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THE INTRINSIC VISCOSITY OF BIOLOGICAL MACROMOLECULES. PROGRESS IN MEASUREMENT, INTERPRETATION AND APPLICATION TO STRUCTURE IN DILUTE SOLUTION

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I. INTRODUCTION

Over the last decade there has been a mini-explosion of interest in hydrodynamics as a tool for probing biomolecular structure and interactions in solution. This has been most notably so with the technique of analytical ultracentrifugation with the appearance of

new instrumentation (Schachman, 1989; Giebeler, 1992; Furst, 1997), scientific papers and a plethora of reviews and books (Harding *et al.*, 1992; Schuster and Laue, 1994). Another area of hydrodynamics which has seen a considerable expansion in analytical applications, is size-exclusion chromatography and its coupling to absolute molecular mass and conformation probes such as laser light scattering (Wyatt, 1992). The hydrodynamic "affinity" technique of surface plasmon resonance has also been developed as a powerful analytical probe for molecular interactions, and there have also been significant advances in both the measurement of translational diffusion by dynamic light scattering (Brown, 1993), and rotational diffusion and relaxation analysis by electro-optic (Porschke and Obst, 1991; Meyer-Almes *et al.*, 1994) and fluorescence anisotropy (Acuna *et al.*, 1987a,b).

Another classical hydrodynamic probe - viscometry - has also been the subject of significant advances, both at the "rheological" or concentrated solution end and at the dilute solution end. With rheology, these advances include an understanding of the structure of gels and colloidal suspensions, particularly with regard to polysaccharides (Lapasin and Pricl, 1995) and large glycoconjugates (Hardingham *et al.*, 1989). Whereas the rheology of concentrated dispersions is of considerable interest to the colloid scientist, pharmacist or food technologist, of more interest to the molecular biophysicist is *dilute solution viscometry* and the structural parameter *the intrinsic viscosity* $[\eta]$.

The intrinsic viscosity is not a new molecular parameter. Einstein considered it for a suspension of spherical particles in 1906 (Einstein, 1906); with a correction five years later, Einstein, 1911). The classical review of its measurement and application, particularly to proteins, appeared almost forty 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), which focussed on linear polymers.

The intrinsic viscosity is also not a true viscosity at all: the dimensions of viscosity are conventionally the "Poise" in c.g.s units ($dyn.cm^{-2}.s$) or the "Pascal second" ($N.m^{-2}.s$) in S.I. units, whereas intrinsic viscosity has reciprocal concentration units: although in the past, units of $dl.g^{-1}$ have been highly popular, the c.g.s. unit of $ml.g^{-1}$ is now preferred, at least in physical biochemistry.

This article considers the considerable progress that has been made in the measurement of $[\eta]$ and its interpretation in terms of molecular conformation, hydration and flexibility of proteins and glycopolymers (including glycoproteins, polysaccharides and nucleic acids) in solution. The traditional measurement of viscosity of a macromolecular solution was by either timing the flow of liquid through a capillary tube, or by recording the force required to rotate one concentric plate with respect to another with the fluid in between. Although instrumentation for both these "capillary and "cone and plate" approaches has become either automatic or semi-automatic with on- or off-line computer data capture and analysis, the basic design has remained the same since the time of Yang's (Yang, 1961) article. Neither are outstandingly attractive however, in protein biochemistry, because of the relatively large quantities of material required: normally greater than 1 ml at concentrations of several mg/ml. Although such quantities are still less than may be required by nuclear magnetic resonance spectroscopy (NMR), they may still be prohibitive, particularly for newly engineered proteins or precious glycoconjugate molecules. As with NMR, measurement at concentrations in excess of a few mgs/ml poses the additional potential problem of molecular aggregation phenomena if structural information about the molecule is being sought. In this respect, parallel measurements with the analytical ultracentrifuge are mandatory to monitor for such complications.

A more radical development has been the appearance of an instrument based on quite different principles (the direct dependence of viscosity on hydrostatic pressure) and is the so-called pressure imbalance or "differential" viscometer. The significance of this is that measurements can be performed at lower concentration and on smaller volumes, making the intrinsic viscosity attractive to the protein biochemist once more. This is for-

tunate because advantage can be taken of developments in hydrodynamic representations of molecular conformation in dilute solution. Although intrinsic viscosity measurement—in common with any other hydrodynamic measurement—cannot give high resolution information about molecular structure in the same way as high-resolution NMR or X-ray crystallography, $[\eta]$ is one of a number of parameters from hydrodynamics and solution light and X-ray scattering for pinpointing the overall configuration of molecules in dilute solution. Also since Yang, developments have progressed beyond the "ellipsoid of revolution" approach to allow both three-dimensional representations via the *general ellipsoid approach* and modelling of complex shapes via the *hydrodynamic bead approximation*. The old problem of molecular hydration still remains and has to be addressed: a hydrodynamic property is not only a measure of the shape of a macromolecule but also the amount of solvent it immobilises, through hydrogen bonding and through physical entrainment. Since Yang, there have also been important developments in the representation of the conformation and flexibility (in terms of parameters such as the Kratky-Porod persistence length, L_p, the Kuhn statistical length, λ^{-1} or the Smidsrød-Haug stiffness parameter B) of linear polymers that are a feature of nucleic acids, filamentous viruses and many glycopolymers, and also branched biopolymers (in terms of the branching parameter g') that are the hallmark of polysaccharides like amylopectin. This article will thus serve the purpose of addressing the progress that has been made in (i) instrumentation; (ii) molecular modelling of quasi-rigid particles such as globular proteins; (iii) the hydration problem, and (iv) molecular modelling of the conformation and flexibility of linear biopolymers such as nucleic acids and many glycopolymers and also branched glycopolymers.

II. THE INTRINSIC VISCOSITY

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 (Fig. 1):

$$\eta = \tau/g \tag{2.1}$$

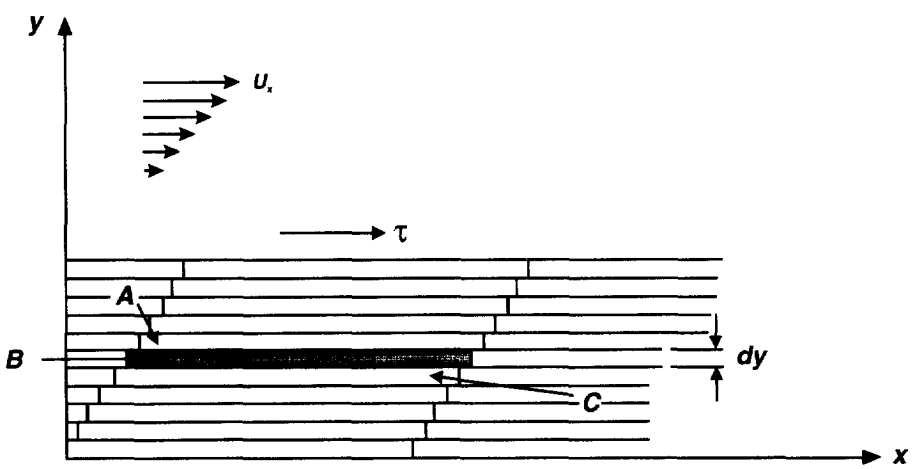

Fig. 1. Shearing of a Newtonian fluid (laminar flow with constant velocity gradient, g). $g = u_x/y$ where u_x is the local mass average velocity of the fluid in the x-direction and $u_y = u_z = 0$. dy is the infinitesimally small thickness of adjacent fluid elements such as A, B and C. Adapted from Tsvetkov *et al.* (1971). See also Van Holde (1985)

An alternative definition of viscosity is in terms of energy dissipation (see e.g., Tsvetkov *et al.*, 1971). Consider the simple case of Fig. 1 and the three adjacent layers A, B and C of infinitesimal thickness dy. The infinitesimal shift, dx in the x-direction of layer C relative to layer A in time t is $dx = t(du_x/dy).dy = tg.dy$. Thus in the centre layer B of thickness dy the work done per unit area in overcoming the resistance to flow through internal friction is $\tau.dx = \tau gt.dy$ for a volume of liquid $dy \times 1$. Substituting for τ from eqn (2.1), the work done, E, in unit time per unit volume due to the directional flow is then

$$E = \eta g^2 \tag{2.2}$$

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 capilliary 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:

$$\rho \frac{\partial \boldsymbol{u}}{\partial t} + \boldsymbol{u}.\nabla \boldsymbol{u} = -\nabla p + \eta \nabla^2 \mathbf{u} + \rho \boldsymbol{F} \tag{2.3}$$

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 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 (2.3) (or its equivalent form in energy dissipation terms), in the appropriate coordinate 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}, where

$$\eta_{rel} = \eta/\eta_o \tag{2.4}$$

$$\eta_{sp} = \eta_{rel} - 1 \tag{2.5}$$

and

$$\eta_{red} = \eta_{sp}/c = (\eta_{rel} - 1)/c \tag{2.6}$$

η is the viscosity of the solution (or dispersion), η_o is the viscosity of the solvent and c is the weight (mass) concentration. The c.g.s. 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

$$\eta_{inh} = (\ln \eta_{rel})/c \tag{2.7}$$

Because of the effects of non-ideality and/or associative phenomena, both η_{red} and η_{inh} will be concentration dependent. The limit as $c \to 0$ 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:

$$[\eta] = \lim_{c \to 0}(\eta_{red}) = \lim_{c \to 0}(\eta_{sp}/c) \tag{2.8}$$

$$[\eta] = \lim_{c \to 0}\eta_{inh} = \lim_{c \to 0}\{(\ln \eta_{rel})/c)\} \tag{2.9}$$

2.2. *Form of the Concentration Extrapolation*

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

$$\eta_{red} = [\eta](1 + K_H[\eta].c) \tag{2.10}$$

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

$$\eta_{red} = [\eta](1 + k_\eta.c) \tag{2.11}$$

(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). K_H, k_η are both generally positive: i.e. a plot of η_{red} versus c usually has a positive slope (Fig. 2).

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

$$\eta_{red} = [\eta](1 + K_{SB}.\eta_{sp}) \tag{2.12}$$

The equivalent concentration dependence relation to eqn (2.7) for the inherent viscosity, $(\ln \eta_{rel})/c$, is the Kraemer (1938) equation

$$(\ln\eta_{rel})/c = [\eta](1 - K_K[\eta].c) \tag{2.13}$$

with a negative slope (Fig. 2) and where K_K is the Kraemer constant.

These equations were put forward over 50 years ago: subsequent attempts have been made to modify and refine them. For example a power-law form of eqn (2.13) has been proposed (Baranov *et al.*, 1987; Krasovskii *et al.*, 1993):

$$(\ln\eta_{rel})/c = ([\eta]c)^\alpha \tag{2.14}$$

and Chee (1985) has suggested other numerical procedures. Other attempts at developing the Huggins and Kraemer relations have centred around estimating $[\eta]$ from measure-

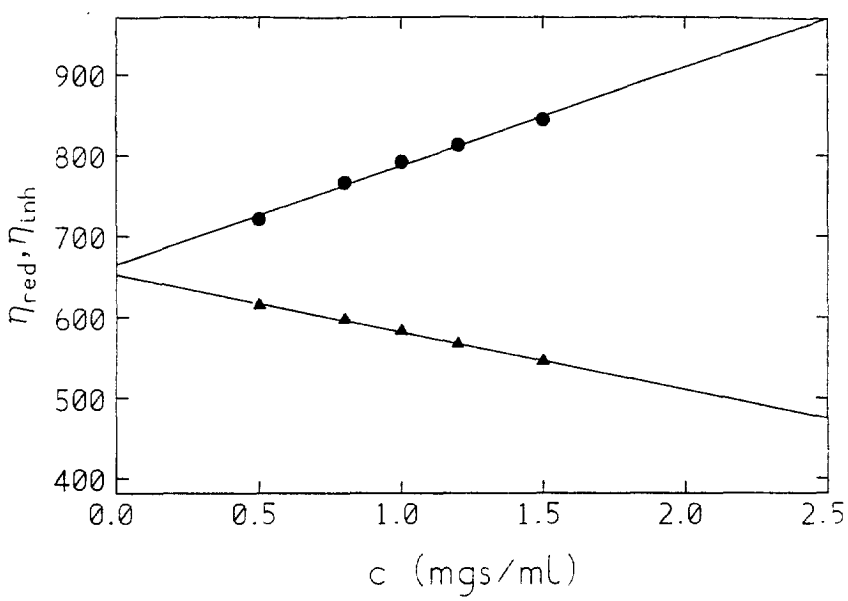

Fig. 2. Huggins and Kraemer extraction methods for intrinsic viscosity. Reduced viscosity η_{red} (ml/g) versus concentration (●) and inherent viscosity η_{inh} $\{=\ln(\eta_{rel})/c\}$ (ml/g) versus concentration (▲) for irradiated (10 kGy) guar in phosphate chloride buffer (pH = 6.8, I = 0.10). The "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)

ment of η_{rel} at a single concentration (Solomon and Ciuta, 1962; Solomon and Gotesmann, 1969; Deb and Chatterjee, 1969; Elliott *et al.*, 1970; Rudin and Wagner, 1975; Ram Mohan Rao and Yaseen, 1986). For example Solomon and Ciuta (1962) proposed a combination of eqn (2.10) and eqn (2.13) to yield the approximate relation

$$[\eta] \simeq (1/c).[2\eta_{sp} - 2\ln(\eta_{rel})]^{1/2} \tag{2.15}$$

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 (see Section 3 below). Deb and Chatterjee (1968) suggested the following alternative relation:

$$[\eta] \simeq (1/c).\left[3\ln(\eta_{rel}) + (3/2)(\eta_{sp}^2) - 3\eta_{sp}\right]^{1/3} \tag{2.16}$$

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

$$[\eta] \simeq (1/2c).[\eta_{sp} - \ln(\eta_{rel})] \tag{2.17}$$

Other workers have attempted to alternatively improve the form of the extrapolation of equations (eqn (2.8)) and (eqn (2.9)). 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: extrapolation methods using η_{sp} as opposed to η_{sp}/c instead would therefore appear to be advantageous. For example, application of l'Hopital's rule to eq. (eqn (2.8)) 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):

$$[\eta] = (d\eta_{sp}/dc)_{c=0} \tag{2.18}$$

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—required by the Huggins, Kraemer and related procedures (eqns (2.10), (2.11), (2.12), (2.13) and (2.14)) is therefore not required. These workers have also demonstrated that non-linear least squares fitting the specific viscosity data versus concentration c to either the polynomial

$$\eta_{sp} = [\eta]c + a_2c^2 + a_3c^3 \tag{2.19}$$

or the relation

$$\eta_{sp} = [\eta]c + b.c^d \tag{2.20}$$

with $[\eta]$, a_2,a_3 or $[\eta]$, b, d the variables gives significantly improved estimates for $[\eta]$, with eq. (2.20) the best (Fig. 3).

III. MEASUREMENT OF INTRINSIC VISCOSITY

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 eqns. (2.15), (2.16) and (2.17) can then be used to extract $[\eta]$ as discussed in Section 2.2.

η_{rel} can be measured in one of three principle ways: 1. using a capillary viscometer; 2. using a plate viscometer (cone and plate or parallel plate or cub and bob); 3. using 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.

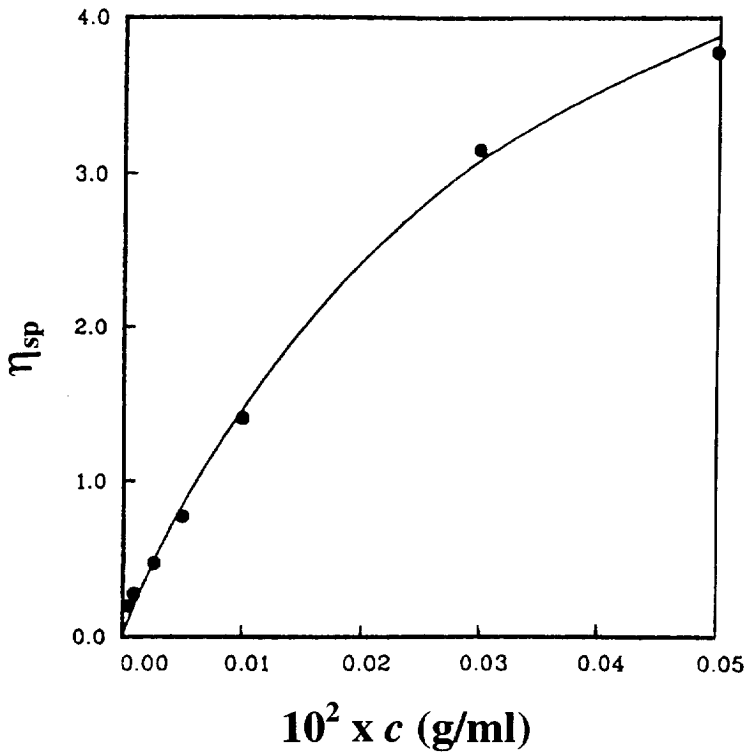

Fig. 3. Specific viscosity η_{sp} versus macromolecular concentration, for aqueous polyacrylamide at 25.0°C. The non-linear least squares line fitted is to $\eta_{sp} = [\eta]c + b.c^d$ where $[\eta]$, b and d are variables. Reproduced, with permission, from Kozicki and Kuang (1996)

3.1. *Capillary Viscometry*

The capillary or "*Ostwald*" viscometer (Ostwald and Malss, 1933) is still the most common viscometer (Fig. 4a) and involves essentially just a piece of glassware—albeit beautifully constructed (see Fig. 5)—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 called an *Ubbelohde* viscometer (Ubbelohde, 1936) can be used which is so 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 (see, e.g. Tanford, 1961) the relative viscosity can, under the appropriate experimental conditions, be given simply by

$$\eta_{rel} = (t\rho/t_o\rho_o) \tag{3.1}$$

where t and t_o are the flow times for the biomolecular solution at a particular concentration c and ρ and ρ_o the corresponding solution and solvent densities. The relative viscosity without the density correction is known as the "kinematic" (as opposed to "dynamic") relative viscosity $\eta'_{rel} = t/t_o$; 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 concentrations are possible with many solutions of polysaccharides and mucus

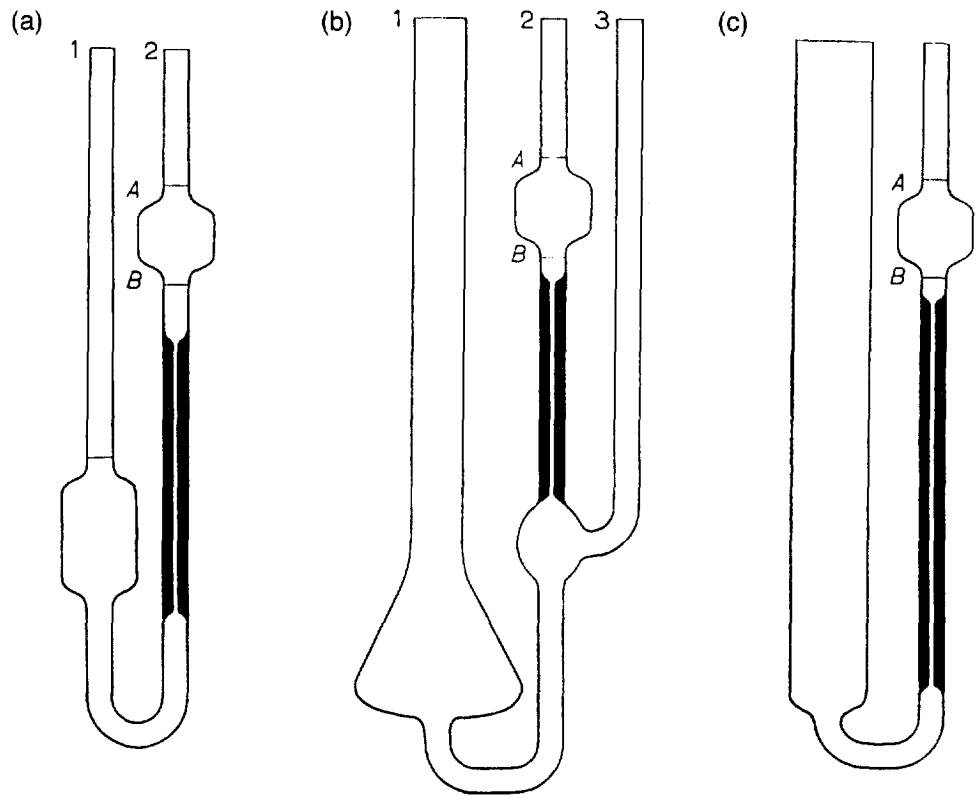

Fig. 4. Schematic Ostwald (a), Ubbelohde (b) and Fox-Flory (c) viscometers (from Kragh, 1961)

glycoproteins 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 (~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:

$$[\eta] = [\eta]' + [(1 - \bar{\nu}\rho_o)/\rho_o] \tag{3.2}$$

or

$$\eta_{red} = \eta_{red}' + [(1 - \bar{\nu}\rho_o)/\rho_o] \tag{3.3}$$

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:

$$\bar{\nu} = (1/\rho_o).(1 - \partial\rho/\partial c) \tag{3.4}$$

ρ_o and ρ can be measured using a mechanical oscillator device as described by Kratky *et al.* (1973). There are two ways in which the precision of η_{rel} can be increased, particularly for measurements at low concentration, both based on increasing the flow time (and hence flow time difference $t - t_o$) for solvent and solution. The first is the method of Szuchet-Derechin and Johnson (1966), which is to add a low concentration of glycerol (~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* (Fig. 5) (Holt and Creeth, 1972), which

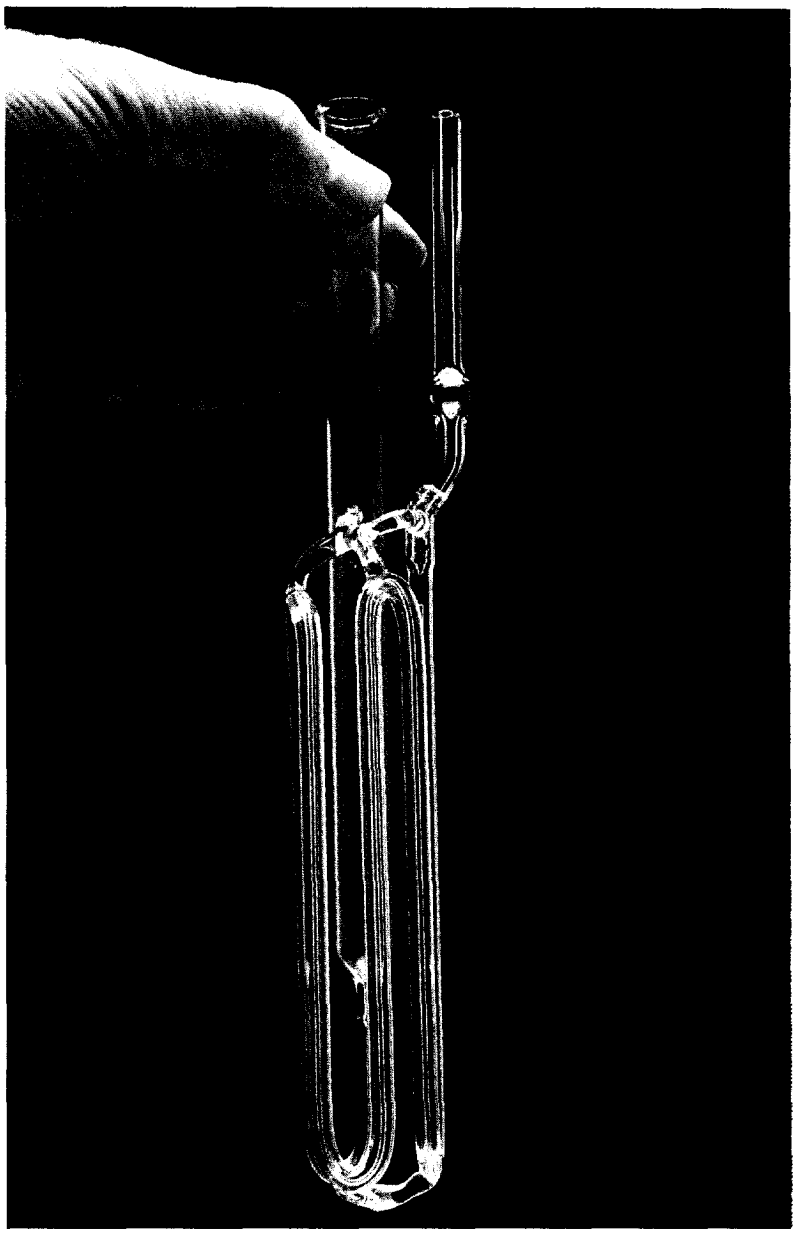

Fig. 5. Extended Ostwald viscometer

increase the flow time difference $(t - t_o)$ 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 (Kragh, 1961) classical article, are still relevant however and should be consulted by any potential user.

3.2. *Plate Viscometers*

With these types 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_o the relative viscosity $\eta_{rel} = \eta/\eta_o$ can be readily found. There are three principal types (Lapasin and Pricl, 1995): *cone and plate, parallel plate* and *cup and bob*. Like capilliary 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 viscosity, 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 capilliary viscometry. The principle 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 in practice, 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.

3.3. *Pressure Imbalance Differential Viscometer*

This uses a fluid analogue of a Wheatstone bridge electrical circuit (Haney, 1985a,b). 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 ($\sim$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 the sample enters the bridge (Fig. 6) 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 related to relative viscosity or the specific viscosity of the solution (Haney, 1985b):

$$\eta_{sp} = 4\Delta P/(P_i - 2\Delta P) \tag{3.5}$$

From knowledge of the concentration the reduced specific viscosity can be obtained. A commercially available instrument is available 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 uv absorbance based—see Section 3.5) 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 2, or, since concentrations can be very small ($\sim$1 mg/ml for globular proteins) the single point $[\eta]$ evaluation formulae can be applied, such as the Solomon-Ciuta (Solomon and Ciuta, 1962; Solomon and Gotesman, 1967) formula eqn (2.15). 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 (see Section 5 below). A popular set-up would thus have this on-line facility plus a separate injection port if monodisperse solutions were being characterised not requiring column separation. A further development (not for SEC) is the so-called Dual Capillary Viscometer (Vicotek Ltd., Houston USA)

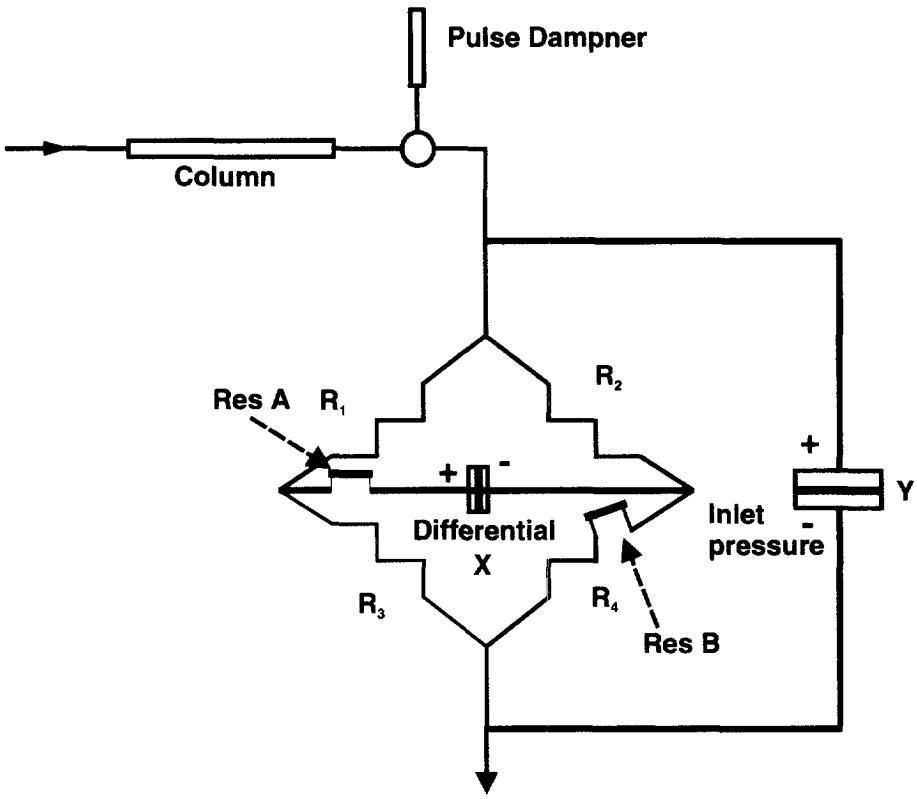

Fig. 6. Principles of the pressure imbalance differential viscometer. Uses a fluid analogue of a Wheatstone bridge electrical circuit to measure differential viscosity directly from the chromatography effluent. For a detailed description see Haney (1985b). At "baseline conditions" (solvent only) the differential pressure across the bridge will be zero because there is solvent in all four capillaries, R_1–R_4. When the biomolecular solution enters the bridge from the column (delay for non-separation, or size-exclusion for separation) it fills capillaries R_1, R_2 and R_3 while solvent from a delay reservoir (Res B) remains in capillary R_4 and prevents the entry of the biomolecular solution. The transducer X measures the differential pressure ΔP and Y measures the inlet pressure P_i. The reservoir Res A located out of the flowstream acts to compensate volume so that any flow rate fluctuations cause equal pressure changes on each side of the differential pressure transducer Y. The difference in viscosity causes a pressure imbalance in the bridge which is proportional to the specific viscosity of the sample solution. From knowledge of the concentration (measured using an off- or on-line refractive index detector), the reduced specific viscosity can be obtained. Alternatively $[\eta]$ can be obtained using the "single point" approximate formulae of eqns (2.15), (2.16), (2.17) and (2.18)). Adapted from Haney (1985b) and Dutta *et al.* (1991)

which operates with just two capillaries (one solvent, one solution) with the same rate of flow.

3.4. *Microviscometry Using EPR and NMR*

Mention should be made of the use of correlation times, τ_c, from electron paramagnetic resonance or nuclear magnetic resonance using a suitable label or probe to measure the "microviscosity" in the vicinity of the probe (Tanford, 1980; Gennaro *et al.*, 1996). This is particularly useful for probing the local viscosity in cytoplasmic or other physiological media and can be used to probe solute concentration, or if this is known, a *local* intrinsic viscosity can be estimated which can be compared with the "macroscopic" intrinsic viscosity $[\eta]$ found by the conventional methods described above. A calibration curve is necessary of τ_c versus viscosity, using sucrose solutions of known concentration. It has been popularly used to study the dependence of cytoplasmic microviscosity on hemoglobin concentration (Morse *et al.*, 1979; Daveloose *et al.*, 1983; Herrmann and Muller, 1986; Endre and Kuchel, 1986; Gennaro *et al.*, 1996).

3.5. *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. It is particularly important for the measurement of intrinsic viscosity not only because of the extrapolation to zero concentration (Section 2.2), but because the concentration is also required for the evaluation of the reduced specific viscosity or inherent viscosity (cf. Equation (2.8), eqn (2.9)). For proteins, the most popular concentration measurement method is by uv 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, 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, $\delta\rho/\delta c$ (or the partial specific volume, $\bar{v}$, see eqn (3.4)) 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 proteins or the phenol-sulphuric acid method for polysaccharides (Ball, 1989). With both refractive index and density methods it is important that 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 polarised light is also a function of concentration and this can be used (see Van Holde, 1985).

A most important point derived from all this is that concentration cannot be measured to an accuracy much greater than $\sim \pm 1\%$: the $[\eta]$ can thus also be measured to no better than $\sim \pm 1\%$, no matter how accurate the 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.

IV. CONFORMATION MODELLING OF $[\eta]$ IN DILUTE SOLUTION

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

$$[\eta] = \nu.v_s \tag{4.1}$$

where ν is a molecular shape parameter known as the viscosity increment (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 $v_s(= V.M/N_A$ 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 which 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

$$v_s = \bar{v} + \delta/\rho_o \tag{4.2}$$

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 (the symbol w rather than δ is often used to

avoid clash in notation with a shape parameter used by Scheraga (1961) based on a combination of $[\eta]$ or ν with the rotatory diffusion coefficient). For its experimental measurement it does however require measurement of v_s (or $\bar{v}$, δ and ρ_o) as well as of course $[\eta]$.

4.2. *The "Hydration", δ*

Opinions vary as to what this parameter actually means—if it is a parameter at all—but it is used to 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 by Kuntz and Kauzmann (1974). Another interesting method was subsequently presented by Rowe (1977), nvolving use of the ratio of the viscosity concentration dependence regression coefficient k_η (eqn (2.11)) with the corresponding parameter k_s from sedimentation velocity in the analytical ultracentrifuge. Rowe (1977) equated the ratio of k_η/k_s to $v_s/\bar{v}$ and this method has been used for example to assess the δ for 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 eqns. (eqn (4.1)) and (eqn (4.2)) and also via analagous 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 to ~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; Harding *et al.*, 1997a).

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 affects can be particularly serious if the macromolecule is a multiply charged "polyelectrolyte". Proteins, DNA, mucin glycoproteins 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 neglible (< 0.01 M): this is the exception rather than the rule for biomolecular systems. Physiological media have ionic strengths > 0.1, largely through the presence of low molecular weight electrolyte, and most physical measurements are accomplished 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 if these interparticle repulsions affects the shape itself of the protein—the "tertiary effect". The latter effect is very small for globular proteins (see 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 a linear molecule (DNA, polysaccharides), the effect can be more significant and is considered in more detail in Section 5.5.

4.4. *Spheres and Ellipsoids of Revolution: the Einstein, Simha and Saito Equations*

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, 1965). It was the pioneering work of Einstein (1906, 1911) {English translation: Einstein (1956)} 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, corrected in the later 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 (Fig. 7), 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 is 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. Jeffery (1922) neglected the effects of Brownian motion ($P_e \sim \infty$) and produced a complicated open solution which predicted a range of possible values of ν for each value of a/b, where a,b are the ellipsoid semi-axes and $a \geq b$. However, for macromolecules Brownian motion is the dominant factor, i.e. $P_e \rightarrow 0$, and Simha (1940) gave the first correct formula:

$$\nu = \frac{1}{a_1 a_2^2}\left\{\frac{2\alpha_0''}{15a_2^2\alpha_0'\beta_0'} + \frac{7}{15a_2^2\alpha_0'} + \frac{2}{5}\left[\frac{\beta_0'(a_1^2+a_2^2)+2\beta_0''}{\beta_0'[2a_1^2a_2^2\beta_0^2+(a_1^2+a_2^2)\beta_0]''}\right]\right\} \tag{4.3}$$

where the α_0' etc are the same elliptic integrals as defined by Jeffery (1922) {see Harding and Cölfen (1995) for these in a form appropriate to the notation of eq. (eqn (4.3))}. 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 eq. (eqn (4.3)) are soluble numerically (and now easily using numerical packages such as the NAG (1986) routine D01GAF), and Fig. 8 shows a plot of ν versus a/b for both prolate and oblate ellipsoids. Simpler approximations are available (Simha, 1940) for the limits of large axial ratios ($a >> b$) where prolate ellipsoids can be approximated by rods and oblate ellipsoids by discs. If p is the axial ratio $(a/b) > 1$, for prolate ellipsoids, this approximation is (for $p > \sim 15$):

$$\nu = p^2/15\{\ln(2p) - 1.5\} + p^2/\{5(\ln(2p) - 0.5)\} + 14/15 \tag{4.4a}$$

and for oblate ellipsoids, again with $p > \sim 15$

$$\nu = 16/\{15p\arctan(1/p)\} \tag{4.4b}$$

For a homologous series of rod-like prolate ellipsoids having the same minor axis (b), equation (eqn (4.4a)) can be further approximated by (Simha, 1945):

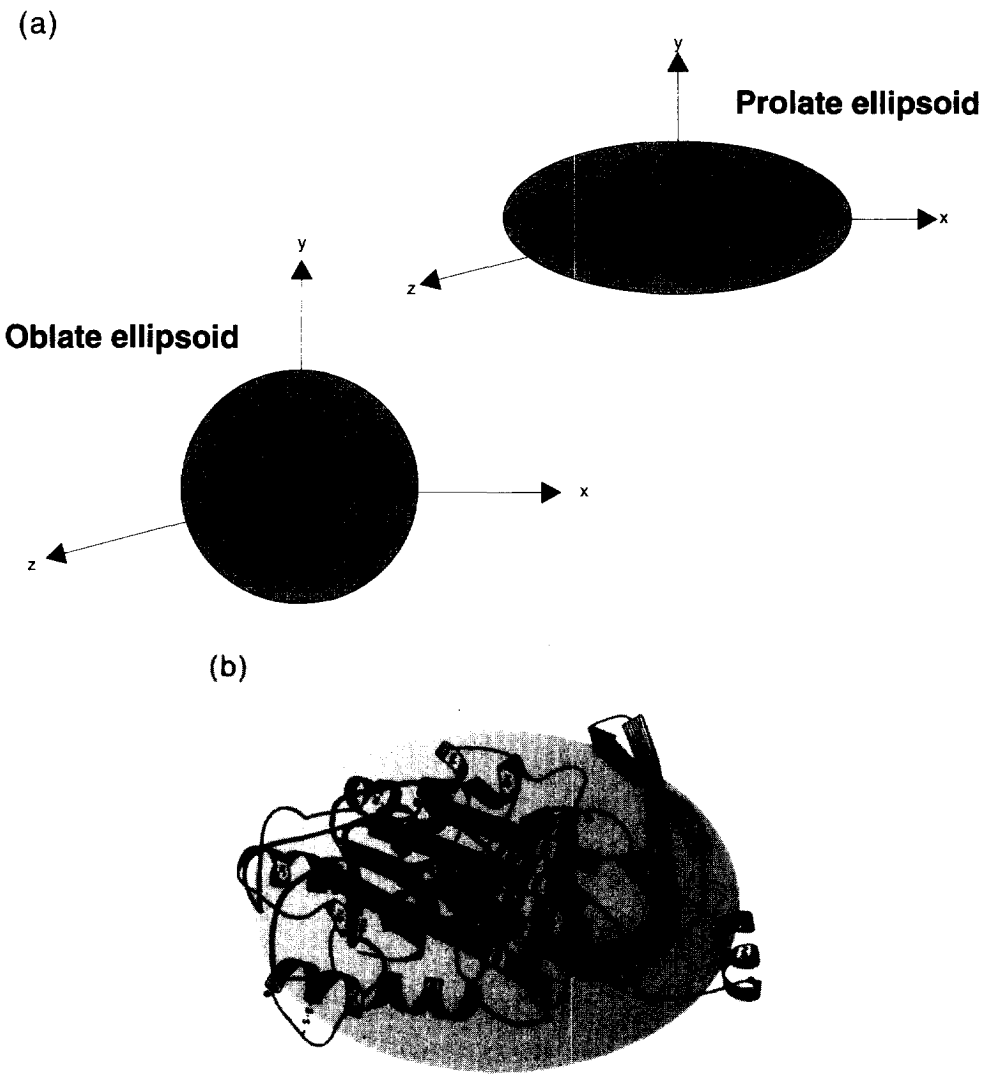

Fig. 7. (a) Prolate and oblate ellipsoids of revolution. The axial ratio in both cases = a/b, where $a \geq b$. (b) Prolate ellipsoid approximation with $(a/b) = 1.5$, to the crystal structure for ovalbumin. Crystal structure: Stein *et al.* (1991). Hydrodynamic shape: Harding (1981b)

$$\nu = 0.233^{1.698} \, (20 \leq p \leq 100) \tag{4.5a}$$

$$\nu = 0.207^{1.732} \, (100 \leq p \leq 300) \tag{4.5b}$$

formulae which are useful for the representation of linear molecules as considered further in Section 5 below. Simha's approximate formulae (eqn (4.4a)) and (eqn (4.4b) are very similar to those developed by Kuhn and Kuhn (1945). However, there is absolutely no need for these approximations now the full equation (eqn (4.3)), along with other useful hydrodynamic shape functions, are available using an easy to use PC routine (ELLIPS2 (Harding *et al.*, 1997b)) which not only covers the case of ellipsoids of revolution, but general tri-axial ellipsoids with two axial ratios (a/b, b/c)—as described below. There is thus also no need now to follow the customary practice of quoting extensive tables of data.

It is impossible however to *invert* equation (eqn (4.3)) 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%:

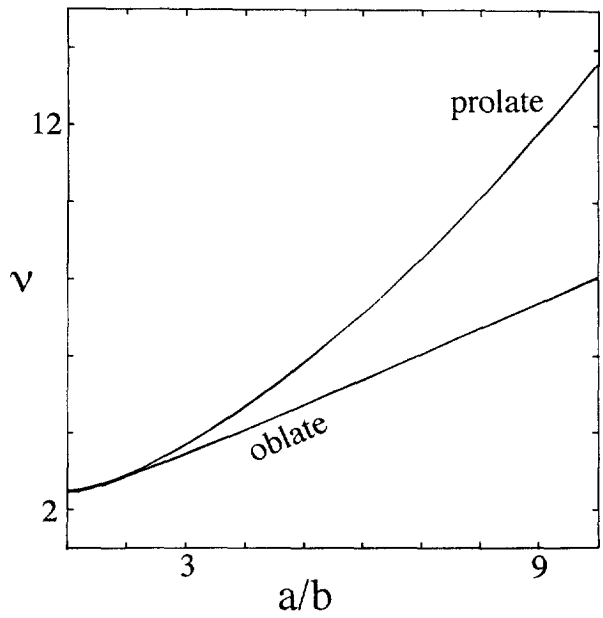

Fig. 8. ν evaluated from the Simha formula (eq. 4.3) plotted against axial ratio (a/b) for prolate and oblate ellipsoids

$$
\begin{aligned}
(a/b) &= -10.71584 + 2.79158\nu + 1.622009\nu^2 + 0.01556169\nu^3 - 0.192997\nu^4 \\
&\quad +0.02060718\nu^5 (\textit{prolate}\ \ 1.1 \le a/b < 2) \\
&= -3.80413 + 2.8712\nu - 0.3908319\nu^2 + 0.03612282\nu^3 - 0.001733981\nu^4 \\
&\quad + 0.0000332711\nu^5 (\textit{prolate}\ \ 2 \le a/b < 10) \\
&= 4.241113 + 0.464459\nu - 0.001981036\nu^2 + 6.111643.10^{-6}\nu^3 - 9.374974.10^{-9}\nu^4 \\
&\quad + 5.478654.10^{-12}.\nu^5 (\textit{prolate}\ \ 10 \le a/b < 100) \\
&= -25.23436 + 10.43327\nu + 2.122294\nu^2 - 0.4294092\nu^3 + 0.05816609\nu^4 \\
&\quad - 0.1960477\nu^5 + 0.04331335\nu^6 (\textit{oblate}\ \ 1.1 \le a/b < 2) \\
&= -5.439531 + 3.883619\nu - 0.6477747\nu^2 + 0.08353639\nu^3 - 0.004945992\nu^4 \\
&\quad + 9.922261.10^{-5}\nu^5 (\textit{oblate}\ \ 2 \le a/b < 10) \\
&= 0.6888919 + 0.9078403\nu + 0.04002417\nu^2 - 1.154619.10^{-3}\nu^3 \\
&\quad + 1.457981.10^{-5}\nu^4 - 6.725495.10^{-8}.\nu^5 (\textit{oblate}\ \ 10 \le a/b < 100)
\end{aligned}
\tag{4.6}
$$

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).

Although the Simha result (eqn (4.3)) is correct, the derivation as originally given by Simha (1940) is wrong, and in fact the correct formula is a result of fortituitous cancellation of errors. According to Simha, as a result of the Brownian motion the suspended ellipsoids would have random orientations and have zero angular velocity, ω: "$\omega = 0$ in Jeffery's formulae 22, 24 and 26". Saito (1951) was the first to question the Simha derivation, although surprisingly however, obtained the same final formula as Simha, and this fact led him to suggest that Simha had made "some errors in calcu-

Table 1. Axial dimensions of some globular proteins based on crystal structures. For some the approximation of two equal axes (axisymmetric ellipsoid) is reasonable, for others it is not

Protein	Dimensions (nm)	Reference
Lipase (*Humicola lanuginosa*)	4.3 × 4.3 × 4.0	Brady *et al.* (1990); Dodson (1995)
Ovalbumin	7.0 × 4.5 × 5.0	Stein *et al.* (1991)
β-Lactoglobulin	8.1 × 4.7 × 4.0	Brownlow and Sawyer (1995)
Myoglobin	4.3 × 3.5 × 2.3	Kendrew *et al.* (1958)
Hemoglobin	6.4 × 5.5 × 5.0	Perutz *et al.* (1960)

lation". Harding *et al.* (1982) later showed that Simha had arrived at the correct result from the wrong assumption by the fortuitous omission of a series of terms, later confirmed by Haber and Brenner (1984). Had Simha really been considering a model in which the ellipsoids had zero rotation as his words may have suggested, the formula should, without the fortuitous omissions, have reduced to $\nu = 4$ as shown by Brenner (1970), and not the correct Einstein value of (5/2).

4.5. *Triaxial Modelling: the Batchelor, Hocquart et al., Rallison, Harding et al., Haber and Brenner Relations*

Although some globular proteins have two axial dimensions approximately equal (Table 1), the ellipsoid of revolution is rather limited in its ability to represent the overall conformation of quasi-rigid macromolecules. The first published attempt to calculate the intrinsic viscosity of dilute suspensions of ellipsoids with three unequal axes, i.e. "general triaxial ellipsoids" (of semi-axes $a \geq b \geq c$, Fig. 9), in the case of dominant Brownian motion is due to Batchelor (1970). Unfortunately, although in his model the particles were randomly oriented, he considered following Jeffery (1922) only the hydrodynamic torque and neglected the Brownian couple. Although his formula yields the correct value for ν of 5/2 in the sphere limit, it leads to serious error at higher axial ratio, and fails to reproduce the correct Simha formula (eqn (4.3)) in the case of axisymmetric ellipsoids.

The first correct formula was given by Hocquart *et al.* (1974) who considered properly, both the hydrodynamic and Brownian torques. Their formula was confirmed by Haber and Brenner (1984) some 10 years later using a similar approach considering the dynamics of the particle movement and also the Einstein scalar energy dissipation method. This formula, in terms of the viscosity increment $\nu = [\eta]/v_s$ is given by

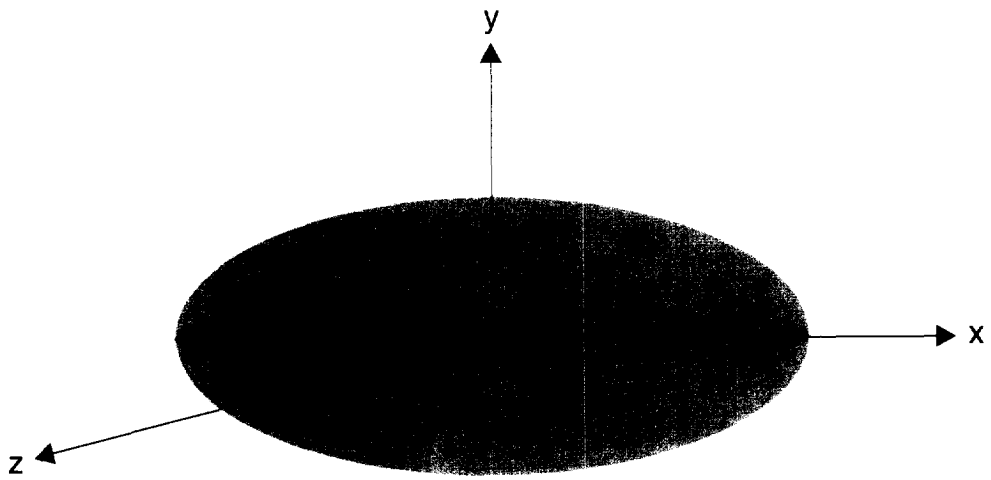

Fig. 9. The general triaxial ellipsoid. Semi-axes are $a \geq b \geq c$. Semi-axial ratios are (a/b, b/c). Limits are (i) sphere (a/b, b/c) = (1,1); (ii) Prolate ellipsoid ($b/c = 1$); (iii) Oblate ellipsoid ($a/b = 1$); Rod ($a/b >> 1$; $b/c = 1$); Disc ($a/b = 1$; $b/c >> 1$); Tape ($a/b >> 1$; $b/c >> 1$)

$$\nu = \frac{1}{abc}\left\{\frac{4(\alpha_7+\alpha_8+\alpha_9)}{15(\alpha_8\alpha_9+\alpha_9\alpha_7+\alpha_7\alpha_8)} + \frac{1}{5}\left[\frac{\alpha_2+\alpha_3}{\alpha_4(b^2\alpha_2+c^2\alpha_3)}+\frac{\alpha_3+\alpha_1}{\alpha^5(c^2\alpha_3+a^2\alpha_1)}+\frac{\alpha_1+\alpha_2}{\alpha_6(a^2\alpha_1+b^2\alpha_2)}\right]\right\}+\varepsilon \tag{4.7}$$

and where the small term ε is given by

$$\varepsilon = -\frac{1}{5abc}\left[\frac{\left(\frac{a^2-b^2}{a^2\alpha_1+b^2\alpha_2}+\frac{b^2-c^2}{b^2\alpha_2+c^2\alpha_3}+\frac{c^2-a^2}{c^2\alpha_3+a^2\alpha_1}\right)^2}{\left(\frac{a^2+b^2}{a^2\alpha_1+b^2\alpha_2}+\frac{b^2+c^2}{b^2\alpha_2+c^2\alpha_3}+\frac{c^2+a^2}{c^2\alpha_3+a^2\alpha_1}\right)}\right] \tag{4.7b}$$

the elliptic integrals α_1 etc are, as with the case for ellipsoids of revolution (eqn (4.3)) given by Jeffery (1922), and in a form appropriate to the notation of eqn (4.7) and eqn (4.7b) by Harding (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 convenient for this.

The term ε on the RHS of eqn (4.7) is identically $=0$ for spheres and ellipsoids of revolution. For other values of (a,b,c) it contributes only $\sim 1\%$ at most to the total value of ν (Harding *et al.*, 1981).

In between the derivations of Hocquart (1974) (missed by Haber and Brenner) and Haber and Brenner (1984) two other interesting approaches were published, throwing different insights into the relation between suspension viscosity and particle dynamics. The approach of Rallison (1978) was to use the Gibbs rotation dyadic (Gibbs and Wilson, 1960; Brenner and Condiff, 1972). This led to algebraic complexity involving inversion of a fourth rank Cartesian tensor and a closed analytical expression similar to eqn (4.7) was not possible, although numerical inversion procedures led to results in exact agreement with the results of eqn (4.7) (Haber and Brenner, 1984). Harding *et al.* (1981) explored the validity of the assumption of particles rotating on average with the same local angular velocity of the fluid, knowing this assumption to be exact only for axisymmetric ellipsoids. This assumption simplifies considerably the calculation of ν, and they found a formula for ν which at worst led to deviations of no more than 1% from Rallison's (Rallison, 1981) numerical procedure. In fact their formula is identical to eqn (4.7) without the small final extra term ε: the magnitude of ε is thus a measure of the deviation of ellipsoids from not rotating with the same angular velocity of the fluid. This term ($=0$ for axisymmetric ellipsoids) asymptotically $\rightarrow 0$ for tapes ($a>>b>>c$). The assumption was thus shown to be excellent for general ellipsoids although no comment can be made on its validity for general particle shapes (Haber and Brenner, 1984).

The PC FORTRAN routine ELLIPS2 (Harding *et al.*, 1997) has been set up to calculate ν using the full form of eqn (4.7) (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 $(a/b, b/c)$.

4.6. *Solving the Uniqueness and Hydration Problem for Ellipsoids*

With both axisymmetric (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 axial ratios $(a/b, b/c)$ for a given value of ν or $[\eta]$, as Fig. 10 illustrates. A further problem is that of the influence of associated solvent: to convert $[\eta]$ to the shape function ν using eqns. (eqn (4.1)) or (eqn (4.2)). the swollen specific volume, v_s or the "hydration" δ is required. Although for

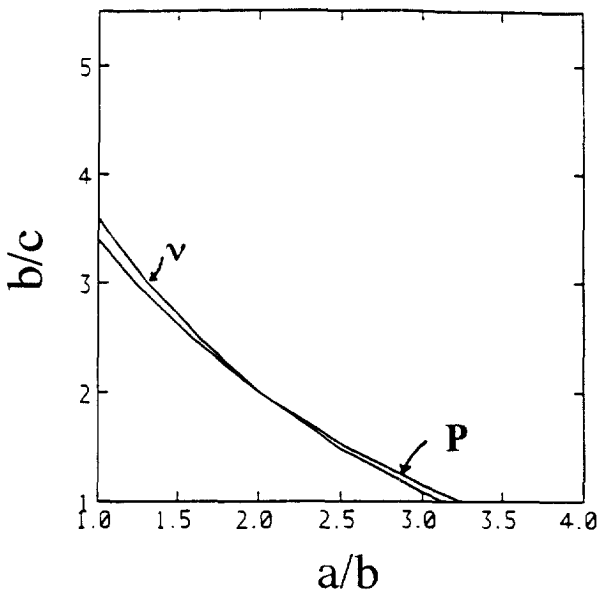

Fig. 10. Line solution of possible values of (a/b b/c) for a given value of ν (=3.803). The line solution for the Perrin translational frictional ratio function, P(=1.130) is also shown

globular proteins there appears to be support for a value for δ of between 0.3 and 0.4 (Section 4.2 above) for other macromolecules it is far less clear to define.

The uniqueness problem and the hydration problem 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:

$$\beta \equiv \frac{[\eta]^{1/3}\eta_0}{M^{2/3}(1-\bar{\nu}\rho_0)100^{1/3}} = \frac{N_A^{1/3}}{(16200\pi^2)^{1/3}}\frac{\nu^{1/3}}{P} \tag{4.8}$$

Unfortunately the β-function proved very insensitive to shape change (Fig. 11a). Fortunately further combinations of ν with other universal shape parameters have proved more successful. These include

(i) the Λ_h function (Harding, 1980a):

$$\Lambda_h = (\eta_0.[\eta].M)/(N_A.k_BT\tau_h) = \nu/(\tau_h/\tau_0) \tag{4.9}$$

(Fig. 11b) where τ_h is the harmonic mean rotational relaxation time (from steady state or time resolved fluorescence anisotropy decays measurements) and τ_h/τ_o is another universal shape parameter, the "harmonic mean rotational relaxation time ratio", with $\tau_o(=\eta_o M v_s/RT)$ the corresponding value for a spherical particle of the same *hydrated* volume and η_o, 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).

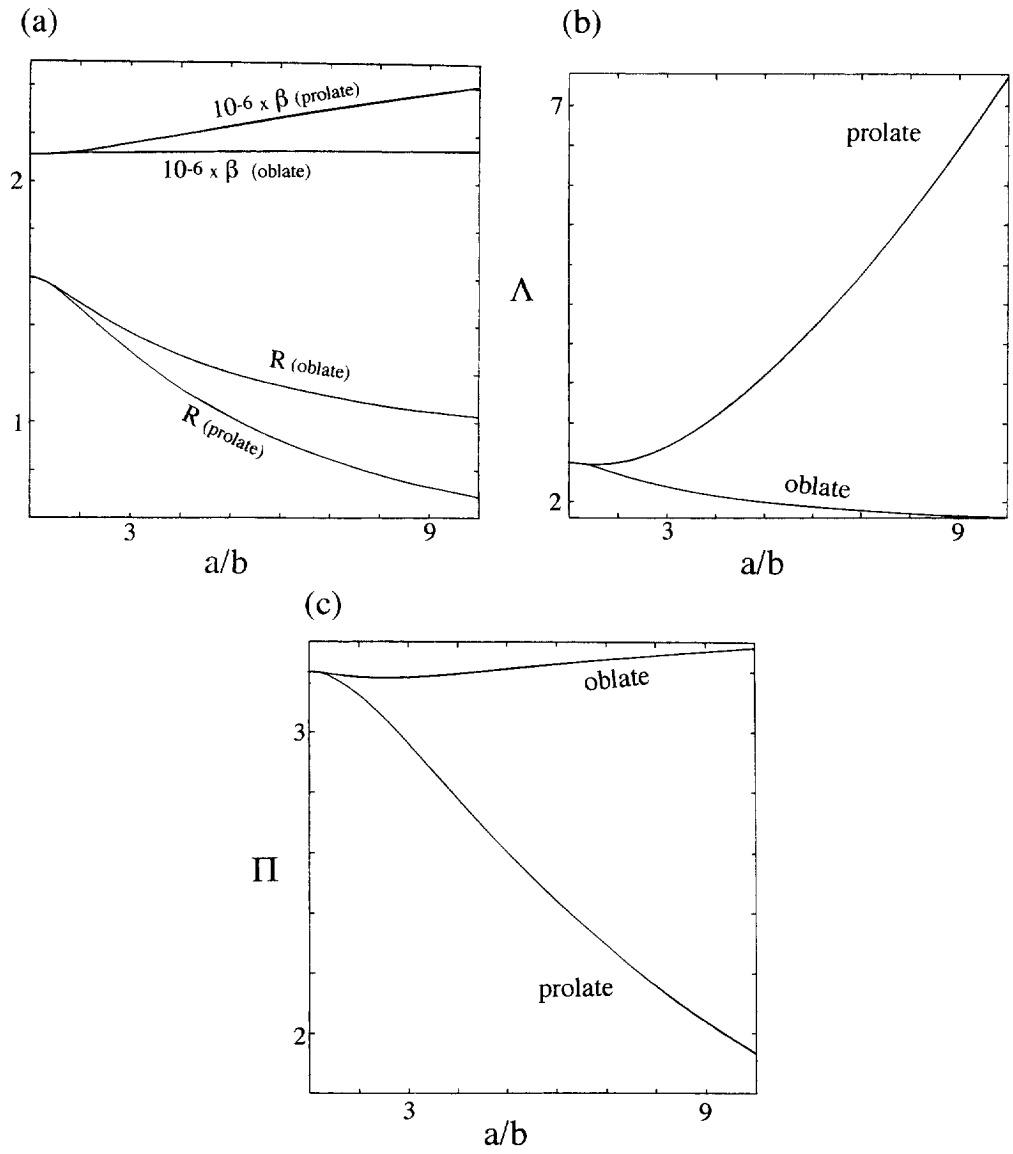

Fig. 11. Hydration independent universal shape functions involving $[\eta]$ for axisymmetric ellipsoids. (a) β and R. (b) Δ_h. (c) Π.

(ii) the Π function (Harding, 1981a):

$$\Pi = \{2BM/[\eta]\} - f(Z,I) = u_{red}/\nu \tag{4.10}$$

(Fig. 11c) 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 $2BM$. Of course for uncharged molecules and proteins at the isoelectric point, $Z = 0$, and $f(Z,I) = 0$

(iii) the Wales-Van Holde (Wales and Van Holde, 1954; Rowe, 1977) parameter

$$R = k_s/[\eta] = 2(1 + P^3)/\nu \tag{4.11}$$

(Fig. 11a) where k_s is the concentration dependence parameter of the sedimentation coefficient $s_{20,w}$ in the limiting relation $s_{20,w} = s^o_{20,w}(1 - k_s c)$ or alternatively $1/s_{20,w} = \{1/s^o_{20,w}\}(1 + k_s c)$. Although the theory behind eqn (4.11) 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 from Fig. 11 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 eqn (4.6), giving (a/b) for a specified value of β, R, Λ_h or Π are available in tabular form (Harding and Cölfen, 1995) and have been directly built in to 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. Figure 10 shows an attempt to combine, for example, ν with the Perrin frictional function, P: the intersection is too shallow (and after allowance for experimental error, the intersection disappears), and both require measurement of v_s or δ. The concept of this graphical combination of hydration independent universal shape functions has been explored in detail by Harding and coworkers (Harding and Rowe, 1982a,b, 1983, 1984; Harding, 1986; Harding, 1987; Harding, 1989; Harding, 1995; Harding *et al.*, 1997). Besides the derivation and availability of ν for triaxial ellipsoids as considered in Section 4.4 above, the Perrin translational friction shape parameter P, together with the rotational relaxation time ratios τ_h/τ_o and τ_i/τ_o (i = 1–5) are all available (see Harding, 1995) and combinations of these with ν has enabled the hydration independent β, R, Λ_h, Λ_i (eqn (4.8), eqn (4.9), eqn. (4.10), and eqn (4.11)) to be specified for the general triaxial case. Combination of ν with the reduced electro-optic decay constants θ^{red}_+ *and* θ^{red}_- (Ridgeway, 1966, 1968) has also enabled the hydration independent "δ_+ and δ_-" universal shape functions to be specified (Harding and Rowe, 1983, 1984):

$$\delta_+ = (6\eta_0/N_A k_B T)[\eta]M \cdot \theta_+ = 6\theta^{red}_+ \nu$$

$$\delta_- = (6\eta_0/N_A k_B T)[\eta]M \cdot \theta_- = 6\theta^{red}_- \nu \tag{4.12}$$

where θ_+ and θ_- are the electro optic decay constants, η_o the viscosity of the solvent at temperature T, and k_B is the Boltzmann constant.

Finally, the evaluation of the excluded volume and reduced excluded volume, u_{red} for general ellipsoids (Rallison and Harding, 1985) has enabled the Π function to be also specified for general ellipsoids (eqn (4.10)). 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). Again, because they are all universal shape functions and hence not dependent on size, specification of the two axial ratios $(a/b, b/c)$ is quite sufficient. 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 Λ_h with R, see Fig. 12 or Π with the radius of gyration based function G) or ELLIPS4 (combining the electro-optic/viscosity based shape functions $\delta_\pm$ with a further shape function of the user's choosing (Harding *et al.*, 1997)).

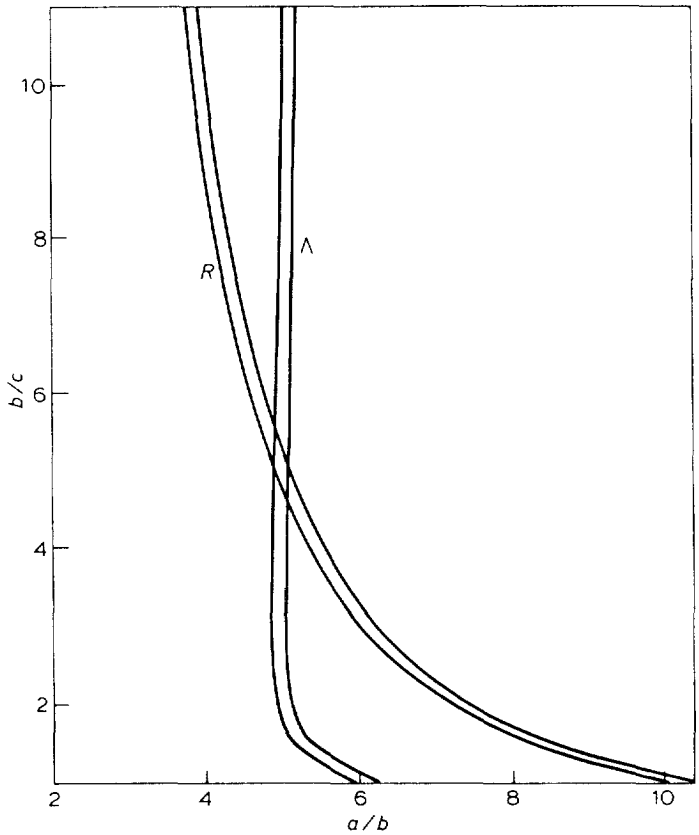

Fig. 12. Use of the intrinsic viscosity based Λ_h–R intersection plot to uniquely fix the triaxial shape of a particle in terms of (a/b, b/c). Based on synthetic data generated for a true (a/b, b/c) = (5.0, 5.0), an allowance for ±1% experimental error in R, ±2% in Λ_h. Reproduced with permission from Harding and Rowe (1982b)

4.7. *The Hydrodynamic Bead Model Approximation: the Bloomfield et al. and Garcia De La Torre et al. Approaches*

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 coworkers (see, 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 (see, 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 shape parameters based on translational and rotational frictional properties.

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 fric-

tional 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 4.5) has to be calculated (Garcia Bernal and Garcia de la Torre, 1980). Furthermore, as with the derivation for ellipsoids (Section 4.5) the calculation must be *orientationally averaged* {in terms of Euler angles (Nakajima and Wada, 1977; Garcia de la Torre and Bloomfield, 1978), or other procedures (Yamakawa *et al.*, 1977)}. Like the Rallison (1978) procedure for triaxial ellipsoids a numerical matrix inversion is required: in this case it 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.

Tsuda (1969, 1970a,b) gave the first expression for $[\eta]$ for a bead array, but his calculation was based on the original Oseen tensor together with a simplified, approximate hydrodynamic treatment that avoids the inversion matrix. This resulted in a loss of accuracy to an extent that depended on the particular geometry of the model (Garcia de la Torre *et al.*, 1983). 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):

$$[\eta] = \frac{N_A}{M\eta_0}\sum_i\sum_j \zeta_i\left[\frac{1}{15}\sum_\alpha (x_i^\alpha - v^\alpha)S_{ij}^{\alpha\alpha}(x_j^\alpha - v^\alpha) + \frac{1}{20}\sum\sum_{\alpha\neq\beta}(x_i^\alpha - v^\alpha)S_{ij}^{\beta\alpha}(x_j^\beta - v^\beta)\right.$$

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

$$+ \Delta_v \qquad (\alpha,\beta = 1,2,3). \tag{4.13}$$

where $\zeta_i = 6\pi\eta_o\sigma_i$ 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 **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 v^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

$$\frac{\partial[\eta]}{\partial v^\alpha} = 0\,(\alpha = 1,2,3), \tag{4.14}$$

which gives a set of three simultaneous linear equations with coefficients combinations of the x_j^α, s and S_{ij}'s. Substitution into eqn (4.13) 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 (Garcia de la Torre, 1989):

$$[\eta] = \frac{N_A\pi}{M}\left(\sum_i \sigma_i R''^2_i\right)\left\{1 + \frac{1}{\left(\sum_i \sigma_i R''^2_i\right)} \times \frac{3}{4}\left[\sum_i\sum_j \sigma_i\sigma_j\left(\left(1 + \frac{a_{ij}}{3}\right)\frac{R''_i R''_j \cos\alpha_{ij}}{R_{ij}}\right.\right.\right.$$

$$\left.\left.\left. + (1 - a_{ij}) \times \frac{4\left(R''^2_i + R''^2_j\right)R''_i R''_j \cos\alpha_{ij} - R''^2_i R''^2_j(1 + 7\cos^2\alpha_{ij})}{10R^3_{ij}}\right)\right]\right\}^{-1} + \Delta_V \tag{4.15}$$

where σ_i,σ_j are the bead radii for particles i, j, $\mathbf{R}_{ij}$ is the distance vector between the centre of beads i and j, and the $a_{ij} = (\sigma_i^2 + \sigma_j^2)/R_{ij}^2$,$\cos\alpha_{ij} = \mathbf{R}''_i\mathbf{R}''_j/R''_i R''_j$.

The viscosity increment ν is simply $(1/v_s) = M/VN_A$ times equation (eqn (4.13)) or (eqn (4.15)) (where V is the hydrated volume of the particle) and as we explained in Section 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 coordinates, whereas the routine SOLPRO (Garcia de la Torre *et al.*, 1997) evaluates ν and other universal shape parameters (including P, τ_h/τ_o, τ_i/τ_o (i = 1–5) β, R, G, Λ_h, Λ_i) described in Section 4.6 above (apart from u_{red} and Π which are not yet available for the bead approximation).

The volume correction term,

$$\Delta_V \sim (5/2)(N_A V/M) \tag{4.16}$$

with V as before the (hydrated) volume of the particle, $= v_s M/N_A = (\bar{v} + \delta/\rho_o)M/N_A$ on the RHS of equations (eqn (4.13)) and (eqn (4.15)) which had been inspired by a similar correction for rotational coefficients (Garcia de la Torre and Rodes, 1983), is essential in models in which one or a few beads have a large fraction of the particle size: e.g. 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 8 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 (eqn (4.3)) (to no worse than ~12% for a range of axial ratios a/b from 1 to 6): much better agreement 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. Table 2 shows results for various modes of assembly of spherical subunits based on eqn (4.13). Nonetheless, epresentations of known ellipsoidal shapes is still not exact and usually leads to overestimations of ν. An improved method incorporating a better volume correction is currently being developed (Garcia de la Torre, 1997).

These models are shown pictorially in Fig. 13. Similar calculations have been performed for the translational and rotational frictional properties (Garcia de la Torre, 1989).

Bead modelling has also been extended to the case of segmental and partial flexibility. There are many interesting biological macromolecules (Yguerabide *et al.*, 1970), whose function critically requires movement between relatively rigid parts of a macromolecule: the classical examples being antibodies, myosin and tRNA. There are three approaches (Garcia de la Torre, 1994): one, the most pertinent for the calculation of intrinsic viscosity is the so-called "rigid-body approach" in which the macromolecule is taken to have rigid domains linked together by a flexible swivel. Each rigid domain could be a single sphere, an ellipsoid, a rod or an array of spheres. The procedure is that intrinsic viscosity or other hydrodynamic properties are calculated for each domain and the relative orientations between them averaged, according to (Garcia de la Torre, 1994)

$$\langle X \rangle = \frac{\int \cdots \int X(q_1, q_2, \ldots) e^{-V(q_1,q_2,\ldots)/kT} d\tau}{\int \cdots \int e^{-V(q_1,q_2,\ldots)/kT} d\tau} \tag{4.17}$$

with the "property" X in this case being the intrinsic viscosity (or viscosity increment), q_1, q_2... are the internal coordinates of the domains, $V(q_1,q_2...)$ the potential energy of

Table 2. Intrinsic viscosity $[\eta]$ (normalised to a spherical particle of the same (hydrated) volume) and viscosity increment ν for oligomeric structures of Fig. 13. Eq. (4.13) with the volume correction of eq. (4.16) as described in the text had been used. The values for ν in column 3 must be regarded as approximate rather than prescriptive as further improvements to the volume correction are being made. Based on values given in Garcia de la Torre (1989)

Oligomer	$[\eta]/[\eta_{sphere}]$	ν
Monomer:		
sphere	1.0	2.5
Dimer:		
linear	1.6	4.1
Trimers:		
linear	2.1	5.3
triangle	1.9	4.7
Tetramers:		
square	2.0	5.1
tetrahedron	2.0	4.9
linear	2.7	6.6
Pentamers:		
pentagon	2.2	5.5
bipiramid	2.0	5.1
Hexamers:		
hexagon	2.4	6.0
octahedron	2.0	5.1
trigonal prism	2.1	5.3
linear	3.9	9.8
Octamer:		
cube	2.2	5.5

the system and $d\tau$ the differential of volume. $[\eta]$ or ν can be calculated to good accuracy using this approach (Wegener, 1985; Garcia de la Torre, 1994). The treatment of more general cases of flexibility will be considered in Section 5 below.

It should be stressed that, even with the improved Rotne-Prager-Yamakawa interaction, tensor bead modelling is *only an approximation* on two counts. (i) In an array of N spheres hydrodynamic interaction is in fact a many-body problem—the pairwise description is an approximation, although fortunately higher order interactions tend to cancel each other (Garcia de la Torre, 1989): this is in contrast to the calculation regime for "whole-body" ellipsoid representations which are exact; (ii) in common with whole-body modelling a calculated structure is only a model for the true structure in solution, although bead modelling does permit a much closer representation, and is the only valid approach for complex shapes such as immunoglobulins.

Despite its greater sensitivity to conformation the $[\eta]$ appears however to be the "most" approximate of the shape predictions for bead representations compared with the translational and rotational frictional based properties, although improvements to the volume correction in equations (eqn (4.13)) and (eqn (4.15)) are being made (Garcia de la Torre, 1997). This, together with the more serious issue of the "hydration" problem of Section 4.6, should serve as a clear warning to those who regard hydrodynamic methods as a potential high-resolution probe as a parallel to X-ray crystallography or nuclear magnetic resonance: this is not the intention, even with bead modelling. Rather, the correct philosophical approach is to use hydrodynamics to give better representations of molecular morphology in solution with the bead approach to oligomeric or complex structures where ellipsoid/triaxial representations are inadequate.

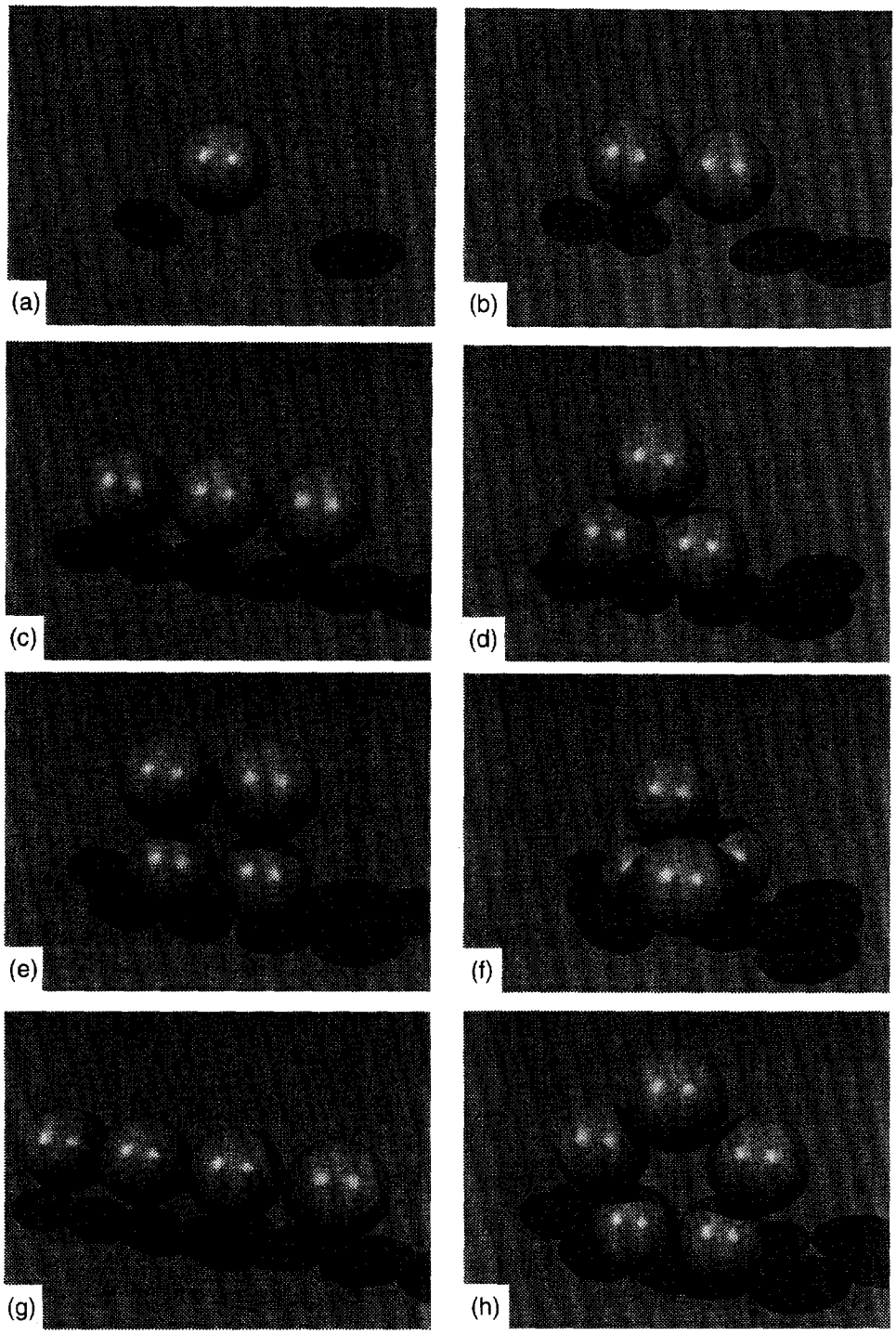

Fig. 13a–h

V. GENERAL CONFORMATION AND FLEXIBILITY ANALYSIS

For many biological macromolecules the rigid particle approach involving ellipsoid or bead analysis is inapplicable. Both types of modelling require stringent assumptions concerning the monodispersity of the macromolecular system being represented. This discounts molecules like polysaccharides and related glycopolymers such as nucleic acids and mucus glycoproteins (mucins), although for fairly rigid systems thereof—for

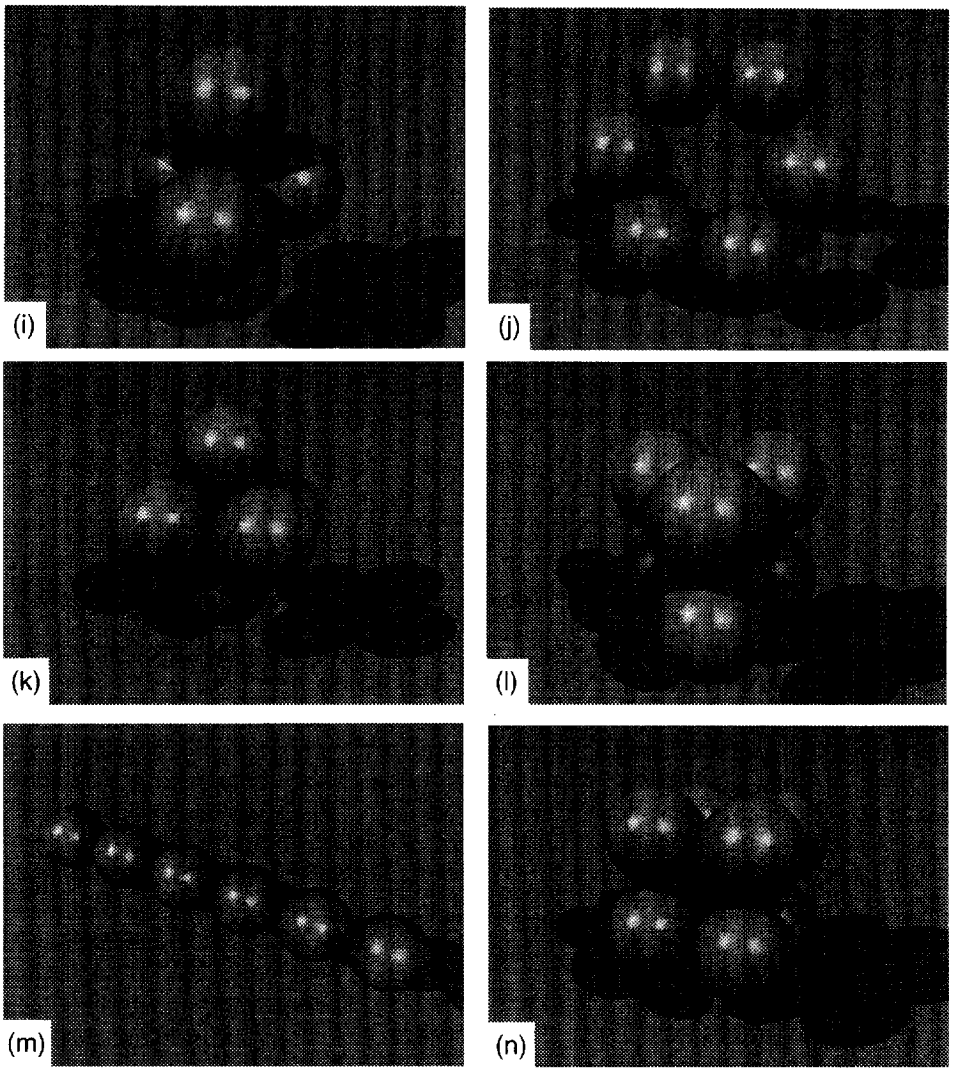

Fig. 13. Bead models for various oligomeric structures with approximately spherical subunits. (a) Monomer sphere, $\nu = 2.5$; (b) Dimer, $\nu = 4.1$; (c) Trimer-linear, $\nu = 5.3$; (d) Trimer-triangle, $\nu = 4.7$; (e) Tetramer-square, $\nu = 5.1$; (f) Tetramer-tetrahedron, $\nu = 4.9$; (g) Tetramer-linear, $\nu = 6.6$; (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 eq. 4.13 with the volume correction of eq. (4.16). An improved volume correction is currently being developed (Garcia de la Torre, 1997)

example highly charged rod-shaped polysaccharides—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 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.

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:

$$[\eta] = K' M^{a} \tag{5.1}$$

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

$$s_{20,w} = K'' M^{b}$$

$$D_{20,w} = K''' M^{-\varepsilon}$$

$$R_g = K'''' M^{c} \tag{5.2}$$

These relations are collectively known as the "Mark-Houwink-Kuhn-Sakurada" relations (Mark, 1938; Houwink, 1940; Kuhn and Kuhn, 1943; 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 monograph's of Tanford (1961), Smidsrød and Andresen (1979), Tsvetkov *et al.* (1971) and Bohdnaecky and Kovar (1982). Values for the other parameters are given in Table 3, 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 3 that the relation between b and ε is trivial (because of their common relation with the frictional coefficient):

$$b + \varepsilon = 1 \tag{5.3}$$

(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 (see e.g., Manaresi *et al.*, 1988; Guaita *et al.*, 1991) that the MHKS relation for viscosity (5.1) is only rigorous where each $[\eta]$ value corresponds to a monodisperse polymer. The same of course applies to the other MHKS relations (5.2). Most of the biological macromolecules to which equations of the type (eqn (5.1) and eqn (5.2)) have been applied are themselves polydisperse—such as polysaccharides, heavily glycosylated protein systems (mucins and glycosaminoglycans) and nucleic acids—and evaluation of the coefficients K' and a would be done after prior fractionation of the sample: each fraction however is likely to have a residual polydispersity, so

Table 3. 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

some caution needs to be expressed. This feature of polydispersity is particularly important since eqn (5.1) has been used as a "relative" method for obtaining molecular weight. A molecular weight obtained from direct application of equation (eqn (5.1)) 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:

$$[\eta] = K' M_w^a (M_w/M_n)^\alpha (M_z/M_w)^\beta \tag{5.4}$$

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, eqn (5.1) 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 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 eqn (2.15)) simultaneously determined. The exponent a thus found along with the exponent c from the R_g relation of eqn (5.2) {which can also, within certain limitations (see Yau, 1990) be found 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 3) or the use of the more refined models described in Section 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 for use on an on-line viscometer and concentration detector alone. The principle of Universal calibration (see, e.g., Harding *et al.*, 1991) is that, for example, separation is based on a relation V_e and the product $[\eta] \times M$ (where V_e is the elution volume) rather than being based on just M alone. Other refinements have been suggested (see for example Horta *et al.*, 1986).

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 most in Norway and Nottingham) places the extremes at the three corners of a triangle—the conformation of a given macromolecules can then be represented by a locus along the perimeter of the triangle (see 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 conformation "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. We have already considered in detail in Section 4 the analysis of the solution conformation of rigid macromolecules using $[\eta]$ in conjunction with other hydrodynamic parameters. We now consider the case of the flexibility of linear biopolymers, such as polysaccharides, mucins, glycosaminoglycans and nucleic acids.

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:

$$[\eta] = [\eta]_\infty + (S.I^{-1/2}) \tag{5.5}$$

where $[\eta]_\infty$ 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 (1971) suggested the use of a modified parameter, B (not to be confused with the 2nd 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

$$S = B.([\eta]_{I=0.1})^{\nu} \tag{5.6}$$

a relation which seems to fit the experimental data for glycopolymers and nucleic acids very well (see Section 6) with the exponent ν (also not to be confused—this time with the viscosity increment of Section 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 eqn (5.5)) as well as $[\eta]_{I=0.1}$ and using a value of $\nu = 1.3$ in eqn (5.6).

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 (see Section 4.3), the conventional reduced viscosity versus concentration plot can depart from its conventional positive slope characteristics (Fig. 2) and the reduced viscosity can decrease with increase in polymer concentration c. A good example is the glycosaminoglycan hyaluronic acid (Fig. 14)

The classical Fuoss-Strauss relation (Fuoss and Strauss, 1948a,b, Fuoss and Strauss, 1949) relation empirically describes this behaviour in the limit $c >> c_s$ where c_s is the salt concentration (g/ml):

$$\eta_{red} \sim c^{-1/2} \tag{5.7}$$

At lower (biopolymer) concentration c the condition $(c >> c_s)$ clearly does not hold for the case of Fig. 14 and a maximum is observed: such maxima have been observed for other systems (Malovikova *et al.*, 1994; Antonietti *et al.*, 1996). A much better representation of the behaviour is the Hess-Klein relation (Hess and Klein, 1983) which in simplified form (Malovikova *et al.*, 1994) is given by

$$\eta_{red} \sim c/[(c/\lambda) + c_s]^{3/2} \tag{5.8}$$

where λ is a function of the charge (valency) of the biopolymer. Equation (eqn (5.8)) is clearly consistent with the appearance of a maximum. Both Rinaudo and coworkers (Malovikova *et al.*, 1994; Roure *et al.*, 1996; 1998; Rinaudo *et al.*, 1997; Miles and Rinaudo, 1997) and Antonietti and coworkers (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.

5.5. *$[\eta]$-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;

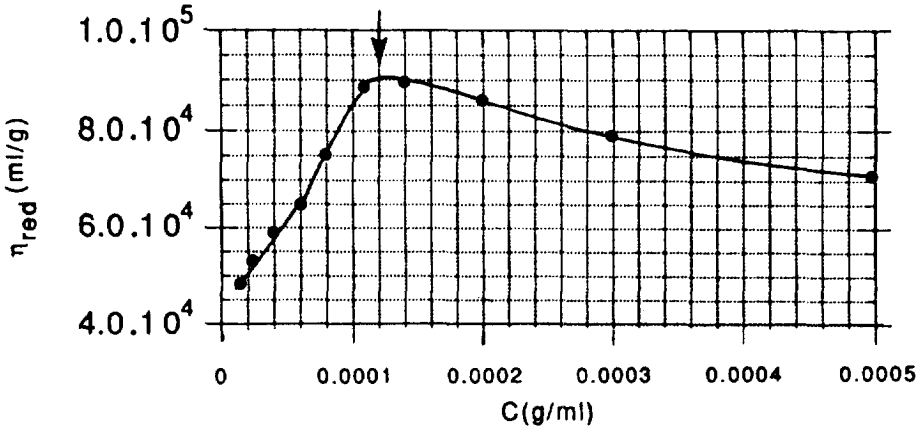

Fig. 14. Reduced viscosity versus concentration plot for a hyaluronic acid preparation in I = 0.0001 M NaCl solution. The arrow indicates the position of the maximum. From Roure *et al.* (1996)

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 (see Tsvetkov *et al.*, 1971; Yamakawa, 1971). This approach, which led to an estimate of a of ~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 inpenetrable 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 coworkers (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; see 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 interactions are preferred over interactions between different parts of the biopolymer, whereas 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 $[\eta]_\theta$. {The reader is warned that the same symbol has also been used in the literature to denote the intrinsic viscosity at zero ionic strength, and also the intrinsic viscosity of the equivalent spherical particle of the same mass}.

Flory and coworkers (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" of a linear molecule:

$$C_\infty = \langle h^2 \rangle / nl^2 \tag{5.9}$$

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 DNA l would represent the distance between intrachain base pairs, which is ~0.34 nm). $C_\infty \geq 1$, with the 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 $C_\infty > \sim 25$–400 (Lapasin and

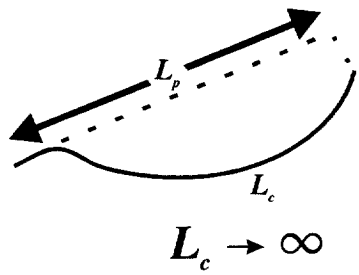

Fig. 15. The persistence length L_p and contour length L_c of a linear macromolecule. L_p corresponds to the average projection (onto a line of the initial direction projected from one end of the macromolecule) that L_c would have in the hypothetical limit that $L_c \rightarrow \infty$

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 residue molecular weight (see 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): see also Ptitsyn and Eizner (1959); Peterlin (1950); Peterlin (1952); Peterlin (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 $l \rightarrow 0$ and the number of segments $n \rightarrow \infty$. The *persistence length* L_p, is the principle 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 $L_c \rightarrow \infty$ (Fig. 15). Thus in the limits $L_c/L_p \rightarrow 0$ and $L_c/L_p \rightarrow \infty$ correspond to a perfectly rigid rod and a perfectly random coil respectively. Alternatively, just $L_p \rightarrow 0$ and $L_p \rightarrow \infty$ 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 (say DNA) *independent* of chain length or molecular weight". An alternative but equivalent parameter (see Tsvetkov *et al.*, 1971 and Fujita, 1990) is the "*Kuhn statistical segment length*" λ^{-1} $(=2L_p)$.

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

$$[\eta] = 100 \times 2.19 \times 10^{23}.(1/M).(L_c\lambda^{-1})^{3/2}.\{1 - 0.89[\ln(x/\lambda^{-1}) + 2.431 - x/d](L_c/\lambda^{-1})^{-1/2}\}^{-1} \tag{5.10}$$

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

$$[\eta] = 100 \times [\pi N_A L_c^3/(90M)].[1/\{\ln(L_c/x) - 2.72 + 0.66(x/d)\} + 3/\{\ln(L_c/x) - 2.72 + 1.33(x/d)\}] \tag{5.11}$$

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 coworkers (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 provides, however, 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:

$$(M^2/[\eta]_0)^{1/3} = A_\eta + B_\eta M^{1/2} \tag{5.12}$$

where

$$A_\eta = A_0 M_L \Phi_{0,\infty}^{-1/3} \tag{5.13}$$

and

$$B_\eta = B_0 \Phi_{0,\infty}^{-1/3} \left(\langle R_0^2 \rangle / M\right)_\infty^{-1/2} \tag{5.14}$$

$\Phi_{o,\infty} = 2.86 \times 10^{23}$ and A_o and B_o 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 (see Stokke and Elgsaeter, 1994). Table 4 lists some useful values of M_L.

An even simpler version of eqn (5.12) 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.

Table 4. Mass per unit length M_L for various biopolymers

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		
cellulose nitrate*	single chain polysaccharide	500–600	a,b	Bohdanecky (1983); Yamakawa and Fujii (1974)
amylose	single chain polysaccharide	790–1400	a,b,c	Yamakawa and Yoshizaki (1980); Stokke *et al.* (1987)
Poly (γ-ethyl-L-glutamate)*	single chain polypeptide	980–1040	a	Bohdanecky (1983); Terbojevich *et al.* (1967)
Poly (ε-carbobenzoxy-L-lysine)*	single chain polypeptide	1450–1680	a	Bohdanecky (1983); Matsuoka *et al.* (1973)
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)
DNA	double helical nucleic acid	1950		
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

The parameter $\Phi_{o,\infty}$ as given by Bohdanecky (1983) is actually a limiting form of the parameter Φ in the well-known fundamental relationship between $[\eta]$ and the dimension of a flexible polymer coil of Flory and Fox (1951):

$$\Phi = \frac{[\eta]M}{\langle h^2 \rangle^{3/2}} = \frac{[\eta]M}{6^{3/2}R_g^3} \tag{5.15}$$

The nature of the parameter Φ has been considered in detail by, for instance, Schmidt and Burchard (1981), Bohdanecky *et al.* (1983), Garcia de la Torre *et al.* (1983) and more recently by Garcia Bernal *et al.* (1991).

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)

$$[\eta] = Q.N_A.M^2/\{M_L(\ln M - \ln M_L - \ln d)\} \tag{5.16}$$

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

$$c^* \sim \chi/[\eta] \tag{5.17}$$

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). Grassley (1980) gave a somewhat higher value (1.08). This formula (with the lower three values for the coefficient χ) also seems to be valid for stiffer structures. For example, it accurately predicts a discontinuity at $c \sim 0.4$–0.8 mg/ml in the Huggins plot for the bacterial polysaccharide xanthan (Fig. 16). Discontinuities for this substance were observed at the same approximately concentration in plots of the sedimentation coefficient and apparent molecular weight versus concentration.

VI. PROTEINS AND POLYPEPTIDES

Table 5 gives a comprehensive list of the intrinsic viscosities of proteins with a clearly defined molecular weight. The table also includes polypeptides (include different molecular weight fractions), collagen sonicates (again of different molecular weight) and glycoproteins such as ovalbumin of modest degree of glycosylation ($< 15\%$)—more heavily glycosylated systems are considered in Section 7.

6.1. *General Conformation Studies*

Table 5 illustrates the principles concerning $[\eta]$-general conformation relationships discussed in Section 5 quite well, and in Table 6 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 (eqn (5.1)). From Table 5, 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 6). Sonicates of the triple-helical protein collagen (Nishihara and Doty, 1958) yield an a of ~1.8 (from a plot of log$[\eta]$ versus logM), consistent with a rigid rod conformation, whereas the protein in its gelatin state adopts a random coil configuration

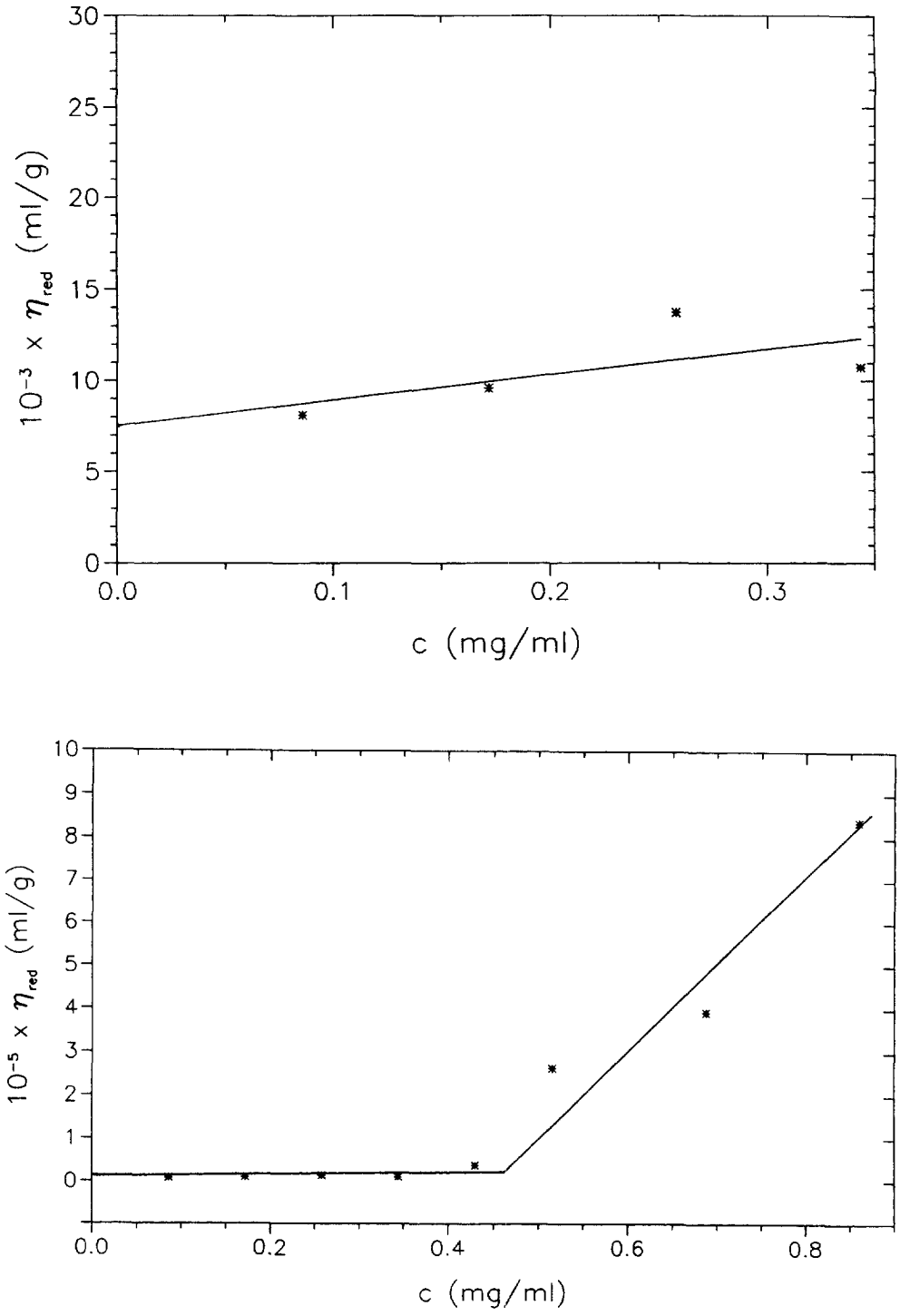

Fig. 16. Reduced viscosity versus concentration, c, plots for xanthan (Keltrol RD), (a) in dilute solution ($c = 0 \rightarrow 0.35$ mg/ml); and (b) in the region $c = 0 \rightarrow 0.9$ mg/ml. From (a), $[\eta] = (7500 \pm 2700)$ ml/g. Predicted c^* from eq. (5.17) = 0.4–0.8 mg/ml. Reproduced with permission from Dhami *et al.* (1995)

with $a = 0.45$–0.88 (Veis, 1964). Gelatin intrinsic viscosity has been subjected to a recent thorough investigation for a range of different preparations and temperatures (Krasovskii *et al.*, 1993).

Table 5. Intrinsic viscosities of proteins and polypeptides

Protein	M (Da)	Conditions	$[\eta]$ (ml/g)	Reference
Aldolase	150 000	pH 7.0, I = 0.21, 25°C	4.0	Stellwagen and Schachman (1962)
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)
Angiotensin converting enzyme	200 000		4.4	Sharma and Singh (1991)
Arachin (11S groundnut globulin)	300000		4.5	Prakash (1994)
Aspartate transcarbamylase	307 000		4.5	Cohlberg *et al.* (1972)
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)	30 000		3.9	Prakash (1994)
Catalase	250 000		3.9	Lovrien (1958)
Chymotrypsinogen A	25 660		2.5	Tanford (1968)
			3.13	Schwert (1951)
Clathrin	180 000	pH 7.5, 20°C	10	Bloom *et al.* (1980)
Collagen	345 000	pH 4.0, 0.06 M 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)
Cytochrome c		pH 7, I = 0.1, 25°C	2.67	Ahmad and Ahmad (1992)
		pH 7, 2.9 M, $CaCl_2$, 25°C	3.60	Ahmad and Ahmad (1992)
		pH 7, 4.25 M, $CaCl_2$, 25°C	14.62	Ahmad and Ahmad (1992)
		pH 7, 3.5 M, GuHCl, 25°C	14.61	Ahmad and Ahmad (1992)
		pH 7, 6 M, LiCl, 25°C	3.49	Ahmad and Ahmad (1992)
		pH 7, 8 M, LiCl, 25°C	14.53	Ahmad and Ahmad (1992)
Fetuin	48 000	pH 6.5, 20°C	6.0–7.8	Putnam (1965); Spiro (1960)
Fibrinogen	330 000		27	Tsao *et al.* (1951)
			25.0	Shulman (1953)
			25.4	Lederer and Schurtz (1972)
			24.0	Nemoto *et al.* (1977)
Fibrinogen trypric subfragment	95 000		7.2	Mihalyi and Godfrey (1963)
Gelatin	383 000		69	Gouinlock *et al.* (1955)
	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)
Hemocyanin	3 750 000		5.6	Van Holde and Cohen (1964)
Hemoglobin	68 000	free solution	3.6	Cohn and Prentiss (1927)
		inside erythrocyte cytoplasm	3.4	Gennaro *et al.* (1996)
Lactate dehydeogenase	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)
Lysozyme	14 320	0.067 M phosphate buffer, pH 6.2, 1% NaCl	2.7	Tanford (1968)
		0.067 M phosphate buffer, pH 6.2, 1% NaCl	2.54	Sugihara (1983)
			3.00	Sophianopoulos *et al.* (1962)
			2.98	Luzzati *et al.* (1961)
Metamerythrin	107 000	Borate buffer, pH 8, 25°C	3.6	Klotz and Keresztes-Nagy (1963)
Mucin peptide (unglycosylated 3 tandem repeats of the MUC1 gene)		pH 7.1 M phosphate, 30°C	7.71	Fontenot *et al.* (1993)
Myoglobin	17 190	pH 7.1, 0.1 M NaCl, 20°C	3.25	Harding (1980b)
			3.15	Wyman and Ingalls (1943)
		pH 8.6	3.54	Pötschke *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, 1959)
Myosin S1 heads	110 000	5°C	6.44	Garrigos *et al.* (1983)
		20°C	6.40	Lowey *et al.* (1969)
Neurophysin monomers	10 000		5.5	Nicolas *et al.* (1980a,b); Rholam and Nicolas (1981)
Neurophysin dimers	20 100		4.3	Harding and Rowe (1982)
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)
Plasminogen	143 000	25°C	8.0	Shulman *et al.* (1958)
Poly (γ-benzyl-L-glutamate)	64 000	In *m*-cresol, 30°C	46	Ookubo *et al.* (1976)
	75000	In *m*-cresol, 30°C	56	Ookubo *et al.* (1976)
	120 000	In *m*-cresol, 30°C	130	Ookubo *et al.* (1976)
	170 000	In *m*-cresol, 30°C	205	Ookubo *et al.* (1976)
Poly (his-ala-glu)	22 000	pH 2.97, 0.2 M NaCl 25°C	8	Goren *et al.* (1977)
		pH 4.99, 0.2 M NaCl 25°C	33	Goren *et al.* (1977)
		pH 9.70, 0.2 M NaCl 25°C	55	Goren *et al.* (1977)
Poly (L-lysine)$_n$ n = 955		pH 6.5, 0.003 M NaBr, 22°C	2.50	Martin *et al.* (1979)
		pH 6.5, 0.001 M NaBr, 22°C	5.10	Martin *et al.* (1979)
PRN60 glycoprotein of the retrovirus feline leukemia virus (FeLV)	6300	pH 7, phosphate buffer, 30°C	7.49	Fontenot *et al.* (1994)
Prothrombin	18 500	0.1 M KCl, 20°C	4.1	Harmison and Seegers (1962)
Ribonuclease A	13 700		3.30	Buzzell and Tanford (1956)
Serum albumin	65 000		3.7	Tanford and Buzzell (1956)
Spectrin hetero-dimers	470 000	pH 7.5, 0.02 M NaCl, 4°C	79	Stokke and Elgsaeter (1981)
	470 000	pH 7.5, 0.1 M NaCl, 4°C	40	
		pH 7.5, 0.1 M NaCl, 25°C	36	Dunbar and Ralston (1981)
Spectrin hetero-tetramers	940 000	pH 7.5, 0.2 M NaCl, 4°C	180	Stokke and Elgsaeter (1981)
		pH 7.5, 0.1 M NaCl, 4°C	78	
		pH 7.5, 0.1 M NaCl, 25°C	70.4	Dunbar and Ralston (1981)
Thrombin	33 400	0.1 M KCl, 20°C	3.8	Harmison and Seegers (1962)
Thyroglobulin	660 000	pH 7.0, 0.01 M KNO_3, 28.1°C	4.7	Creeth and Knight (1965); Edelhoch (1960)
		pH 9.0, 0.01 M KNO_3, 28.1°C	8.5	
		pH 11.0, 0.01 M KNO_3, 28.1°C	14.3	
Tropomyosin	93 000		52	Tsao *et al.* (1951)

Table 6. MHKS parameters for proteins and polypeptides

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	1660	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	Tanford (1967)
			0.64	Van Kleef *et al.* (1978)
Hyperhemoglobin			0.46	Pötschke *et al.* (1996)
			0.39	Pötschke *et al.* (1996)
Poly-L-glutamate	0.2 M NaCl, pH 4.3–7.3		1.0	Morcellet and Loucheux (1976)
Poly (γ-benzyl-L-glutamate)	in *m*-cresol solvent		1.6	Rha and Pradipasena (1986).
	dimethyl formamide	0.14	1.75	Van Holde (1985)
	dichloroacetic acid	28	0.87	Doty *et al.* (1956)
Spectrin	0.1 M NaCl, pH 7.5		0.93	Dunbar and Ralston (1981)

*: this equation is given in the form $[\eta] = K'_n{}^{a}$, where n is the number of amino acid residues

A similar value is obtained ($a \sim 1.6$) to that for collagen if log[η] versus logM is plotted for the data of another helical molecule, poly (γ-benzyl-L-glutamate) in *m*-cresol solvent (Rha and Pradipasena, 1986). A similar value is also obtained in dimethyl formamide as solvent ($a \sim 1.75$) and also corresponds to the helical form (Van Holde, 1985) whereas in dichloro-acetic acid (Doty *et al.*, 1956) the polypeptide adopts a coiled conformation ($a \sim 0.87$). Data for the glycoprotein human spectrin also corresponds to a coil, with $a \sim 0.93$ (Dunbar and Ralston, 1981). Poly-L-glutamate (Morcellet and Loucheux, 1976) has (stiff) coil like properties ($a \sim 1.0$) in contrast to poly (γ-benzyl-L-glutamate). Data collected for globular 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 (1967) suggested the following relation for proteins in the random coil state:

$$[\eta](ml/g) = 0.716n^{0.67} \tag{6.1}$$

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

The sensitivity of intrinsic viscosity measurement for monitoring denaturation or more subtle changes of conformation of proteins has long been known, as illustrated in Fig. 17. In Table 5 and Table 6 data for the polypeptide poly (his-ala-glu) has been included at three different pH's: 2.97, 4.99, 9.70: it can be seen that the intrinsic viscosity dramatically changes with corresponding [η]'s of 8, 33 and 55 ml/g respectively: this

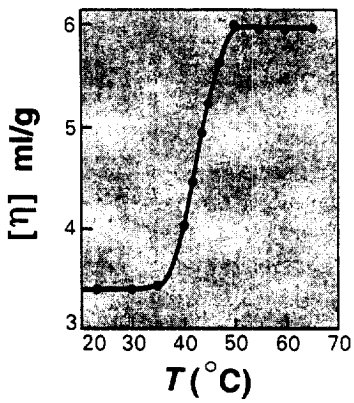

Fig. 17. Increase in [η] during thermal denaturation of ribonuclease at pH 2.8. The process is reversible. From Van Holde (1985)

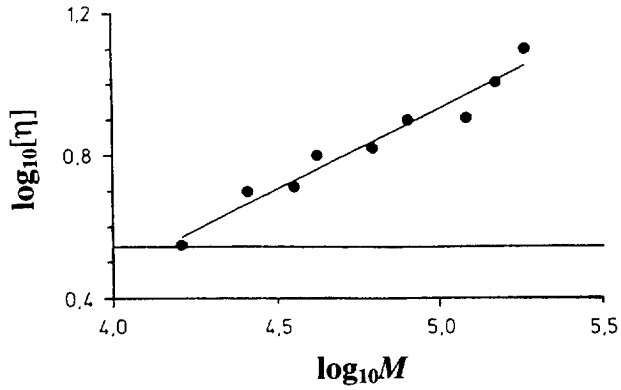

Fig. 18. MHKS [η] versus M plot for cross-linked "hyperpolymers" of myoglobin. a = 0.39. From Pötschke *et al.* (1996)

shows the dramatic effect of ionisation of the COOH group in the glutamic acid residues whose $pK_a \sim 4.3$ and deionisation of the histidine $NH3^+$ residue ($pK_a \sim 6$). The increase in charge leads to much stronger repulsive forces through the tertiary electroviscous effect (Section 4.3), with the polypeptide adopting a more rod shape conformation. For *globular* proteins the effect is much less dramatic as shown in the classical data of Tanford and Buzzell (1956) where over a wide range of both pH (from 4.3–10.5) and ionic strength I, (from 0.01–0.5 M) the [η] remains approximately constant at a value of (3.9 ± 0.3) ml/g.

An interesting observation is that of Gennaro *et al.* (1996) who demonstrated by using electron-paramagnetic resonance correlation time τ_c measurements of re-sealed erythrocytes incorporating a 4-maleimido-2,2′,6,6′-tetramethyl-piperidine-oxyl spin-label (after suitable calibration studies on the τ_c of the label in the presence of sucrose solutions of known viscosity) that the [η] of hemoglobin in the erythrocyte is comparable to the [η] of hemoglobin in free solution which had been measured over half a century earlier (Table 5, Table 6).

In the desire to develop artificial oxygen carriers in humans, Pötschke *et al.* (1996) have constructed "hyperpolymers" of myoglobin, and also hyperpolymers of hemoglobin. These hyperpolymers consist of multiple units of the native protein chemically cross-linked together. Both hyperhemoglobin and hypermyoglobin give MHKS a exponents consistent with a random coil (0.46 and 0.39 respectively). Figure 18 shows the MHKS plot for myoglobin polymers in the molecular weight range 17 100–180 000.

Another interesting recent study was that of Shinagawa *et al.* (1993), who re-examined the classical work of Reynolds and Tanford (1970) on the extent of binding of SDS to proteins and its affect on protein conformation as manifested by both reduced and intrinsic viscosity. Their data showed that the amount of SDS binding to protein under saturation conditions was not in fact fixed at 1.4 g/g protein, but was in the range 1.0 to 2.2 g/g; depending on the concentration of buffer used. Increases in the ionic strength of the buffer caused a decrease in the value of the Huggins constant K_H, a decrease ascribed to an increase in flexibility of the linear complex and down to an $I \sim 45$ mM typical [η] dependence on $I^{-1/2}$ was observed (cf. Section 5.3).

6.2. *Ellipsoid Modelling Studies*

The earlier modelling of macromolecular conformation of proteins from measurements of [η] was largely based on simple ellipsoids of revolution and using ν directly from eqns (eqn (4.1)) and (eqn (4.2)) with eqn. (eqn (4.3)) or (eqn (4.4a)–eqn (4.5a)) 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 [η], along with the sedi-

Table 7. Axial ratios of proteins from k_s and intrinsic viscosity [η] measurements

Protein	k_s (ml/g)	[η] (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, 1992; Harding and Johnson, 1985b).

a: Miller and Golder (1952); b: Holt (1970); c: Harding (1981b); d: Baldwin (1957); e: Tanford and Buzzell (1956); f: Advani *et al.* (1997); g: Townend *et al.* (1960); h: Nishihara and Doty (1958); i: Creeth and Knight (1965).

mentation coefficient could be represented by the extremes of axial ratio (a/b) of ~2.5 (hydration δ = 1.24) and ~1.0 (δ = 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 [η] with another hydrodynamic parameters has involved the R, Π and Λ_h (Section 4.6), rather than the β-function because of the latter's insensitivity to shape. Table 7 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 collagen is approximately a rigid rod, we can model the molecule as a rigid prolate ellipsoid of large axial ratio, and

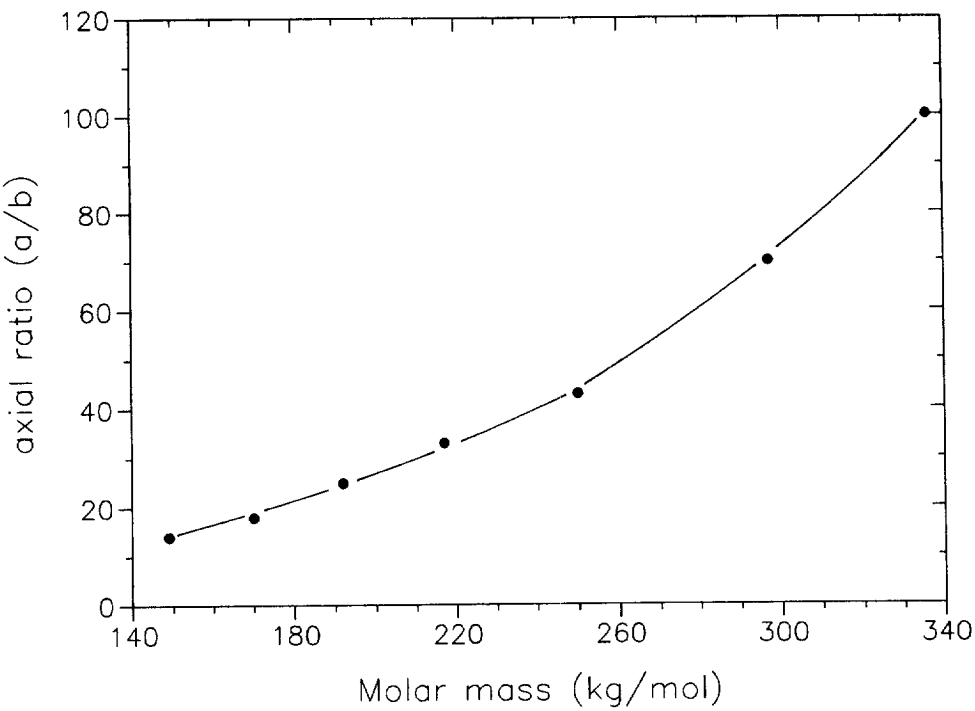

Fig. 19. Axial ratio of collagen sonicates estimated from the Wales-Van Holde ratio, R as a function of molecular weight (Reprinted with permission from Harding, 1995)

Table 8. Axial ratios of three proteins from the Π function

Protein	Π	axial ratio (a/b)*	Ref.
hemoglobin	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.

use the known dependence of R on axial ratio (a/b) to evaluate the change of (a/b) with molecular weight and Fig. 19 shows the increase in axial ratio is approximately linear with molecular weight for $M < 260\,000$.

Table 8 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 10 years later by X-ray crystallography (Stein *et al.*, 1991) (Fig. 7b)

Table 9 gives three examples of the application of the Λ_h function, which involves a combination of $[\eta]$ with the steady state fluorescence anisotropy harmonic mean relaxation time, τ_h. The first example is applicable to the tryptic subfragment of fibrinogen: combining the value for $[\eta]$ of 7.2 ml/g with that of 178 ns for τ_h (giving $\Lambda_h = 4.74 \pm 0.17$) Harding (1980a) deduced a value for (a/b) of 6.8 for the equivalent prolate ellipsoid. Incidentally, if the ν function had been used directly assuming no hydration a value for (a/b) of approximately 8 would have been predicted (Mihalyi and Godfrey, 1963). In further examples, Rholam and Nicolas (1981) obtained values of $\Lambda_h = 3.16$ and 2.69 respectively for both monomers and dimers of the neural protein neurophysin. Corresponding values of the (a/b) are 4.2 and 2.8 respectively showing that in going from monomer → dimer, the association of the subunits is likely to be side-by-side rather than an end-to-end process.

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 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 tri-axial ellipsoid of semi-axes $a \geq b \geq c$ and 2 axial ratios (a/b, b/c). Harding and Rowe (1982b) have applied the graphical intersection method of Section 4.3 using the Λ_h and R functions for triaxial ellipsoids to neurophysin monomers and dimers and essentially confirmed the conclusions of Rholam and Nicolas (1981) who used the cruder prolate ellipsoid of revolution model to represent the mode of association of the subunits: the association is a side-by side rather than end-to-end process. Figure 20 shows the Λ_h-R intersection plots for monomer and dimer with axial ratios (a/b, b/c) ~ (4.1, 1) for the monomer and (a/b, b/c) ~ (2.5, 2.7) for the dimer. A small correction to the original value of $[\eta]$ given by Rholam and Nicolas (1981) for the neurophysin dimer was necessary, and was obtained by using a more realistic value for the Huggins constant K_H in the extraction of $[\eta]$ for monomer (unaffected) and dimer (Harding and Rowe, 1982b).

In another application of the triaxial model Harding (1987) has used the intersection of Π with the radius of gyration shape function G to show that despite the segmental

Table 9. Axial ratios of three proteins from the Λ_h function

Protein	Λ_h	axial ratio (a/b)*	Ref.
Fibrinogen: tryptic subfragment	4.74	6.8	Harding (1980)
Neurophysin monomer	3.16	4.2	Rholam and Nicolas (1981)
Neurophysin dimer	2.69	2.8	Rholam and Nicolas (1981)

*of the equivalent prolate ellipsoid.

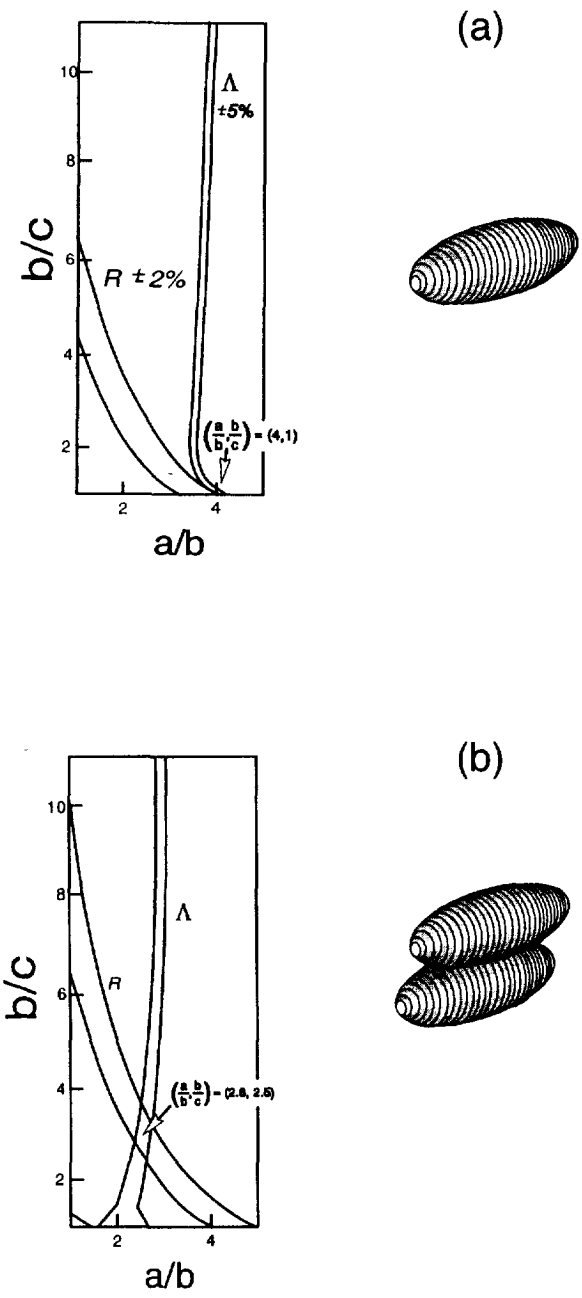

Fig. 20. Λ_h–R intersection plots for (a) neurophysin monomers, and (b) dimers. After Harding and Rowe (1982b) and using the PC routine ELLIPS3 of Harding *et al.* (1997)

flexibility of myosin (dimers), the overall conformation of a rod of axial ratio (a/b, b/c)~ (80, 1) is faithfully reproduced.

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

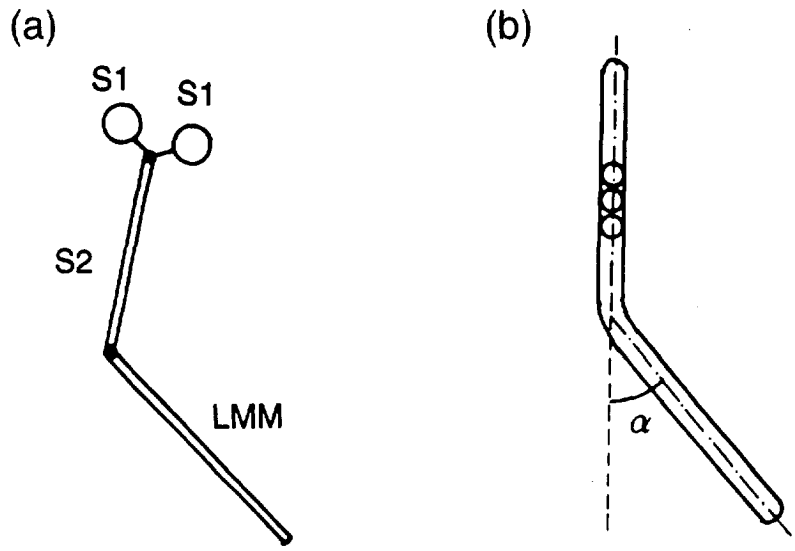

Fig. 21. (a) Simple "broken rod" model for a myosin molecule. (b) Broken region between the S2 and LMM regions can be modelled as an array of beads and inter-arm angle α. From Garcia de la Torre (1994)

molecular flexibility compared with rigid triaxial ellipsoids (Byron, 1995), with two potential regions of flexibility (Garcia de la Torre, 1994) as Fig. 21a illustrates: the link between the LMM and S2 regions (LMM and S2 are collectively referred to as the "myosin rod") and the link between S2 and the S1 heads. The rigid parts can themselves be modelled as an array of spherical beads (Fig. 21b).

There is still, however, considerable disagreement in terms of the extent of myosin flexibility, with some works suggesting there may be a large flexibility within the rod (Highsmith *et al.*, 1982; Cardinaud and Bernengo, 1985; Iniesta *et al.*, 1988), whereas others indicating 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 (Section 4.7) or whether it is more evenly distributed as a worm-like cylinder (Section 5.5). Garcia de la Torre (1994) has given three sources for uncertainty, (i) the length of the rod is a sensitive parameter needed for the modelling, and values ranging from 144 to 156 nm have been assumed; (ii) large discrepancies with relaxation times from rotational frictional measurements (birefringence or fluorescence anisotropy); (iii) the existence of two different theoretical approaches (the "rigid body" and "Wegener" (Wegener, 1985) approaches of Section 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 approximately 0.50 (Table 10) and the optimum rod (contour) length, L_c is indeed 144 nm.

Fibrinogen is another example of an elongated protein molecule whose conformation has been represented by viscosity-based bead models. Lopez Martinez *et al.* (1984) have taken advantage of the excellent agreement in published values for $[\eta]$ (Table 5) and combined this information with rotational diffusion and translational frictional information to examine the validity of molecular models proposed for fibrinogen. Both

Table 10. Broken rod modelling of myosin (from Garcia de la Torre, 1994)

Property	Experimental	Predicted for $L_c = 144$ nm	Predicted for $L_c = 150$ nm	Predicted for $L_c = 156$ nm	Flexibility parameter Q
Intrinsic viscosity, $[\eta]$ (ml/g)	265	290	355	370	0.42
Radius of gyration, R_g (nm)	38	41	43	45	0.70

the "dodecahederon" model of Koppel (1966) and the "three nodule" model proposed by Hall and Slayter (1959) on the basis of electron microscopy were ruled out, whereas a cylindrical model gave the best agreement with the data.

VII. GLYCOPOLYMERS AND NUCLEIC ACIDS

As the extent of glycosylation of a protein increases, the departure from classical rigid particle hydrodynamics to the realm of flexibility and semi-flexible conformations, and the relations described in Section 5 (MHKS exponents, Wales-Van Holde parameter, flexible coil theory) become relevant. Although there are no glycosidic bonds present, we make no apologies for regarding nucleic acids as "glycopolymers" because of their large repeating sugar content (deoxyribose or ribose), and indeed application of a worm-like coil theory has enabled us to obtain a fairly detailed picture of the conformation and flexibility of these molecules in solution. The most fundamental parameter describing the general conformation is the MHKS a exponent and Table 11 gives a comprehensive list for a range of polysaccharides, mucin glycoprotein and DNA. 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 glycopolymers represented in Table 11 have MHKS a values in the random coil range (0.5–0.8). This includes not only the classical random coils such as pullulan, but also mucin glycoproteins, and is consistent with the analagous "linear random coil" or "swollen coil array" models proposed for general mucin structure in solution (see Sheehan and Carlstedt, 1989). This contrasts with the conformation of the native mucin backbone peptide from the "*MUC*-1" gene {the $[\eta]$ of 7.71 ml/g (Table 5) is consistent with a rod-shape}. Like mucins, DNA also appears to adopt a coil shape conformation with the value from Crothers and Zimm (1965) of $a = 0.66$, based on collected data. These workers also observed that a better representation of the $[\eta]$-molecular weight relation could be obtained by a slight departure from the MHKS relation of eqn (5.1):

$$[\eta] = 0.1371M^{0.665} - 5 \tag{7.1}$$

with $[\eta]$ in ml/g (and M of course in Da or g/mol). A similar relation was presented for the sedimentation coefficient, s:

$$s = 0.01517M^{0.445} + 2.7 \tag{7.2}$$

with s in Svedbergs.

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 11. 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 affect 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 a molecular structure based around the MHKS a coefficient, other MHKS coefficients can be used such as the sedimentation b coefficient (see Harding, 1995 for a review) and the Wales-Van Holde parameter R ($= k_s/[\eta]$), as described in Section 5.1, with values of ~1.6 signifying a spheroidal domain (either

Table 11. MHKS parameters for Glycopolymers

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 (manA/gulA[a] = 1.8)	0.01 M NaCl, 20°C	4.8	1.15	Smidsrød (1970)
	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)
	DP[b] 100–10000, 5% cadoxin, 25% ethylene-diamine,	1800	0.77	Henley (1962)
Cellulose	0.35 m NaOH, 25°C			
Chitosan	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)
di-iodotyrosine Dextran	pH 7, I = 0.30		0.24	Errington *et al.* (1992)
	I = 0.2	13.71	0.665	Crothers and Zimm (1965); Doty *et al.* (1958); Kawade and Watanabe (1956); Iso and Watanabe (1957); Burgi and Hershey (1961)
DNA				
Ethyl-cellulose	in Ethyl acetate, 20°C $M_w < 80\,000$	28.2	1.00	Samsonova and Frenkel (1958)
	in Ethyl acetate, 20°C $M_w < 90\,000$	32.7	1.00	Moore and Brown (1958)
	in Ethyl acetate, 20°C $M_w < 150\,000$	10.7	0.89	Moore and Brown (1958)

Ethylhydroxyethyl-cellulose	in water, 25°C	370	0.80	Manley (1956)
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); Doublier and Launay, 1981)
		380	0.723	Robinson *et al.* (1982)
Hyaluronic acid	pH 6.8, I = 0.10, M_w 30 000–2 × 10^6	35480	0.48	Clarke and Harding (1997)
	pH 6, 0.2 M NaCl	228	0.82	Cleland and Wang (1970)
	pH 6, 0.5 M NaCl	318	0.78	Cleland and Wang (1970)
		10000	0.70	Brown (1961); Uda and Meyerhoff (1961); Brown *et al.* (1963); Savage (1965); Wirick (1986); Hodges *et al.* (1979)
Hydroxyethylcellulose				
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 (1968); Hodges *et al.* (1979)
		3160	0.55	Senti *et al.* (1955)
Mucin glycoprotein (human cervical)				Sheehan and Carlstedt (1984)
Mucin glycoprotein (pig gastric)	pH 6.8, I = 0.10	2218	0.46	Clarke and Harding (1997)
Mucin glycoprotein (pig colonic)	pH 6.8, I = 0.30		1.0	Jumel *et al.* (1997)
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 < 50 000	0.0013	1.7	Yanaki *et al.* (1980)
	M_w > 50 000	0.92	1.2	Yanaki *et al.* (1980)
	in DMSO[e]	2230	0.69	Yanaki *et al.* (1980)
Succinoglycan	M_w < 10^6.0.1 M NaCl	6.5	1.4	Gravanis *et al.* (1982)
Xanthan	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		1.32	Liu and Norisuye (1988)
	M_w > 150 000. 0.01 M NaCl		0.95	Liu and Norisuye (1988)
	0.5% NaCl	63	0.93	Muller *et al.* (1984)

Data partly taken from Lapasin and Pricl (1995). a: ManA-mannuronic acid, GulA-guluronic acid; b: DP-degree of polymerization; c: DD-degree of deacetylation; d: DM-degree of methoxylation; e: dimethylsulphoxide

Table 12. Glycopolymer gross conformation from the Wales-Van Holde relation

Glycopolymer	$R(=k_s/[\eta])$	Conformation	Reference
Alginates	0.6	Extended	Ball (1989)
Amylopectin (in DMSO)	1.5	spheroidal	Fronimos (1991)
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)
Mucin glycoprotein (human cervical)	1.5	Random coil	Sheehan and Carlstedt (1994)
Pullulans	1.4	Random coil	Kawahara *et al.* (1994)
Xanthan	0.3	Rigid rod	Dhami *et al.* (1995)

DMSO: dimethylsulfoxide.

a compact sphere or random coil) and low values ($\rightarrow$0.2) indicating a rigid rod conformation. Table 12 summarizes some findings.

Once the general conformation has been determined 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 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 ~70:1 was inferred on the basis of both the Π-(eqn (4.10)) and the R-functions (eqn (4.11)). Or in the case of more coiled structures, more detailed information about the flexibility of the molecule in terms of the characteristic 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, as described in Section 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. C_∞ (Section 4.5). For example, for uncharged polysaccharides C_∞ for the randomly coiled pullulan has been shown to be ~4 (Buliga and Brant, 1987; Kato *et al.*, 1984), whereas the more stiffer guar was shown to have a value of ~13 (Robinson *et al.*, 1982) {corresponding a values are ~0.65 and ~1 (Table 11)}. For polyelectrolytes the Smidsrød stiffness parameter, B has had popular application (see 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 the 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 (degree of substitution) values $0.5 \rightarrow 1.0$, whereas for pectin the chain became considerably stiffer as DS changed from $0.58 \rightarrow 0.89$ with B decreasing from $0.052 \rightarrow 0.005$ (Smidsrød and Haug, 1971). By far however, the most

Table 13. Persistence lengths L_p for Glycopolymers

Glycopolymer	L_p (nm)	Reference
Pullulan	1.2–1.9	Muroga *et al.* (1987)
Heparin	2.0–2.1	Stivala *et al.* (1968)
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)
DNA	45	Gray *et al.* (1967)
Xanthan ($M = 1.8 \times 10^6$ Da, $I = 0.1$ M)	40	Muller *et al.* (1986)
Xanthan ($M = 1.8 \times 10^6$ Da, $I = 10^{-5}$ M)	210	Muller *et al.* (1986)
Schizophyllan	115–200	Plaschina *et al.* (1985); Yanaki *et al.* (1981); Richardson and Ross-Murphy (1987); Murphy (1987); Carriere *et al.* (1986); 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.

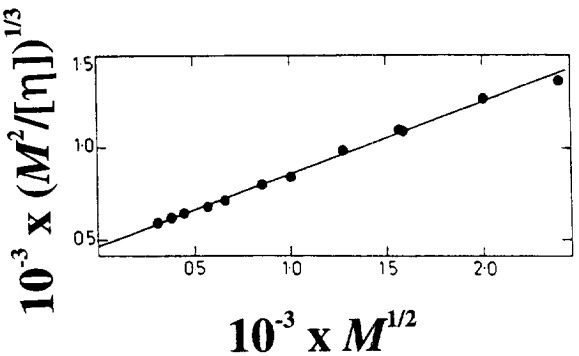

Fig. 22. Three-parameter Bohdanecky worm-like coil plot of $(M/[\eta])^{1/3}$ vs. $M^{1/2}$ for schizophyllan. $M_L = 1900$ Da nm^{-1}; $\lambda^{-1} = 274$ nm; $d = 1.6$ nm. From Bohdanecky (1983)

popular parameter representing chain flexibility has been as noted above the persistence length, L_p, with the theoretical limits of 0 for a completely random chain and ∞ for a completely rigid rod (practically the range goes from $\sim 1 \rightarrow 200$ nm). Table 13 gives the L_p for a collection of glycopolymers ranging from the randomly coiled pullulan ($L_p \sim 1.2$–1.9 nm) to the extra-rigid triple-helical schizophyllan ($L_p \sim 185$–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.* (1996) showed that the L_p for xanthan was only consistent with a double-helical structure (see also Stokke and Elgsaeter, 1994).

In the case of DNA, Sharp and Bloomfield (1968) extended the fundamental MHKS-based work of Crothers and Zimm (1965) to evaluate the Kuhn statistical segment length λ^{-1} ($= 2L_p$) and the hydrodynamic or "Stokes" diameter of, d, from the $[\eta]$-M data: values of $\lambda^{-1} = 54$ nm ($L_p = 27$ nm) and $d \sim 8$ nm were obtained, although these differ from estimates from the *s-M* dependence: Gray *et al.* (1967) obtained values of $\lambda^{-1} = 90$ nm ($L_p = 45$ nm) and $d \sim 2.7$ nm and Hearst and Stockmayer (1962) obtained values of $\lambda^{-1} = 72$ nm ($L_p = 36$ nm) and $d \sim 3.5$ nm using equations for the sedimentation coefficient s analagous to eqn (5.10) for $[\eta]$.

Using the simplified "3 parameter" representation for worm-like cylinders, Bohdanecky (1983) has applied equations (eqns (5.12), (5.13) and (5.14)) and the plot of $(M/[\eta])^{1/3}$ vs $M^{1/2}$ to data for the rod-shaped molecule schizophyllan (Yanaki *et al.*, 1980) (Fig. 22).

VIII. VIRUSES

The classical demonstration of Markham (1951) that the large tomato bushy stunt virus, TBSV ($M = 10.7 \times 10^6$) had a value for the intrinsic viscosity, $[\eta]$ of 3.44 ml/g, comparable to those for globular proteins of three molecular weight magnitudes smaller, confirmed that $[\eta]$ was completely independent of molecular weight (MHKS $a = 0$) for quasi-rigid spheroidal macromolecules and macromolecular assemblies. In another classical study, Boedtker and Simmons (1958) found a value for $[\eta]$ of 36.7 ml/g for the rod-shape tobacco mosaic virus, a value though significantly less than rod-shape polysaccharides such as xanthan (~ 7000 ml/g) and also DNA (~ 5000 ml/g). In a later study on turnip yellow mosaic virus, TYMV ($M = 5.7 \times 10^6$ Da), Harding and Johnson (1985b) obtained a value of (5.3 ± 0.3) ml/g, in which they used the incorporation of a small amount (3%) of glycerol suggested by Szuchet-Derechin and Johnson (1966) to facilitate measurements below 5 mg/ml (Section 3.1). This gave a Wales Van Holde ratio $R = k_s/[\eta] = 1.5$, in good agreement with R values for other spherical and globular particles (Creeth and Knight, 1965; Harding and Rowe, 1982a; Rowe, 1977). The higher value of $[\eta]$ corresponds to a significant degree of solvent association, as confirmed by measure-

ments of the sedimentation coefficient s, the translational diffusion coefficient, D and the second thermodynamic virial coefficient, B {from both combining k_s with the corresponding coefficient k_d from diffusion measurements (Harding and Johnson, 1985a), and sedimentation equilibrium reciprocal apparent molecular weight versus concentration plots}, which all yielded an estimate for the swollen specific volume (eqn (4.1)) $v_s \sim 1.8$ ml/g, once again expounding the virtue of combining more than one hydrodynamic measurement together.

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REFERENCES

Acuna, A. U., Gonzalez Rodriguez, J., Lillo, M. P. and Naqvi, K. R. (1987a) *Biophys. Chem.* **26,** 55–61.
Acuna, A. U., Gonzalez Rodriguez, J., Lillo, M. P. and Naqvi, K. R. (1987b) *Biophys. Chem.* **26,** 63–70.
Advani, M., Harding, S. E. and Rowe, A. J. (1997) Mss. in preparation.
Ahmad, Z. and Ahmad, F. (1992) *Indian J. Chem.* **31B,** 874–879.
Ahn, K. H., Schrag, J. L. and Lee, S. J. (1993) *J. Non-Newtonian Fluid Mech.* **50,** 349–373.
Antonietti, M., Briel, A. and Forster, S. (1996) *J. Chem. Phys.* **105,** 7795–7807.
Axelos, M. A. V., Thibault, J. F. and Lefebvre, J. (1989) *Int. J. Biol. Macromol.* **11,** 186–191.
Ball, A. (1989) PhD Dissertation, University of Nottingham, U.K.
Banks, W. and Greenwood, C. T. (1969) *Eur. Polym. J.* **5,** 649–658.
Banks, W. and Greenwood, C. T. (1968) *Carbohyd. Res.* **7,** 349–356.
Banks, W. and Greenwood, C. T. (1975) *Starch and its Components.* Edinburgh University Press, Edinburgh.
Baranov, V. G., Frenkel, S. Ya. and Agranova, S. A. (1987) *Vysokomol. Soedin.* **29B**(10), 745–750.
Bareiss, R. E., Chiantore, O. and Guaita, M. (1982) *Makromol. Chem.* **183,** 951–962.
Batchelor, G. W. (1970) *J. Fluid Mech.* **41,** 545–570.
Berkovich, L. A., Timofeyeva, M. P., Tsyurupa, M. P. and Davankov, V. A. (1980) *Polym. Sci. USSR* **22,** 2009–2018.
Berth, G., Anger, H. and Linow, F. (1977) *Die Nahrung* **21,** 939–950.
Biver, C., Lesec, J., Allain, C., Salome, L. and Lecourtier, J. (1986) *Polym. Comm.* **27,** 351–353.
Bloom, W. S., Schook, W., Feageson, E., Ores, C. and Puszkin, S. (1980) *Biochim. Biophys. Acta* **598,** 447–455.
Bloomfield, V. A., Dalton, W. O. and Van Holde, K. E. (1967a) *Biopolymers* **5,** 135–148.
Bloomfield, V. A., Garcia de la Torre, J. and Wilson, R. W. (1979) In *Electro-optics and Dielectrics of Macromolecules and Colloids*, ed. B. R. Jennings, pp. 183-195. Plenum Press, New York.
Bloomfield, V. A., Van Holde, K. E. and Dalton, W. O. (1967b) *Biopolymers* **5,** 149–159.
Boedtker, H. and Simmons, N. S. (1958) *J. Am. Chem. Soc.* **80,** 2550–2556.
Bohdanecky, M. (1983) *Macromolecules* **16,** 1483–1492.
Bohdanecky, M. (1996) *Macromolecules* **29,** 2265–2268.
Bohdanecky, M. and Kovar, J. (1982) *Viscosity of Polymer Solutions.* Polymer Science Library 2, Elsevier, Amsterdam.
Bohdanecky, M. and Netopilik, M. (1993) *Makromol. Chem. Rapid Commun.* **14,** 383–386.
Bohdanecky, M., Petrus, V., Porsch, B. and Sundelof, L. O. (1983) *Makromol. Chem.* **184,** 309–319.
Brady, J. F. and Durlovsky, L. J. (1988) *Phys. Fluids* **31,** 717–727.
Brenner, H. (1958) *Phys. Fluids* **1,** 338–346.
Brenner, H. (1970) *J. Coll. Int. Sci.* **32,** 141–158.
Brenner, H. and Condiff, D. W. (1972) *J. Coll. Int. Sci.* **41,** 228–274.
Brinkman, H. C. (1947a) *Proc. Acad. Amsterdam* **50,** 618–625.
Brinkman, H. C. (1947b) *Physica* **13,** 447–448.
Brinkman, H. C. (1947c) *Appl. Sci. Research* **A1,** 27–35.
Brown, W. (1961) *Arkiv. Kemi* **18,** 227–284.
Brown, W. (1993) *Dynamic Light Scattering, The Method and Some Applications.* Oxford University Press.
Brown, W. and Henley, D. (1964) *Makromol. Chem.* **79,** 68–88.
Brown, W., Henley, D. and Ohman, J. (1963) *Makromol. Chem.* **64,** 49–67.
Brown, W., Henley, D. and Ohman, J. (1964) *Arkiv. Kemi* **22,** 189–206.
Buliga, G. S. and Brant, D. A. (1987) *Int. J. Biol. Macromol.* **9,** 71–76.
Burchard, W. (1963) *Mackromol. Chem.* **64,** 110–125.
Burgi, E. and Hershey, A. D. (1961) *J. Mol. Biol.* **3,** 458–472.
Buzzell, J. G. and Tanford, C. (1956) *J. Phys. Chem.* **60,** 1204–1207.
Byron, O. (1995) *Prog. Coll. Polym. Sci.* **99,** 82–86.

Carriere, C. J., Schindlauer, G., Muller, M. F., Schrag, J. L. and Ferry, J. D. (1986) *J. Rheol.* **30,** 359–366.
Chee, K. K. (1985) *J. Appl. Polym. Sci.* **30,** 2607–2614.
Clarke, P. and Harding, S. E. (1997) mss in preparation.
Cleland, R. L. and Wang, J. L. (1970) *Biopolymers* **9,** 799–810.
Cohlberg, J. A., Pigiet, V. P. and Schachman, H. K. (1972) *Biochemistry* **11,** 3396–3411.
Cohn, E. J. and Prentiss, A. M. (1927) *J. Gen. Physiol.* **8,** 619–639.
Courts, A. and Stainsby, G. (1958) In *Recent Advances in Gelatin and Glue Research*, ed. G. Stainsby, pp. 100. Pergamon New York.
Coviello, T., Kajiwara, K., Burchard, W., Dentini, N. and Crescenzi, V. (1986) *Macromolecules* **19,** 2826–2831.
Cowie, J. M. G. (1963) *Makromol. Chem.* **59,** 189–200.
Creeth, J. M. and Knight, C. G. (1965) *Biochim. Biophys. Acta* **102,** 549–558.
Crothers, D. M. and Zimm, B. H. (1965) *J. Mol. Biol.* **12,** 525–536.
Curry, J. F. and Krause, S. (1991) *Biopolymers* **31,** 1677–1687.
Das, B. K., Agawal, S. K. and Khan, M. Y. (1991) *Biochim. Biophys. Acta* **1076,** 343–350.
Daveloose, D., Fabre, G., Berleur, F., Testylier, G. and Leterrier, F. (1983) *Biochim. Biophys. Acta* **763,** 41–49.
Davisson, E. O., Gibson, D. M., Raym, B. R. and Vestling, C. S. (1953) *J. Phys. Chem.* **57,** 609–613.
Deb, P. C. and Chatterjee, S. R. (1968) *Indian J. Appl. Chem.* **31,** 121–125.
Debye, P. (1946) *J. Chem. Phys.* **14,** 636–639.
Debye, P. and Bueche, A. N. (1948) *J. Chem. Phys.* **16,** 573–579.
Dhami, R., Harding, S. E., Jones, T., Hughes, T., Mitchell, J. R. and To, K.-M. (1995) *Carbohyd. Polym.* **27,** 93–99.
Dickinson, E. (1992) *An Introduction to Food Colloids*, p.72. Oxford University Press, U.K.
Dobkowski, Z. (1981) *Eur. Polym. J.* **17,** 1131–1144.
Dobkowski, Z. (1984) *Eur. Polym. J.* **20,** 265–267.
Doty, P., Bradbury, J. H. and Holzer, A. M. (1956) *J. Am. Chem. Soc.* **78,** 947–954.
Doty, P., McGill, B. B. and Rice, S. A. (1958) *Proc. Natl. Acad. Sci. USA* **44,** 432–438.
Doublier, J. L. and Launay, B. (1976) In *Proc. 7th Intl. Congress Rheology*, ed. C. Klason, and J. Kubat, pp. 532-533. Gothenburg, Sweden.
Doublier, J. L. and Launay, B. (1981) *J. Text. Stud.* **12,** 151–172.
Dreveton, E., Monot, F., LeCourtier, J., Ballerini, D. and Choplin, L. (1996) *J. Ferment. Bioeng.* **82,** 272–276.
Dunbar, J. C. and Ralston, G. B. (1981) *Biochim. Biophys. Acta* **667,** 117–184.
Dutta, P. K., Hammons, K., Willibey, B. and Haney, M. A. (1991) *J. Chromatog.* **536,** 113–121.
Edelhoch, H. (1960) *J. Biol. Chem.* **235,** 1326–1334.
Einstein, A. (1906) *Ann. Physik.* **19,** 289–305.
Einstein, A. (1911) *Ann. Physik.* **34,** 591–593.
Einstein, A. (1956) *Investigations on the Theory of the Brownian Movement*, 36-62.
Eizner, Yu. E. and Ptitsyn, O. B. (1962) *Vysokomolekul. Soedin.* **4,** 1725–1731.
Elias, H.-G., Bareiss, R. and Watterson, J. G. (1973) *Adv. Polym. Sci.* **11,** 111–204.
Elliott, J. H., Horowitz, K. H. and Hoodock, T. (1970) *J. Appl. Polym. Sci.* **14,** 2947–2951.
Endre, Z. H. and Kuchel, P. W. (1986) *Biophys. Chem.* **24,** 337–356.
Errington, N., Harding, S. E. and Rowe, A. J. (1992) *Carbohyd. Polym.* **17,** 151–154.
Errington, N., Harding, S. E., Varum, K. M. and Illum, L. (1993) *Int. J. Biol. Macromol.* **15,** 113–117.
Flory, P. J. (1953) *Principles of Polymer Chemistry*. Cornell Univ. Press, New York.
Flory, P. J. and Fox, T. G., Jr. (1951) *J. Am. Chem. Soc.* **73,** 1904–1908.
Flory, P. J. and Krigbaum, W. R. (1950) *J. Chem. Phys.* **18,** 1086–1092.
Fontenot, J. D., Tjandra, N., Bu, D., Ho, C., Andrews, P. C. and Montelaro, R. C. (1994) *J. Biomol. Struct. and Dynamics* **11,** 821–836.
Fontenot, J. D., Tjandra, N., Bu, D., Ho, C., Montelaro, R. C. and Finn, O. J. (1993) *Cancer Research* **53,** 5386–5394.
Freire, J. J. and Garcia de la Torre, J. (1992) In *Analytical Ultracentrifugation in Biochemistry and Polymer Science*, ed. S. E., Harding, A. J., Rowe, and J. C. Horton, Chap. 19. Royal Soc. Chem., Cambridge, U.K.
Fronimos, P. (1991) M. Phil Dissertation, Univ. Leicester, U.K.
Fujita, H. (1988) *Macromolecules* **21,** 179–185.
Fujita, H. (1990) *Polymer Solutions*. Elsevier, Amsterdam.
Fuoss, R. M. and Strauss, J. (1948a) *J. Polym. Sci.* **3,** 246–255.
Fuoss, R. M. and Strauss, J. (1948b) *J. Polym. Sci.* **3,** 603–604.
Fuoss, R. M. and Strauss, J. (1949) *J. Polym. Sci.* **4,** 96–120.
Furst, A. (1997) *Eur. Biophys. J.*, **25**, 3-7-310.
Garcia Bernal, J. M. and Garcia de la Torre, J. (1980) *Biopolymers* **19,** 751–766.
Garcia Bernal, J. M. and Garcia de la Torre, J. (1981) *Biopolymers* **20,** 129–139.
Garcia Bernal, J. M., Tirado, M. M., Freire, J. J. and Garcia de la Torre, J. (1991) *Macromolecules* **24,** 593–598.
Garcia de la Torre, J. (1989) In *Dynamic Properties of Biomolecular Assemblies*, ed. S. E., Harding, and A. J. Rowe, Chap. 1. Royal Soc. Chem., Cambridge UK.
Garcia de la Torre, J. (1994) *Eur. Biophys. J.* **23,** 307–322.
Garcia de la Torre, J. (1997) Mss in preparation.
Garcia de la Torre, J. and Bloomfield, V. A. (1977) *Biopolymers* **16,** 1747–1763.
Garcia de la Torre, J. and Bloomfield, V. A. (1978) *Biopolymers* **17,** 1605–1627.
Garcia de la Torre, J. and Bloomfield, V. A. (1981) *Quart. Rev. Biophys.* **14,** 83–139.
Garcia de la Torre, J., Carrasco, B. and Harding, S. E. (1997) *Eur. Biophys. J.*, **25**, (in press).

Garcia de la Torre, J., Lopez Martinez, M. C., Tirado, M. M. and Freire, J. J. (1984) *Macromolecules* **17,** 2715–2720.
Garcia-Molina, J. J., Lopez-Martinez, M. C. and Garcia de la Torre, J. G. (1990) *Biopolymers* **29,** 883–900.
Garcia de la Torre, J., Navarro, S., Lopez Martinez, M. C., Diaz, F. G. and Lopez Cascales, J. J. (1994) *Biophys. J.* **67,** 530–531.
Garcia de la Torre, J. and Rodes, V. (1983) *J. Chem. Phys.* **79,** 2454–2460.
Garrigos, M., Morel, J. E. and Garcia de la Torre, J. (1983) *Biochemistry* **22,** 4961–4969.
Gennaro, A. M., Luquita, A. and Rasia, M. (1996) *Biophys. J.* **71,** 389–393.
Gibbs, J. W. and Wilson, E. N. (1960) *Vector Analysis.* Dover, New York.
Giebeler, R. (1992) In *Analytical Ultracentrifugation in Biochemistry and Polymer Science*, ed. S. E., Harding, A. J. Rowe, and J. C. Horton, Chap. 2. Royal Soc. Chem. Cambridge, UK.
Goren, H. J., Grandan, L., Jay, A. N. L. and Lotan, N. (1977) *Biopolymers* **16,** 1541–1555.
Gouinlock, E. V., Jr., Flory, P. J. and Scheraga, H. A. (1955) *J. Polym. Sci.* **16,** 383–395.
Granath, K. A., Stromberg, R. and De Belder, A. N. (1969) *Die Starke* **21,** 251–256.
Grassley, W. W. (1980) *Polymer* **21,** 258–262.
Gravanis, G., Milas, M., Rinaudo, M. and Tinland, B. (1982) *Carbodyd Res.* **160,** 259–265.
Gray, H. B., Jr., Bloomfield, V. A. and Hearst, J. E. (1967) *J. Chem. Phys.* **46,** 1493–1498.
Guaita, M., Chiantore, O., Munari, A., Manaresi, P., Pilati, F. and Toselli, M. (1991) *Eur. Polym. J.* **27,** 385–388.
Guth, E. and Gold, O. (1938) *Phys. Rev.* **53,** 322.
Haber, S. and Brenner, H. (1984) *J. Coll. Int. Sci.* **97,** 496–514.
Hall, C. E. and Slayter, H. S. (1959) *J. Biophys. Biochem. Cytol.* **5,** 11–15.
Haney, M. A. (1985a) *American Laboratory* **17,** 41–56.
Haney, M. A. (1985b) *American Laboratory* **17,** 116–126.
Happel, J. and Brenner, H. (1973) *Low Reynolds Number Hydrodynamics*, (2nd edition). Martinus Nijhoff, Dordrecht, The Netherlands.
Harding, S. E. (1980a) *Biochem. J.* **189,** 359–361 (Also Vol). 189 corrigenda (correction in the formula for τ_h).
Harding, S. E. (1980b) *IRCS Med. Sci.* **8,** 610.
Harding, S. E. (1981a) *Int. J. Biol. Macromol.* **3,** 340–341.
Harding, S. E. (1981b) *Int. J. Biol. Macromol.* **3,** 398–399.
Harding, S. E. (1987) *Biophys. J.* **51,** 673–680.
Harding, S. E. (1989) In *Dynamic Properties of Biomolecular Assemblies*, ed. S. E. Harding, and A. J., Rowe, Chap. 2. Royal Society of Chemistry, Cambridge, UK.
Harding, S. E. (1995) *Biophys. Chem.* **55,** 69–93.
Harding, S. E. and Cölfen, H. (1995) *Analyt. Biochem.* **228,** 131–142.
Harding, S. E., Dampier, M. and Rowe, A. J. (1981) *J. Coll. Int. Sci.* **79,** 7–13.
Harding, S. E., Dampier, M. and Rowe, A. J. (1982) *Biophys. Chem.* **15,** 205–208.
Harding, S. E., Day, K., Dhami, R. and Lowe, P. M. (1997a) *Carbohydrate Polym.* **32,** 81–87.
Harding, S. E., Horton, J. C. and Cölfen, H. (1997b) *Eur. Biophys. J.* **25,** 333–346.
Harding, S. E. and Johnson, P. (1985a) *Biochem. J.* **231,** 543–547.
Harding, S. E. and Johnson, P. (1985b) *Biochem. J.* **231,** 549–555.
Harding, S. E. and Rowe, A. J. (1982a) *Int. J. Biol. Macromol* **4,** 160–164.
Harding, S. E. and Rowe, A. J. (1982b) *Int. J. Biol. Macromol* **4,** 357–361.
Harding, S. E. and Rowe, A. J. (1983) *Biopolymers* **22,** 1813–1829.
Harding, S. E. and Rowe, A. J. (1984) *Biopolymers* **23,** 843.
Harding, S. E., Rowe, A. J. and Creeth, J. M. (1983) *Biochem. J.* **209,** 893–896.
Harding, S. E., Rowe, A. J. and Horton, J. C. (1992) eds., *Analytical Ultracentrifugation in Biochemistry and Polymer Science.* Royal Soc. Chem., Cambridge, UK.
Harding, S. E., Vårum, K. M., Stokke, B. T. and Smidsrød, O. (1991) *Adv. Carbohyd. Analysis* **1,** 63–144.
Hardingham, T. E., Hughes, C., Mow, V. C. and Lai, W. M. (1989) In *Dynamic Properties of Biomolecular Assemblies*, ed. S. E. Harding, and A. J. Rowe, Chap. 16. Royal Soc. of Chem., Cambridge, U.K.
Harmison, G. R. and Seegers, W. H. (1962) *J. Biol. Chem.* **237,** 3074–3076.
Hearst, J. E. (1963) *J. Chem. Phys.* **38,** 1062–1065.
Hearst, J. E. (1964) *J. Chem. Phys.* **40,** 1506–1509.
Hearst, J. E. and Tagami, Y. (1965) *J. Chem. Phys.* **42,** 4149–4151.
Hearst, J. E. and Stockmayer, W. H. (1962) *J. Chem. Phys.* **37,** 1425–1433.
Henley, D. (1962) *Ark. Kemi* **B18,** 327.
Hess, W. and Klein, R. (1983) *Adv. Phys.* **32,** 173–283.
Hermans, J. J. (1949) In *Colloid Science*, ed. M. Kruyt, Vol. 2. Elsevier, Amsterdam.
Herrmann, A. and Muller, P. (1986) *Biochim. Biophys. Acta* **885,** 80–87.
Hocquart, R., Cressely, R. and Leray, J. (1974) *J. Chimie Physique.* **71,** 1256–1262.
Hodges, K. L., Kester, W. E., Widerrich, D. L. and Grover, J. A. (1979) *Analyt. Chem.* **51,** 2172–2176.
Holt, J. C. (1970) PhD Dissertation, Univ. London.
Holt, J. C. and Creeth, J. M. (1972) *Biochem. J.* **129,** 665–676.
Holtzer, A. and Lowey, S. (1959) *J. Am. Chem. Soc.* **81,** 1370–1377.
Horta, A., Saiz, E., Barrales-Rienda, J. M. and Galera Gomez, P. A. (1986) *Polymer* **27,** 139–146.
Houwink, R. (1940) *J. Prakt. Chem.* **157,** 15–18.
Huggins, M. L. (1942) *J. Am. Chem. Soc.* **64,** 2716–2718.
Hvidt, S., Henry, F., Greaser, M. L. and Ferry, J. D. (1982) *Biochemistry* **21,** 4064–4073.
Iso, K. and Watanabe, I. (1957) *J. Chem. Soc. Japan, Pure Chem. Sect.* **78,** 1268–1272.
Jackson, C., Barth, H. G. and Yau, W. W. (1991) *Abstracts Am. Chem. Soc.* **202**(2), 97.
Jeffery, G. B. (1922) *Proc. Roc. Soc., London* **A102,** 161–179.
Jumel, K. (1994) PhD Dissertation, University of Nottingham, U.K.

Jumel, K., Harding, S. E., Mitchell, J. R., To, K.-M., Hayter, I., O'Mullane, J. E. and Ward-Smith, S. (1996) *Carbohyd. Polym.* **29,** 105–109.
Kawade, Y. and Watanabe, I. (1956) *Biochim. Biophys. Acta* **19,** 513–523.
Kawahara, K., Ohta, K., Miyamoto, H. and Nakamura, S. (1984) *Carbohyd. Polym.* **4,** 335–356.
Kirkwood, J. G. (1967) *Macromolecules*. Gordon and Breach, New York.
Kirkwood, J. G. and Riseman, J. (1948) *J. Chem. Phys.* **16,** 565–573.
Kirkwood, J. G. and Riseman, J. (1949) *J. Chem. Phys.* **17,** 442–446.
Kitamura, S., Takeo, K., Kuge, T. and Stokke, B. T. (1991) *Biopolymers* **31,** 1243–1255.
Klotz, I. M. and Keresztes-Nagy, S. (1963) *Biochemistry* **2,** 445–452.
Koppel, G. (1966) *Nature* **212,** 1608–1609.
Kozicki, W. and Kuang, P. Q. (1996) *Can. J. Chem. Eng.* **74,** 429–432.
Kragh, A. M. (1961) In *A Laboratory Manual of Analytical Methods of Protein Chemistry (including polypeptides), Vol. 3, Determination of the Size and Shape of Protein Molecules*, ed. P. Alexander, and R. J. Block, Chap. 5. Pergamon Press, Oxford, U.K.
Kraemer, E. O. (1938) *Ind. Eng. Chem.* **30,** 1200–1203.
Kramers, H. A. (1946) *J. Chem. Phys.* **14,** 415–424.
Krasovskii, A. N., Mnatsakanov, S. S., Guseva, E. G., Andreeva, A. I., Maryanina, E. V. and Ustinova, L. N. (1993) *Russian J. Appl. Chem.* **66,** 671–679.
Kratky, O., Leopold, H. and Stabinger, H. (1973) *Meth. Enzymol.* **27D,** 98–110.
Kratky, O. and Porod, G. (1949) *Rev. Trav. Chim. Pay-Bas.* **68,** 1106–1109.
Kuhn, W. and Kuhn, H. (1945) *Helv. Chim. Acta* **28,** 97–127.
Kuhn, W., Kuhn, H. and Buchner, P. (1951) *Ergeb. Exact. Naturwiss* **25,** 1–108.
Kuntz, I. D., Jr. (1971) *J. Am. Chem. Soc.* **93,** 514–516.
Kuntz, I. D., Jr. and Kauzmann, W. (1974) *Adv. Prot. Chem.* **28,** 239–345.
Kurata, M. and Stockmayer, M. H. (1963) *Adv. Polym. Sci.* **3,** 196–312.
Krigbaum, W. and Flory, P. J. (1953) *J. Polym. Sci.* **9,** 381–384.
Lapasin, R. and Pricl, S. (1995) *Rheology of Industrial Polysaccharides, Theory and Applications*. Blackie, London.
Liu, W. and Norisuye, T. (1988) *Int. J. Biol. Macromol.* **10,** 44–50.
Launay, B., Doublier, J. L. and Cuvelier, G. (1986) In *Functional Properties of Food Macromolecules*, ed. J. R. Mitchell, and D. A. Ledward, Chap. 1. Elsevier Applied Science, London.
Lavrenko, P. N., Linow, K. J., Gornitz, E. (1992) In *Analytical Ultracentrifugation in Biochemistry and Polymer Science*, ed. S. E., Harding, A. J. Rowe, and J. C. Horton, Chap. 28. Royal Soc. Chem., Cambridge UK.
Lederer, K. and Schurtz, J. (1972) *Biopolymers* **11,** 1989–1993.
Lopez Martinez, M. C. and Garcia de la Torre, J. (1983) *Biophys. Chem.* **18,** 269–279.
Lopez Martinez, M. C., Rodes, R. and Garcia de la Torre, J.[??] (1984) *Int. J. Biol. Macromol.* **6,** 261–265.
Lovrien, R. E. (1958) PhD Thesis, State Univ. of Iowa, USA.
Lowey, S., Slayter, H. S., Weeds, A. G. and Baker, H. (1969) *J. Mol. Biol.* **42,** 1–29.
Luzzati, V., Witz, J. and Nicolaieff, A. (1961) *J. Mol. Biol.* **3,** 367–378.
Malovikova, A., Milas, M., Rinaudo, M. and Borsali, R. (1994) In Macro-ion Characterization, *Am. Chem. Soc. Symp. Ser.*, ed. K. S. Schmitz, **548**, Chap. 24.
Manley, R. (1956) *Ark. Kemi* **9,** 519–581.
Mark, H. (1938) *Der feste Korper*, p. 103. Hirzel, Leipzig, Germany.
Markham, R. (1951) *Discuss. Faraday Soc.* **11,** 221–227.
Martin, N. B., Tripp, J. B., Shibata, J. H. and Schurr, J. M. (1979) *Biopolymers* **18,** 2127–2133.
Matsuoka, M., Norisuye, T., Teramoto, A. and Fujita, H. (1973) *Biopolymers* **12,** 1515–1532.
McCammon, J. A., Deutch, J. M. and Felderhoff, B. U. (1975) *Biopolymers* **14,** 2613–2623.
Manaresi, P., Munari, A., Pilati, F. and Marianucci, E. (1988) *Eur. Polym. J.* **6,** 575–578.
Meyer-Almes, F.-J., Heumann, H. and Porschke, D. (1994) *J. Mol. Biol.* **236,** 1–6.
Milas, M., Rinaudo, M. and Tinland, B. (1985) *Polym. Bull.* **14,** 157–164.
Milas, M. and Rinaudo, M. (1997) *Curr. Trends. Polym. Sci.* **2,** 47–67.
Mihalyi, E. and Godfrey, J. (1963) *Biochim. Biophys. Acta.* **67,** 90–103.
Miller, G. L. and Golder, R. H. (1952) *Arch. Biochem. Biophys.* **36,** 249–258.
Morcellet, M. and Loucheux, C. (1976) *Biopolymers* **15,** 1857–1862.
Moore, W. R. and Brown, A. (1958) *J. Appl. Chem.* **8,** 363–367.
Morris, E. R. and Ross-Murphy, S. B. (1981) In *Techniques in Carbohydrate Metabolism*, ed. D. H. Northcote, **B310**, p. 1-46. Elsevier, Amsterdam[??].
Morse, P. D., II, Lusczakoski, D. M. and Simpson, D. A. (1979) *Biochemistry* **18,** 5021–5029.
Muller, G., Lecourtier, J., Chauveteau, G. and Allain, C. (1984) *Makromol. Chem. Rapid Comm.* **5,** 203–208.
Muller, G., Anrhourrache, M., Lecourtier, J. and Chauvetau, G. (1986) *Int. J. Biol. Macromol.* **8,** 167–172.
Muroga, Y., Yamada, Y., Noda, I. and Nagasawa, M. (1987) *Macromolecules* **20,** 3003–3006.
Muzzarelli, R. A. A. (1977) *Chitin*. Pergamon Press, Oxford, U.K.
NAG (1986) *NAG manual*. Numerical Algorithms Group, Oxford, UK.
Nakajima, H. and Wada, Y. (1978) *Biopolymers* **16,** 875–893.
Neely, W. B. (1963) *J. Polym. Sci. A.* **1,** 311–320.
Nemoto, N., Nestler, F. H. M., Schrag, J. L. and Ferry, J. D. (1977) *Biopolymers* **16,** 1957–1969.
Nestler, F. H. M., Hvidt, S., Ferry, J. D. and Veis, A. (1983) *Biopolymers* **22,** 1747–1758.
Nicolas, P., Batelier, G., Rholam, M. and Cohen, P. (1980a) *Biochemistry* **19,** 3565–3573.
Nicolas, P., Camier, M., Lauber, M., Masse, M. J., Möhring, J. and Cohen, P. (1980b) *Proc. Natl. Acad. Sci. USA* **77,** 2587–2591.
Nishihara, T. and Doty, P. (1958) *Proc. Nat. Acad. Sci. USA* **44,** 411–417.
Norisuye, T., Yanaki, T. and Fujita, H. (1980) *J. Polym. Sci.-Polym. Phys. Ed.* **18,** 547–558.

Oncley, J. L. (1941) *Ann. New York Acad. Sci.* **41,** 121–150.
Ookubo, N., Komatsubara, M., Nakajima, H. and Wada, Y. (1976) *Biopolymers* **15,** 929–947.
Oseen, C. W. (1927) *Hydrodynamik*. Academishes Verlag, Leipzig, Germany.
Ostwald, W. and Malss, H. (1933) *Koll. Z.* **63,** 61–77.
Pals, D. T. and Hermans, J. J. (1952) *Rec. Trav. Chim. Pays-Bas* **71,** 433–467.
Papanagopoulos, D. and Dondos, A. (1995) *Polymer* **36,** 369–372.
Patel, J. R., Patel, C. K. and Patel, R. D. (1967) *Die Starke* **19,** 330–335.
Pavlov, G. M., Korneeva, E. V. and Yevlampieva, A. (1994) *Int. J. Biol. Macromol.* **16,** 318–323.
Pavlov, G. M., Harding, S. E. and Rowe, A. J. (1997a) *Trends Analyt. Chem.* **16,** 401–405.
Pavlov, G. M., Harding, S. E. and Rowe, A. J. (1997b) Mss. in preparation.
Perkins, S. J. (1986) *Eur. J. Biochem.* **157,** 169–180.
Peterlin, A. (1948) *Les Grosses Molecules en Solution*. P. 70, Paris.
Peterlin, A. (1950) *J. Polym. Sci.* **5,** 473–482.
Peterlin, A. (1952) *J. Polym. Sci.* **8,** 173–185.
Peterlin, A. (1960) *J. Chem. Phys.* **33,** 1799–1802.
Phelps, R. A. and Cann, J. R. (1956) *Arch. Biochem. Biophys.* **61,** 51–71.
Plashchina, I. G., Semenova, M. G., Braudo, E. E. and Tolstuguzov, V. B. (1985) *Carbohyd. Pol.* **5,** 159–179.
Porschke, D. and Obst, A. (1991) *Rev. Sci. Instrum.* **62,** 818–820.
Pötschke, H., Barnikol, W. K.R., Kirste, R. G. and Rosenbaum, M. (1996) *Macromol. Chem. Phys.* **197,** 1419–1437.
Prakash, V. (1994) *J. Sci. Indust. Res.* **53,** 684–691.
Ptitsyn, O. B. and Eizner, Yu. E. (1959) *Zh. tekh. Fiz.* **29,** 1117–1134.
Ptitsyn, O. B. and Eizner, Yu. E. (1962) *Dokl. Acad. Nauk, SSSR* **142,** 134–136.
Putnam, F. W. (1965) In *The Proteins*, ed. H. Neurath, pp. 153. Academic Press, New York.
Rallison, J. M. (1978) *J. Fluid Mech.* **84,** 237–263.
Rallison, J. M. and Harding, S. E. (1985) *J. Coll. Int. Sci.* **103,** 284–289.
Ram Mohan Rao, M. V. and Yaseen, M. (1986) *J. Appl. Polym. Sci.* **31,** 2501–2508.
Reddy, G. V., Petrus, V. and Bohdanecky, M. (1990) *Makromol. Chem. Rapid Comm.* **11,** 355–358.
Reilly, P. M., van der Hoff, B. M. E. and Ziogas, M. (1979) *J. Appl. Polym. Sci.* **24,** 2087–2100.
Reynolds, J. A. and Tanford, C. (1970) *Proc. Natl. Acad. Sci. USA* **66,** 1002–1007.
Rha, C. K. and Pradipasena, P. (1986) In *Functional Properties of Food Macromolecules*, ed. J. R. Mitchell, and D. A. Ledward, Chap. 2. Elsevier Applied Science, London.
Rholam, P. and Nicolas, P. (1981) *Biochemistry* **20,** 5837–5843.
Richardson, R. K. and Ross-Murphy, S. B. (1987) *Int. J. Biol. Macromol.* **9,** 257–264.
Ridgeway, D. (1966) *J. Am. Chem. Soc.* **88,** 1104–1112.
Ridgeway, D. (1968) *J. Am. Chem. Soc.* **90,** 18–22.
Rinaudo, M., Roure, I., Milas, M. and Malovikova, A. (1997) *Int. J. Polym. Anal. Charact.* **4,** 57–69.
Ring, S. G., l'Anson, K. J. and Morris, V. J. (1985) *Macromolecules* **18,** 182–188.
Roberts, G. A.F. and Domszy, J. G. (1982) *Int. J. Biol. Macromol.* **4,** 374–377.
Robinson, G., Ross-Murphy, S. B. and Morris, E. R. (1982) *Carbohyd. Res.* **107,** 17–32.
Rotne, J. and Prager, S. (1969) *J. Chem. Phys.* **50,** 4831–4837.
Roure, I., Rinaudo, M. and Milas, M. (1996) *Ber. Bunsenges. Phys. Chem.* **100,** 703–706.
Roure, I., Rinaudo, M., Milas, M. and Frolini, E. (1998) *Polymer* (in press).
Rowe, A. J. (1977) *Biopolymers* **16,** 2595–2611.
Rowe, A. J. (1992) In *Analytical Ultracentrifugation in Biochemistry and Polymer Science*, ed. S. E., Harding, A. J., Rowe, and J. C. Horton, Chap. 21. Royal Soc. Chem., Cambridge, UK.
Rudin, A. and Wagner, R. A. (1975) *J. Appl. Polym. Sci.* **19,** 3361.
Rupley, J. A. and Careri, G. (1991) *Adv. Prot. Chem.* **41,** 37–172.
Rupley, J. A., Gratton, E. and Careri, G. (1983) *Trends Biochem. Sci.* **8,** 18–22.
Sabater de Sabates, A. (1979) PhD Thesis, University of Paris XI-ENSIA.
Saito, N. (1951) *J. Phys. Soc. Jap.* **6,** 297–301.
Sakurada, I. (1940) *Kasen Koenshu* **5,** 33.
Sakurada, I. (1941) *Kasen Koenshu* **6,** 177.
Samsonova, T. I. and Frenkel, S. Ya. (1958) *Koll. Zh.* **20,** 67–78.
Sato, T., Norisuye, T. and Fujita, H. (1984) *Macromolecules* **17,** 2696–2700.
Savage, A. B. (1965) In *Encyclopedia of Polymer Science and Technology*, ed. H. F., Mark, N. G. Gaylor, and N. M. Bikales, pp. 459-519. Interscience, New York.
Schachman, H. K. (1989) *Nature* **941,** 259–261.
Scheraga, H. A. and Mandelkern, L. (1953) *J. Am. Chem. Soc.* **75,** 179–184.
Schmidt, M. and Burchard, W. (1981) *Macromolecules* **14,** 210.
Schulz, G. V. and Blaschke, F. (1941) *J. Prakt. Chem.* **158,** 130–135.
Schuster, T. M. and Laue, T. M. (1994) eds. *Modern Analytical Ultracentrifugation*. Birkhauser, Boston, USA.
Schwert, G. W. (1951) *J. Biol. Chem.* **190,** 799–806.
Senti, F. R., Hellman, N. N., Ludwig, N. H., Babcock, C. E., Tobin, R., Glass, C. A. and Lamberts, B. L. (1955) **17,** 527-546.
Sharma, M. and Singh, U. S. (1991) *Andrologia*. **23,** 315–319.
Sharman, W. R., Richards, E. L. and Malcolm, G. N. (1978) *Biopolymers* **17,** 2817–2833.
Sharp, P. and Bloomfield, V. A. (1968) *J. Chem. Phys.* **48,** 2149–2155.
Shaw, D. J. (1980) *Introduction to Colloid and Surface Chemistry*. 3rd Edn. Butterworth, London.
Sheehan, J. K. and Carlstedt, I. (1984) *Biochem. J.* **217,** 93–101.
Sheehan, J. K. Carlstedt, I. (1989) In *Dynamic Properties of Biomolecular Assemblies*, ed. S. E. Harding, and A. J. Rowe, Chap. 17. Royal Soc. Chem., Cambridge, UK.
Shinagawa, S., Kameyama, K. and Takagi, T. (1993) *Biochim. Biophys. Acta* **1161,** 79–84.

Shulman, S. (1953) *J. Am. Chem. Soc.* **75,** 5846–5852.
Shulman, S., Alkjaersig, N. and Sherry, S. (1958) *J. Biol. Chem.* **233,** 91–97.
Simha, R. (1940) *J. Phys. Chem.* **44,** 25–34.
Smidsrød, O. (1970) *Carbohyd. Res.* **13,** 359–372.
Smidsrød, O. and Andresen, I. L. (1979) *Biopolymerkjemi.* Tapir, Trondheim, Norway.
Smidsrød, O. and Haug, A. (1971) *Biopolymers* **10,** 1213–1227.
Snoeren, T. H.M., Van Markwizk, B. and Van Montfort, R. (1980) *Biochim. Biophys. Acta* **622,** 268–276.
Solomon, O. F. and Ciuta, I. Z. (1962) *J. Appl. Polym. Sci.* **6,** 683–686.
Solomon, O. F. and Gotesman, B. S. (1967) *Makromol. Chem.* **104,** 177–184.
Sophianopoulos, A. J., Rhodes, C. K., Holcomb, D. N. and Van Holde, K. E. (1962) *J. Biol. Chem.* **237,** 1101–1112.
Sousa, I. M.N., Morgan, P. J., Mitchell, J. R., Harding, S. E. and Hill, S. E. (1996) *J. Agric. Food Chem.* **44,** 3018–3021.
Spiro, R. G. (1960) *J. Biol. Chem.* **235,** 2860–2869.
Spotorno, B., Piccinini, L., Tassara, G., Ruggiero, C., Nardini, M., Molina, F. and Rocco, M. (1997) *Eur. Biophys. J.* **25,** 373–384.
Stein, P. E., Leslie, A. G.W., Finch, J. T. and Carrel, R. W. (1991) *J. Mol. Biol.* **221,** 941–959.
Stellwagen, E. and Schachman, H. K. (1962) *Biochemistry.* **1,** 1056–1069.
Stivala, S. S., Herbst, M., Kratky, O. and Pilz, I. (1968) *Arch. Biochem. Biophys.* **127,** 795–802.
Stockmayer, W. H. and Fixman, M. (1963) *J. Polym. Sci.* **C1,** 137–141.
Stokke, B. T. and Elgsaeter, A. (1981) *Biochim. Biophys. Acta.* **640,** 640–645.
Stokke, B. T. and Elgsaeter, A. (1994) *Micron* **25,** 469–491.
Stokke, B. T., Elgsaeter, A. and Smidsrød, O. (1989a) *Biopolymers* **28,** 617–637.
Stokke, B. T., Elgsaeter, A. and Smidsrød, O. (1989b) *Am. Chem. Soc. Symp. Ser.* **396,** 145–156.
Sugihara, T. (1983) *Inhibition of Lysozyme by Detergents.* Research report of Gifer University, Japan.
Squire, P. G. and Himmel, M. E. (1979) *Arch. Biochem. Biophys.* **196,** 165–177.
Swanson, E., Teller, D. C. and de Haen, C. (1980) *J. Chem. Phys.* **72,** 1623–1628.
Szuchet-Derechin, S. and Johnson, P. (1966) *Eur. Polym. J.* **2,** 115–128.
Tanford, C. (1955) *J. Phys. Chem.* **59,** 798–799.
Tanford, C. (1961) *Physical Chemistry of Macromolecules*, Chap. 6. John Wiley & Sons, Inc., New York.
Tanford, C. (1968) *Adv. Prot. Chem.* **23,** 121–282.
Tanford, C. (1980) *The Hydrophobic Effect: Formation of Micelles and Biological Membranes*, 2nd, edn. John Wiley and Sons, Inc., New York.
Tanford, C. and Buzzell, J. G. (1956) *J. Phys. Chem.* **60,** 225–231.
Tanford, C., Kawahara, K. and Lapanje, S. (1967) *J. Am. Chem. Soc.* **89,** 729–736.
Tashiro, Y., Mochizuki, Y., Ogawa, H., Mizuno, H. and Iso, N. (1996) *Fisheries Science* **62,** 80–83.
Terbojevich, M., Peggion, E., Cosani, A., D'Este, G. and Scoffone, E. (1967) *Eur. Polym. J.* **3,** 681–689.
Townend, R., Weinberger, L. and Timasheff, S. N. (1960) *J. Am. Chem. Soc.* **82,** 3175–3179.
Tsao, T.-C., Bailey, K. and Adair, G. S. (1951) *Biochem. J.* **49,** 27–36.
Tsuda, K. (1969) *Bull. Chem. Soc. Japan* **42,** 850.
Tsuda, K. (1970a) *Rheol. Acta* **9,** 509–516.
Tsuda, K. (1970b) *Polym. J.* **1,** 616–631.
Tsvetkov, V. N., Eskin, V. E. and Frenkel, S. Ya. (1971) *Structure of Macromolecules in Solution* (translated from Russian). Volume 1, Chapter 2. National Lending Library for Science and Technology, Boston, U.K.
Ubbelohde, L. (1936) *Oel und Kohle* **42,** 949–974.
Uda, K. and Meyerhoff, G. (1961) *Makromol. Chem.* **47,** 168–184.
Van Holde, K. E. (1985) *Physical Biochemistry* (2nd edn), Chap. 7. Prentice Hall, Englewood Cliffs, New Jersey, USA.
Van Holde, K. E. and Cohen, L. B. (1964) *Biochemistry* **3,** 1803–1808.
Van Kleef, F. S. M., Boskamp, J. V. and Van den Tempel, M. (1978) *Biopolymers* **17,** 225–235.
Veis, A. (1964) *The Macromolecular Chemistry of Gelatin.* Academic Press, New York.
Vidakovic, P., Allain, C. and Rondelez, F. (1982) *Macromolecules* **15,** 1571–1580.
Wales, M. and Van Holde, K. E. (1954) *J. Polym. Sci.* **14,** 81–86.
Wang, W., Bo, S., Li, S. and Qin, W. (1991) *Int. J. Biol. Macromol.* **13,** 281–285.
Ward, A. G. and Saunders, P. R. (1958) In *Rheology* ed. F. R. Eirich, Vol. II, pp. 313. Academic Press, New York.
Wegener, W. A. (1985) *Macromolecules* **18,** 2522–2530.
Whittington, S. G. and Glover, R. M. (1972) *Macromolecules* **5,** 55–58.
Wilson, R. W. and Bloomfield, V. A. (1979a) *Biopolymers* **18,** 1205–1211.
Wilson, R. W. and Bloomfield, V. A. (1979b) *Biopolymers* **18,** 1543–1549.
Wirick, M. G. (1968) *J. Polym. Sci.* **A1**(6), 1965–1974.
Woodward, J. R., Phillips, D. R. and Fincher, G. B. (1983) *Carbohyd. Polym.* **3,** 143–156.
Wyatt, P. J. (1992) In *Laser Light Scattering in Biochemistry*, ed. S. E., Harding, D. B. Sattelle, and V. A. Bloomfield, Chap. 3. Royal Soc. Chem., Cambridge, UK.
Wyman, J., Jr. and Ingalls, E. N. (1943) *J. Biol. Chem.* **147,** 297–318.
Yamakawa, H. (1970) *J. Chem. Phys.* **53,** 436–443.
Yamakawa, H. (1971) *Modern Theory of Polymer Solutions.* Harper and Row, New York.
Yamakawa, H. (1977) *Macromolecules* **10,** 692–696.
Yamakawa, H. (1984) *Ann. Rev. Phys. Chem.* **35,** 23–47.
Yamakawa, H. and Fujii, M. (1976) *J. Chem. Phys.* **64,** 5222–5228.
Yamakawa, H. and Yoshizaki, T. (1980) *Macromolecules* **13,** 633–643.
Yamakawa, H., Yoshizaki, T. and Fujii, M. (1977) *Macromolecules* **10,** 934–943.

Yanaki, T., Norisuye, T. and Fujita, H. (1980) **13**, 1462-1466.
Yanaki, T., Kojima, T. and Norisuye, T. (1981) *Polym. J.* **13,** 1135–1143.
Yang, J. T. (1958a) *J. Am. Chem. Soc.* **80,** 1783–1788.
Yang, J. T. (1958b) *J. Am. Chem. Soc.* **80,** 5139–5146.
Yang, J. T. (1961) *Adv. Protein Chem.* **16,** 323–400.
Yguerabide, J., Epstein, H. and Stryer, L. (1970) *J. Mol. Biol.* **51,** 573–590.
Zhou, H.-X. (1995) *Biophys. J.* **69,** 2298–2303.
Zimm, B. H. (1956) *J. Chem. Phys.* **24,** 269–278.