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Hydrodynamic Properties of a Polyisoprene/Poly(oxyethylene) Block Copolymer

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ABSTRACT: The usefulness of combining various types of analytical ultracentrifuge measurements (sedimentation and flotation velocity, sedimentation and flotation equilibrium), together with intrinsic viscosity information for the characterization of an amphiphilic block copolymer system, is described. A block poly(ethylene oxide)/polyisoprene/poly(ethylene oxide) copolymer was studied in two solvents: water (in which the copolymer forms micelles) and chloroform (in which the copolymer remains as unimers). In the latter solvent the copolymers exhibit flotation behavior under the influence of a centrifugal field. In chloroform the polydispersity of the unimers appears to be a quasi-continuous type rather than of a discrete type with a weight-average molecular weight of 35 000 ± 2000 g/mol. The value for the Wales–van Holde parameter, $k_s/[\eta]$, of ~1.1 suggests an extended rather than a compact conformation for the unimer, at least in $CHCl_3$. The (apparent) weight-average molecular weight of the micelles in water is ~(15.0 ± 1.0) × 10^6, suggesting a unimer association number of the order of 400. From this value for the molecular weight and the intrinsic viscosity an estimate for the weight-average hydrodynamic radius of 42 ± 3 nm for the micelles is obtained.

Introduction

The solution behavior of amphiphilic block copolymers is currently of interest in a variety of medical and commercial applications. Many of these copolymers are micelle forming in aqueous solvent, and, depending on the specific nature of the polymeric components and the stability of the micelles, they have potential to be engineered for use for example as carrier systems for targeting drugs.[1] These copolymers have already been used to modify the surface characteristics and clearance behavior of microspheres.[2]

The solution properties of block copolymers have previously been studied by using a wide range of techniques,[3] principally dynamic light scattering,[4,5] electron microscopy,[6] and gel permeation chromatography.[7] Tuzar and co-workers[3,5] and Kotaka et al.[8] have demonstrated the utility of sedimentation velocity and sedimentation equilibrium analyses, respectively, for these systems.

The purpose of this present study is to demonstrate the utility of using a combination of ultracentrifuge measurements (sedimentation velocity, flotation velocity, sedimentation equilibrium, flotation equilibrium), together with intrinsic viscosity information for the characterization of an amphiphilic block copolymer of the "ABA" type (poly(ethylene oxide)/polyisoprene/poly(ethylene oxide)) dispersed in two solvents: one micelle forming (water); the other maintaining the unimer form (chloroform). Specifically, we (1) compare the transport behavior under the influence of an ultracentrifugal field (sedimentation coefficients and concentration-dependence behavior) and investigate the cosedimentation behavior of a hydrophobic dye (Sudan Black) with the micelle forms and (2) compare the low-speed equilibrium behavior (weight-average molecular weights of unimers and micelles). These data, together with intrinsic viscosity information, are used to investigate the size, shape, and polydispersity of the block copolymer unimers and the size, polydispersity, and unimer association number of the micelles.

Materials and Methods

Copolymer. Poly[(ethylene oxide)-*b*-isoprene-*b*-(ethylene oxide)] block copolymer ("Copo 9") was obtained from Professor Riess and Dr. Abou Madi (ENSC, Mulhouse, France) under a collaborative agreement with Ciba-Geigy. (The chemical composition of the polymer, as determined by NMR was as follows: 65.8%; H, 10.1%; O, 23.4%. From these data, a corresponding mean isoprene content of ~34.5% was evaluated.) *Approximate* values for apparent molecular weight averages (relative to polystyrene standards) as estimated by size-exclusion chromatography of the copolymer in tetrahydrofuran at 30.0 °C were $M_n \sim 17\,000$, $M_w \sim 25\,000$, and $M_z \sim 30\,000$, with polydispersity indices $M_w/M_n \sim 1.4$ and $M_z/M_w \sim 1.2$ (from ref 9).

Solvents. Two solvents were used: deionized distilled water and chloroform. **(i) Water**: Copo 9 stock solutions were prepared up to a concentration of ~10.0 mg/mL. The method of dissolution consistently used was to heat the solution to 80.0 °C for 30 min followed by stirring the solution overnight at room temperature, leaving a nonturbid solution. **(ii) Chloroform**: Copo 9 readily dissolved at ambient temperature.

We stress that much care was taken in the preparation of solutions to ensure consistency, as a precaution against any influence on the nature of the dissolution conditions on the observed properties.

Partial Specific Volume ($\bar{v}$). This was measured, at 25.00 ± 0.01 °C with water as the solvent, in the standard way by using automatic precision densimetry[10] and cesium chloride solutions as standards.[11]

A value for $\bar{v}$ of 0.921 ± 0.006 mL/g was obtained. The assumption was made that this value was the same in both solvents and constant in the range 13.5–25.0 °C.

Sedimentation Velocity. An MSE Centriscan 75 analytical ultracentrifuge was used equipped with scanning Schlieren and absorption optics and a monochromator. All experiments were performed at 20.0 °C and at a rotor speed of 47 000 rpm. Apparent sedimentation coefficients $s_{c(20,w)}$ at given concentrations, c (corrected for radial dilution), were obtained from the Schlieren scans off-line by using a computer digitizing tablet (in all that follows we use for convenience the symbol s_c in place of $s_{c(20,w)}$).

Additional sedimentation velocity experiments were performed with the inclusion of the hydrophobic dye "Sudan Black" to specifically identify micelles in the sedimenting species. It is considered[12] that water-insoluble dyes such as Sudan Black are solubilized by micelles and become incorporated into the hydrophobic core. A ~7.0 mg/mL solution of Copo 9 in water was equilibrated for ~4 h with an excess of Sudan Black (the

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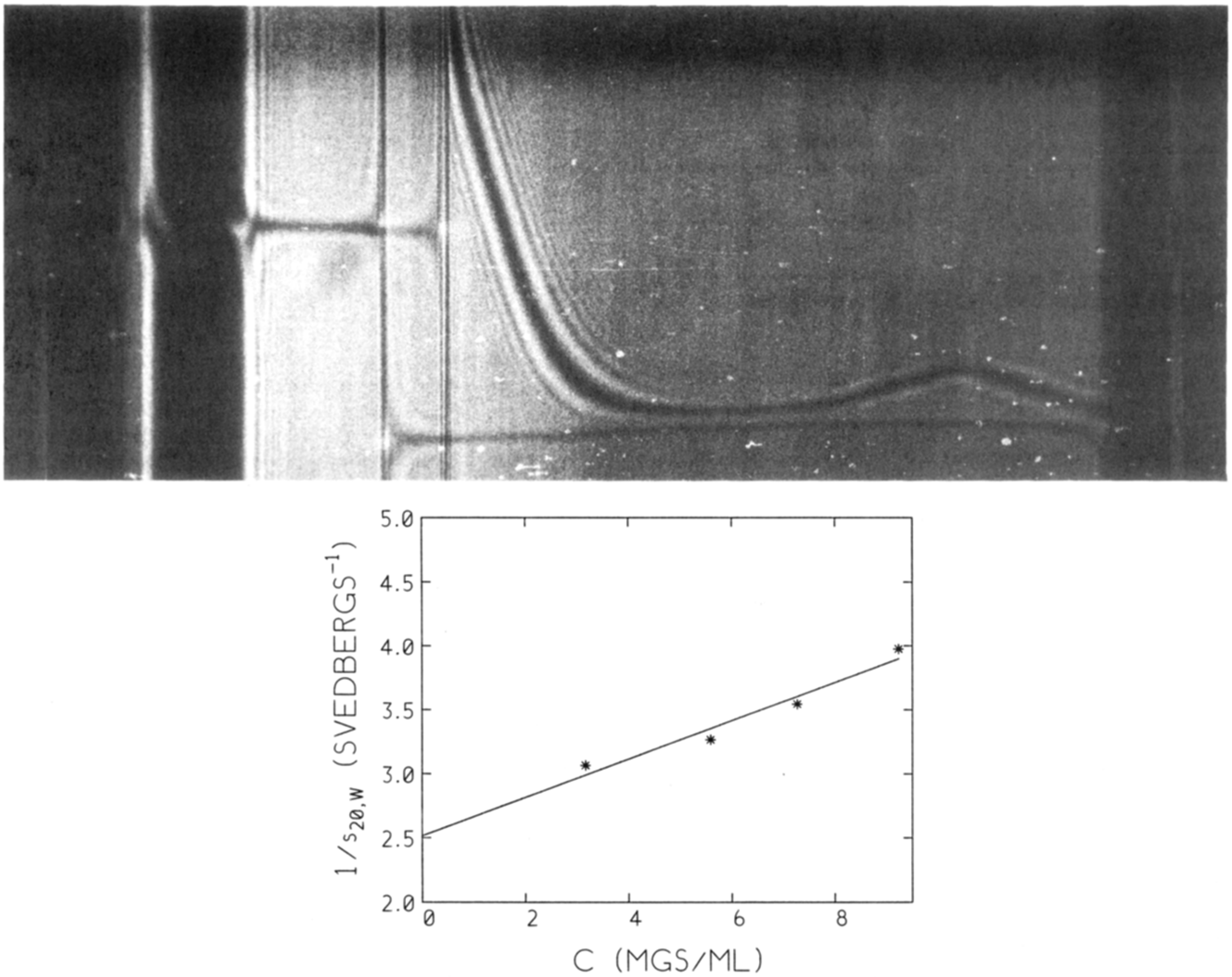

Figure 1. Flotation velocity of Copo 9 in $CHCl_3$. (a) Schlieren flotation velocity diagram from the MSE Mk. II. A 12-mm Kel-F cell was used; loading concentration $c = 8.9$ mg/mL. Rotor speed = 35 160 rpm. Temp = 13.5 °C. The direction of flotation is from right to left. (b) Plot of the reciprocal of the sedimentation coefficient, s_c (after correction to standard conditions—water at 20.0 °C) versus true sedimenting concentration, c.

resulting solution was unfiltered). Run conditions were identical with those where conventional Schlieren optics were used except that scanning absorption optics were employed with the monochromator set at 587 nm.

Flotation Velocity. Due to the large refractive index difference between the solvent ($CHCl_3$) and the Copo9 itself, conventional "optical" (phase plate) Schlieren optics were used[13] on an MSE Mk. II analytical ultracentrifuge. All runs were at 13.5 °C (in order to minimize the effects of evaporation) and a rotor speed of 35 160 rpm. Each solution was loaded into 12-mm, 4° Kel-F single-sector Beckman cells with adaptors to fit MSE rotors. A total of 7–10 frames were used for each determination of $s_{c(T,b)}$, the sedimentation coefficient in this solvent at temperature, T, at a given sedimenting concentration c (mg/mL). Concentrations were corrected for radial dilution effects.

Copo 9 has a density less than that of $CHCl_3$ (i.e., the buoyancy term $(1 - \bar{v}\rho_0)$, where ρ_0 is the solvent density, is negative), and hence the direction of movement is in the opposite sense to the direction of the ultracentrifugal field: $s_{T,b}$ values are negative. After correction to standard conditions[14] (water at 20.0 °C), the $s_{c(20,w)}$ values are positive:

$$s_{c(20,w)} = \frac{(1 - \bar{v}\rho_{20,w})}{(1 - \bar{v}\rho_{T,b})} \frac{\eta_{T,b}}{\eta_{20,w}} s_{c(T,b)} \qquad (1)$$

In eq 1, $\rho_{T,b}$ refers to the solution density and $\eta_{T,b}$ to the solvent viscosity. As above for conventional sedimentation velocity, in all that follows we use the symbol s_c in place of $s_{c(20,w)}$. In calculations we follow the common procedure of using solvent densities. This has no effect on the value of s_c extrapolated to infinite dilution (s), and a simple correction is available for the concentration-dependence regression factor, k_s, in the equation

$$\frac{1}{s_c} = \frac{1}{s}(1 + k_s c) \qquad (2)$$

with $k_s(\text{true}) = k_s(\text{meas}) - \bar{v}$ (see, e.g., ref 15).

Sedimentation Equilibrium. Copo 9 in Water. A Beckman Model E analytical ultracentrifuge was used, employing Rayleigh interference optics, a 5-mW He–Ne laser light source (λ = 632.18 nm), and an RTIC temperature-measuring system. Due to the high molecular weight of the micelles being studied, a low equilibrium speed (1961 rpm) was used to assist resolution of the fringes near the cell base. Even at this speed, near-meniscus-depletion conditions were obtained. Nonetheless, the meniscus concentration remained measurable and was obtained by mathematical manipulation of the fringe data.[16] The solution was loaded at a concentration c^0 of $\sim$2.0 mg/mL (to ensure $c >$ critical micelle concentration) into 30-mm path length double-sector cells to a column height of 2 mm, and the temperature was maintained at a constant 20.0 °C. Since thermodynamic nonideality may not be negligible at this concentration the molecular weights so found will be apparent values.

Whole cell (i.e., whole solute distribution) apparent weight-average molecular weights, M_w^0, were extracted by using the limiting value at the cell base of a particularly directly determinable point average (the "star" average, M^*; see ref 16); an independent estimate for the initial loading concentration was not required. Apparent point weight-average molecular weights were obtained by using sliding-strip quadratic fit procedures to the observed fringe data.[17]

Table I
Hydrodynamic Parameters for Copo 9 in Unimer and Micelle-Forming Conditions

	$10^{13}s$, s	k_s, mL/g	$[\eta]$, mL/g	$10^{-6}M_w^0$, g/mol	f/f_0	$10^{-6}\beta$	$k_s/[\eta]$	r_H,[a] nm
Copo 9 in $CHCl_3$ (unimer)	0.40 ± 0.02	59.5 ± 9.6	52.5 ± 0.5	0.035 ± 0.002	2.50 ± 0.05	2.3 ± 0.1	1.1 ± 0.1	
Copo 9 in H_2O (micelles)	25.6 ± 1.0[b] 35.0 ± 3.0[c]		32.1 ± 0.8	15.0 ± 1.0				40.0 ± 3.0

[a] Estimated from $[\eta]$ and M_w^0. [b] Major component. [c] Minor component.

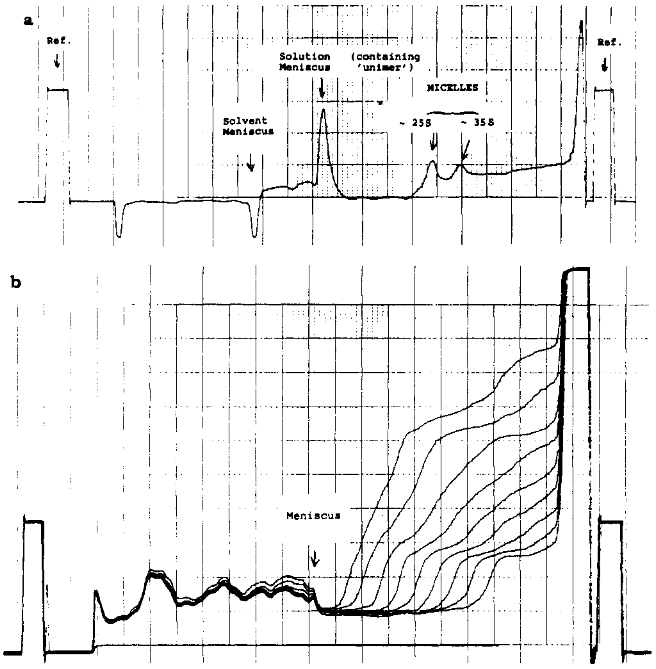

Figure 2. Sedimentation velocity of Copo 9 in water. (a) Scanning Schlieren sedimentation velocity diagram from the MSE Centriscan. A 10-mm standard cell was used, c = 2.0 mg/mL. Rotor speed = 47 000 rpm. Temp = 20.0 °C. The direction of sedimentation is from left to right. (b) Scanning absorption (λ = 587 nm) sedimentation velocity diagrams of Copo 9 solution in water in the presence of the water-insoluble dye Sudan Black. Scan intervals 3 min. $c \sim 7.4$ mg/mL. Rotor speed = 37 000 rpm.

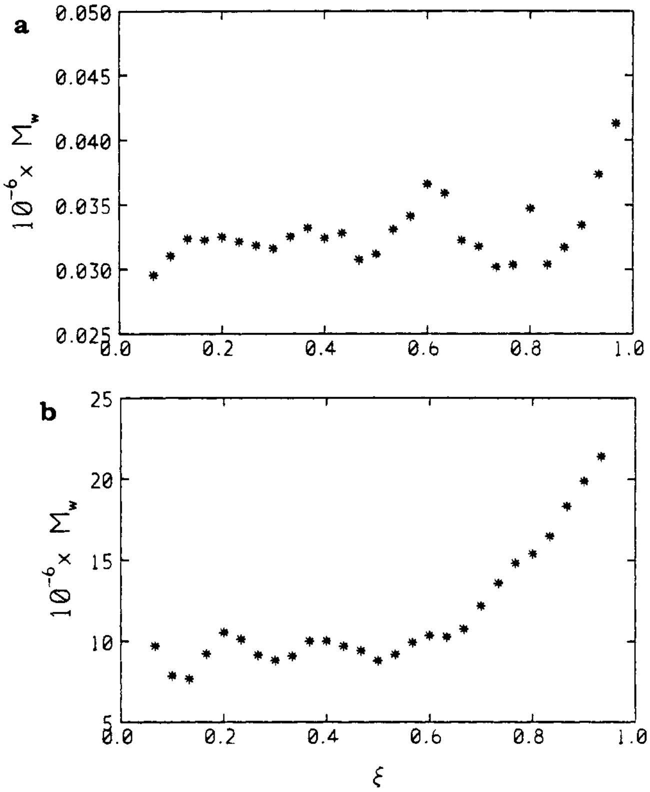

Figure 3. Point weight-average molecular weights (M_w) of Copo 9. (a) Copo 9 in $CHCl_3$ (flotation equilibrium). Rotor speed = 19 130 rpm. Temp = 20.0 °C. Loading concentration $c^0 \sim 1.0$ mg/mL. (b) Copo 9 in water (sedimentation equilibrium). Rotor speed = 1961 rpm. Temp = 20.0 °C. $c^0 \sim 2.0$ mg/mL. $\xi = (r^2 - a^2)/(b^2 - a^2)$, where r is the radial displacement at any given point in the cell and a and b are the corresponding values at the meniscus and cell base, respectively.

Flotation Equilibrium. Copo 9 in $CHCl_3$. The Beckman Model E ultracentrifuge was used as above, except that inert Kel-F centerpieces were employed. Determinations were made at 19 130 rpm and a c^0 of ~1.0 mg/mL. Other details were as above except that M_w^0 is obtained for the case of flotation equilibrium from the limiting value of the M^* point average at the cell *meniscus*.[18]

Intrinsic Viscosity Determination. Kinematic reduced specific viscosities in both water and $CHCl_3$ as solvents, η_{red}', were determined by using extended Ostwald viscometers[19] timed automatically or manually, in a bath thermostatically maintained at 25.00 ± 0.01 °C. Dynamic intrinsic viscosities, $[\eta]$, were obtained from the corresponding kinematic values, $[\eta']$, by using the correction formula of Tanford[20]

$$[\eta] = \frac{(1 - \bar{v}\rho_0)}{\rho_0} + [\eta'] \tag{3}$$

where ρ_0 is the density of the solvent.

Results and Discussion

Hydrodynamic Parameters. Copo 9 in Chloroform. For Copo 9 in $CHCl_3$ a single Schlieren peak was evident from flotation velocity in the analytical ultracentrifuge (Figure 1a): any polydispersity of the sample present appeared therefore to be of a "quasi-continuous" type rather than of a "discrete" (monomer, dimer, trimer, etc.) type. After correction to standard conditions (water at 20.0 °C), the reciprocal sedimentation coefficient data were fitted as a first approximation by linear regression (Figure 1b), yielding s and k_s values of (0.40 ± 0.02) × 10^{-13} s and (59.5 ± 9.6) mL/g, respectively. A value for the kinematic intrinsic viscosity, $[\eta']$, of 52.8 ± 0.5 mL/g was obtained, corresponding to a value for the (dynamic) intrinsic viscosity, $[\eta]$, of 52.5 ± 0.5 mL/g using the Tanford[20] correction formula (eq 3).

Hydrodynamic Parameters. Copo 9 in Water. For the copolymer in water as solvent the situation is quite different (Table I). Sedimentation velocity diagrams corresponding to solute concentrations above ~2.0 mg/mL (the minimum concentration necessary for satisfactory optical Schlieren records) clearly demonstrate more than one sedimenting species (Figure 2a). Two principle forms were seen: an $s_c \sim (25 \pm 1) \times 10^{-13}$ s form and an apparently[21] smaller amount of an $s_c \sim (35 \pm 1) \times 10^{-13}$ s form. These "25S" and "35S" forms *may* correspond to micelles of different size. At a relatively high loading concentration (~5.0 mg/mL) a small amount of a much slower sedimenting species, of $s \sim 0.5 \times 10^{-13}$ s, was visible, corresponding to the unimer form (cf. results for Copo 9 in $CHCl_3$). Further experiments with the copolymer in the presence of Sudan Black and the use of scanning absorption optics (Figure 2b)—showing two distinct sedimenting boundaries—strongly support the view that the 25S and 35S forms were micelles. Reduced kinematic viscosities yielded, by using eq 3, a (dynamic) intrinsic viscosity, $[\eta]$, of (32.1 ± 0.8) mL/g with no evidence of a transition to unimer form down to a concentration of ~0.7 mg/mL.

Molecular Weight Determination. Flotation equilibrium experiments on Copo 9 unimers in $CHCl_3$ at a loading concentration of ~1.0 mg/mL gave near-linear plots of ln J vs r^2 (where r is the radial displacement at a given point in the ultracentrifuge cell) with negative slope. A whole cell (apparent) weight-average molecular weight, M_w^0, of 35 000 ± 2000 g/mol was obtained from the limiting value of the M^* point average at the cell meniscus.[18] Plots of the point weight-average molecular weight as a function of radial displacement (Figure 3a) demonstrated that polydispersity was not great (although such effects can be masked by thermodynamic nonideality; see, e.g., refs 22 and 23).

Conventional sedimentation equilibrium on the copolymer in water at a loading concentration of ~2.0 mg/mL (to ensure a predominance of micellar forms) again yielded a quite different situation (Figure 3b). Throughout most of the cell an apparent point average molecular weight of $\sim(9.5 \pm 2.0) \times 10^6$ was evident although near the cell base higher molecular weight material was evident ($M_w \sim 22.0 \times 10^6$). The apparent weight-average molecular weight over the whole solute distribution, M_w^0, of $(15.0 \pm 1.0) \times 10^6$ was obtained from the limiting value of the M^* function[16] at the cell base. From this data an estimate for the "association number" for the micelles (assuming M_w^0 for Copo 9 in water represents a weight average of micellar forms only) of ~400 is obtained. This will be a lower limit because of the likely greater effects of thermodynamic nonideality on the micelle M_w^0 value.

It is also possible to estimate a (weight-average) hydrodynamic radius, r_H, of the micelles from the formula linking the viscosity increment, ν, the intrinsic viscosity, $[\eta]$, and the molecular weight, M_r: $\nu = \{[\eta]M_r\}/\{N_A V_e\}$ (see ref 14), where V_e is the effective volume (mL) and N_A Avogadro's number. For spheres ν is simply the Einstein[24,25] value of 2.5, and hence

$$r_H = \left(\frac{3[\eta]M_r}{10\pi N_A}\right)^{1/3} \tag{4}$$

When M_r is used in eq 4 as the weight-average molecular weight of $\sim 15.0 \times 10^6$, a value for r_H of ~42 ± 3 nm is obtained.

Gross Conformation of Copo 9 Unimers in $CHCl_3$. Table I gives the frictional ratio, f/f_0 (calculated from the sedimentation coefficient), as well as the (dynamic) intrinsic viscosity, $[\eta]$. Both f/f_0 and $[\eta]$ are relatively sensitive functions of gross conformation (via the Perrin function, P, and viscosity increment, ν, respectively), particularly $[\eta]$. Their relatively high values 2.50 ± 0.05 and 52.5 ± 0.5) mL/g are symptomatic of either a highly solvated (i.e., flexible coiled) macromolecule or a more rigid, less solvated but asymmetric structure. To obtain a more unambiguous model for the gross conformation requires the use of compound "solvation-independent" functions.[26] One function that has been popularly used in the past—particularly in the study of types of biopolymer where a "rigid" model is considered appropriate—has been the Scheraga–Mandelkern β function[27] (Table I). This shape parameter (obtained from combination of s and $[\eta]$ data), although solvation independent, is virtually insensitive to the conformation of a macromolecule and in this instance is similarly of little use.[26]

One solvation-independent parameter that appears to be of value for this type of system is the relatively underused Wales–van Holde parameter, $k_s/[\eta]$ (see refs 16, 26, and 28–30). The value of ~1.1 obtained for Copo 9 is somewhat less than that predicted for a random coil or a compact sphere (1.6–1.66) and would suggest as expected a more extended conformation (i.e., a structure with somewhat limited flexibility) under these solvent conditions.

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