

$$g(1)(\tau) = \langle E^*(t)E(t + \tau)\rangle =$$

$$\lim_{T \to \infty} \frac{1}{T} \int_0^T E^*(t)E(t + \tau)\mathrm{d}\tau \qquad (6)$$

where $E(t)$ is the time dependent electric field of the scattered light, and $E^*(t)$ is the complex conjugate of $E(t)$. $E(t)$ is related to the intensity of the scattered light by

$$I(t) = E^*(t)E(t) \qquad (7)$$

For the case of semi-dilute polymer solutions, the relationship between $G(2)(\tau)$ and $g(1)(\tau)$ is given by the homodyne autocorrelation function [9], thus

$$G(2)(\tau) = B[1 + \beta|g(1)(\tau)|^2] \qquad (8)$$

where B is the baseline and the pre-exponential factor β is an instrumental correction factor having a value between 0 and 1. A typical plot of the scattered electric field autocorrelation function is shown in Fig. 3.

The analysis of the measured autocorrelation function, $G(2)(\tau)$, is a critical step in a dynamic light-scattering experiment. In the simplest case, that of a monodisperse suspension of nearly spherical particles, *e.g.* polystyrene latex spheres, the autocorrelation function can be fitted to a single exponential function. In this case,

$$g(1)(\tau) = A \exp(-\Gamma\tau) + B \qquad (9)$$

where A is the spectral amplitude and B is the baseline.

In the case of semi-dilute polymer solutions, a number of relaxation processes occur simultaneously, and a single exponential approach is not satisfactory. These processes include center-of-mass motion of a single chain, center-of-mass motion of groups of chains and changes in the conformation of individual chains. Therefore, one is forced to use a multi-exponential approach to resolve the different relaxation modes present. In this study we will use the CONTIN method [10,11]. CONTIN fits the electric field autocorrelation function to a series of exponential functions,

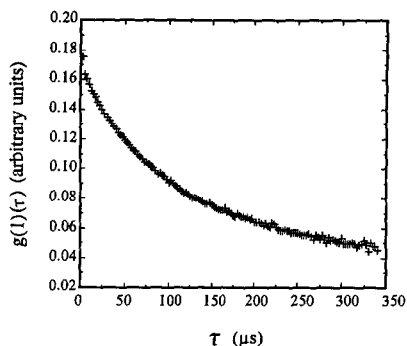$$g(1)(\tau) = \int_{j=1}^{N_j} G(\Gamma_j)\exp(-\Gamma_j\tau) + 1 \qquad (10)$$

Fig. 3. Representative plot of the scattered electric field auto-correlation function, $g(1)(\tau)$. Data are from a solution of 0.006 g/ml HEC in TBE buffer at 30°C. The scattering angle is 90°.
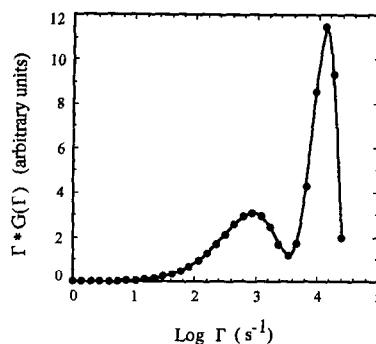

Fig. 4. Representative plot of the distribution of relaxation times. Data are from a solution of 0.01 g/ml HEC in TBE buffer at 30°C. The scattering angle is 90°.

where $G(\Gamma)$ is the normalized rate distribution, 1 is a constant and N_j is the number of exponentials used in the sum. Note that if $N_j = 1$, eqn. 10 reduces to eqn. 9. In this study 31 exponentials were used to extract values for Γ_j from measurements of $g(1)(\tau)$.

MATERIALS AND METHODS

The light-scattering instrument used in these studies was purchased from Brookhaven Instruments Corporation (Holtsville, NY, USA). The light source is a Lexel Model 95-2 argon ion laser (Palo Alto, CA, USA) whose incident polarization is perpendicular to the scattering plane. The sample cell is immersed in an index matching liquid (toluene) in order to reduce the reflection of the incident beam from the surface of the sample cell. All measurements were made at a scattering angle of 90° and the sample time was 2.5 μs. Data for each measurement were accumulated over a period of 5 min. The temperature was controlled at 30.0 $\pm$ 0.1°C using a Lauda Model RM6 circulating water bath (Westbury, NY, USA). The correlator is a Brookhaven BI-2030 real-time 136-channel digital correlator.

As mentioned before, the measured autocorrelation functions were analyzed using the LaPlace inversion program CONTIN [10,11].

The buffer used in all experiments was 89 mM tris(hydroxymethyl)aminomethane, 89 mM boric acid and 5 mM ethylenediaminetetraacetic acid (TBE buffer) at pH 8.1. Varying amounts of (hydroxyethyl) cellulose was added to the TBE buffer to make up the polymer solutions. All the solutions

were filtered through a 0.2-μm nylon filter (Fisher Scientific, Pittsburgh, PA, USA) to remove any dust particles and were allowed to sit for between 18 and 24 h to insure complete dissolution of the polymer.

RESULTS AND DISCUSSION

A plot of the distribution of relaxation times of an HEC solution having a concentration of 0.006 g/ml is shown in Fig. 4. This plot is typical of these seen at other polymer concentrations above the overlap concentration. The origin of the modes has been determined in a previous report from this laboratory using solutions of polyacrylamide [9]. The slow mode (small Γ) was attributed to the center-of-mass motion of unentangled single polymer chains, while the fast mode (large Γ) was attributed to the dynamics of the entangled polymer network (co-
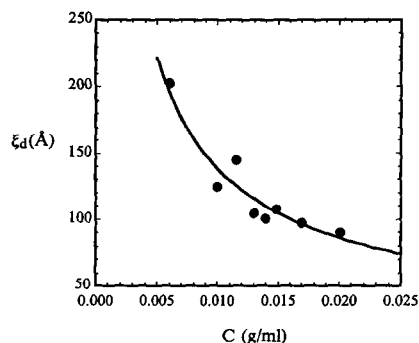

Fig. 5. Plot of the measured dynamic correlation length, ξ_d, as a function of HEC concentration. The solid curve is a plot of eqn. 11 where $a = 4.22$ Å and $v = 0.75$.
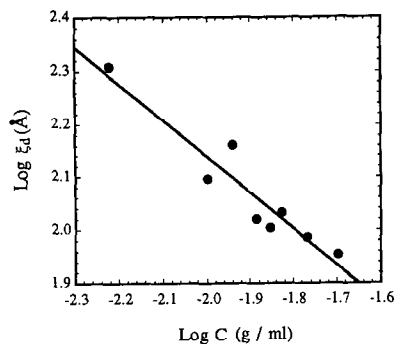
Fig. 6. Concentration dependence of the dynamic correlation length for HEC solutions at 30°C (0.006–0.02 g/ml). The straight line is a linear least-squares fit where $\xi_d(\text{Å}) = 6.0C^{-0.68}$.

Fig. 7. Plot of the measured dynamic correlation length, ξ_d, as a function of HEC concentration. The solid curve is a plot of eqn. 12.

operative diffusion). In this work, because we are concerned with the polymer network and not individual polymer chains, we are interested exclusively in the cooperative diffusion (fast) component of the relaxation spectrum.

Fig. 5 shows plot of the measured correlation length, ξ_d, as a function of polymer concentration. The solid curve is the predicted value of ξ_d based on the scaling theory,

$$\xi_d = aC^{-\nu} \tag{11}$$

where a is the polymer segment length and C is the polymer concentration (in this case the volume fraction and the concentration are assumed to be equivalent) and ν is a constant whose value depends on the solvent quality. For a good solvent, $\nu = 0.75$. The value of a used in constructing the curve in Fig. 5 is 4.22 Å. This value was determined using intrinsic viscosity measurements [3] in the TBE buffer. All the points in Fig. 5 are above the experimentally measured value of the overlap threshold of approximately 0.4%.

In order to determine the experimentally measured value of the exponent in eq. (11) and the polymer segment length, a, a plot of log ξ_d vs. log C was constructed (Fig. 6). The slope of the solid line is −0.68, indicating that $\nu = 0.68$ in this system, in good agreement with the value of 0.75 predicted from theory. The zero intercept of the curve is 0.775,
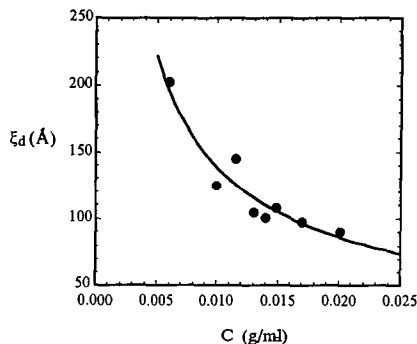
indicating a segment length of 6.00 Å. This value is higher than the value measured by intrinsic viscosity, 4.22 Å, but well within the margin of error for these measurements. Based on these measurements, for the polymer–solvent system considered here,

$$\xi(\text{Å}) = 6.0C^{-0.68} \tag{12}$$

Fig. 7 compares the experimental data with the curve described by eqn. 12. Thus, comparing Figs. 5 and 7, it can be seen that these data are in close agreement with the predictions of scaling theory.

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