polymer communications

Aggregation in solutions of poly(γ-benzyl-L-glutamate) monitored by static and dynamic light scattering

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We have studied dilute and semidilute solutions of poly(γ-benzyl-L-glutamate) in 1,2-dichloroethane by static and dynamic light scattering. The aggregational behaviour of the semiflexible polymer is evidenced in both static and dynamic scattering, and is sensitive to the temperature and the polypeptide molecular weight. In particular, both the reciprocal specific scattering intensity and the effective decay rate of the dynamic correlations exhibit a minimum as a function of the concentration. The slope of effective diffusion coefficient *versus* the square of the scattering vector displays an anomalous maximum as a function of the concentration which can also be attributed to aggregation. The experimental evidence is compatible with head-to-tail dimerization of the polymer as the concentration increases.

(Keywords: poly(γ-benzyl-L-glutamate); light scattering; aggregation; semidilute solutions)

Introduction

By lowering the temperature of a solution of flexible polymers, we decrease the excluded-volume effect since the attractive dispersion interaction among polymer segments manifests itself more strongly. In the classical two-parameter picture[1] this effect is confined to segment pairs so the θ temperature is defined by vanishing excluded volume, irrespective of whether a particular pair belongs to one chain or refers to two segments belonging to two respective chains. Theoretical arguments[2] and recent careful experiments on polystyrene[3] show that the binary collision model is too simple as one approaches the θ temperature from above. Higher-order interactions have to be accounted for but these represent a slight, though subtle amendment. By contrast, for rigid cylindrical macromolecules interacting by hard-core repulsion and dispersion forces, it has been argued[4] that the whole virial series may break down well above the θ temperature at which the binary excluded volume between two rods vanishes. The source of this anomaly may be traced back to the coupling of orientational to translational degrees of freedom. In effect, the attractive interaction is short-ranged so that two or more very close rods attract one another strongly, only when they are virtually parallel.

These considerations motivate an experimental investigation of attractive forces in solutions of stiff polymers. Unfortunately, well-characterized, ideally rod-like macromolecules interacting by van der Waals forces are hard to come by. Poly(γ-benzyl-L-glutamate) (PBLG) in helicogenic solvents has two drawbacks: it is semiflexible and the chains have a significant dipolar interaction. Nevertheless, it is our polymer of choice for it is widely used as a model for typically stiff macromolecules. It has been known for quite some time that helical PBLG chains may display noticeable attractive interactions; in fact, they aggregate more often than not in various solvents[5–9]. We present a preliminary outline of an extensive light scattering investigation of PBLG in 1,2-dichloroethane (DCE) chosen for its intermediate dielectric properties so it should be possible to study 'good' and 'poor' solvent regimes.

Experimental

Three samples of PBLG (Sigma) were vacuum dried and used without further purification. The molecular weights were determined by Sigma in the solvent dimethylformamide (DMF) using low angle laser light scattering to be 45 150, 70 000 and 105 600 g mol^{-1}, henceforth referred to as samples 1, 2 and 3, respectively. The solvent DCE was distilled before use.

Static light scattering experiments at zero scattering angle were performed with a Chromatix KMX-6 low angle laser light scattering photometer whose light source was a He–Ne laser at a wavelength of 633 nm. The sample cell consisted of two thick silica windows and a Teflon spacer. The sample was filtered through 0.2 μm Fluoropore (FG) filters on being introduced into the cell. Measurements were done within 30 min of filtration, with the samples flowing slowly through the sample cell. The quantity measured was the ratio of the scattered to the transmitted radiant power. The Rayleigh factor, R_0, was computed from this ratio and the geometric parameters of the instrument. No reference to external standards or arbitrary calibration factors was required. The scattered intensities were expressed in terms of Kc/R_0, with c the polymer concentration (in g ml^{-1}) and K a constant, which contains the refractive index n and the refractive index increment dn/dc. The latter was determined at 633 nm for sample 3 in DCE with a

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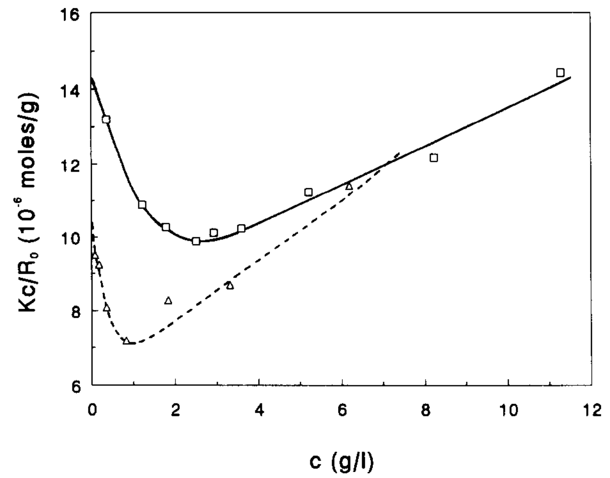

Figure 1 Inverse specific intensity Kc/R_0 at zero scattering angle as a function of concentration for PBLG in DCE, at 25°C: (□) sample 2; (△) sample 3. The curves are guides to the eye

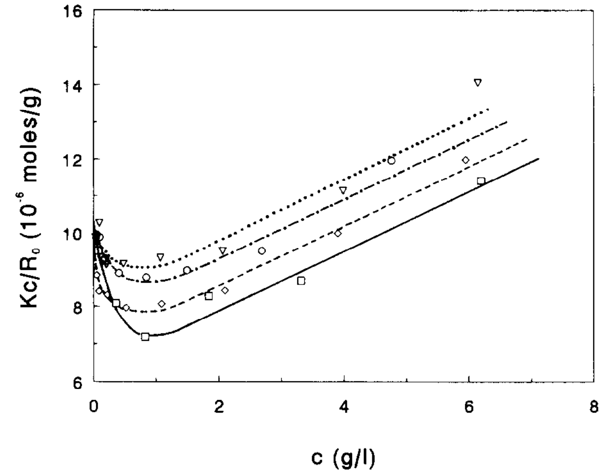

Figure 2 As in *Figure 1* but only for sample 3 at the following temperatures: (□) 25°C; (◇) 30°C; (○) 43.5°C; (▽) 50°C

Chromatix KMX-16 laser differential refractometer and assumed to be independent of the molecular weight. The values of $n = 1.4448$ and $dn/dc = 0.111$ ml g^{-1} yielded $K = 1.05 \times 10^{-7}$ cm^2 mol g^{-2}.

Dynamic light scattering experiments were performed with a spectrometer consisting of an ALV goniometer and an ALV 3000 correlator. The light source was an Ar-ion laser (Spectra Physics, 2000 series) at the 514.5 nm line. Typically, laser power was between 250 mW and 325 mW. Scattering was done at angles between 30° and 150°. The intensity–time correlation functions were recorded with the time axis logarithmically spaced. In this so called 'multi τ' mode the sample time in the first 16 channels was 0.8 μs. Every eight channels the sample time was doubled, reaching a total time for the correlation function of several seconds. For each correlation function several short runs were summed together. For the results focused on here, it was reasonable to apply a third-order cumulant analysis to the data, so as to obtain an effective decay rate Γ. PBLG was allowed time to dissolve completely. Within 24 h, stock solutions were filtered through 0.2 μm Fluoropore (FG) filters into clean sample cells. The solutions were then either diluted by adding filtered solvent or concentrated by evaporating solvent under a filtered N_2 flow. Measurements were always done within 1 h of these operations.

Results

Static light scattering at zero scattering angle. For samples 2 and 3, the inverse specific intensity Kc/R_0 at zero scattering angle has a minimum as a function of the concentration (*Figure 1*), which is in agreement with previous measurements by Gerber and Elias[7]. Extrapolations of Kc/R_0 to zero concentration do yield the correct molecular weights: $M = 70\,000$ g mol^{-1} for sample 2 and $M = 95\,000$ g mol^{-1} for sample 3. At high, semidilute concentrations both curves become linear within experimental error. If we tentatively extrapolate the linear asymptotes down to zero concentration, we may associate apparent molecular weights, M_{app}, with the intercepts. The ratios M_{app}/M are about the same: 1.76 for sample 2 and 1.70 for sample 3. The slopes of the asymptotes define the respective apparent second virial coefficients: $A_{2,\mathrm{app}} = 2.6 \times 10^{-4}$ ml mol g^{-2} for sample 2 and 3.9×10^{-4} ml mol g^{-2} for sample 3.

In *Figure 2* we show the temperature dependence of Kc/R_0 *versus* c for sample 3. The depth of the minimum and the ratio M_{app}/M decrease with increasing temperature although the slopes of the curves at higher concentrations are essentially parallel within the error margin. This behaviour may be universal for it was also seen for PBLG in DMF by Gerber and Elias[7].

Dynamic light scattering at zero scattering angle. The concentration dependence of the effective diffusion coefficient Γ/q^2 extrapolated down to zero scattering vector is shown in *Figure 3*. For all three samples this plot is similar to that of the inverse intensity Kc/R_0. In fact, the concentration at which Γ/q^2 is a minimum corresponds to the respective static value. As in the static case, the slopes of the curves at higher concentrations are less steep the lower the molecular weight.

Dynamic light scattering as a function of q^2. Over the whole range of scattering angles, Γ/q^2 is linear in q^2 with a slope equal to E. As shown in *Figure 4*, E has a maximum as a function of the concentration at about the same position at which both the inverse intensity

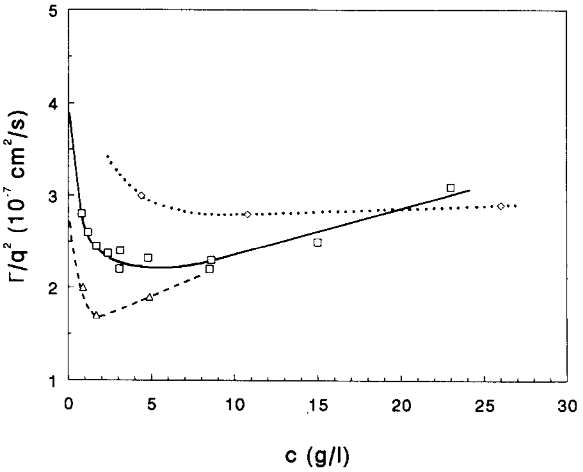

Figure 3 Decay rate Γ/q^2 at zero scattering angle as a function of concentration, at 25°C: (◇) sample 1; (□) sample 2; (△) sample 3. The curves are guides to the eye

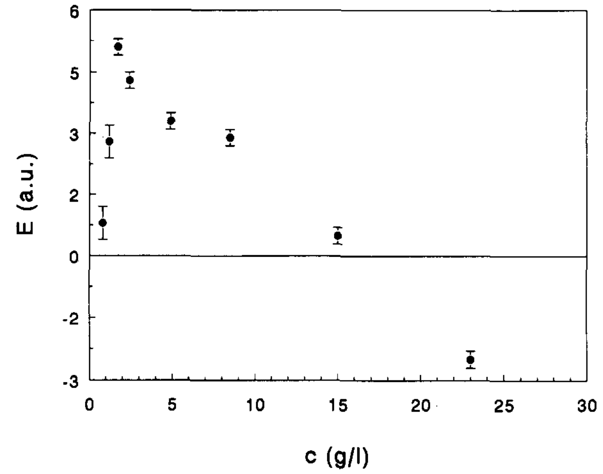

Figure 4 Dependence of the slope E of Γ/q^2 *versus* q^2 on concentration for sample 2, at 25°C

(*Figure 1*) and the effective diffusion coefficient (*Figure 3*) exhibit a minimum.

Discussion

We now reason qualitatively why *Figures 1–4* are consistent with the hypothesis that PBLG dimerizes head-to-tail in DCE, with the equilibrium:

$$\text{two monomers} \leftrightarrows \text{one dimer}$$

shifting from left to right as we increase the concentration.

1. The contour lengths of our PBLG molecules are all smaller than the persistence length, so we regard the chains as essentially rigid slender rods. The minimum in *Figure 1* is not compatible with the scattering predicted for non-aggregating rods interacting by attractive forces[4].
2. In very dilute DCE solution, PBLG is not aggregated since its molecular weight agrees with the value measured in the much better solvent DMF under the same conditions: PBLG is known to aggregate to a much lesser extent in the latter[7]. The apparent molecular weights are in between the monomer and dimer values.
3. The $A_{2,\mathrm{app}}$ values are consistent with the second virial coefficient[1] (3.4×10^{-4} ml mol g^{-2}) calculated for head-to-tail dimers of diameter 1.55 nm as expected to pertain to the PBLG helix. They are not consistent with the coefficient expected for sideways dimerized PBLG which is about half this value.
4. The second virial coefficient for two monomers may be positive or negative; a positive coefficient is not in conflict with the phenomenon of aggregation[4]. The absolute magnitude of the negative slopes apparent in *Figures 1* and *2*, is substantially greater than $A_{2,\mathrm{app}}$ so we suspect the binary collisions between monomers at low concentrations are swamped by the aggregation process, at least with regard to the scattering by the solutions.
5. As the temperature is lowered, the monomer–dimer equilibrium shifts from left to right (*Figure 2*), which seems reasonable if the equilibrium is determined by an interplay of dispersion forces and configurational entropy. $A_{2,\mathrm{app}}$ is independent of the temperature to a good approximation, which is in accord with argument 3 where we surmised that $A_{2,\mathrm{app}}$ is dominated by hard core interactions.
6. In *Figure 3* the effective diffusion initially decreases with increasing concentration which may be ascribed in large part to a lengthening of the macromolecules.
7. An examination of the theory of Doi *et al.*[10] as applied to rods aggregating head-to-tail, proves that as we add polymer to the solution starting from infinite dilution, E must be positive and increase quite fast at first, then reach a maximum and decrease rather gradually, ultimately changing sign. This agrees with *Figure 4*. Sideways aggregation would lead to E decreasing monotonically.

Concluding remarks

Our scattering experiments have all been carried out within 24 h of sample preparation and were reproducible on this condition. However, we have also found other complicated aggregational phenomena for PBLG in DCE beginning to be discernible after about a week. In a future publication we shall discuss these temporal phenomena as well as present a more exhaustive analysis of the regime focused on here.

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