1 Dilute solution viscometry of food biopolymers

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1.1 Introduction

Viscosity is relevant from a food perspective for two reasons. First, it is an important functional property of foods, and understanding how biopolymer concentration, shape, size and polydispersity affects this is industrially very important. Second, we can turn the first point on its head to say that viscometric measurements on food biopolymers, either in highly purified form or in controlled mixtures of highly purified materials, allow us to probe fundamental molecular properties of the food macromolecule (conformation in dilute solution, molecular weight, molecular weight distribution and interaction properties). Since viscosity properties of biopolymers under highly concentrated and gel systems is covered under the general term 'rheology' and dealt with elsewhere in this volume, this article will focus on the dilute solution viscosity properties of food biopolymers (proteins and polysaccharides), and in particular, the measurement and use of a molecular parameter known as the intrinsic viscosity, a parameter which underpins the whole of the viscosity behaviour of food and other dispersions. For a more general form of this review concerning other biopolymer dispersions the reader is referred to another article by the author (Harding, 1997).

Dilute solution viscometry, like rheology, has been the subject of significant advances, both at the 'rheological' or concentrated solution end and at the dilute solution end. The intrinsic viscosity itself however, is not a new molecular parameter. Einstein considered it for a suspension of spherical particles in 1906 (with a correction in 1911). The classical review of its measurement and application, particularly to proteins, appeared almost 40 years ago (Yang, 1961) and a corresponding treatise focusing mainly on the theory for linear macromolecules appeared almost 30 years ago (Yamakawa, 1971). A more recent treatise was the highly useful text of Bohdanecky and Kovar (1982) again focusing on linear polymers.

The intrinsic viscosity is also not a true viscosity at all: the dimensions of viscosity are conventionally the 'Poise' in cgs units ($dyn \cdot cm^{-2} \cdot s$) or the 'Pascal second' ($N \cdot m^{-2} \cdot s$) in SI units, whereas intrinsic viscosity has reciprocal concentration units: although in the past units of $dl \cdot g^{-1}$ have been highly popular, the cgs unit of $ml \cdot g^{-1}$ is now the preferred, simply because it is consistent with the units generally used for other solution measurements.

This article will thus serve the purpose of addressing the progress that has been made in:

(a) instrumentation
(b) molecular modelling of quasi-rigid particles such as globular food proteins
(c) the hydration problem
(d) molecular modelling of the conformation and flexibility of linear biopolymers, which is the hallmark of food polysaccharides.

1.2 The intrinsic viscosity

1.2.1 Definitions

The viscosity of a fluid is a measure of its resistance to flow. Formally, the (shear) viscosity coefficient, η (or μ) of a fluid is defined as the shearing stress, τ (or σ) per unit rate of shear, g (other common notations are G or β) via Newton's formula:

$$\eta = \tau/g. \tag{1}$$

An alternative definition of viscosity is in terms of energy dissipation (e.g. Tsvetkov *et al.*, 1971):

$$E = \eta g^2, \tag{2}$$

where E is the work done in unit time per unit volume due to the directional flow. A Newtonian fluid is one where the viscosity coefficient η is not a variable with shear rate: macromolecular solutions approximate Newtonian fluids at slow or creeping velocities, u, as found in, for example, capillary viscometers. More formally, if the fluid is also incompressible the equation of motion for the fluid can be described by the following form of the Navier–Stokes equation:

$$\rho\left(\frac{\partial \mathbf{u}}{\partial t} + \mathbf{u} \cdot \nabla \mathbf{u}\right) = -\nabla p + \eta \nabla^2 \mathbf{u} + \rho \mathbf{F}, \tag{3}$$

where $\partial/\partial t$ is the time rate of change at a fixed point in the fluid, p is the hydrostatic pressure the fluid would be supporting if it was at rest at its local density ρ and temperature T and $\mathbf{F}$ is the external body force per unit mass (in the absence of any other forces this will be from the acceleration due to gravity). Equation 3 (or its equivalent form in energy dissipation terms), in the appropriate co-ordinate systems and boundary conditions forms the basis of the calculation of the effect of dissolving or dispersing macromolecular solute on the viscous flow properties of a fluid (Happel and Brenner, 1973).

In practical terms, the effect of the dissolved/dispersed macromolecular solute on a solution is given by the relative viscosity, (η_{rel}) or the reduced viscosity (or 'reduced specific viscosity'), (η_{red}):

$$\eta_{rel} = \eta/\eta_0 \tag{4}$$

$$\eta_{sp} = \eta_{rel} - 1 \tag{5}$$

and

$$\eta_{red} = \eta_{sp}/c = (\eta_{rel} - 1)/c, \tag{6}$$

where η is the viscosity of the solution (or dispersion), η_0 is the viscosity of the solvent and c is the weight (mass) concentration. As indicated above the cgs system of units is preferred, so the unit of reduced viscosity is ml/g, although the traditional unit of dl/g is still in use. A related term is the inherent viscosity (η_{inh}) or ($|\eta|$) which is defined by:

$$\eta_{inh} = (\ln \eta_{rel})/c. \tag{7}$$

Because of the effects of non-ideality and/or associative phenomena, both η_{red} and η_{inh} will be concentration dependent. The limit as $c \rightarrow 0$ of both η_{red} and η_{inh} is defined as the intrinsic viscosity $[\eta]$, presumably so named because it is an intrinsic function of the dissolved/dispersed macromolecule:

$$[\eta] = \lim_{c \rightarrow 0}(\eta_{red}) = \lim_{c \rightarrow 0}(\eta_{sp}/c) \tag{8}$$

$$[\eta] = \lim_{c \rightarrow 0} \eta_{inh} = \lim_{c \rightarrow 0}\{(\ln \eta_{rel})/c)\}. \tag{9}$$

1.2.2 Form of the concentration extrapolation

The following equations have been given to describe the dependence of η_{red} and η_{inh} with concentration, correct to first order in concentration (i.e. dilute solutions). The most popular of these is the Huggins (1942) equation:

$$\eta_{red} = [\eta](1 + K_H[\eta] \cdot c) \tag{10}$$

where K_H is the (dimensionless) Huggins constant. A variant is the form:

$$\eta_{red} = [\eta](1 + k_H \cdot c), \tag{11}$$

(Rowe, 1977) and so the concentration dependence parameter has the same units (ml/g) as the equivalent parameters from sedimentation velocity (k_s) and translational diffusion (k_d) respectively. K_H and k_H are both generally positive, i.e. a plot of η_{red} versus c usually has a positive slope (Figure 1.1).

Another form is due to Schulz and Blaschke (1941):

$$\eta_{red} = [\eta](1 + K_{SB} \cdot \eta_{sp}). \tag{12}$$

The equivalent concentration dependence relation to equation 7 for the inherent viscosity, $(\ln \eta_{rel})/c$, is the Kraemer (1938) equation:

$$(\ln \eta_{rel})/c = [\eta](1 - K_K[\eta] \cdot c), \tag{13}$$

with a negative slope (Figure 1.1) and where K_K is the Kraemer constant.

These equations were put forward over 50 years ago and subsequent attempts have been made to modify and refine them. For example a power-law form of equation 13 has been proposed (Baranov *et al.*, 1987; Krasovskii *et al.*, 1993):

$$(\ln \eta_{rel})/c = ([\eta]c)^{\alpha}, \tag{14}$$

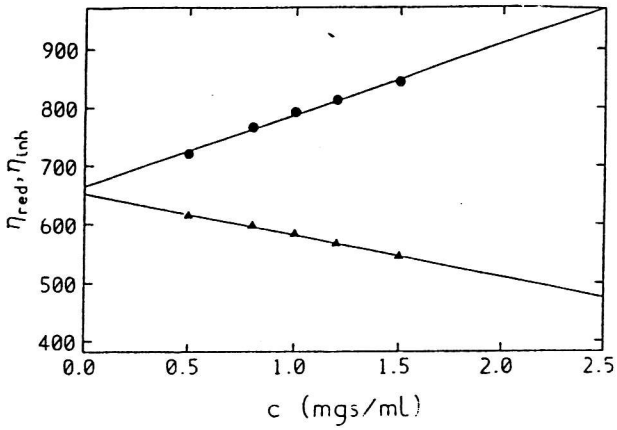

Figure 1.1 Huggins and Kraemer extraction methods for intrinsic viscosity. Reduced viscosity η_{red} (ml/g) versus concentration (●) and inherent viscosity η_{inh} $\{=\ln(\eta_{rel})/c\}$ (ml/g) versus concentration (▲) for irradiated (10 k Gy) guar in phosphate chloride buffer (pH = 6.8, $I = 0.10$). The 'common' intercept gives $[\eta]$, the slopes are $K_H[\eta]^2$ and $K_K[\eta]^2$. K_H is the Huggins constant and K_K the Kraemer constant, respectively (from Jumel, 1994).

and Chee (1985) has suggested other numerical procedures. Other attempts at developing the Huggins and Kraemer relations have centred around estimating $[\eta]$ from measurement of η_{rel} at a single concentration (Solomon and Ciuta, 1962; Solomon and Gotesmann, 1967; Deb and Chatterjee, 1968; Elliot *et al.*, 1970; Rudin and Wagner, 1975; Ram Mohan Rao and Yaseen, 1986). For example, Solomon and Ciuta (1962) proposed a combination of equations 10 and 13 to yield the approximate relation:

$$[\eta] \simeq (1/c) \cdot [2\{\eta_{sp} - \ln(\eta_{rel})]^{1/2}, \tag{15}$$

a relation which is also known also as the 'Solomon–Gotesmann' (1969) equation. This has been popular with pressure imbalance types of viscometers coupled on-line to a gel filtration (size exclusion chromatography) column (section 1.3 below). Deb and Chatterjee (1969) suggested the following alternative relation:

$$[\eta] \simeq (1/c) \cdot [3 \ln(\eta_{rel}) + (3/2)(\eta_{sp}^2) - 3\eta_{sp}]^{1/3}, \tag{16}$$

and more recently Ram Mohan Rao and Yaseen (1986) gave a more simplified form:

$$[\eta] \simeq (1/2c) \cdot [\eta_{sp} - \ln(\eta_{rel})]. \tag{17}$$

Other workers have attempted instead to improve the form of the extrapolation of equations 8 and 9. For example, Reilly *et al.* (1979) have pointed out that when η_{sp} or $\ln \eta_{sp}$ is divided by the solution concentration, the error in the quotient caused by error in the relative viscosity measurement is magnified at low concentration, therefore extrapolation methods using η_{sp} as opposed to η_{sp}/c would appear to be advantageous. For example, application of l'Hopital's rule to

equation 8 provides an alternative method for evaluation of the intrinsic viscosity in terms of the derivative $d\eta_{sp}/dc$ at zero concentration (Kozicki and Kuang, 1996):

$$[\eta] = (d\eta_{sp}/dc)_{c=0} \tag{18}$$

i.e. the limiting slope at $c = 0$ of η_{sp} plotted versus c. Kozicki and Kuang (1996) have pointed out that (0, 0) is an experimental point and hence extrapolation outside the range of data – as required by the Huggins, Kraemer and related procedures (equations 10–14) – is therefore unnecessary. These workers have also demonstrated that non-linear least squares fitting the specific viscosity data versus concentration c to either the polynomial:

$$\eta_{sp} = [\eta]c + a_2c^2 + a_3c^3, \tag{19}$$

or in the relation:

$$\eta_{sp} = [\eta]c + b \cdot c^d, \tag{20}$$

with $[\eta]$, a_2, a_3 or $[\eta]$, b, d as the variables gives significantly improved estimates for $[\eta]$, with equation 20 the best.

1.3 Experimental measurement

This requires measurement of the relative viscosity η_{rel} and concentration, c. A plot of either the reduced specific viscosity, $\eta_{red} = \eta_{sp}/c$ versus concentration, or just η_{sp} versus concentration, or manipulation of equations 15–17 can then be used to extract $[\eta]$ as discussed in section 1.2.2.

η_{rel} can be measured in one of three principal ways using a capillary viscometer, a plate viscometer (cone and plate or parallel plate or cup and bob), or a so-called 'pressure-imbalance' differential method. One often neglected feature is the importance of accurate concentration measurement for the subsequent evaluation of $[\eta]$: this will also be considered.

1.3.1 Capillary viscometry

The capillary or 'Ostwald' viscometer (Ostwald and Malss, 1933) is still the most common viscometer and involves essentially just a piece of glassware – albeit beautifully constructed (Figures 1.2a and 1.2b) – suspended in a constant temperature environment.

The principle is simple: measurement of the time for a volume of liquid (solution or solvent) to flow through the capillary in the vertically aligned viscometer. This measurement is performed for the solvent and then the biomolecular solution at one or more concentrations. To facilitate measurement at a series of concentrations where the dilutions can be performed *in situ*, a modified form (Figure 1.2c) called an Ubbelohde viscometer (Ubbelohde, 1936) can be

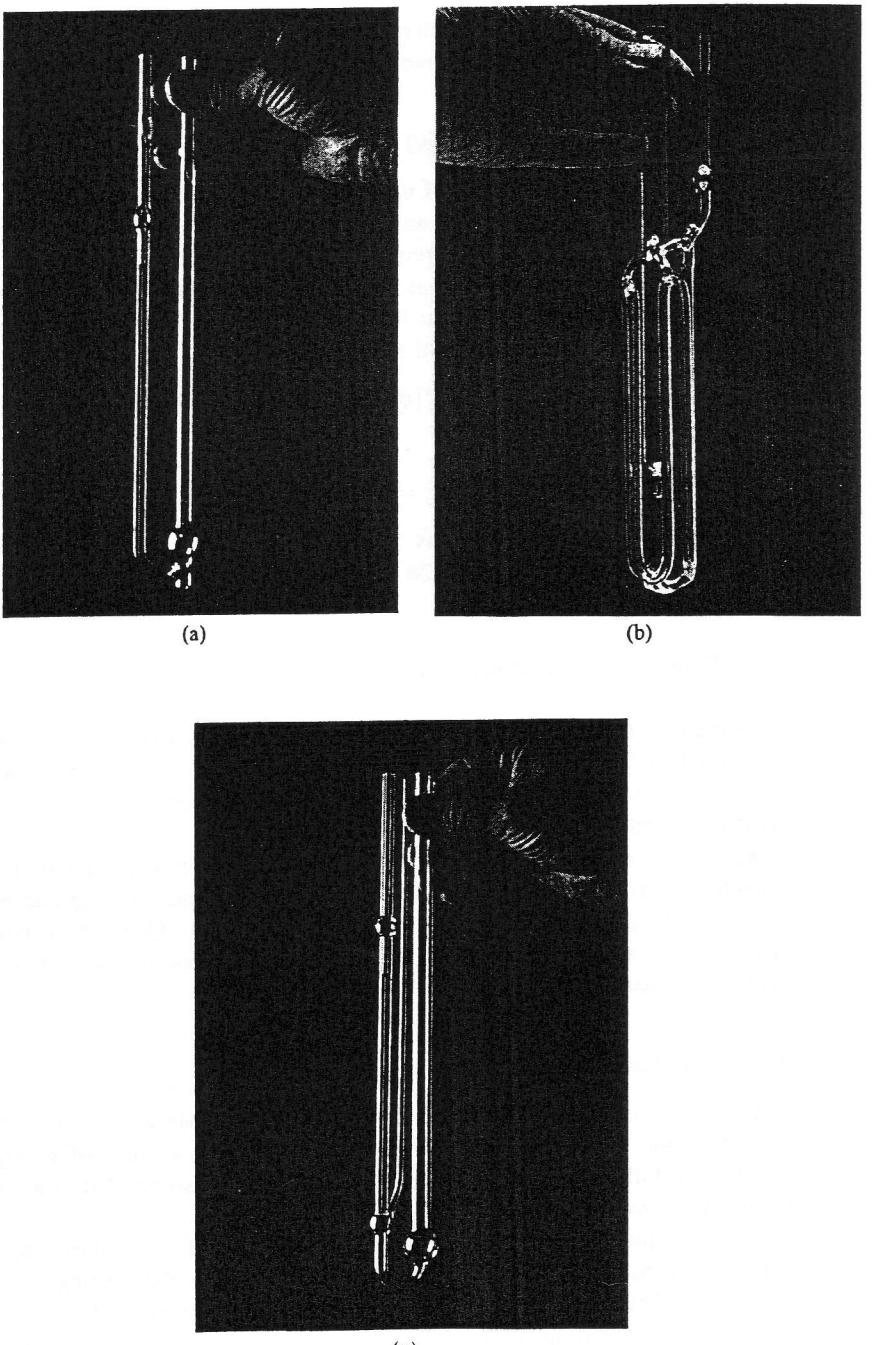

Figure 1.2 Ostwald (a) and extended Ostwald (b) and Ubbelohde (c) viscometers.

used which is designed so that the head of liquid when the flow time is being measured is independent of the amount of solution in the viscometer: progressive dilutions can then be made directly in the viscometer. However, if a macromolecule degrades or denatures appreciably during a series of measurements, this type of viscometer should not be used. Kragh (1961) discusses the advantages and practical limitations of both this and the conventional Ostwald.

From Poiseuille's law (Tanford, 1961) the relative viscosity is given simply by:

$$\eta_{rel} = (t\rho/t_0\rho_0), \tag{21}$$

where t and t_0 are the flow times for the biomolecular solution at a particular concentration c and ρ and ρ_0 the corresponding and solvent densities. The relative viscosity without the density correction is known as the 'kinematic' (as opposed to 'dynamic') relative viscosity $\eta'_{rel} = t/t_0$; subsequent derived parameters: η'_{sp}, η'_{red}, $\ln(\eta'_{rel})/c$ and $[\eta]'$ are the corresponding kinematic quantities. To a reasonable approximation, for concentrations <1 mg/ml $\eta_{rel} \sim \eta'_{rel}$. Although measurements at such low concentration are possible with many solutions of polysaccharides which have large relative viscosities, for globular proteins and globular macromolecular assemblies (even large spheroidal plant viruses) this is not generally possible since the relative viscosities are too small ($\sim$1.003 or less). However it is not necessary to measure solution density at each concentration since the correction of Tanford (1955) can be applied:

$$[\eta] = [\eta]' + [(1 - \bar{\nu}\rho_0)/\rho_0] \tag{22}$$

or

$$\eta_{red} = \eta'_{red} + [(1 - \bar{\nu}\rho_0)/\rho_0], \tag{23}$$

where $\bar{\nu}$ is the partial specific volume of the macromolecule. Of course if this latter parameter ($\bar{\nu}$) is not known for the solvent conditions being used, or cannot be calculated from the chemical composition of the macromolecule (Perkins, 1986) then solution density measurements are required:

$$\bar{\nu} = (1/\rho_0) \cdot (1 - \partial\rho/\partial c). \tag{24}$$

ρ_0 and ρ can be measured using a mechanical oscillator device as described by Kratky *et al.* (1973). There are two ways the precision with which η_{rel} can be increased, particularly for measurements at low concentration, both based on increasing the flow time (and hence flow time difference $t - t_0$) for solvent and solution. The first is the method of Szuchet-Derechin and Johnson (1966) which is to add a low concentration of glycerol ($\sim$3%) to the solvent and solution: this has permitted the measurement of protein relative viscosities at concentrations $<$4 mg/ml. The second way is to use specially designed extended Ostwald viscometers (Figure 1.2b) (Holt and Creeth, 1972) which increase the flow time difference ($t - t_0$) by extending the length of the capillary (the same result can

in principle be obtained by decreasing the capillary radius but this increases problems of capillary blockage). In a further development Booij *et al.* (1991) have described a multiple bulb viscometer with different volumes and different capillary lengths between them, facilitating shear rate dependence studies of the intrinsic viscosity.

Measurement of flow times is now done automatically using photosensors, and a commercial example is the Schott–Geräte (Hofheim, Germany) system. Because solvent viscosity is such a sensitive function of temperature, a controlled water bath (to within at least 0.01 °C) and accurate temperature measurement (using, for example, an accurately calibrated platinum resistance thermometer) are necessary. Other practical details (kinetic energy correction, guarding against capillary blockage, effect of alignment and other errors) described in Kragh's (1961) classical article are still however relevant and should be consulted by any potential user.

1.3.2 Plate viscometers

With this type of viscometer the solution is placed in a space between two plates and one is moved at constant speed relative to the other which is held by a torsion wire on which the viscous drag will exert a torque: measurement of the torque change with increase in speed (and hence shear rate) gives the viscosity η of the solution. If this is repeated for the solvent η_0 the relative viscosity $\eta_{rel} = \eta/\eta_0$ can be readily found. There are three principal types (Lapasin and Pricl, 1995): cone and plate, parallel plate and cup and bob. Like capillary viscometry, measurement is now automated and an example of a commercial system is the CS Rheometer from Bohlin instruments (Lund, Sweden). Although all permit (after appropriate calibration) the evaluation of absolute, η, and the investigation of the effect of shear rate on η (and hence the measurement of non-Newtonian behaviour), for dilute solution work the accuracy is considerably less than for capillary viscometry. The principal limitation is that to measure the very small torsions at dilute solution conditions it is necessary to have a very narrow gap between the plates: it is practically very difficult to maintain a uniform separation when one plate is moving relative to another (Kragh, 1961), and this puts a lower limit for accurate measurement of $\eta_{rel} \sim 1.01$. For a detailed consideration of the application of these methods, the reader is referred to Lapasin and Pricl (1995) and references cited therein.

1.3.3 Pressure imbalance differential viscometer

This uses a fluid analogue of a Wheatstone bridge electrical circuit (Haney, 1985a,b). It is referred to as a 'differential viscometer' since it measures relative viscosity directly. It is also highly sensitive, permitting the accurate measurement of low relative viscosities and hence measurements at low concentration

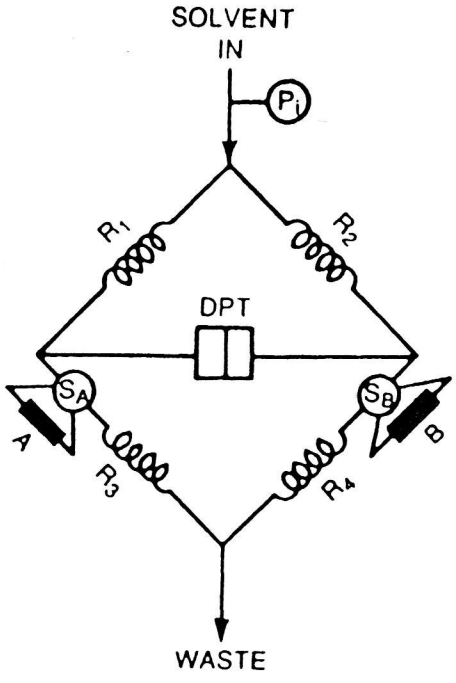

Figure 1.3 Simplified schematic of pressure imbalance differential viscometer. Uses fluid analogue of a Wheatstone bridge electrical circuit to measure differential viscosity directly from the chromatography effluent. R_1–R_4 capillaries. A, B solvent reservoirs. P_i: inlet pressure. DPT: differential pressure transducer. From Haney *et al.* (1985a,b).

(~1 mg/ml for globular proteins). At baseline conditions the differential pressure across the bridge will be zero because there is solvent in all four capillaries. When sample solution enters the bridge (Figure 1.3) it fills capillaries R_1, R_2 and R_3 while solvent from a delay reservoir remains in capillary R_4. The difference in viscosity between the solvent in R_4 and solution in R_3 causes a pressure imbalance ΔP in the bridge which from Poiseuille's law can be relative viscosity or specific viscosity of the solution (Haney, 1985b):

$$\eta_{sp} = 4\Delta P(P_i - 2\Delta P). \tag{25}$$

From knowledge of the concentration the reduced specific viscosity can be obtained. A commercially available instrument is from Viscotek Ltd (Houston, USA).

Besides its great sensitivity at high dilution and rapidity of measurement, solution can be injected continuously via a flow cell; it can thus be fitted on-line to a concentration detector (refractive index or u.v. absorbance-based – section 1.3.4) for converting η_{rel} to reduced specific viscosities. It can also be fitted on-line to a multi-angle laser light scattering detector (Wyatt, 1992) so that the (weight average) molecular weight (M_w) can also be obtained.

Either η_{sp}/c can be obtained and plotted versus c to obtain $[\eta]$ as described in section 1.2.1, or, since concentrations can be very small (~1 mg/ml for globular proteins) the single point $[\eta]$ evaluation formulae can be applied, such as the Solomon–Ciuta (1962; Solomon–Gotesman, 1967) formula (equation 15). Use of this latter equation is particularly valuable for polydisperse materials (the hallmark of polysaccharides and other heavily glycosylated systems) if the system is coupled not only to a concentration and molecular weight detector but also downstream from size-exclusion chromatography (SEC) columns (Dutta *et al.*, 1991; Jackson *et al.*, 1991): the $[\eta]$ versus M_w relationships can then be readily described (section 1.5.1 below). A popular set-up would thus have this on-line facility plus a separate injection port if monodisperse solutions were being characterized not requiring column separation. A further development (not for SEC) is the so-called Dual Capillary Viscometer (Viscotek Ltd, Houston, USA) which operates with just two capillaries (one solvent, one solution) with the same rate of flow.

1.3.4 Concentration measurement

Concentration errors are more often than not the principal limiting factor to which the accuracy of a macromolecular parameter – molecular weight, sedimentation coefficient, diffusion coefficient or intrinsic viscosity – can be measured by hydrodynamics. It is particularly important for the measurement of intrinsic viscosity not only because of the extrapolation to zero concentration (section 1.2.2) but particularly because the concentration is also required for the evaluation of the reduced specific viscosity or inherent viscosity (cf. equations 8 and 9). For proteins, the most popular concentration measurement method is by measurement of u.v. absorbance at 278 nm. The extinction coefficient is required from prior measurement (and hence itself from accurate concentration measurement!) or can be estimated from the amino-acid composition (Perkins, 1986). A more general method, which is not just limited to proteins, is based on measurement of the solution refractive index, n, measurement using differential refractometry (Wyatt, 1992). The refractive increment, dn/dc is required (which again requires accurate concentration measurement).

By analogy the density, ρ, of the macromolecular solution can be measured (Kratky *et al.*, 1973): concentration can be calculated from this so long as the density increment, $\partial\rho/\partial c$ (or the partial specific volume, $\bar{v}$, see equation 24) is known. $\bar{v}$, like ε for proteins, can be calculated from knowledge of the composition of the macromolecule (Perkins, 1986). Alternatively chemical methods for concentration measurement can be used, such as the Kjehldahl method for food proteins or the phenol-sulphuric acid method for food polysaccharides (Ball, 1989). With both refractive index and density methods it is important the concentration of non-macromolecular solutes in the solvent is the same for both the macromolecular solution and the reference solvent: careful dialysis with allowance (by weight measurement) for loss of water is recommended. For

polysaccharides that are optically active, the extent of rotation of polarized light is also a function of concentration and this can also be used (Van Holde, 1985).

A most important point from all this is that concentration cannot be measured to an accuracy much greater than ~1%: the $[\eta]$ can thus also be measured to no better than $\sim \pm 1\%$, no matter how accurate measurement of relative (or specific) viscosity is. This fact is sometimes forgotten when attempts to obtain detailed information about biomolecular structure in solution are made.

1.4 Determination of food biopolymer conformation in dilute solution

1.4.1 The viscosity increment, ν

There are two molecular contributions to the intrinsic viscosity: one from shape, the other from size or volume, as summarized by the relation:

$$[\eta] = \nu \cdot v_s, \tag{26}$$

where ν is a molecular shape parameter known as the viscosity increment (see, e.g., Yang, 1961) and v_s (ml/g) is known as the swollen specific volume: an anhydrous macromolecule will essentially expand when suspended or dissolved in solution because of solvent association, and v_s ($=V \cdot M/N_A$ where V is the swollen volume (ml), M the molecular weight (Da or g/mol) and N_A is Avogadro's number) is a measure of such (aqueous) solvent associated with the macromolecule, and is defined as the volume of the macromolecule in solution per unit anhydrous mass of macromolecule. This 'associated' solvent as we consider in more detail below can be regarded as that which is either chemically attached or physically entrained by the macromolecule. v_s can be related to a popular term called the 'hydration' δ, by the relation:

$$v_s = \bar{v} + \delta/\rho_0. \tag{27}$$

The viscosity increment ν is referred to as a 'universal shape function' (Garcia de la Torre *et al.*, 1997; Harding *et al.*, 1997b) since, unlike $[\eta]$, it can be directly related to the shape of a particle independent of volume. For its experimental measurement is does however require measurement of v_s (or $\bar{v}$, δ and ρ_0) as well as of course $[\eta]$.

1.4.2 The 'hydration' δ

Opinions vary as to what this parameter actually means – if it is a parameter at all – but it represents the amount of solvent 'associated' with the macromolecule and includes 'chemically bound' via hydrogen bonds and 'physically entrained' solvent. The 'monolayer' concept sometimes propagated is however without proper justification and it is therefore safer to regard 'hydration' as simply the level to which aqueous solvent can be added to a dry macromolecule beyond

which there is no change in a macromolecular property other than dilution of the sample (Rupley and Careri, 1991).

Various techniques have been used to assign values for δ, particularly for globular proteins and have been considered in some detail elsewhere by Kuntz and Kauzmann (1974). Another interesting method was subsequently presented by Rowe (1977) involving use of the ratio of the viscosity concentration dependence regression coefficient k_η (equation 11, with the corresponding parameter k_s from sedimentation velocity in the analytical ultracentrifuge. Rowe (1977) equated the ratio of k_η/k_s to v_s/v and this method has been used for example to assess the δ for the meat protein myosin (Byron, 1995).

For globular proteins a value of 0.3–0.4 has been inferred from nuclear magnetic resonance (Kuntz, 1971), infra red spectroscopy (Rupley *et al.*, 1983) and computer simulation. It is possible to assign a value for δ from viscosity measurements via equations 26 and 27 and also via analogous relations for the translational frictional ratio (from sedimentation coefficient or translational diffusion coefficient measurements) if the shape of the macromolecule is known. For example, by approximating crystal structures of globular proteins as ellipsoids of revolution, Squire and Himmel (1979) showed that apparent hydration values calculated from the sedimentation or diffusion data varied greatly (from ~0.1–~1, with a mean, from over 20 proteins studied of ~0.54). Zhou (1995) later claimed that this discrepancy with the other treatments was due to inadequacy of the crude ellipsoid of revolution as a model for the molecular surface, and that using a more refined approach based on relating the intrinsic viscosity to the capacitance and polarizability of a protein estimated from its atomic structure, a value of 0.3–0.4 for δ is returned apparently consistent with the other techniques. This narrow range must not however be regarded as prescriptive for all biomolecular types, particularly the larger and highly expanded polysaccharides and glycoconjugates which can have δ values >50 (Harding *et al.*, 1983; 1997a).

1.4.3 Effect of molecular charge

In addition to shape and 'hydration', if the biomolecule possesses electrostatic charge this can also affect the intrinsic viscosity. These effects can be particularly serious if the macromolecule is a multiply charged 'polyelectrolyte'. Proteins and many polysaccharides are all polyelectrolytes. This electrostatic contribution will be strongly dependent on the pH of the solution (relative to the pK_a of the charged groups) and the ionic strength, I (in mol l^{-1} or 'M') of the solution. The polyelectrolyte itself will only make a significant contribution to I under conditions where the presence of low molecular weight electrolyte is negligible (<0.01 M): this is the exception rather than the rule for food systems, and from a molecular characterization standpoint most physical measurements are done buffered and in the presence of low molecular electrolyte to an I of 0.01 or above.

For compact globular proteins, there will be three distinct 'electroviscous' contributions (Shaw, 1980; Dickinson, 1992): one from the resistance of the diffuse double (electrostatic) layer surrounding the protein – the 'primary effect'; another from repulsion between the double layers of different protein molecules – the 'secondary effect'; another of these interparticle repulsions affects the shape of the protein itself – the 'tertiary effect'. The latter effect is very small for globular proteins (Tanford and Buzzell, 1956) and the first two are only significant at very low ionic strength. In water, for example, the contribution to the solution I is entirely from the polymer: hence as the polymer concentration decreases, the I will decrease which results in an increase in $[\eta]$. For linear molecules (many polysaccharides) the effect can be more significant and is considered in more detail in section 1.5.5.

1.4.4 Spheres and ellipsoids of revolution

The effect of a suspended particle is to increase the energy dissipation during bulk flow because the extra stresses acting over its surfaces are doing work (Happel and Brenner, 1973). It was the pioneering work of Einstein (1906; 1911) who, based on scalar energy dissipation arguments and with the assumption that the suspension behaved macroscopically as an isotropic incompressible Newtonian continuum, was able to evaluate a value of 5/2 for the parameter $\nu\,(=[\eta]/v_s)$ for a suspension of non-interacting randomly distributed spheres. (His 1906 paper contained an error that was corrected in the later 1911 paper.) Brenner (1958) obtained the same result using an improved derivation 'avoiding a rather unusual integration over the surface of a large, vaguely defined spherical surface concentric with the particle'.

When attention turned to ellipsoids of revolution (3-D ellipsoids but with the restriction of two of the three axes equal, Figure 1.4) the calculation became considerably more complicated because of two opposing effects: the hydrodynamic shear which tends to align the ellipsoids in the direction of flow, and Brownian motion which tends to randomize particle orientations. The relative effects of the two are represented by the rotary Peclet number, $P_e = g/D_r$, where g is the shear rate and D_r is the effective rotational diffusion coefficient of the macromolecule. For macromolecules Brownian motion is the dominant factor, i.e. $P_e \to 0$, and Simha (1940) gave the first correct formula:

$$\nu = \frac{1}{a_1 a_2^2}\left\{\frac{2\alpha_0''}{15a_2^2\alpha_0'\beta_0'} + \frac{7}{15a_2^2\alpha_0'} + \frac{2}{5}\left[\frac{\beta_0'(a_1^2 + a_2^2) + 2\beta_0''}{\beta_0'[2a_1^2a_2^2\beta_0^2 + (a_1^2 + a_2^2)\beta_0'']}\right]\right\}, \quad (28)$$

where the α_0', etc. are elliptic integrals as defined by Jeffery (1922). See Harding and Cölfen (1995) for these in a form appropriate to the notation of equation 28. For prolate ellipsoids $a_1 = a$, $a_2 = b$ and for oblate ellipsoids $a_1 = b$ and $a_2 = a$ with $a > b$ in both cases. The elliptic integrals in equation 28 are soluble numerically (and now easily using numerical packages such as the NAG (1986) routine D01GAF), and Figure 1.5 shows a plot of ν versus a/b for both prolate

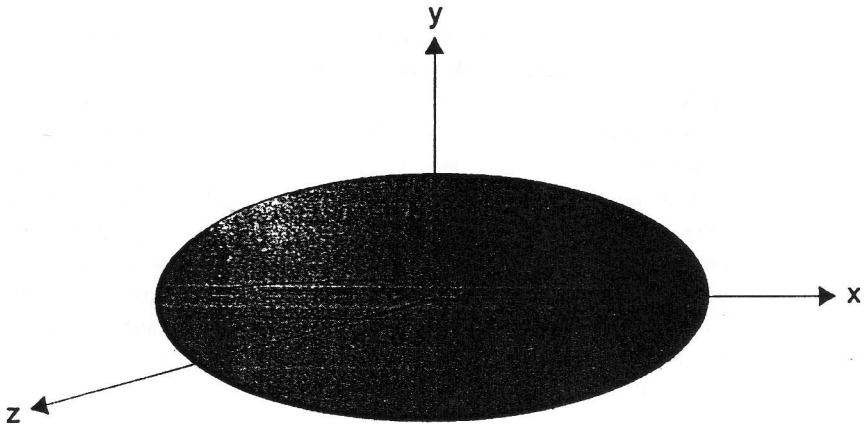

Figure 1.4 Ellipsoid representation for the solution conformation of quasi-rigid biopolymers. Semi-axes are $a \geq b \geq c$. The extremes are (i) sphere (a/b, b/c) = (1,1); (ii) Prolate ellipsoid (b/c = 1); (iii) Oblate ellipsoid (a/b = 1); (iv) Rod ($a/b \gg 1$; b/c = 1); (v) Disc (a/b = 1, $b/c \gg 1$); (vi) Tape ($a/b \gg 1$; $b/c \gg 1$).

and oblate ellipsoids. Simpler approximations are available although there is absolutely no need for these now the full equation 28, along with other useful hydrodynamic shape functions are available using an easy to use PC routine (ELLIPS2 (Harding *et al.*, 1997b)) which covers also general triaxial ellipsoids with two axial ratios (a/b, b/c) – as described below. There is also no need now to follow the customary practice of quoting extensive tables of data.

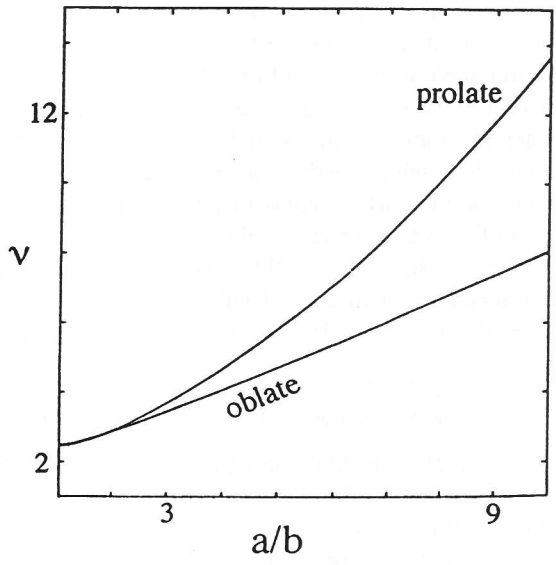

Figure 1.5 ν evaluated from the Simha formula (equation 28) plotted against axial ratio (a/b) for prolate and oblate ellipsoids.

It is impossible however to *invert* equation 28 directly to specify (a/b) in terms of ν. However a simple polynomial approximation has been found (Harding and Cölfen, 1995) which is accurate to within 1%, and the PC QUICKBASIC algorithm ELLIPS1 (Harding *et al.*, 1997b) has been set up to perform these calculations and other inversions of hydrodynamic shape functions (from sedimentation, exclusion volume, and rotary diffusion). These formulae, for ν, are:

$$
\begin{aligned}
(a/b) &= -10.71584 + 2.79158\nu + 1.622009\nu^2 + 0.01556169\nu^3 \\
&\quad - 0.192997\nu^4 + 0.02060718^5 \qquad \text{(prolate } 1.1 \leq a/b < 2\text{)} \\
&= -3.80413 + 2.8712\nu - 0.3908319\nu^2 + 0.03612282\nu^3 \\
&\quad - 0.001733981\nu^4 + 0.0000332711\nu^5 \qquad \text{(prolate } 2 \leq a/b < 10\text{)} \\
&= 4.241113 + 0.464459\nu - 0.001981036\nu^2 + 6.111643.10^{-6}\nu^3 \\
&\quad - 9.374974.10^{-9}\nu^4 + 5.478654.10^{-12} \cdot \nu^5 \quad \text{(prolate } 10 \leq a/b < 100\text{)} \\
&= -25.23436 + 10.43327\nu + 2.122294\nu^2 - 0.4294092\nu^3 \\
&\quad + 0.05816609\nu^4 - 0.1960477\nu^5 + 0.04331335\nu^6 \\
&\qquad\qquad \text{(oblate } 1.1 \leq a/b < 2\text{)} \\
&= -5.439531 + 3.883619\nu - 0.6477747\nu^2 + 0.08353639\nu^3 \\
&\quad - 0.004945992\nu^4 + 9.922261.10^{-5}\nu^5 \qquad \text{(oblate } 2 \leq a/b < 10\text{)} \\
&= 0.6888919 + 0.9078403\nu + 0.04002417\nu^2 - 1.154619.10^{-3}\nu^3 \\
&\quad + 1.457981.10^{-5}\nu^4 - 6.725395.10^{-8} \cdot \nu^5 \quad \text{(oblate } 10 \leq a/b < 100\text{)}
\end{aligned}
\tag{29}
$$

Although the Simha result (equation 28) is correct, the derivation as originally given by Simha (1940) is wrong, and in fact the correct formula is a result of fortuitous cancellation of errors (Saito, 1951), a discrepancy resolved some 40 years later (Harding *et al.*, 1982).

1.4.5 Triaxial modelling

Although some globular proteins have two axial dimensions approximately equal, and indeed the ellipsoid of revolution representation can be very reasonable to the overall conformation (Figure 1.6) it can be rather limited in its ability to represent the overall conformation of most quasi-rigid macromolecules. The formula corresponding to Simha's for general triaxial ellipsoids, with the restriction of two equal axes removed, was first given by Hocquart *et al.* (1974), and independently confirmed by Rallison (1978) and Harding *et al.* (1981) who used different approaches. This formula, in terms of the viscosity increment

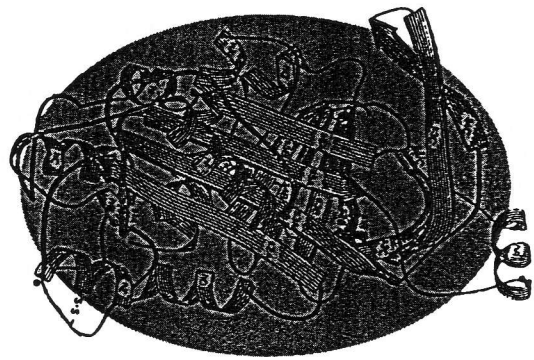

Figure 1.6 Prolate ellipsoid approximation with (a/b) = 1.5, to the crystal structure for ovalbumin. Crystal structure: Stein *et al.* (1991). Hydrodynamic shape (based on $[\eta]$ with other parameters): Harding (1981b).

$\nu = [\eta]/v_s$ is given by:

$$\nu = \frac{1}{abc}\left\{\frac{4(\alpha_7 + \alpha_8 + \alpha_9)}{15(\alpha_8\alpha_9 + \alpha_0\alpha_7 + \alpha_7\alpha_8)} + \frac{1}{5}\left[\frac{\alpha_2 + \alpha_3}{\alpha_4(b^2\alpha_2 + c^2\alpha_3)} + \frac{\alpha_3 + \alpha_1}{\alpha_5(c^2\alpha_3 + a^2\alpha_1)} + \frac{\alpha_1 + \alpha_2}{\alpha_6(a^2\alpha_1 + b^2\alpha_2)}\right]\right\} + \varepsilon \tag{30}$$

and where the small term ε is given by:

$$\varepsilon = -\frac{1}{5abc}\left[\frac{\left(\frac{a^2 - b^2}{a^2\alpha_1 + b^2\alpha_2} + \frac{b^2 - c^2}{b^2\alpha_2 + c^2\alpha_3} + \frac{c^2 - a^2}{c^2\alpha_3 + a^2\alpha_1}\right)^2}{\left(\frac{a^2 + b^2}{a^2\alpha_1 + b^2\alpha_2} + \frac{b^2 + c^2}{b^2\alpha_2 + c^2\alpha_3} + \frac{c^2 + a^2}{c^2\alpha_3 + a^2\alpha_1}\right)}\right] \tag{30b}$$

the elliptic integrals α_1, etc. are, as with the case for ellipsoids of revolution (equation 28) given by Jeffery (1922), and also in a form appropriate to the notation of equations 30 and 30b by Harding and Cölfen (1995). Unlike for ellipsoids of revolution however, the use of high-speed computers is mandatory (rather than just highly useful) for the numerical solution of the elliptic integrals for the general triaxial case, and we have found the NAG (1986) routine D01GAF again highly useful in this context.

The term ε on the RHS of equation 30 identically = 0 for spheres and ellipsoids of revolution and asymptotically $\rightarrow 0$ for tapes ($a \gg b \gg c$). For other values of (a, b, c) it contributes only ~1% at most to the total value of ν (Harding *et al.*, 1981). The PC FORTRAN routine ELLIPS2 (Harding *et al.*, 1997b) has been set up to calculate ν using the full form of equation 30 along with other universal shape parameters such as P (from the frictional ratio), u_{red} (from the exclusion volume), G (from the radius of gyration), θ_{red} (from electro-optic decay), for either a user specified (a, b, c) or, since all these are universal functions which depend on shape only (and not size), just $(a/b, b/c)$.

1.4.6 Solving the uniqueness and hydration problems for ellipsoids

With both axisymmetic (ellipsoid of revolution) and triaxial ellipsoid modelling there is a uniqueness problem. A value of ν will specify two ellipsoids of revolution axial ratios (one for a prolate the other for an oblate). For a triaxial ellipsoid the situation is worse: there is a line solution of possible values of the two ratios (a/b, b/c) for a given value of ν or $[\eta]$, as Figure 1.7 illustrates. A further problem is that of the influence of associated solvent: to convert $[\eta]$ to the shape function ν using equation 26 or 27 the swollen specific volume, v_s or the 'hydration' δ is required. Although for globular proteins there appears to be support for a value for δ of between 0.3 and 0.4 (section 1.4.2 above) for other macromolecules it is far less clear to define.

The uniqueness and hydration problems can both be addressed by the combination of ν with other hydrodynamic parameters.

The earliest attempt to tackle the hydration problem for ellipsoids of revolution was by Oncley (1941) who suggested a graphical combination of ν with the shape contribution (called the Perrin or 'P' function) to the frictional ratio. This was followed in 1953 with formulae given by Flory (1953) and Scheraga and Mandelkern (1953) describing an analytical combination of ν with P to yield a function β, which, with $[\eta]$ in ml/g is given by:

$$\beta \equiv \frac{[\eta]^{1/3}\eta_0}{M^{2/3}(1-\bar{v}\rho_0)100^{1/3}} = \frac{N_A^{1/3}}{(16200\pi^2)^{1/3}}\frac{\nu^{1/3}}{P}. \tag{31}$$

Unfortunately the β-function proved very insensitive to shape change (Figure 1.8a), however, further combinations of ν with other universal shape

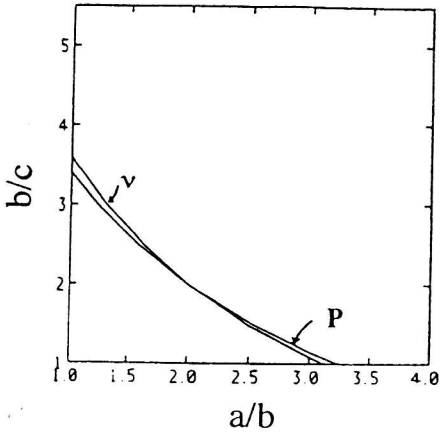

Figure 1.7 Line solution of possible values of (a/b, b/c) for a given value of ν (=3.803). The line solution for the Perrin translational frictional ratio function, P (=1.130) is also shown. This combination is clearly not a good one because of the shallowness of the intersection and the dependence on assumed hydration values for the two functions. It does form the basis though of other related combinations of hydration independent functions involving $[\eta]$, as explained in the text (and Figure 1.13).

parameters have proved more successful. These include the Λ_h function (Harding, 1980a):

$$\Lambda_h=(\eta_0 \cdot [\eta] \cdot M)/(N_A \cdot k_B T\tau_h) = \nu/(\tau_h/\tau_0) \quad (32)$$

(Figure 1.8b) where τ_h is the harmonic mean rotational relaxation time (from steady state or time resolved fluorescence anisotropy decay measurements) and τ_h/τ_0 is another universal shape parameter, the 'harmonic mean rotational relaxation time ratio', with τ_0 $(=\eta_0 M v_s/RT)$ the corresponding value for a spherical particle of the same hydrated volume and η_0, T, the solvent viscosity and temperature of the anisotropy measurements. Similar hydration independent shape functions, Λ_i are available corresponding to the time resolved anisotropy decay times τ_i (i = 1–3 for ellipsoids of revolution, i = 1–5 for general particles) (Garcia de la Torre *et al.*, 1997; Harding *et al.*, 1997b).

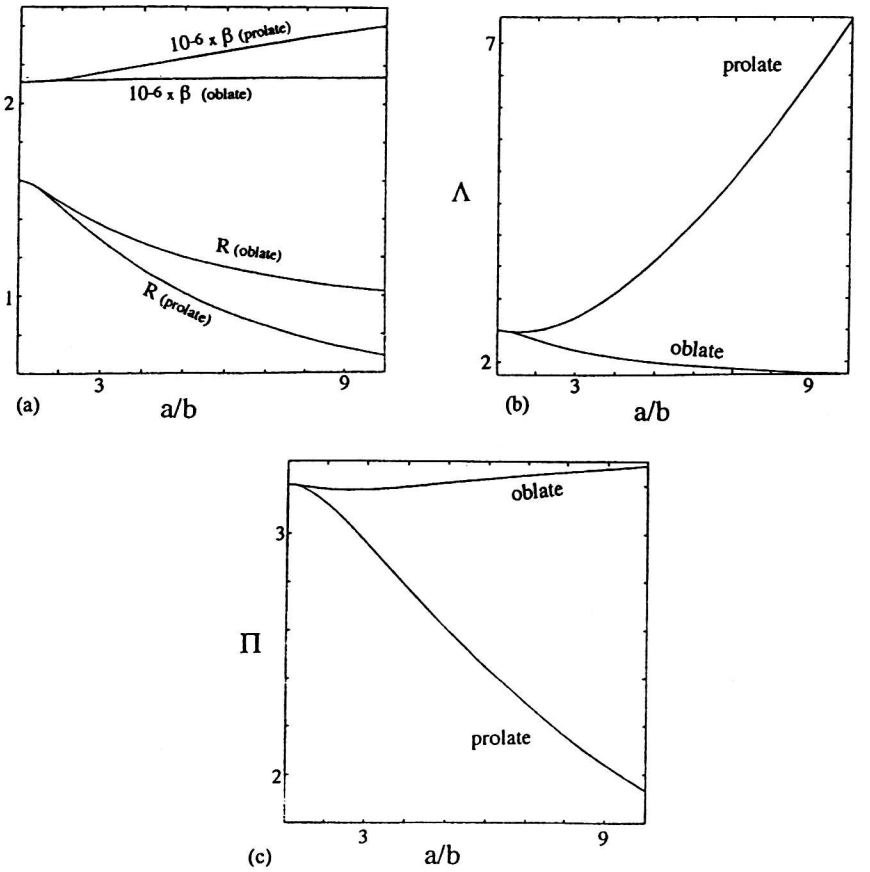

Figure 1.8 Hydration independent universal shape functions involving $[\eta]$ for axisymmetric ellipsoids. (a) β and R. (b) Λ_h. (c) Π.

The Π function (Harding, 1981a):

$$\Pi = \{2BM/[\eta]\} - \{f(Z, I)/[\eta]M\} = u_{red}/v, \quad (33)$$

(Figure 1.8c) with u_{red} the reduced excluded volume (Rallison and Harding, 1985), B (ml·mol·g^{-2}) the thermodynamic (or 'osmotic pressure') second virial coefficient (from osmotic pressure, light scattering or sedimentation equilibrium), and $f(Z, I)$ is a function of the charge (valency), Z, on a macromolecule and the ionic strength I (mol·ml^{-1}). At sufficient ionic strengths, the $f(Z, I)$ term becomes negligible compared with $2BM^2$. Of course for uncharged molecules and proteins at the isoelectric point, $Z = 0$, and $f(Z, I) = 0$.

The Wales–Van Holde (1954; Rowe, 1977) parameter:

$$R = k_s/[\eta] = 2(1 + P^3)/v, \quad (34)$$

(Figure 1.8a) where k_s is the concentration dependence parameter of the sedimentation coefficient $s_{20,w}$ in the limiting relation $s_{20,w} = s^0_{20,w}(1 - k_s c)$ or $1/s_{20,w} = \{1/s^0_{20,w}\}(1 + k_s c)$. Although the theory behind equation 34 is less rigorous than that for Π, it does have a strong experimental basis (Creeth and Knight, 1965; Rowe, 1977; 1992; Lavrenko *et al.*, 1992) and appears to give the correct value for spheres (Brady and Durlovsky, 1988). To apply k_s in this way it is important that charge contributions to k_s are absent or if the macromolecule is a polyelectrolyte, charge contributions are suppressed by working in a solvent of sufficient ionic strength.

It can be seen form Figure 1.8(b) and (c) that both Λ_h and Π have the added advantage that, except at low axial ratio (<2), a value of Λ_h or Π will uniquely specify a prolate or an oblate ellipsoid. Polynomial inversion formulae, similar to equation 29, giving (a/b) for a specified value of β, R, Λ_h or Π are available in tabular form (Harding and Colfen, 1995) and have been directly built into the PC algorithm ELLIPS1 (Harding *et al.*, 1997b),

For triaxial ellipsoids there is no analytical or numerical combination of (universal) shape functions that can uniquely specify a triaxial shape, via the two axial ratios $(a/b, b/c)$. Instead a graphical inversion procedure is necessary involving a combination of two or more universal shape functions, and the concept of this graphical combination of hydration independent universal shape functions has been explored in detail by Harding and co-workers (Harding and Rowe, 1982a,b; 1983; 1984; Harding, 1987; 1989; 1995; Harding *et al.*, 1997b). Whereas the PC routine ELLIPS2 evaluates the complete set of hydration dependent and hydration independent universal shape functions, for user specified values of $(a/b, b/c)$ or (a, b, c), the performance of the reverse procedure, i.e. obtaining a unique value of $(a/b, b/c)$ for a macromolecule from a combination of universal shape parameters (using the graphical intersection procedure) has been built into the program ELLIPS3 (combining for example, Λ with R, or Π, using the excluded volume worked out for triaxial ellipsoids by Rallison and Harding (1985) with the radius of gyration based function G) or

ELLIPS4 (combining electro-optic/viscosity-based shape functions (Harding *et al.*, 1997b)).

1.4.7 *The hydrodynamic bead model approximation: the Bloomfield* et al. *and Garcia de la Torre* et al. *approaches*

This is potentially very useful for representing the solution conformation of multi-subunit food proteins such as the soya and pea globulins, but in the past there have been some difficulties with calculating the intrinsic viscosity of such structures, difficulties which are now being resolved.

The pioneering work for representing the shapes of complex but quasi-rigid macromolecules was done by Bloomfield *et al.* (1967a,b). Their idea was to model a macromolecule as an array of spheres or 'beads' and from approximate calculations based on the interaction tensor between these spheres the hydrodynamic properties of macromolecules of arbitrary shape could be approximately calculated. The main restrictions of this early work were the approximate nature of the interaction tensor used (the so-called Burgers–Oseen tensor) and the limited computational power available at that time (bearing in mind computation time $\sim N^3$ where N is the numbers of beads in a model). Aided with the assistance of an improved interaction tensor and the huge advances in computational capabilities, Bloomfield, Garcia de la Torre and their co-workers (e.g. Garcia de la Torre and Bloomfield, 1978; Wilson and Bloomfield, 1979a,b; Bloomfield *et al.*, 1979; Garcia de la Torre and Bloomfield, 1981; Garcia Bernal and Garcia de la Torre, 1980; Garcia de la Torre, 1989; Garcia de la Torre *et al.*, 1994; Garcia de la Torre *et al.*, 1997) and others (e.g. McCammon *et al.*, 1975) have thence considerably extended the power of this methodology for the calculation of the intrinsic viscosity (and hence the viscosity increment, ν) and other related hydrodynamic shaped parameters based on translational and rotational frictional properties. Spherical bead models and their viscosity increments for a range of oligomeric structures are given in Figure 1.9.

In common with rotational frictional coefficients the intrinsic viscosity is a much more sensitive function of bead geometry than translational friction (from sedimentation and translational diffusion measurements). However, also in common with rotational frictional coefficients its calculation is more difficult compared to the translational frictional property (Garcia de la Torre and Bloomfield, 1981) because the calculation is origin sensitive: in the case of $[\eta]$ the so-called 'viscosity centre' of the particle (the point which gives the minimum energy dissipation in the calculations – cf. section 1.4.5) has to be calculated (Garcia Bernal and Garcia de la Torre, 1980). Furthermore, as with the derivation for ellipsoids (section 1.4.5) the calculation must be orientationally averaged in terms of Euler angles (Nakajima and Wada, 1978; Garcia de la Torre and Bloomfield, 1978), or other procedures (Yamakawa *et al.*, 1977). A numerical matrix inversion is required: this is a so-called 'supermatrix', **Q** containing

$N \times N$ blocks (N = the number of beads) each of dimension 3×3. In the inversion procedure, Garcia de la Torre and Bloomfield (1981) have shown that the modified interaction tensor of Rotne and Prager (1969) and Yamakawa (1970), later modified by Garcia de la Torre and Bloomfield (1977) for beads of different size, need to be used rather than the original Oseen (1927) interaction tensor (which fails to take into account the finite sizes of the beads) to avoid singularities. The first expression, without singularities, for $[\eta]$ and using the modified Rotne–Prager–Yamakawa interaction tensor was given by Nakajima and Wada (1978) which, after a small correction given by Garcia de la Torre and Bloomfield (1981), and a volume correction, Δ_V subsequently added by Garcia de la Torre (1989):

$$[\eta] = \frac{N_A}{M\eta_0} \sum_i \sum_j \zeta_i \left[\frac{1}{15} \sum_\alpha \left(x_i^\alpha - \upsilon^\alpha \right) S_{ij}^{\alpha\alpha} \left(x_j^\alpha - \upsilon^\alpha \right) \right.$$

$$+ \frac{1}{20} \sum_{\alpha \neq \beta} \sum \left(x_i^\alpha - \upsilon^\alpha \right) S_{ij}^{\beta\alpha} \left(x_j^\beta - \upsilon^\beta \right) - \frac{1}{30} \sum_{\alpha \neq \beta} \sum \left(x_i^\alpha - \upsilon^\alpha \right) S_{ij}^{\alpha\beta} \left(R_j^\beta - \upsilon^\beta \right)$$

$$\left. + \frac{1}{20} \sum_{\alpha \neq \beta} \sum \left(x_j^\alpha - \upsilon^\alpha \right) S_{ij}^{\beta\beta} \left(x_j^\alpha - \upsilon^\alpha \right) \right] + \Delta_V \quad \left(\alpha_1, \beta = 1, 2, 3 \right), \tag{35}$$

where $\zeta_i = 6\pi\eta_0\sigma_i$ is the Stoke's law friction coefficient for a bead i of radius σ_i, S_{ij} are the elements of the inverse of the supermatrix $\mathbf{Q}$, $\mathbf{R}_j$ is the distance vector between the viscosity centre of the particle and the centre of the ith bead, and x_i^α and υ^α are, respectively, the coordinates of bead i and the viscosity centre in a body-fixed frame of reference. From the energy minimisation criterion referred to above the position of the viscosity centre is obtained by imposing the condition:

$$\frac{\partial[\eta]}{\partial \upsilon^\alpha} = 0 \qquad (\alpha = 1, 2, 3), \tag{36}$$

which gives a set of three simultaneous linear equations with coefficients combinations of the x_j^αs and S_{ij}s. Substitution into equation 35 then gives $[\eta]$. An approximate form of this has been given by Garcia de la Torre and Bloomfield (1981), again with a volume correction Δ_V subsequently added by Garcia de la Torre (1989). The viscosity increment ν simply $(1/v_s) = M/VN_A$ times equation 35 (where V is the hydrated volume of the particle) and as we explained in section 1.4.1, is dependent only on the shape and not the size of the particle or model (i.e. it is a 'universal' shape function). The PC routine HYDRO (Garcia de la Torre *et al.*, 1994), and a parallel routine BEAMS (Spotorno *et al.*, 1997) evaluates $[\eta]$ for a given set of (absolute) bead co-ordinates, whereas the routine SOLPRO (Garcia de la Torre *et al.*, 1997) evaluates ν and other universal shape parameters described in section 1.4.6 above (apart from u_{red} and Π which are not yet available for the bead approximation).

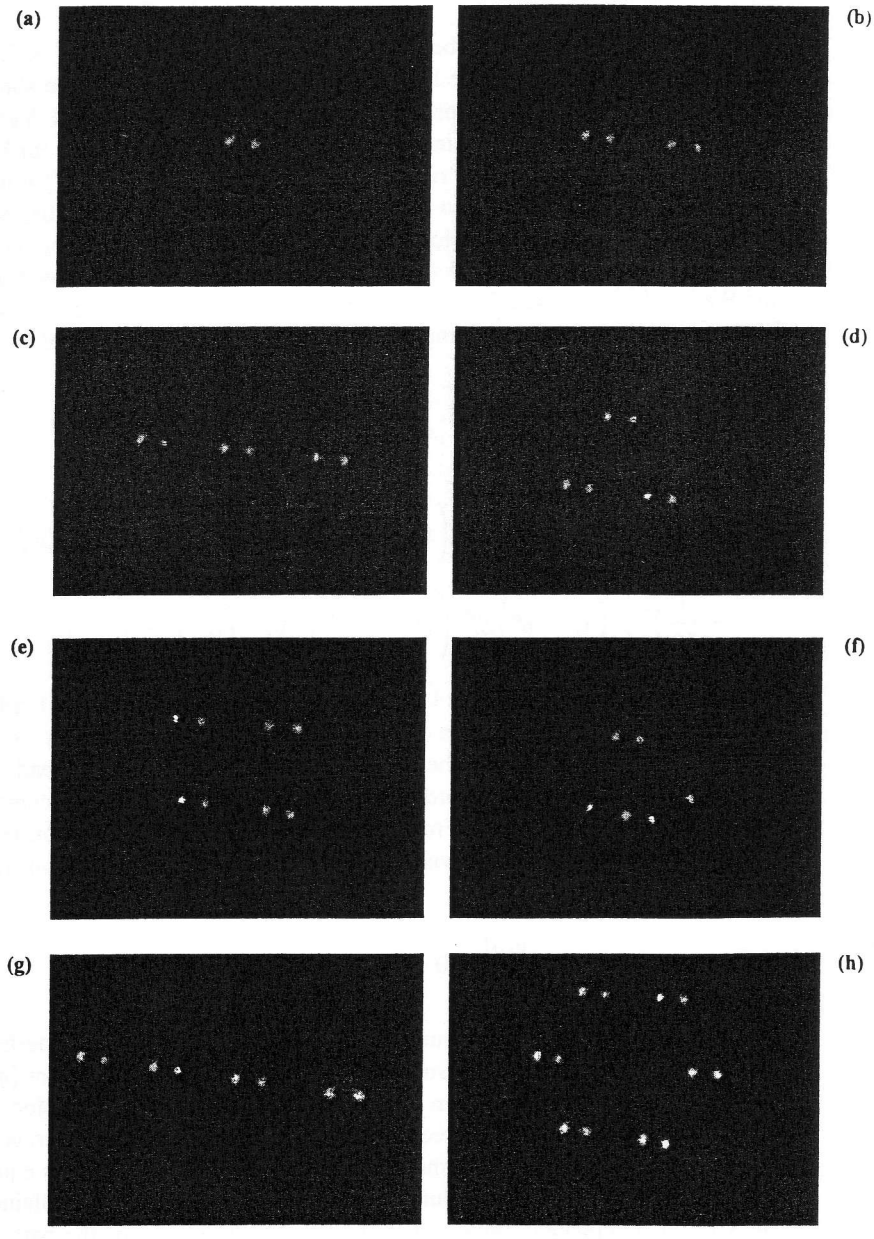

Figure 1.9 Bead models for various oligomeric structures with ~spherical subunits. (a) Monomer sphere, $\nu = 2.5$ (b) Dimer, $\nu = 4.1$ (c) Trimer-linear, $\nu = 5.3$ (d) Trimer-triangle, $\nu = 4.7$ (e) Tetramer-square, $\nu = 5.1$ (f) Tetramer-tetrahedron, $\nu = 4.9$ (g) Tetramer-linear, $\nu = 6.6$ (h) Pentamer-pentagon, $\nu = 5.5$ (i) Pentamer-bipiramid, $\nu = 5.1$ (j) Hexamer-hexagon, $\nu = 6.0$ (k) hexamer octahedron, $\nu = 5.1$ (l) hexamer trigonal prism, $\nu = 5.3$ (m) hexamer linear, $\nu = 9.8$ (n) octamer cube, $\nu = 5.5$. Values for ν are based on equation 37 with the volume correction of equation 35. An improved volume correction is currently being developed (Garcia de la Torre, 1997).

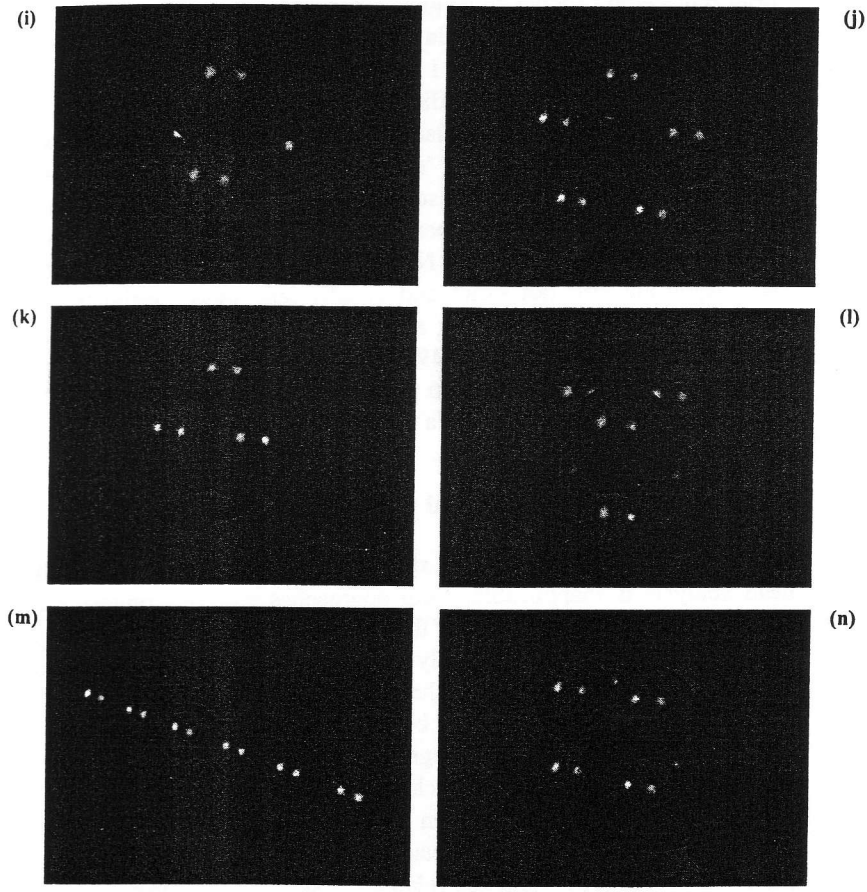

Figure 1.9 (*continued*)

The volume correction term,

$$\Delta_V = (5/2)\,(N_A V/M), \tag{37}$$

with V as before the (hydrated) volume of the particle, $= v_s M/N_A = (\bar{v}+\delta/\rho_0)M/N_A$ on the RHS of equation 35, which had been inspired by a similar correction for rotational coefficients (Garcia de la Torre and Rhodes, 1983), is essential in models in which one or a few beads have a large fraction of the particle size, for example, oligomeric protein structures consisting of two or more approximately spherical subunits. Without this correction for example, for a single sphere, a value of $\nu = 0$ is returned instead of the correct Einstein value of (5/2). Garcia Bernal and Garcia de la Torre (1981) had earlier suggested that each subunit should itself be represented as an array of eight smaller spheres arranged as a cube. Lopez Martinez and Garcia de la Torre (1983) then showed that bead model representations of prolate ellipsoids, with the central spherical

bead replaced by such a cube, gave reasonable agreement with the exact values known from the Simha formula (equation 28) (to no worse than ~12% for a range of axial ratios a/b from 1 to 6): much better arrangement was found for the translational frictional coefficient modelled in this way. The 'eight-sphere cube' approach also gave similar results (but requiring dramatically less computer time) to the 'raspberry' or 'shell' model approach of Swanson *et al.* (1980) who modelled the surface of each subunit as an array of 126 small spherical beads. For models with many beads of similar sizes (chain like structures) this correction term is insignificant. Nonetheless, representations of known ellipsoidal shapes are still not exact and usually lead to overestimations of ν. An improved method incorporating a better volume correction is currently being developed (Garcia de la Torre, 1997).

Similar calculations have been performed for the translational and rotational frictional properties (Garcia de la Torre, 1989).

1.5 General conformation and flexibility analysis

For many food biopolymers the rigid particle approach involving ellipsoid or bead analysis is inapplicable. Both approaches require stringent assumptions concerning the monodispersity of the macromolecular system being represented. This discounts molecules like polysaccharides although for fairly rigid systems thereof – for example, highly charged rod-shaped molecules like xanthan – ellipsoidal axial ratios can still be applied in a ball-park sort of way. For *molecules where approximate rigidity in the overall molecular morphology* cannot be reasonably assumed such as these, we have to use intrinsic viscosity and other hydrodynamic probes in a much more general sort of way. We can however take advantage of molecular polydispersity – especially if it is of a quasi-continuous type – and use relations describing the dependence on molecular weight with intrinsic viscosity (and other hydrodynamic properties) known as the Mark–Houwink–Kuhn–Sakurada relations, together with the Wales–Van Holde ratio, $k_s/[\eta] = R$, to distinguish between classes of particle conformation (for example, between the three extremes of compact sphere, rigid rod and random-coil). The $[\eta]$-molecular weight dependency can then be further developed to give a more quantitative description of particle flexibility. As a very approximate guide, the Huggins constant itself, K_H, has been used as a rough guide to the general conformation of a biopolymer: for solid uncharged spheres it can be as high as ~2 (Guth and Gold, 1938; Tanford, 1961) with lower values for more extended shapes, whereas for flexible biomolecules a value of ~0.35 can be expected, a value which is slightly higher in poor solvents.

1.5.1 Mark–Houwink–Kuhn–Sakurada and Wales–Van Holde relations

For molecules which can exist with a variety of molecular weights, the relation between $[\eta]$ and M is one of the most important properties (Tanford, 1961). The

following relation was first suggested by Mark (1938) and independently by Houwink (1940) as an empirical relation between the two parameters:

$$[\eta] = K'M^a, \tag{38}$$

where K' and a both depend on the polymer conformation, with the latter more easy to define. Similar relations exist for other hydrodynamic properties:

$$\begin{aligned} s_{20,w} &= K''M^b \\ D_{20,w} &= K'''M^{-\varepsilon} \\ R_g &= K''''M^c. \end{aligned} \tag{39}$$

These relations are collectively known as the 'Mark–Houwink–Kuhn–Sakurada' relations (Mark, 1938; Houwink, 1940; Kuhn and Kuhn, 1945; Sakurada, 1940; 1941; see also Bohdanecky and Kovar, 1982) and the exponents a, b, c, $-\varepsilon$ are known as the 'Mark–Houwink–Kuhn–Sakurada' exponents (or just 'MHKS' or 'Mark–Houwink' exponents) and can be obtained from simple double-logarithmic representations. The values of the viscosity exponent a are 0, 0.5–0.8 and ~1.8 for spherical, random-coil and rod conformations respectively, as described, for example, in the monographs of Tanford (1961), Smidsrød and Andresen (1979), Tsvetkov *et al.* (1971) and Bohdanecky and Kovar (1982). Values for the other parameters are given in Table 1.1, along with the Wales–Van Holde ratio (Wales and Van Holde, 1954; Creeth and Knight, 1965; Lavrenko *et al.*, 1992), R, of the concentration dependence parameter of the sedimentation coefficient, k_s, to $[\eta]$.

It can be seen from Table 1.1 that the relation between b and ε is trivial (because of their common relation with the frictional coefficient):

$$b + \varepsilon = 1, \tag{40}$$

(Elias *et al.*, 1973). Relations between b or ε with a have also been proposed but these are model dependent (e.g. non-draining random coils) and not universally valid (Kurata and Stockmayer, 1963; Reddy *et al.*, 1990).

It has also been pointed out (e.g. Manaresi *et al.*, 1988; Guaita *et al.*, 1991) that the MHKS relation for viscosity (38) is only rigorous where each $[\eta]$ value corresponds to a monodisperse polymer. The same of course applies to the other MHKS relations (39). Most of the biological macromolecules to which equations of the type 38 and 39 have been applied are themselves polydisperse –

Table 1.1 MHKS coefficients and the Wales–Van Holde ratio for general conformation types

Conformation	a	b	ε	c	$R = k_s/[\eta]$
Compact sphere	0	0.667	0.333	0.333	~1.6
Rigid rod	1.8	0.15	0.85	1.0	~0.2*
Random coil	0.5–0.8	0.4–0.5	0.5–0.6	0.5–0.6	~1.6

* Depends on axial ratio.

such as polysaccharides – and evaluation of the coefficients K' and a would be performed after prior fractionation of the sample: each fraction however is likely to have a residual polydispersity so some caution needs to be expressed. This feature of polydispersity is particularly important since equation 38 has been used as a 'relative' method for obtaining molecular weight. A molecular weight obtained from direct application 38 is often referred to as a 'viscosity average' (Tanford, 1961), M_v. For values of $a < 1$ (Tsvetkov *et al.*, 1971) $M_n < M_v < M_w$, where M_n and M_w are the number and weight average molecular weights respectively. For $a > 1$, $M_v > M_w$. More recently, attempts have been made to correlate $[\eta]$ directly with M_n, M_w, M_z, etc. (Dobkowski, 1981; 1984; Bareiss *et al.*, 1982; Manaresi *et al.*, 1988). For example, Manaresi *et al.*, (1988) have proposed a relation:

$$[\eta] = K' M_w^a (M_w/M_n)^{\alpha} (M_z/M_w)^{\beta}, \tag{41}$$

which has been shown to work for synthetic polymers (polystyrenes in various solvents) provided that the ratio M_z/M_w is not too high (Guaita *et al.*, 1991).

For most practical purposes, equation 38 is taken to be a reasonable approximation, with M taken as M_w, and is particularly popular with the use of microviscometers coupled on-line to size-exclusion chromatography separation systems, a concentration detector and an absolute molecular weight detection system (multi-angle laser light scattering), as described in section 1.3.3 (Haney, 1985a,b; Dutta *et al.*, 1991; Jackson *et al.*, 1991): each volume 'slice' leaving the column has its weight average molecular weight (by the light scattering detector) and intrinsic viscosity (via the microviscometer and appropriate application of equation 15) simultaneously determined. The exponent a thus found along with the exponent c from the R_g relations of equation 39 (which can also, within certain limitations, be obtained from the same set of measurements if the light scattering detector is of the multi-angle type (Wyatt, 1992)) can be used to specify the conformation either in terms of conformation type (Table 1.1) or the use of the more refined models described in section 1.5.5 below. The inclusion of the light scattering detector on-line also permits the testing of so-called 'Universal calibration procedures' for obtaining molecular weights from size exclusion chromatography from use of an on-line viscometer and concentration detector alone. The principle of Universal calibration (see Harding *et al.*, 1991) is that, for example, separation is based on a relation between V_e and the product $[\eta] \times M$ (where V_e is the elution volume) rather than being used on just M alone. Other refinements have been suggested (e.g. Horta *et al.*, 1986).

1.5.2 Representations of conformation type

Various graphical ways of representing the relation between the three conformation extremes (sphere, rod, coil) have been presented. One, the Haug triangle (seemingly popular only in Norway and Nottingham) places the extremes at the three corners of a triangle – the conformation of given macromolecules can then

be represented by a locus along the perimeter of the triangle (Smidsrød and Andresen, 1979). A more recent improved alternative has been given in terms of 'Conformation Zones' (Pavlov *et al.*, 1997a,b) A–E, with A = extra rigid rod, B = rigid rod, C = semi-flexible coil, D = random coil, E = compact sphere or heavily branched macromolecule. The current assignment of a zone based on sedimentation analysis alone (Pavlov *et al.*, 1997a) is now being extended to a complementary procedure based on measurement of $[\eta]$ and M and mass per unit length, M_L alone (Pavlov *et al.*, 1997b).

Having established the conformation type (sphere, rod, coil or a confirmation 'zone' A–E) via simple application of the MHKS or Wales–Van Holde relations, more detail about the conformation can be sought. For example, if it is rod-like, its length and dimensions can be sought; if it is sphere-like, its radius; if it is a coil, its flexibility; if its conformation is between a sphere and rod or disk (an ellipsoid) its axial ratio.

1.5.3 Smidsrød–Haug stiffness parameter, B

This is probably the simplest index for flexibility of a biopolymer, but applies only to polyelectrolytes. For polyelectrolytes Pals and Hermans (1952) had proposed the following relation between intrinsic viscosity and ionic strength.

$$[\eta] = [\eta]_\infty + (S \cdot I^{-1/2}), \tag{42}$$

where $[\eta]$ is the intrinsic viscosity at infinite ionic strength and with S a parameter which could be used as a comparative criterion of stiffness for polymers, but only for those of the same molecular weight, M and solvent counterion environment (Smidsrød, 1970; Smidsrød and Haug, 1971). To avoid this restriction, Smidsrød and Haug suggested the use of a modified parameter, B (not to be confused with the second thermodynamic virial coefficient, B): by comparing stiffnesses at a fixed ionic strength I (typically 0.1 M NaCl) the necessity of comparing biopolymers of the same M and even the necessity of knowing M is avoided. The 'Smidsrød' stiffness parameter B is defined by:

$$S = B \cdot ([\eta]_{I=0.1})^{\nu} \tag{43}$$

a relation which seems to fit the experimental data for glycopolymers and nucleic acids very well (section 1.6) with the exponent ν (also not to be confused – this time with the viscosity increment of section 1.3) fitting within the range (1.3 ± 0.1): B can thus be evaluated from measurement of S (via an $[\eta]$ versus I plot and equation 42) as well as $[\eta]_{I=0.1}$ and using a value of $\nu = 1.3$ in equation 43.

1.5.4 Polyelectrolyte behaviour at low ionic strength

For polyelectrolytes – such as a nucleic acid or a polyanionic polysaccharide in a solution where the concentration of low molecular weight electrolyte (salt,

etc.) is too small to suppress charge effects (section 1.4.3) the conventiona reduced viscosity versus concentration plot can depart from its conventiona positive slope characteristics (Figure 1.1) and the reduced viscosity can decreas with increase in polymer concentration c. A good representation of the behaviour is the Hess–Klein relation (Hess and Klein, 1983) which in simplified form (Malovikova *et al.*, 1994) is given by:

$$\eta_{red} \sim c/[(c/\lambda) + c_s]^{3/2}, \tag{44}$$

where λ is a function of the charge (valency) of the biopolymer and is consistent with the appearance of a maximum observed in these plots for charged polysaccharides. Both Rinaudo and co-workers (Malovikova *et al.*, 1994; Roure *et al.*, 1996) and Antonietti and co-workers (Antonietti *et al.*, 1996) have recently examined the nature of this maximum in some detail, the former for polysaccharides, the latter for spherical synthetic macromolecules.

1.5.5 [η]-M dependencies and the flexibility of linear biopolymers

Early attempts on the representation of a linear coil were based on a so-called 'free draining coil' model (Debye, 1946; Kramers, 1946; Peterlin, 1948; 1950; Hermans, 1949; Kuhn *et al.*, 1951) in which a macromolecule is represented by a linear chain of interconnected beads acting essentially independently of each other, followed by a summation of all their effects (Tsvetkov *et al.*, 1971; Yamakawa, 1971). This approach, which led to an estimate of a of $\sim$1, was later modified to incorporate hydrodynamic interaction (Brinkman, 1947a,b,c; Kirkwood and Riseman, 1948; 1949; Debye and Bueche, 1948; Kirkwood, 1967) and led to a range of possible values for a of 0.5–1.0 a range which represents the extremes of impenetrable coil (i.e. non-free draining where the solvent in the interior of the coil moves with the biomolecule) and a completely permeable free draining coil. Essentially the same result was obtained by Zimm (1956) based on a bead-spring or sub-chain model which took into account Brownian motion effects. Flory and co-workers (Flory and Krigbaum, 1950; Flory and Fox, 1951; Krigbaum and Flory, 1953) questioned the interpretation of values of $a > 0.5$ for a coil as due to partial or complete permeability, and proposed instead an alternative explanation in terms of swelling or (intramolecular) exclusion volume effects. Based on this theory the predicted range for a for coils is $0.5 < a < 0.8$, a range subsequently vindicated experimentally (Flory, 1953; also Ahn *et al.*, 1993). The concept of 'theta' solvents was also developed. In opposition to intramolecular exclusion volume effects are attractive effects: a 'good solvent' is one in which solvent-biopolymer interaction are preferred over interactions between different parts of the biopolymer, whereas in a 'poor solvent' intrachain (and inter-chain) biopolymer interactions predominate: this serves to effectively 'shrink' the molecule in opposition to the excluded volume effect. Under certain solvent conditions, known as 'θ-temperature' or 'θ-solvent' conditions these effects can effectively cancel giving rise to pseudo-ideal

behaviour. The intrinsic viscosity at these 'theta conditions' is represented by the symbol $[\eta]_0$.

Flory and co-workers (Flory and Krigbaum, 1950; Flory and Fox, 1951; Flory, 1953) also provided the basis for estimating the characteristic ratio C_∞ of a linear biopolymer which is a measure of the conformation restriction or 'stiffness':

$$C_\infty = \langle h^2 \rangle / nl^2, \tag{45}$$

where $\langle h^2 \rangle$ is the mean square end to end distance, n is the number of segments in the chain and l the length of each segment or residue (e.g. for a polysaccharide l would represent the saccharide residue length). $C_\infty \geq 1$, with equality holding only for a perfectly flexible chain. In practical terms, flexible coils appear to have values of $C_\infty \sim 1$–10 whereas very stiff polymers have $C_\infty \gtrsim 25$–400 (Lapasin and Pricl, 1995). Following Stockmayer and Fixman (1963) C_∞ can be estimated from the intercept of a plot of $[\eta]/M^{1/2}$ versus $M^{1/2}$ together with knowledge of the residue length l and the residue molecular weight (e.g. Robinson *et al.*, 1982).

Arguably a more useful representation of linear flexibility is in terms of the persistence length of the equivalent worm-like chain, a representation first proposed by Kratky and Porod (1949): also Ptitsyn and Eizner (1959); Peterlin (1950; 1952; 1960) and more recently Bohdanecky and Kovar (1982) and Fujita (1990). In this model, developed largely to give better representations of the conformation of DNA, the polymer chain is taken as continuous: effectively the segment length $l \to 0$ and the number of segments $n \to \infty$. The persistence length L_p, is the principal parameter, defined (Tvetskov *et al.*, 1971; Yamakawa, 1971; Fujita, 1990; Freire and Garcia de la Torre, 1992) as the average projection length along the initial direction of a chain of (contour) length L_c and in the limit of $L_c \to \infty$ (Figure 1.10). Thus the limits $L_c/L_p \to 0$ and $L_c/L_p \to \infty$ correspond to a perfectly rigid rod and a perfectly random coil respectively. Alternatively, just $L_p \to 0$ and $L_p \to \infty$ correspond to a perfect coil and perfect rod respectively. As Freire and Garcia de la Torre (1992) have said 'apart from its precise definition, the persistence length, L_p gives an indication of the length scale for which correlation between separate parts of the chain begin to disappear – it takes a given value for a given macromolecule independent of chain

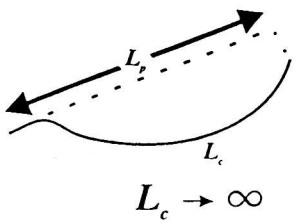

Figure 1.10 The persistence length L_p and contour length L_c of a linear macromolecule. L_p corresponds to the average projection (onto a line of the initial direction projected from one end of the macromolecule) that L_c would have in the hypothetical limit that $L_c \to \infty$.

length or molecular weight'. An alternative but equivalent parameter (Tsvetko *et al.*, 1971; Fujita, 1990) is the 'Kuhn statistical segment length' $\lambda^{-1}(= 2L_p)$.

Hearst (1963, 1964) and Hearst and Tagami (1965) provided expressions fo $[\eta]$ for both extremes: for the random coil ($L_c/L_p \rightarrow \infty$):

$$[\eta] = 100 \times 2.19 \times 10^{23} \cdot (1/M) \cdot (L_c\lambda^{-1})^{3/2} \cdot \{1 - 0.89[\ln(x/\lambda^{-1}) + 2.431 - x/d](L_c/\lambda^{-1})^{-1/2}\}^{-1}, \quad (46$$

and for the rigid rod ($L_c/L_p \rightarrow 0$):

$$[\eta] = 100 \times [\pi N_A L_c^3/(90M)] \cdot [1/\{\ln(L_c/x) - 2.72 + 0.66(x/d)\} + 3/\{\ln(L_c/x) - 2.72 + 1.33(x/d)\}], \quad (47)$$

with $[\eta]$ in ml/g and where d is the hydrodynamic diameter of a segment of length x. More general relations have been given by Eizner and Ptitsyn (1962), Ptitsyn and Eizner (1962) and Sharp and Bloomfield (1968).

Such worm-like modelling is referred to as 'two parameter' representations of flexibility – that is to say in terms of the contour length L_c and the persistence length L_p. The desire to represent a wider range of conformations and flexibilities – particularly helical structures – was noted by Yamakawa (1971) and in response to this the helical worm-like coil model, was developed by Yamakawa and co-workers (Yamakawa and Fujii, 1976; Yamakawa, 1977; Yamakawa, 1984; Yamakawa and Yoshizaki, 1980). The helical worm-like coil model involves five conformation parameters: the contour length L_c, a bending force constant, a twisting force constant, and two parameters representing the centroid helix. Extraction of so many parameters, however, provides a considerable strain on the experimental data. Consequently limiting cases with a reduced number of parameters have been developed. For example, Bohdanecky (1983) gave an approximate form in terms of three conformation parameters: L_c (or the mass per unit length $M_L = M/L_c$), λ^{-1} {or $2L_p$: use of either λ^{-1} or L_p seems to be one of personal preference (Fujita, 1990)} and the hydrodynamic diameter of the cylinder or chain, d. In simplified form, the Bohdanecky (1983) relation is:

$$(M^2/[\eta]_0)^{1/3} = A_\eta + B_\eta M^{1/2}, \quad (48)$$

where

$$A_\eta = A_0 M_L \Phi_{0,\infty}^{-1/3} \quad (49)$$

and

$$B_\eta = B_0 \Phi_{0,\infty}^{-1/3} (\langle R_0^2 \rangle / M)_\infty^{-1/2}, \quad (50)$$

and with the parameter $\Phi_{0,\infty} = 2.86 \times 10^{23}$. A_0 and B_0 are tabulated functions of d/λ^{-1} (Bohdanecky, 1983). Thus a plot of $(M^2/[\eta])^{1/3}$ vs. $M^{1/2}$ provides the basis for obtaining M_L, λ^{-1} and d. The mass per unit length M_L can either be used as a variable parameter in the analyses or used as a fixed parameter on the basis of other measurements such as from 'static' (i.e. classical or 'total intensity') light scattering or from electron microscopy (Stokke and Elgsaeter, 1994). Table 1.2 lists some useful values of M_L.

Table 1.2 Mass per unit length M_L for various polysaccharides

Biopolymer	Biopolymer type	M_L Da nm^{-1}	Method	Reference
pullulan	single chain polysaccharide	340	a	Kawahara *et al.* (1984)
methyl cellulose	single chain polysaccharide	360		
amylose	single chain polysaccharide	790–1400	a,b,c	Yamakawa and Yoshizaki (1980) Stokke *et al.* (1987)
xanthan	double helical polysaccharide	1700–2000	a,b,c	Sato *et al.* (1984) Coviello *et al.* (1986) Stokke *et al.* (1989a,b) Kitamura *et al.* (1991)
schizophyllan	triple helical polysaccharide	1900–2100	a,b	Bohdanecky (1983) Yanaki *et al.* (1980)

* Non-aqueous solvent

a: Viscometry or sedimentation analysis. b: Light scattering. c: Electron microscopy.

An even simpler version of equation 50 has been proposed by Bohdanecky and Netopilik (1993), and using this type of treatment Bohdanecky in a very recent paper (Bohdanecky, 1996) addressed an anomaly raised by Fujita (1988; 1990) as to why under theta solvent conditions, it is observed experimentally for many polymers that the MHKS coefficient a remains constant at ~0.5 (i.e. the non-draining coil limit) over a broad range of molecular weights, instead of increasing from 0.5 to 1 as the chain length decreases.

Close to the rod limit, Freire and Garcia de la Torre (1992) have highlighted the limitations of the worm-like coil theories and provided the motivation for the elucidation of theories for rigid cylinders. The most recent equation is that of Garcia-Molina *et al.* (1990):

$$[\eta] = Q \cdot N_A \cdot M^2/\{M_L(\ln M - \ln M_L - \ln d)\}, \qquad (51)$$

with N_A Avogadro's number and the coefficient $Q = 0.015$ ($[\eta]$ in ml/g; M_L in Da nm^{-1}).

1.5.6 Critical overlap concentration, c: the dilute solution limit*

In connection with the behaviour of coil-shaped molecules, the critical overlap concentration c^* has been used as a parameter representing the upper limit of dilute solution behaviour. Above this concentration the influence of overlapping molecular domains becomes significant. Vidakovic *et al.* (1982) have proposed the approximation:

$$c^* \sim \chi/[\eta], \qquad (52)$$

with $\chi = 0.58$. Launay *et al.* (1986) based on polysaccharides and Papanagopoulos and Dondos (1995) for polystyrene in ethyl acetate gave the same formulae differing only relatively slightly in the value of χ (0.33 and 0.5 respectively). This formula seems also to be valid for stiffer structures. For example, it accurately predicts a discontinuity at $c \sim 0.4$–0.8 mg/ml in the

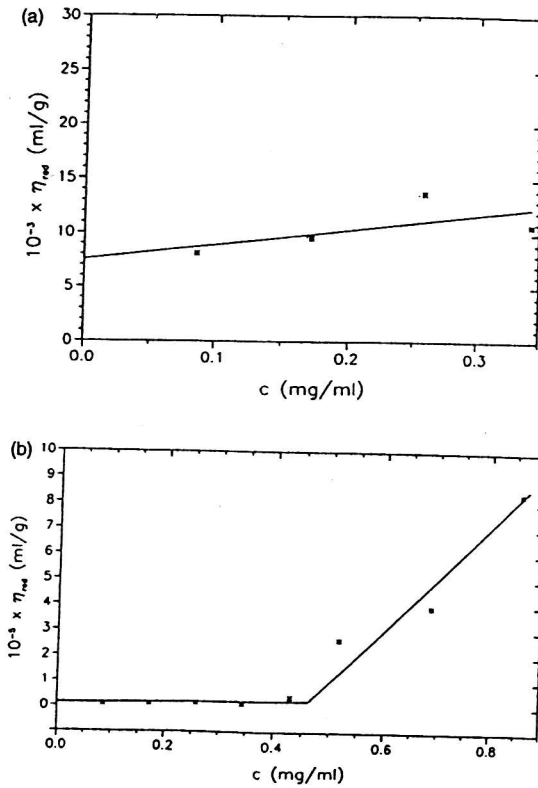

Figure 1.11 Reduced viscosity versus concentration, c, plots for xanthan (*Keltrol RD*) (a) in dilute solution (c = 0 → 0.35 mg/ml) and (b) in the region $c = 0 \rightarrow 0.9$ mg/ml. From (a), $[\eta] = (7500 \pm 2700)$ ml/g. Predicted c^* from equation 52 = 0.4–0.8 mg/ml. Reproduced with permission from Dhami *et al.* (1995).

Huggins plot for the bacterial polysaccharide xanthan (Figure 1.11). Discontinuities for this substance were observed at the same approximate concentration in plots of the sedimentation coefficient and apparent molecular weight versus concentration.

1.6 Applications to food biopolymers

Table 1.3 gives a list of the intrinsic viscosities of food and seed proteins with a clearly defined molecular weight. The table also includes collagen sonicates (again of different molecular weight).

1.6.1 General conformation studies

Table 1.3 illustrates the principles concerning $[\eta]$–general conformation relationships discussed in section 1.5 quite well, and in Table 1.4 we have collected

together data for an homologous series of proteins and polypeptides and their corresponding Mark–Houwink–Kuhn–Sakurada (MHKS) a (and K′) coefficients (equation 38). From Table 1.3, globular proteins are seen to have relatively small $[\eta]$s in the range 2.5–6 ml/g with little dependence on molecular weight (corresponding to an MHKS exponent $a = 0$ of Table 1.4). Sonicates of the triple-helical protein collagen (Nishihara and Doty, 1958) yield an a of ~1.8 (from a plot of log $[\eta]$ versus log M), consistent with a rigid rod conformation, whereas the protein in its gelatin state adopts a random coil configuration with $a = 0.45$–0.88 (Veis, 1964). Gelatin intrinsic viscosity has been the subject of a recent thorough investigation for a range of different preparations and temperatures (Krasovskii *et al.*, 1993).

Data collected for globular food proteins denatured by 8 M urea or 6 M guanidine hydrochloride (Tanford *et al.*, 1967; Van Kleef *et al.*, 1978) yield an $a \sim 0.68$, consistent with a random coil conformation, as shown earlier by Yang (1958a,b). Tanford (1968) suggested the following relation for proteins in the random coil state:

$$[\eta]\ (\text{ml/g}) = 0.716\, n^{0.67}, \tag{53}$$

where n is the number of amino acids in the protein.

1.6.2 Ellipsoid modelling studies

The earlier modelling of macromolecular conformation of proteins from measurement of $[\eta]$ was largely based on simple ellipsoids of revolution and using ν directly from equations 26 and 27 with equation 28 or approximate forms thereof together with an assumed value for the hydration δ of ~0.2–0.35 g/g (Tanford, 1961). Garrigos *et al.* (1983), for example, have examined the conformation of the S1 heads of myosin using a prolate ellipsoid model and showed that the $[\eta]$, along with the sedimentation coefficient could be represented by the extremes of axial ratio (a/b) of ~2.5 (hydration $\delta = 1.24$) and ~1.0 ($\delta = 2.02$). These workers have attempted to combine this information with images of 'pear-shaped molecules' from electron microscopy and with solution X-ray scattering data to propose a prolate ellipsoidal molecule with the hydration unevenly distributed into a hole at one end.

Use of the hydration independent shape functions that avoid δ through the combination of $[\eta]$ with another hydrodynamic parameters has involved the R, Π and Λ_h (section 1.4.6) rather than the β-function because of the latter's insensitivity to shape. Table 1.5 shows a number of proteins whose shapes have been determined using the R-function.

Since we know from the MHKS a exponent (= 1.8) that collegen is ~ a rigid rod we can model the molecule as a rigid prolate ellipsoid of large axial ratio, and use the known dependence of R on axial ratio (a/b) to evaluate the change of (a/b) with molecular weight and Figure 1.12 shows the increase of axial ratio is ~ linear with molecular weight for $M < 260\,000$.

Table 1.3 Intrinsic viscosities of food and seed proteins

Protein	M (Da)	Conditions	$[\eta]$ (ml/g)	Reference
α_{s2}-Casein	23 000	pH 6.7, I = 0.01, 20 °C	11.3	Snoeren *et al.* (1980)
		pH 6.7, I = 0.05, 20 °C	11.4	Snoeren *et al.* (1980)
		pH 6.7, I = 0.15, 20 °C	13.7	Snoeren *et al.* (1980)
		pH 6.7, I = 0.30, 20 °C	12.1	Snoeren *et al.* (1980)
		pH 6.7, I = 0.60, 20 °C	13.7	Snoeren *et al.* (1980)
α-globulin (11S sesame seed globulin)	300 000		3.5	Prakash (1994)
Arachine (11S groundnut globulin)	300 000		4.5	Prakash (1994)
β-Lactoglobulin dimers BSA – see serum albumin	36 000	pH 7.0, I = 0.10, 25 °C	2.9	Advani *et al.* (1997)
Brassin M (oilseed rape 11S globulin)	300 000		3.8	Prakash (1994)
Brassin R (mustard seed 11S globulin)	300 000		3.8	Prakash (1994)
Carmin (11S safflower seed globulin)	300 000		3.9	Prakash (1994)
Catalase	250 000		3.9	Lovrien (1958)
Collagen	345 000	pH 4.0, 0.06M NaCl, 17 °C	1270	Nestler *et al.* (1983)
	364 000		1250	Nishihara and Doty (1958)
				Creeth and Knight (1965)
Collagen sonicates	336 000		1075	Nishihara and Doty (1958)
	297 000		865	Nishihara and Doty (1958)
	250 000		625	Nishihara and Doty (1958)
	217 000		495	Nishihara and Doty (1958)
	192 000		400	Nishihara and Doty (1958)
	170 000		325	Nishihara and Doty (1958)
	149 000		245	Nishihara and Doty (1958)
Conalbumin	75 500	pH 6.7, I = 0.02, 25 °C	3.5	Phelps and Cann (1956)
		pH 6.0, I = 0.15, 25 °C	3.8	Phelps and Cann (1956)
		pH 5.3, I = 0.15, 25 °C	4.0	Phelps and Cann (1956)
		pH 3.0, I = 0.10, 25 °C	8.4	Phelps and Cann (1956)
		pH 3.0, I = 0.07, 25 °C	11.0	Phelps and Cann (1956)
		pH 3.1, I = 0.02, 25 °C	16.0	Phelps and Cann (1956)

Conbrassin M (2S mustard seed globulin)	15 000		5.4	Prakash (1994)
Conbrassin R (2S rapeseed globulin)	15 000		7.27	Prakash (1994)
Concarmin (2S safflower seed globulin)	15 000		6.50	Prakash (1994)
Conhelianthin (2S sunflower seed globulin)	15 000		7.28	Prakash (1994)
Consesamin (2S sesame seed globulin)	15 000		4.10	Prakash (1994)
Gelatin	383 000		69	Gouinlock *et al.* (1995)
	320 000		88	Courts and Stainsby (1958)
		at pH 5.08 (pI)	42	Ward and Saunders (1958)
Glycinin (11S soybean globulin)	300 000		4.6	Prakash (1994)
Gossypin (11S cottonseed globulin)	300 000		4.0	Prakash (1994)
Helianthin (11S sunflower seed globulin)	300 000		3.75	Prakash (1994)
Hemoglobin	68 000	free solution	3.6	Cohn and Prentiss (1927)
Lactate dehydrogenase	138 000	pH 7.8, I = 0.1, 25 °C	3.9	Davisson *et al.* (1953)
Linin (11S linseed globulin)	300 000		3.7	Prakash (1994)
Lupin protein isolate	390 000	pH 7.6, 0.5 M phosphate, 25 °C	6.8	Sousa *et al.* (1996)
		pH 7.0, 0.01 M phosphate, 25 °C	7.2	Sousa *et al.* (1996)
Myoglobin	17 190	pH 7.1, 0.1 M NaCl, 20 °C	3.25	Harding (1980b)
		pH 8.6	3.15	Wyman and Ingalls (1943)
			3.54	Potschke *et al.* (1996)
Myosin dimers	474 000	pH 6.7, 0.55 M KCl, 5 °C	217	Emes and Rowe (1978)
			234	Holtzer and Lowey (1956, 1969)
Myosin S1 heads	110 000	5 °C	6.44	Garrigos *et al.* (1983)
		20 °C	6.40	Lowey *et al.* (1969)
Ovalbumin	45 000		3.5	Holt (1970)
Ovomucoid		pH 7.0, 25 °C	5.48	Das *et al.* (1991)
		pH 4.6, 25 °C	5.54	Das *et al.* (1991)
		6 M GuHCl, 25 °C	9.94	Das *et al.* (1991)
Tropomyosin	93 000		52	Tsao *et al.* (1951)

Table 1.4 MHKS parameters for food proteins

Protein	Conditions/Comments	$10^4 \times K'$ (for $[\eta]$ in ml/g)	a	Reference
Collagen			1.8	Nishihara and Doty (1958)
Gelatin			0.45–0.88	Veis (1964)
	Water at the isoelectric point	1600	0.885	Pouradier and Venet (1950)
Globular proteins		–	0	
Denatured proteins	6 M GuHCl or 8 M urea, +0.1 M β-mercaptoethanol	7160*	0.67 0.64	Tanford (1967) Van Kleef *et al.* (1978)
Poly-L-glutamate	0.2 M NaCl, pH 4.3–7.3		1.0	Morcellet and Loucheux (1976)

* This equation is given in the form $[\eta] = K'n^a$, where n is the number of amino acid residues.

Table 1.6 shows the axial ratios (a/b) of three proteins worked out by the Π function (Harding, 1981a). It is particularly interesting to note that the overall shape of the ovalbumin molecule from both the R- and Π-functions found in 1981 (Harding, 1981b) is almost exactly as found some ten years later by X-ray crystallography (Stein *et al.*, 1991) (Figure 1.6).

The ellipsoid of revolution approximation to hydrodynamic structure assumes a protein can be reasonably modelled by a three-dimensional shape with two of the perpendicular axes equal axes (length $2b$) and the final perpendicular longer axis (length $2a$), a shape specified by a single axial ratio (a/b). As noted in section 1.4.3, a much better representation of molecular shape can be obtained if the restriction of two equal axes is removed to give a general triaxial ellipsoid of

Table 1.5 Axial ratios of food proteins from k_s and intrinsic viscosity $[\eta]$ measurements

Protein	k_s (ml/g)	$[\eta]$ (ml/g)	R $\{=k_s/[\eta]\}$	axial ratio (a/b)*	Reference
ovalbumin	5.45	3.49	1.56	1.5	a,b,c
bovine serum albumin	5.4	3.9	1.38	2.3	d,e
β-lactoglobulin (B) {dimer}	4.6	2.86	1.61	1.0	f,g
collagen (374 kDa)	265	1250	0.212	>100	h,i
sonicates: 336 kDa	250	1075	0.232	100	h
297 kDa	227	865	0.262	70	h
250 kDa	202	625	0.323	43	h
217 kDa	182	495	0.368	33	h
192 kDa	166	400	0.415	25	h
170 kDa	154	325	0.474	18	h
149 kDa	142	245	0.580	14	h

* Of the equivalent prolate ellipsoid. k_s values are normally corrected for 'radial dilution' and to 'solution density' (see Rowe, 1977; Harding and Johnson, 1985).
a: Miller and Golder (1952); b: Holt (1970); c: Harding (1981b); d: Baldwin, (1957); e: Tanford and Buzzell, (1956); f: Advani *et al.* (1957); g: Townend *et al.* (1960); h: Nishihara and Doty (1958); i: Creeth and Knight (1965).

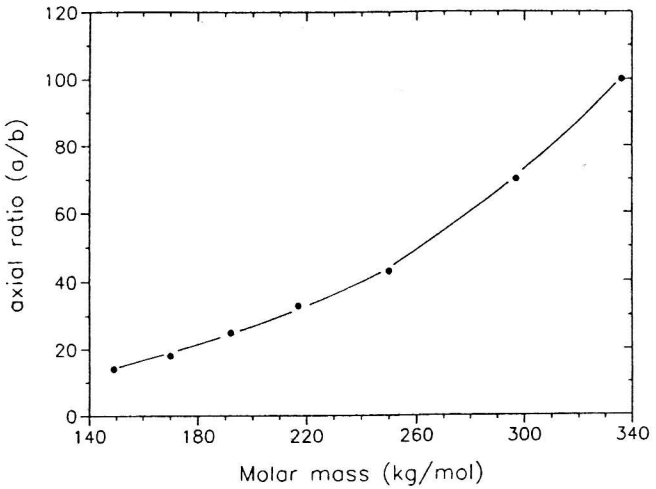

Figure 1.12 Axial ratio of collagen sonicates estimated from the Wales–Van Holde ratio, R as a function of molecular weight (Harding, 1995).

semi-axes $a \geq b \geq c$ and 2 axial ratios (a/b, b/c). As an example Harding (1987) has used the intersection of Π with the radius of gyration shape function G to show that despite the segmental flexibility of myosin (dimers) the overall conformation of a rod of axial ratio (a/b, b/c) ~ (80, 1) is faithfully reproduced (Figure 1.13).

1.6.3 Bead modelling

Although it is encouraging that general ellipsoid modelling reproduces the overall rod-shape conformation of the myosin molecule, it does not provide any information about the nature of any kinks or bends in the rod and nothing about possible flexible regions in the macromolecule: myosin is in fact a good example where *bead modelling* can be successfully applied to viscosity data and is indeed more appropriate for representing molecular flexibility compared with rigid triaxial ellipsoids (Byron, 1995), with two potential regions of flexibility (Garcia de la Torre, 1989). There is however considerable disagreement in terms of the

Table 1.6 Axial ratios of three food proteins form the Π function

Protein	Π	Axial ratio (a/b)*	Reference
haemoglobin	3.20	1.0	Tanford and Buzzell (1956)
ovalbumin	3.18	1.0–2.0	Harding (1981b)
myosin	0.47	80	Harding (1987)

* Of the equivalent prolate ellipsoid

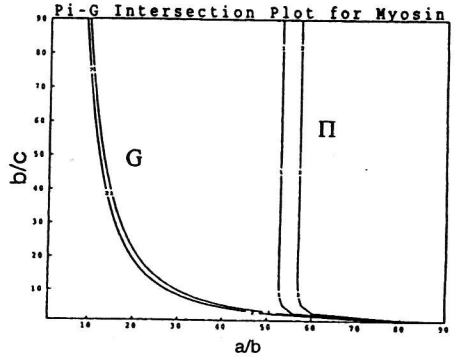

Figure 1.13 Use of the intrinsic viscosity based Π-*G* intersection plot (allowing for experimental error) to uniquely fix the overall triaxial shape of a particle in terms of (*a*/*b*, *b*/*c*). Based on data for myosin (from Harding, 1987). Computer output from the PC routine ELLIPS3. Predicted axial ratios: (*a*/*b*, *b*/*c*) ~ (80, 1).

extent of flexibility, with some works suggesting there may be large flexibility within the rod (Highsmith *et al.*, 1982; Cardinaud and Bernengo, 1985; Iniesta *et al.*, 1988), whereas others indicate that the rod is nearly rigid (Hvidt *et al.*, 1982; Curry and Krause, 1991). There is also uncertainty as to whether the flexibility – if present – is largely localized to one or two flexible joints or whether it is more evenly distributed as a worm-like cylinder (section 1.5.5). Garcia de la Torre (1994) has given three sources for uncertainty:

(a) The length of the rod is a sensitive parameter needed for the modelling, and values ranging from 144 to 156 nm have been assumed.
(b) Large discrepancies with relaxation times from rotational frictional measurements (birefringence or fluorescence anisotropy).
(c) The existence of two different theoretical approaches (the 'rigid body' and 'Wegener' (1985) approaches of section 1.4.7) and some confusion as to notation.

In an attempt to reconcile these difficulties, Garcia de la Torre (1994) has examined data from $[\eta]$ and R_g for which there is general acceptance (unlike the rotational data) and shown that the flexibility parameter Q for the myosin rod is ~0.50 and the optimum rod (contour) length, L_c is indeed 144 nm.

However, it is probably fair to say that bead modelling has yet to fulfil its potential, particularly in the area of the modelling of multi-subunit proteins such as the soya bean and related globulins. As noted in section 1.4.7, the viscosity parameters ν and $[\eta]$ are particularly difficult to calculate compared to other hydrodynamic parameters and a simple representation of 1 sphere = 1 subunit does not yield optimal results. However, in work to be published shortly Carrasco *et al.* (1998) have shown that the arrangement of the subunits of 12S globulins from the sunflower protein can be successfully represented if each

subunit is represented by either a cubic array of smaller beads or the surface of each subunit is represented by a shell of smaller beads.

1.6.4 Food polysaccharides

The most fundamental experimental parameter describing the general conformation of food polysaccharides is the MHKS a exponent and Table 1.7 gives a comprehensive list for a range of polysaccharides (although some, like chitosan, are not yet universally food grade approved). The parameter K' is included as well since the MHKS expression is often used to obtain molecular weights from measured intrinsic viscosities.

It can be seen that the bulk of the polysaccharides represented in Table 1.7 have MHKS a values in the random coil range (0.5–0.8). Low values of a (<0.5) tend to indicate significant branching or an approach to the compact sphere limit of $a = 0$ for the glycopolymers of Table 1.7. There are very few reported values significantly below the lower limit for completely random coils ($a = 0.5$), two exceptions being hydroxyethyl starch ($a = 0.35$) and DIT- (di-iodotyrosine) dextran: with the latter, the effect of incorporation of the label appeared to make the molecule effectively more compact by accentuating the effect of branching of the native dextran. At the other end of the scale a number of charged and particularly helical saccharides have a values >1, particularly succinoglycan, xanthan and the triple-helical schizophyllan. With the latter, the a value illustrates the effect of chain length on the overall conformation, with the 'extra-rigid rod' characteristics at lower chain length ($M_w < 50\,000$), with $a = 1.7$, reverting to a more flexible rod at larger molecular weights ($a = 1.2$). Similar behaviour has been observed for xanthan (Milas *et al.*, 1985; Liu and Norisuye, 1988).

In support of conclusions on molecular structure based around the MHKS a coefficient, other MHKS coefficients can be used such as the sedimentation b coefficient (reviewed in Harding, 1995) and the Wales–Van Holde parameter $R\,(=k_s/[\eta])$, as described in section 1.5.1, with values of ~ 1.6 signifying a spheroidal domain (either a compact sphere or random coil) and low values ($\rightarrow 0.2$) indicating a rigid rod conformation. Table 1.8 summarizes some findings.

Once the general conformation has been sorted out for a glycopolymer by MHKS and/or the Wales–Van Holde treatments, more sophisticated analyses can then be applied. If the glycopolymer is a rigid rod-like structure (such as schizophyllan or xanthan) then the rigid particle ellipsoid or bead theories of section 1.4, although derived mainly for protein work, can be applied. For example, in a recent study on xanthan by Dhami *et al.* (1995) a rod of aspect ratio $\sim 70 : 1$ was inferred on the basis of both the Π- (equation 33) and the R-functions (equation 34). Or in the case of more coiled structures, more detailed information about the flexibility of the molecule in terms of the characterisitc ratio, C_∞, the persistence length L_p (or the Kuhn statistical segment length, λ^{-1}) for a worm-like coil, the helical parameters from the Yamakawa–Fujii helical worm-like coil model or the polyelectrolyte stiffness parameter B can be sought,

Table 1.7 MHKS parameters for polysaccharides

Glycopolymer	Conditions/Comments	$10^4 \times K'$ (for $[\eta]$ in ml/g)	a	Reference
Agar	0.1 M KCl, 65 °C	875	0.68	Tashiro *et al.* (1996)
Alginate	0.01 M NaCl, 20 °C	4.8	1.15	Smidsrød (1970)
(manA/gulA[a] = 1.8)	0.1 M NaCl, 20 °C	20	1.0	Smidsrød (1970)
	1 M NaCl, 20 °C	91	0.87	Smidsrød (1970)
	$I \rightarrow \infty$, 20 °C	120	0.84	Smidsrød (1970)
Amylose		132	0.68	Burchard (1963)
	0.2 N KOH	69.2	0.78	Banks and Greenwood (1969)
	0.33 M KCl	1150	0.50	Banks and Greenwood (1968)
	0.33 M KCl	1120	0.50	Banks and Greenwood (1975)
	0.5 M KCl	550	0.53	Cowie (1963)
Carboxymethylamylose	37.5 °C	252	0.64	Patel *et al.* (1967)
Carboxymethylcellulose (Na^+)	0.005 M NaCl	72	0.95	Brown and Henley (1964)
	0.2 M NaCl	430	0.74	Brown and Henley (1964)
	0.01 M NaCl		0.92	Brown *et al.* (1964)
	0.05 M NaCl	190	0.82	Morris and Ross-Murphy (1981)
	$I \rightarrow \infty$	1900	0.60	Morris and Ross-Murphy (1981)
Chitosan[b]	0.2 M CH_3COOH/ CH_3COONa, DD[c] = 58%, 25 °C		1.14	Errington *et al.* (1993)
	0.2 M CH_3COOH/0.1 M CH_3COONa, DD = 69%, 30 °C	1.04	1.12	Wang *et al.* (1991)
	0.2 M CH_3COOH/0.1 M CH_3COONa, DD = 84%, 30 °C	14.24	0.96	Wang *et al.* (1991)
	0.2 M CH_3COOH/0.1 M CH_3COONa, DD = 91%, 30 °C	65.89	0.88	Wang *et al.* (1991)
	0.2 M CH_3COOH/0.1 M CH_3COONa, DD = 100%, 30 °C	168	0.81	Wang *et al.* (1991)
	0.2 M CH_3COOH/0.1 M NaCl 4 M urea		0.71	Muzzarelli (1977)
	0.2 M CH_3COOH/0.2 M NaCl	18.1	0.93	Roberts and Domszy (1982)
	CF_3COOH		0.3	Berkovich *et al.* (1980)
	1% CH_3COOH/2.8% NaCl		0.15	Berkovich *et al.* (1980)
	1% CH_3COOH/2% LiCl		0.19	Berkovich *et al.* (1980)
Dextran		0.87	0.50	Neely (1963)
			0.51	Senti *et al.* (1955)

continued

Table 1.7 *Continued*

Glycopolymer	Conditions/Comments	$10^4 \times K'$ (for $[\eta]$ in ml/g)	a	Reference
Galactomannans		0.168	0.98	Doublier and Launay (1976)
	20 °C	670	0.80	Sharman *et al.* (1978)
			0.98	Doublier and Launay (1981)
Gellan (deesterified)	0.025 M tetramethylammonium-chloride, 25 °C	74.8	0.91	Dreveton *et al.* (1996)
Guar gum		7.76	0.98	Doublier and Launay (1976, 1981)
		380	0.723	Robinson *et al.* (1982)
Hydroxyethylcellulose		10 000	0.70	Brown (1961);
		95.4	0.87	Uda and Meyerhoff (1961); Brown *et al.* (1963); Savage (1965); Wirick (1988); Hodges *et al.* (1979);
Hydroxyethylstarch		2910	0.35	Granath *et al.* (1969)
Hydroxypropylmethyl-cellulose	pH 6.5, $I = 0.10$, 25 °C		0.50	Jumel *et al.* (1996)
	water, 25 °C		0.41	Jumel *et al.* (1996)
Locust bean gum	mannose/galactose ratio = 1	80.2	0.79	Sabater de Sabates (1979)
Methylcellulose		2800	0.63	Brown (1961); Uda and Meyerhoff (1961); Brown *et al.* (1963); Savage (1965); Wirick (1988); Hodges *et al.* (1979);
		3160	0.55	Senti *et al.* (1955)
Pectins	DM[d] 70% in H_2O	216	0.79	Berth *et al.* (1977)
	DM 30–72%, 0.1 M NaCl		0.8	Axelos *et al.* (1989)
Pullulan	pH 6.8, $I = 0.10$	235	0.659	Clarke and Harding (1997)
		236	0.658	Kawahara *et al.* (1984)
		258	0.646	Buliga and Brant (1987)
Schizophyllan	$M_w < 50000$	0.0013	1.7	Yanaki *et al.* (1980)
	$M_w > 50000$	0.92	1.2	Yanaki *et al.* (1980)
	in DMSO[e]	2230	0.69	Yanaki *et al.* (1980)

continued

Table 1.7 *Continued*

Glycopolymer	Conditions/Comments	$10^4 \times K'$ (for $[\eta]$ in ml/g)	a	Reference
Succinoglycan	$M_w < 10^6$ · 0.1 M NaCl	6.5	1.4	Gravanis *et al.* (1987)
Xantan	$M_w > 310\,000$ · 0.1 M NaCl	170	1.14	Milas *et al.* (1985)
	$M_w > 150\,000$ · 0.01 M NaCl		1.2	Liu and Norisuye (1988)
	$M_w > 150\,000$ · 0.01 M NaCl	63	1.32	Liu and Norisuye (1988)
	$M_w > 150\,000$ · 0.01 M NaCl		0.95	Liu and Norisuye (1988)
	0.5% NaCl		0.93	Muller *et al.* (1984)

Data partly taken from Lapasin and Pricl (1995).
a: ManA – mannuronic acid, GulA – guluronic acid; b: not yet FDA approved;
c: DD – degree of deacetylation; d: DM – degree of methoxylation; e: dimethylsulphoxide.

as described in section 1.5.4. The least popularly applied appears to have been the characteristic ratio, C_∞, and measurements have largely been based on radius of gyration rather than from intrinsic viscosity measurements (section 1.4.5). For example, for uncharged polysaccharides C_∞ for the randomly coiled pullulan has been shown to be ~4 (Buliga and Brant, 1987), whereas the stiffer guar was shown to have a value of ~13 (Robinson *et al.*, 1982); corresponding a values are ~0.65 and ~1 (Table 1.7). For polyelectrolytes the Smidsrød stiffness parameter, B, has had popular application (Lapasin and Pricl, 1995), with low values of B indicating a stiff backbone and *vice versa*. Use of B has demonstrated, for example, the variable effect of degree of substitution of charged groups on a glycopolymer chain. For example, the extent or 'degree' of substitution (DS) by CH_3COO^- groups had little effect on carboxymethylcellulose ($B \sim 0.045$–0.065) for DS values 0.5→1.0, whereas for pectin the chain became considerably stiffer as DS changed from 0.58→0.89 with B decreasing from 0.052→0.005 (Smidsrød and Haug, 1971). By far however the most popular parameter representing chain flexibility has been the persistence length, L_p, with the theoretical limits of 0 for a completely random chain and ∞ for a

Table 1.8 Gross conformation of food polysacharides from the Wales–Van Holde relation

Glycopolymer	R ($=k_s[\eta]$)	Conformation	Reference
Alginates	0.6	Extended	Ball (1989)
β-glucans	0.4	Extended	Woodward *et al.* (1983)
Chitosan	0.2	Rigid rod	Errington *et al.* (1993)
κ-carrageenan	0.9	Extended coil	Harding *et al.* (1997a)
Mannan (yeast)	1.3	Random coil	Pavlov *et al.* (1994)
Pullulans	1.4	Random coil	Kawahara *et al.* (1994)
Xanthan	0.3	Rigid rod	Dhami *et al.* (1995)

Table 1.9 Persistence lengths L_p for food polysaccharides

Glycopolymer	L_p (nm)	Reference
Pullulan	1.2–1.9	Muroga *et al.* (1987)
Amylose	2.8	Ring *et al.* (1985)
Cellulose*	7.0	Whittington and Glover (1972)
Pectin (DE = 0.69)	30	Plaschina *et al.* (1985)
Pectin (DE = 0)	34	Plaschina *et al.* (1985)
Xanthan (M = 1.8 × 10^6Da, I = 0.1 M)	40	Muller *et al.* (1986)
Xanthan (M = 1.8 × 10^6Da, I = 10^{-5} M)	210	Muller *et al.* (1986)
Schizophyllan	115–200	Plaschina *et al.* (1985); Yanaki *et al.* (1981); Richardson and Ross-Murphy (1987); Carriere *et al.* (1985); Norisuye *et al.* (1980); Yanaki *et al.* (1980);
Scleroglucan	180 ± 30	Biver *et al.* (1986)

* In cadoxen; DE: degree of esterification (of COO^- groups); I: ionic strength.

completely rigid rod (practically the range goes from ~1→200 nm). Table 1.9 gives the L_p for a collection of glycopolymers ranging from the randomly coiled pullulan (L_p ~ 1.2–1.9 nm) to the extra-rigid triple-helical schizophyllan (L_p ~ 185–200 nm). In an extensive study on the latter, Yanaki *et al.* (1980) showed that the polysaccharides schizophyllan and scleroglucan have essentially the same triple-helical structure in solution. In an extensive study using intrinsic viscosity with electron microscopy data Stokke *et al.* (1998) showed that the L_p for xanthan was only consistent with a double-helical structure (see also Stokke and Elgsaeter, 1994).

Finally, using the simplified 'three-parameter' representation for worm-like cylinders, Bohdanecky (1983) has applied equations 48–50 and the plot of $(M/[\eta])^{1/3}$ vs $M^{1/2}$ to data for the rod-shaped molecule schizophyllan (Yanaki *et al.*, 1980).

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