MODELLING BIOLOGICAL MACROMOLECULES IN SOLUTION:

THE GENERAL TRI-AXIAL ELLIPSOID

by

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To Anne
'... God, in the beginning form'd Matter in solid, massy, hard, impenetrable, moveable particles ...'

Abstract

Hydrodynamic shape functions for modelling biological macromolecules in solution in terms of an ellipsoid of revolution model are reviewed. Several new, hitherto unpublished shape functions whose experimental determination does not require knowledge of the swollen molecular volume in solution, are given. The limitations and inadequacies of this model are explained. The viscosity increment $\nu$ for a dilute dispersion of tri-axial ellipsoids of semi-axes $a > b > c$, under dominant Brownian motion is derived and an explicit expression in terms of $a$, $b$ and $c$ is given. Knowledge of the viscosity increment alone is not sufficient to uniquely determine the axial ratios $(a/b, b/c)$ because (i) in order to determine $\nu$, knowledge of the swollen volume in solution is required and (ii) a particular value for $\nu$ has a line solution of possible values for $(a/b, b/c)$. (i) is dealt with by combining $\nu$ with the tri-axial frictional ratio function $P$ to give the tri-axial $R$ function and (ii) by combining graphically the $R$ line solution with $\delta_+$ and $\delta_-$ swelling independent line solutions. The experimental determination of $\delta_+$ and $\delta_-$ requires the resolution of a 2-term electric birefringence decay into its component relaxation times; current data analysis techniques are however not satisfactory for resolving close relaxation times (as for globular proteins) with the current experimental precision. It is however shown by exhaustive computer simulation that using a new $R$-constrained non-linear least squares iterative analysis this is now possible. It is thus concluded that the general tri-axial ellipsoid as a model for the gross conformation of biological macromolecules in solution can now be employed.
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PREFACE
Preface

There are two basic approaches for determining the gross conformation of a biological macromolecule in solution. One is to assume a structure (generally an array of spheres of varying sizes) and then calculate its hydrodynamic properties, for example the intrinsic viscosity, sedimentation coefficient, translational diffusion coefficient, and then see how much these predicted properties differ from the experimentally determined properties for the unknown structure. The model is then successively changed or 'refined' until the predicted properties converge to agree with the actual properties. This method has been developed by Bloomfield, Garcia de la Torre and co-workers (Bloomfield et al, 1967, Garcia de la Torre & Bloomfield, 1977a,b,c, 1978, Wilson & Bloomfield, 1979a,b, Garcia Bernal & Garcia de la Torre, 1980). There is however a serious drawback in that the final calculated structure may not be the only one which gives these properties.

The alternative approach is to calculate the structure directly from the known hydrodynamic properties. Some general model must of course be assumed, but although the models available from this approach are less precise (the most general before the commencement of this study being an ellipsoid with two equal axes) it does not suffer from the uniqueness problem. This approach was first developed by Stokes (1851, 1880) in terms of a simple spherical model calculated from the translational frictional property and the rotational frictional property and again for a spherical model by Einstein (1906 - with a correction in 1911) from the viscosity property. Although the current state of theoretical, experimental and data analysis techniques allows use of the '2 equal axes' ellipsoid ("ellipsoid of revolution"), it is clear from a simple perusal of
crystallographic models that for many biological structures this model is a very poor approximation to the true structure.

The aim of this thesis is thus twofold: the first is to review all the current ellipsoid of revolution shape functions (in which some new, hitherto unpublished functions are given) and the second is to develop the current theoretical and data analysis techniques to show that with current experimental precision the restriction of two equal axes on the ellipsoid model can now, in principle at least, be dispensed with to allow use of the more general "tri-axial ellipsoid" model.

I would like to take this opportunity to express my deepest gratitude to Dr. A.J. Rowe for his expert guidance and supervision during the course of this study.

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CHAPTER 1

The Mass, Size and Shape of Macromolecules in Solution: The Ellipsoid of Revolution Model
1.1. **Macromolecular Structure in Solution**

The concept of a unique structure for a biological macromolecule in solution and in crystallized form has only relatively recently been established beyond dispute. Prior to the work of Svedberg the view was commonly taken (Sorensen, 1930) that proteins and other macromolecules exist in solution not as unique structures but as dissociable complexes containing possibly several components, that the equilibrium state was dependent on circumstances (for example the composition of the solution) and any components precipitated are not necessarily to be identified with those occurring in solution. Researchers were consequently surprised at the ultracentrifuge results of Svedberg and his co-workers (Svedberg & Pedersen, 1940) which strongly suggested the molecular homogeneity of many protein systems. Thus, in striking contrast to the polydispersity of many polymer systems (such as carbohydrates, rubber or polystyrene) it was deduced that carefully prepared protein solutions contain one, or at the most a few, different molecular species. This deduction was derived mainly from the observation that boundary spreading observed in the sedimentation of protein solutions could be identified with separately measured translational diffusion coefficients. Bresler and Talmud (1944) suggested however that a monodisperse protein really contains a distribution of molecular weights with a sharply defined maximum. This surmise is, on the other hand, strongly opposed by the immunological properties of proteins (Alexander & Johnson, 1949) together with the overwhelming evidence now available from protein crystallography (Kendrew et al, 1958, Perutz et al, 1960, Blake et al, 1965, Feldman, 1976) which support the idea of discrete individual structures.
X-ray crystallography is by far the most accurate method for determining these structures. Unfortunately this technique is also the most laborious, requiring several researchers working for a period of months to determine the structure of a single globular protein. The calculated structures are also of the 'fossilized' form of the macromolecule which may not necessarily be the same in solution. There are many techniques available, such as nuclear magnetic resonance, electron spin resonance, fluorescence and other spectroscopic techniques which can give much detailed information about the dynamic properties of localized regions of macromolecules in solution (for example, the active sites of enzymes are being extensively studied). These techniques cannot however give information as to the overall macromolecular mass, size and shape. For this one needs to consider the hydrodynamic properties of solutions of the macromolecule (although scattering phenomena can also give useful information), which allows determination of the molecular weight, simple 'hydrodynamically equivalent' mathematical models for the structure and also the size (including the swelling due to solvent association) of the macromolecule.

1.1.1. Mass

The 'inertial mass' of a body can be defined as the quantity of matter in it, or as the ratio of the force applied to its acceleration (Newton's 2nd Law of Motion). For a macromolecule we conveniently express the mass by the 'Molecular Weight' \( M_r \) which is defined as the ratio of the mass of the macromolecule to that of one sixteenth of an oxygen \( O_{16} \) atom, and is expressed in grams.

The mass of fluid displaced by a macromolecule in a solution will
equal the product of the volume displaced and the density of the solution
\[(\frac{M_r \bar{v}}{N_A})\rho_0\], where \(M_r\) is the molecular weight, \(N_A\) Avogadro's number, \(\rho_0\) the
solution density and \(\bar{v}\) the partial specific volume of the macromolecule,
i.e. the volume increase when unit mass (generally one gram) of solute is
added to an infinite volume of the solvent at constant temperature and
pressure

\[\bar{v} = \left[\frac{3\bar{v}}{3m}\right]_{T,P}\]  

(1)

The 'Archimedean mass' (i.e. the buoyant mass) of a macromolecule
(Van Holde, 1971) in solution will equal the true mass minus the mass of
the fluid displaced:

\[M_r = \frac{M_r}{N_A} - \left[\frac{M_r}{N_A} \bar{v}\right] \rho_0 = \frac{M_r}{N_A} (1 - \bar{v} \rho_0)\]  

(2)

The molecular weight of a macromolecular solute can be measured from many
methods, for example sedimentation velocity and translational diffusion,
osmosis, light or x-ray scattering, or most precisely from a sequence
analysis. A recent review of these methods is given by Rowe (1978).
The partial specific volume can be found either from a concentration
determination followed by a densimetric analysis (Kratky et al, 1969,
1973), or for a protein, from Traube's rule (Rowe, 1978). This rule may
possibly also be applicable to nucleic acids (Pearce et al, 1975).

1.1.2. Size

The size of a rigid macromolecule in solution will differ from that
in the anhydrous state because of associated solvent. The hydrodynamic
or swollen specific volume \(\bar{v}_s\), will now comprise of the partial specific
volume, $\bar{v}$, the bound solvent that adheres to the hydrophilic particle surface, and 'entrained’ solvent which may be trapped in the various cavities and indentations in the macromolecule (Figure 1). The ratio $\bar{v}_s / \bar{v}$ is known as the 'swelling' of the macromolecule and is equal to unity if the macromolecule is anhydrous and compact in solution. The swollen specific volume can be simply related to the "effective" hydrodynamic volume $V_e$ i.e. the swollen volume of a single macromolecule in a homogeneous solution:

$$V_e = \frac{\bar{v}_s \cdot M_r}{N_A}$$

(3)

1.1.3. Shape

Owing to the difficulties in developing theoretical relationships between the shape of a macromolecule and experimentally measurable parameters, only rather simple 'hydrodynamically equivalent' models are currently available, the boundaries of which can be described by a simple mathematical equation; these are (Figure 2) rods, discs and ellipsoids of revolution (Tanford, 1961).

An ellipsoid of revolution is formed by rotating an ellipse either about the major axis (prolate ellipsoid) or about the minor axis (oblate ellipsoid) and thus has the necessary restriction that two of the three axes must be equal. In the limit of large axial ratio, a prolate ellipsoid (2 minor axes, 1 major) becomes a good approximation to a rod whilst an oblate ellipsoid (2 major axes, 1 minor) becomes a good approximation to a disc. Consequently, physical biochemists have tended to use the ellipsoid of revolution model to determine the hydrodynamically equivalent shape of a rigid macromolecule in solution.

It should be made clear at this stage that many macromolecules cannot be modelled by any of these rigid structures as they have no preferred
structure in solution: these 'randomly coiled' macromolecules can only be represented by probability configurations. Many other macromolecules have a well defined rigid structure but cannot be reasonably modelled, judging from the x-ray models at least, by any ellipsoid. The L-shaped Transfer RNA molecule is an outstanding example (Kim, 1974).

1.2. The Hydrodynamic Properties of a Macromolecular Solution

The hydrodynamic properties of a macromolecular solution, which are used to determine these structures, can be conveniently divided into three broad classes:

(i) The viscosity property, which concerns the effect of the dissolved macromolecule on the bulk motion of the fluid when a shear gradient is applied.

(ii) The translational frictional property, which concerns the movement of the macromolecule through its solution when some form of external force is applied. This can be a centrifugal field in a sedimentation experiment or a concentration gradient (i.e. a gradient of chemical potential) in a translational diffusion experiment.

(iii) The rotational frictional property, which concerns the disorienting effect on the macromolecule by the local Brownian motion of the surrounding solvent molecules.

1.3. The Viscosity Property of a Macromolecular Solution

The viscosity of a fluid is a measure of its resistance to flow and may be simply defined for a simple shearing flow* (Figure 3) in terms of the shearing stress $\sigma$ and the shear rate $G$:

$$\sigma = nG$$

* For the equations describing a more general flow see Batchelor (1967).
where \( \eta \) is known as the viscosity coefficient. If \( \eta \) is a proportionality constant independent of the shear rate the fluid is said to be Newtonian. However, if the constituent molecules show preferred orientations, this will alter the retarding forces between adjacent fluid elements and hence the internal friction or viscosity coefficient. This non-Newtonian effect will occur in solutions containing highly asymmetric or easily deformable molecules and at high shear rates (Batchelor, 1967); this forms the basis of flow birefringence experiments (see 1.5.3). For characterizing the macromolecule in solution we can set the conditions (i.e. very low shear rates) so that the Newtonian condition prevails, whereas the chemical engineer would be more interested in the general flow properties.

Using equation (4) we can simply relate the viscosity coefficient to the energy dissipation during flow. Writing \( \sigma \) as a tangential force per unit area \((F/A)\) and the shear rate as the velocity gradient \((\frac{dx}{dt})/\Delta y\):

\[
\frac{F}{A} = \eta \frac{1}{\Delta y} \frac{dx}{dt}
\]

Multiplying both sides by \( G \):

\[
\frac{F \ dx}{A \Delta y \ dt} = \eta \ G^2
\]

Since \( A \Delta y \) is the volume of the element under consideration, then

\[
\langle \frac{dW}{dt} \rangle = G^2 \eta
\]

(5)

where \( \langle dW/dt \rangle \) is the mean energy dissipated per unit volume.

The effect of dissolved or suspended macromolecules which are assumed to occupy a volume \( \phi \) of fluid, is to disturb the streamlines of the fluid motion and to reduce the volume of the fluid in which the same overall deformation takes place. Thus the internal friction, the viscosity
coefficient and hence the energy dissipated is increased. This increase can be represented by:

\[ \langle \frac{dW}{dt} \rangle_{\text{inc}} = G^2 (\eta - \eta_0) = G^2 \eta_0 v^\phi \]  

(6)

where \( \eta \) is the viscosity coefficient of the solution and \( \eta_0 \) that of the solvent. Rewriting:

\[ \eta = \eta_0 \left( 1 + v^\phi \right) \]  

(7)

Here \( v \) is defined as the viscosity increment and is a function of the shape of the particle. Again, rewriting equation (7):

\[ \frac{\eta}{\eta_0} - 1 = \eta_{\text{sp}} = v^\phi \]

(8)

where \( \eta_{\text{sp}} \) is the specific viscosity. This equation only applies to an infinitely dilute solution in which no solute-solute interactions occur. For finite concentrations:

\[ \eta_{\text{sp}} = v^\phi + v_1^\phi^2 + v_2^\phi^3 + \ldots \ldots \]

or, replacing \( \phi \) by \( c \bar{V}_s \), where \( c \) is the concentration and \( \bar{V}_s \) the swollen specific volume:

\[ \eta_{\text{red}} = \frac{\eta_{\text{sp}}}{c} = c \bar{V}_s v_1^\phi^2 + c \bar{V}_s v_2^\phi^3 c + \ldots \ldots \]

where \( \eta_{\text{red}} \) is the reduced specific viscosity. As the concentration approaches zero, \( \eta_{\text{red}} \) tends to a limiting value, known as the intrinsic viscosity, [\( \eta \)]. This can therefore be found by extrapolating a plot of \( \eta_{\text{red}} \) versus concentration to infinite dilution, and, if the swollen specific volume, \( \bar{V}_s \) is known (section 1.1.2.), \( v \) can also be found:

\[ v = \frac{[\eta]}{\bar{V}_s} = \frac{[\eta]M}{\bar{V}_s e N_A} \]  

(8)
An approximate value for $\nu$ can be estimated for 'globular' proteins by using the partial specific volume $\bar{\nu}$ and assuming that $\bar{\nu}_g/\bar{\nu}$ is $\sim 1.4$ for globular proteins. A full review of the experimental techniques for determining the intrinsic viscosity, $[\eta]$ is given by Yang (1961).

Einstein (1906, 1911) was the first to determine an explicit value for $\nu$ for a specific particle shape, i.e. a sphere, by solving the equations of motion for the flow using spherical harmonics. His assumptions were:

(i) the particles are large enough compared to the solvent molecules so that the surrounding fluid can be regarded as a continuum and Euler's (Batchelor, 1967) equations concerning the change of flow through specific volume elements rather than the complicated Lagrange equations for particle motion can be used,

(ii) the dimensions of the particles are however considered very much less than the spatial variations in the velocity flow field,

(iii) the flow rates are small enough so that squared terms concerning the velocity (and hence normal stress effects) can be neglected and that the inertia or mass forces can be neglected.

Using these assumptions and considering the increase in the average dissipation of energy per unit volume, he found that $\nu = 2.5$, and was independent of particle size. This result has been confirmed experimentally for polystyrene latex spheres by Cheng & Schachman (1955).

Jeffrey (1922) attempted to extend this theory to find $\nu$ as a function of axial ratio for ellipsoids of revolution, using ellipsoidal harmonics to solve the equations for the fluid flow. Owing to the non-isotropic nature of ellipsoids, the hydrodynamic torques on the ellipsoids were shown to have two effects:
(a) the first effect tends to make the particle rotate on average with the local undisturbed angular velocity of the fluid,
(b) the second effect tends to orient the minor axis parallel to the flow for prolate ellipsoids and perpendicular to the flow for oblate ellipsoids. As a result, the fluid is no longer isotropic and an energy dissipation analysis fails to give a unique value for the axial ratio for a given value of $\nu$ (Brenner, 1972a). However, if the particles are sufficiently small the randomising effect of the Brownian motion of the surrounding solvent molecules counteracts the orientational tendency of the hydrodynamic torque (b) so that the particles are randomly oriented (Simha, 1940) and rotate on average with the local angular velocity of the fluid. The solution is then statistically isotropic, allowing an energy dissipation analysis to be used to obtain an unambiguous solution for $\nu$ in terms of the axial ratio for prolate and oblate ellipsoids of revolution. Simha (1940) was thus able to obtain a formula which has been shown to give good agreement with experiment (Mehl, Oncley & Simha, 1940):

\[
\nu = \frac{1}{ab^2} \left\{ \frac{2a''}{15b^2a'_0 b'_0} + \frac{7}{15b^2a'_0} + \frac{2}{5} \left( \frac{\beta'_0 (a^2 + b^2) + 2\beta''_0}{\beta'_0 [2a^2b^2 \beta'_0 + (a^2 + b^2) \beta''_0]} \right) \right\}
\]

where $a, b, b$ are the three semi-axes of the ellipsoid ($b > a$ for oblate and $b < a$ for prolate), and the $a'_0$ etc. which depend on $a$ and $b$ are elliptic integrals given by Jeffrey (1922) (Appendix I). This relation could be solved numerically for both cases and a table of values for $\nu$ as a function of axial ratio was given by Mehl, Oncley & Simha (1940).
The function is plotted in Figure 4. In the limit of large axial ratio $p (=b/a)$

$$v \sim \frac{1}{15(\ln(2/p)-3/2)} + \frac{1}{5(\ln(2/p)-1/2)} + \frac{14}{15} \quad \text{(prolate)}$$

$$v \sim \frac{16}{15} \tan^{-1}(p) \quad \text{(oblate)}$$

These formulae agree with the independent derivations of Kuhn & Kuhn (1945) and Kirkwood (1967).

Simha apparently did not assume that the particles were on average rotating with the local angular velocity of the fluid but with zero angular velocity. This objection was raised by Saito (1951) who however obtained exactly the same result (equation 9) despite assuming particles on average rotating with the same local angular velocity of the fluid. He suggested that Simha "probably made some error in his calculation" without actually finding it. We will show in the next Chapter that Simha had apparently arrived at the correct result by making the wrong assumption and then missing out a whole series of terms in his calculation.

1.4. The Translational Frictional Property of Macromolecular Solutes

The ease with which a macromolecule moves through its solution under the influence of an applied external force field will depend on its shape and size. The coefficient generally used to describe this ease is the frictional coefficient, $f$, defined as the ratio of the frictional force to the terminal velocity of the particle. Stokes (1851) using spherical harmonics again and assumptions similar to Einstein's (section 1.3) derived the well-known relation between the frictional coefficient $f$ and...
the radius \( R \) of a spherical particle:

\[ f = 6\pi \eta_0 R \] (11)

where \( \eta_0 \) is the viscosity of the solvent. Perrin (1936) and independently Herzog, Illig and Kudar (1934) extended Stokes equation to cover the case of general ellipsoidal particles:

\[ f = \frac{2}{(abc)^{1/3}} \int_0^\infty \frac{d\lambda}{[(a^2+\lambda)(b^2+\lambda)(c^2+\lambda)]^{1/2}} \] (12)

where \( f_0 \) is the corresponding coefficient for a sphere of the same volume:

\[ f_0 = 6\pi \eta_0 (abc)^{1/3} = 6\pi \eta_0 \left( \frac{3V_e}{4\pi} \right)^{1/3} \] (13)

\( V_e \) is the molecular swollen volume, defined in section 1.1.2. The integral in equation (12) is elliptic and could only be solved for the special case of ellipsoids of revolution. For prolate ellipsoids (\( p = (b/a) < 1 \)):

\[ \frac{f}{f_0} = \frac{1 - p^2}{p^{2/3} \tan^{-1} \left( \frac{p^2 - 1}{2} \right)} \] (14a)

and for oblate ellipsoids (\( p = (b/a) > 1 \))

\[ \frac{f}{f_0} = \frac{p^2 - 1}{p^{2/3} \tan^{-1} \left( \frac{p^2 - 1}{2} \right)} \] (14b)

and can easily be plotted as a function of axial ratio (Figure 5). The translational frictional ratio \( f/f_0 \) can be measured experimentally either from a translational diffusion experiment, where the driving force is a concentration gradient, or from ultracentrifugation, where the driving force is a centrifugal field.
1.4.1. Translational Diffusion

The translational diffusion coefficient, $D$, is related to the frictional coefficient, $f$, at a particular particle concentration, $c$, by the relation:

$$D_c = \frac{kT}{f} \left\{ 1 + c \frac{\partial \gamma}{\partial c} \right\}$$

(van Holde, 1971), where $\gamma$ is the 'activity coefficient', a measure of the concentration gradient. Extrapolating $D_c$ to infinite dilution gives the Einstein relation (Einstein 1905, Tanford, 1961):

$$D = \frac{kT}{f}$$

(16)

By assuming the concentration gradient to be in one direction only, and applying Fick's laws (Tanford, 1961) for a two-component system, a simple relation for finding $D$ experimentally can be derived, in terms of the area under, $A$, and the maximum height of, $H$, a concentration gradient $(dc/dx)$ versus distance ($x$) curve:

$$\left( \frac{A}{H} \right)^2 = 4\pi D_c t$$

Thus a plot of $(A/H)^2$ versus time, $t$, in a 'free diffusion of a sharp boundary experiment' will give $D_c$ from the gradient (Tanford, 1961, van Holde, 1971). $D_c$ can be extrapolated to infinite dilution after repeating the procedure for several solute concentrations. Unfortunately, few laboratories have the apparatus required for an accurate determination of $D$ using this method. A fast and accurate method for determining diffusion coefficients has been developed using quasi-elastic laser light scattering (Chu, 1974, Cummins & Pike, 1973, Berne & Pecora, 1974); the fluctuations of solute particles from the equilibrium state are a function of the diffusion...
coefficients and with adequate instrumentation for signal analysis can be time-resolved.

From equation (16), the frictional ratio can be found from the translational diffusion coefficient using the relation

$$\frac{f}{f_0} = \frac{D_o}{D}$$

(17)

where $D_o$ is the translational diffusion coefficient for a sphere of the same volume and molecular weight:

$$D_o = \frac{kT}{f_o} = \frac{kT}{6\pi\eta_o}\left(\frac{4\pi}{3Ve}\right)^{1/3}$$

(18)

1.4.2. Sedimentation Velocity

In a sedimentation velocity experiment, using an analytical ultracentrifuge (van Holde, 1971), the macromolecules quickly attain the terminal velocity, whence

$$\frac{M_r}{N_A}(1 - \vec{v}\rho_o)\omega^2r = f \frac{dr}{dt}$$

where $\rho_o$ is the solution density, $r$ the distance from the centre of rotation of the solution/solvent boundary, $\omega$ the speed of rotation and $M_r(1 - \vec{v}\rho_o)/N_A$ the 'buoyant mass' defined in section 1.1.1. Rearranging:

$$\frac{M_r(1 - \vec{v}\rho_o)}{N_A f} = \frac{dr/dt}{\omega^2r} = s_c$$

(19)

where $s_c$ is the sedimentation coefficient at a particular solute concentration. In a sedimentation velocity experiment the movement of the boundary between solution and solvent is monitored as a function of time using the property of change of refractive index with change in
concentration, hence optical techniques such as scanning Schlieren optics or ultra-violet absorption can be used (Lloyd, 1974). If we rearrange and integrate equation (19) we find that

\[ s_c = \frac{1}{\omega^2} \frac{\Delta \ln r}{\Delta t} \]

thus by plotting \( \log r \) versus \( t \) and knowing the angular velocity \( \omega \), \( s_c \) can be found from the gradient. The sedimentation coefficient \( s_c \) is a function of solute concentration, thus is normally extrapolated to infinite dilution to give the sedimentation coefficient, \( s \), which is characteristic of any macromolecular solute. From equation (19) it can be seen that the frictional ratio \( f/f_o \) will be given by

\[ \frac{f}{f_o} = \frac{s_o}{s} \]

where \( s_o \) is the sedimentation coefficient for a compact sphere of the same molecular weight and volume. From equations (19) and (13):

\[ s_o = \frac{M_T (1 - \bar{v} \rho_o)}{N_A f_o} = \frac{M_T (1 - \bar{v} \rho_o)}{N_A 6\pi \eta_o} \cdot \left( \frac{4\pi}{3V_o} \right)^{1/3} \]

and thus the frictional ratio can be found, provided \( s, M_T, \bar{v}, \rho_o, \eta_o \) and the swollen molecular volume, \( V_o \) are known:

\[ \frac{f}{f_o} = \frac{M_T (1 - \bar{v} \rho_o)}{N_A 6\pi \eta_o s} \left( \frac{4\pi}{3V_o} \right)^{1/3} \]

1.5. The Rotational Frictional Property of Macromolecular Solutes

The ability of a macromolecule to rotate under the influence of the local Brownian motion of the neighbouring solvent molecules will depend on its size and shape. By analogy with the translational frictional
coefficient, we can define, for rotation about a specific particle axis, a rotational frictional coefficient, \( \zeta_i \), as the torque which must be applied to cause the particle to rotate with unit angular velocity. For a general ellipsoidal particle there will be three rotational frictional coefficients corresponding to rotation about each of the three axes; for an ellipsoid of revolution there will be two, and for a spherical particle, one. Each rotational frictional coefficient can be related to a rotational diffusion constant by analogy with the Einstein relation (1905) (equation 16):

\[
\theta_i = \frac{kT}{\zeta_i}
\]

(21)

where \( \theta_i \) is defined as the ratio of the mean squared angular displacement of the axis to the time elapsed (Tanford, 1961). In a typical rotational frictional experiment an initial orientation of the macromolecule is produced by some external field. If, for example, the macromolecules in a solution are oriented with their "a" axis parallel to an orienting field and the field is suddenly removed, the macromolecules will then relax due to the Brownian motion and tend to assume a random configuration by rotating about the b and c axes. We therefore conveniently define a rotational relaxation time in terms of the rotational diffusion constants (\( \theta_a, \theta_b, \theta_c \) about the b, c axes respectively) by

\[
\theta_a = \frac{1}{\theta_c + \theta_b}
\]

(22a)

There will be similar relations describing relaxation of the b and c axes:

\[
\theta_b = \frac{1}{\theta_c + \theta_a} \quad ; \quad \theta_c = \frac{1}{\theta_a + \theta_b}
\]

(22b,c)
By analogy with the translational frictional case, Stokes (1880) using spherical harmonic solutions of the equations of motion with the boundary condition that the fluid in contact with the particle rotates with the same angular velocity (i.e. the 'no-slip' condition) derived an equation linking the rotational frictional coefficient with its radius, R:

\[ \zeta = 8\pi \eta_0 R^3 \]  

(23)

Edwardes (1893) extended this equation to the case of general ellipsoidal particles. After a correction for a numerical error (Perrin, 1934), these are:

\[ \zeta_a = \frac{16\pi \eta_0}{3} \frac{b^2 + c^2}{b^2 b_0 + c^2 c_0} \]

\[ \zeta_b = \frac{16\pi \eta_0}{3} \frac{c^2 + a^2}{c^2 c_0 + a^2 a_0} \]

\[ \zeta_c = \frac{16\pi \eta_0}{3} \frac{a^2 + b^2}{c^2 c_0 + a^2 a_0} \]  

(24)
where again the \( a \), etc. are elliptic integrals defined by Jeffrey (1922) - see Appendix I. The expressions on the right hand side of equations (24) are functions not only of shape but of volume as well; the corresponding rotational frictional ratios however, are not.

\[
\frac{\tau_a}{\tau_o} = \frac{\theta_a}{\theta_o} = \frac{2}{3abc} \frac{b^2 + c^2}{b^2 b_o + c^2 \gamma_o}
\]

\[
\frac{\tau_b}{\tau_o} = \frac{\theta_b}{\theta_o} = \frac{2}{3abc} \frac{c^2 + a^2}{c^2 \gamma_o + a^2 a_o}
\]

\[
\frac{\tau_c}{\tau_o} = \frac{\theta_c}{\theta_o} = \frac{2}{3abc} \frac{a^2 + b^2}{a^2 a_o + b^2 b_o}
\]

(25)

where \( \tau_o = 8 \pi \eta_o abc \) & \( \theta_o = kT/\tau_o \) are the corresponding coefficients for spheres of the same volume, and can be found experimentally only if the swollen molecular volume, \( V_e \) is known:

\[
\tau_o = 6\eta_o V_e \Rightarrow \theta_o = kT/6\eta_o V_e
\]

(26a,b)

The corresponding rotational relaxation time ratios are:

\[
\frac{\theta_a}{\theta_o} = \frac{2}{\left[ \frac{\theta_b}{\theta_o} + \frac{\theta_c}{\theta_o} \right]}
\]

\[
\frac{\theta_b}{\theta_o} = \frac{2}{\left[ \frac{\theta_c}{\theta_o} + \frac{\theta_a}{\theta_o} \right]}
\]

\[
\frac{\theta_c}{\theta_o} = \frac{2}{\left[ \frac{\theta_a}{\theta_o} + \frac{\theta_b}{\theta_o} \right]}
\]

(27a)

where \( \theta_o = 1/2 \theta_o \).

(27b)
Unfortunately, as for the translational frictional coefficients, the elliptic integrals could only be solved analytically for the special case of ellipsoids of revolution of semi-axes $a, b=c$ (Gans, 1928, Perrin, 1934). Although numerically equivalent, Gans uses the less manageable 'eccentricity' ($\epsilon = 1 - b/a$) rather than the axial ratio ($p = b/a$), hence the equations of Perrin are generally used:

$$\frac{\tau_a}{\tau_o} = \frac{\theta_o}{\theta_a} = \frac{2}{3} \frac{(1 - p^2)}{(1 - p^2 S)}$$

$$\frac{\tau_b}{\tau_o} = \frac{\theta_o}{\theta_b} = \frac{2}{3} \frac{(1 - p^4)}{p^2 [S(2 - p^2) - 1]}$$

(28a)

where

$$S = (1 - p^2)^{-\frac{3}{2}} \varepsilon \tan^{-1} \left[ \frac{1}{\sqrt{[1 - (p^2 - 1)\varepsilon^2]}} \right]$$

for a prolate ellipsoid ($p<1$), and

$$S = (p^2 - 1)^{-\frac{3}{2}} \tan^{-1} \left[ \frac{1}{\sqrt{[1 - (p^2 - 1)\varepsilon^2]}} \right]$$

for an oblate ellipsoid ($p>1$).

The rotational diffusion ratio $\theta_i/\theta_o$ ($i=a,b$) can be related to experimental parameters using equations (26b):

$$\frac{\theta_i}{\theta_o} = 6n_o \frac{V}{kT} e^{\theta_i}$$

(28b)
The corresponding rotational relaxation time ratios were also given by Perrin (1934) but contained an error of sign involving $S$. The correct result was given by Koenig (1975):

$$\frac{\theta_a}{\theta_o} = \frac{\theta_o}{\theta_b} = \frac{2(1 - p^4)}{3p^2[S(2-p^2) - 1]}$$

$$\frac{\theta_b}{\theta_o} = \frac{2}{\left(\frac{\theta_a}{\theta_o} + \frac{\theta_b}{\theta_o}\right)} = \frac{4(1 - p^4)}{3[p^2S(1-2p^2) + 1]}$$

(29a)

These may be related to experimental parameters by combining equations (26b, 27b):

$$\frac{\zeta_i}{\zeta_o} = \frac{kT}{3n_0V_o e^{\theta_i}}$$

(29b)

All these functions ($\zeta_i/\zeta_o = \theta_o/\theta_o$, $\phi_i/\theta_o$) are plotted as functions of axial ratio in Figure 6. It should also be pointed out that, like the translational functions the rotational diffusion coefficients and relaxation times are functions of concentration (Riddiford & Jennings, 1967) and should be extrapolated to infinite dilution. The same is also true for the harmonic mean relaxation time, the birefringence decay constants and the fluorescence depolarisation relaxation times mentioned below. The various experimental methods for determining all these shape parameters will now be discussed.

1.5.1. **Dielectric Dispersion**

The capacity of a condenser filled with a solution of the macromolecule is measured as a function of the applied sinusoidal voltage across it
The 'dielectric increment' or increase in the dielectric constant, \( \varepsilon \), due to the presence of the solute is given by

\[
\Delta \varepsilon = \varepsilon - \varepsilon_0 = \frac{C}{C_v} - \frac{C_0}{C_v}
\]

(30)

where \( \varepsilon_0 \) is the dielectric constant of the solvent and \( C, C_0 \) and \( C_v \) are the capacities of respectively, the solution, solvent and vacuo.

At sufficiently small frequencies, the dipolar macromolecules can keep pace with the alternating field, and the dielectric constant will remain at its 'static' value. At sufficiently high fields, the rotation of the macromolecule about a particular axis will no longer follow the field and its contribution, \( \Delta \varepsilon_\infty \) to the dielectric constant is that of a non-polar substance (Oncley, 1940); thus over a certain critical range characteristic of the size and shape of the macromolecule, the dielectric constant decreases as the frequency increases. The frequency corresponding to the mid-point of the dispersion curve is known as the 'critical frequency'. For a general particle with three rotational relaxation times \( \rho_a, \rho_b, \rho_c \), there will be three critical frequencies:

\[
\nu_a = 2\pi \rho_a \quad ; \quad \nu_b = 2\pi \rho_b \quad ; \quad \nu_c = 2\pi \rho_c
\]

(31)

For an ellipsoid of revolution there will be two (since \( \rho_b = \rho_c \)) or one, either if the dipolar axis is parallel to the rotation axis of symmetry or for a spherical particle. Typical dielectric dispersion curves for ellipsoids of revolution of various axial ratios are shown in Figure 7 (from Oncley, 1940)

Even in the most favourable case, \( \theta = 45^\circ \), resolution is poor for axial ratios less than 9 (Squire, 1978). Application of this method is
also limited by the fact that, due to electrode polarization, only solutions of low ionic strength can be used, thus restricting the use to proteins of high solubility.

1.5.2. Electric Birefringence

Polarized light incident on a solution of macromolecules oriented by an electric field will be split into perpendicular components because the refractive index will be different for directions parallel and perpendicular to the electric dipole moment (Benoit, 1951). The solution is then said to be birefringent and the amount of birefringence will depend on the nature and concentration of the macromolecules.

The decay due to Brownian motion of the birefringence when the field is suddenly switched off is most interesting since this will be independent of the electric properties of the macromolecules (apart from the initial amplitude of the decay) but dependent on their size and asymmetry, assuming the solution to be homogeneous. The solution must be rendered homogeneous by, say, ultracentrifugation for removing larger impurities, followed by gel filtration for fine purification. The number of terms in the exponential decay will be dependent on the particle asymmetry, assuming that the particles are small enough so that the Rayleigh - Gans - Debye scattering theory applies (i.e. particle dimensions less than \( \lambda/20 \)). Ridgeway (1966, 1968) has shown that a general particle will have two relaxation times, \( \tau_+ \), \( \tau_- \) or two decay constants, \( \theta_+ (=1/6\tau_+) \), \( \theta_- (=1/6\tau_-) \):

\[
\Delta n = \frac{N}{2n_L} \left( A_+ e^{-6\theta_+ t} + A_- e^{-6\theta_- t} \right)
\]

where \( \Delta n \) is the birefringence, \( N \) is the number density of particles in
suspension, $n_0$ the refractive index of the solvent and $A_+$ complicated expressions depending on the initial particle orientations and their dielectric and diffusion properties. Unfortunately, although Ridgeway provided relationships linking $\theta_+^2 \Delta$ with the size and shape of general tri-axial ellipsoids (see Chapters 3 and 4), only one relaxation time has been resolved from the experimental exponential decays for homogeneous solutions. Thus the method has been restricted to ellipsoids of revolution ($A_- = 0$) for which Benoit (1951) had shown previously that, for an initial birefringence $n_0$,

$$\Delta n = \frac{N}{2n_2} A e^{-6\theta_+^2 t} = n_0 e^{-6\theta_+^2 t}$$

(33)

assuming the electric dipole axis coincides with the rotational axis of symmetry. For spherical particles there would be no birefringence.

1.5.3. Flow Birefringence

The aligning field can also be produced, if the macromolecules are highly asymmetric, by large flow velocity gradients in the annular space between two concentric cylinders, one rotary and one stationary (van Holde, 1971, Squire, 1978). The orientation of the macromolecules will again be opposed by rotational Brownian motion, and for a constant shear rate, there will be an equilibrium distribution of orientation states. Results for early studies are discussed by Cerf and Scheraga (1952) and by Tanford (1961). This method has the advantage that the steady state birefringence can now be used to derive shape parameters, since this will be independent of the electric properties of the macromolecule. However, the method has the serious disadvantage in that relaxation experiments are virtually impossible,
and also the use is restricted to highly asymmetric molecules (Squire, 1978).

1.5.4. Fluorescence Depolarization

This method applies to those macromolecules that possess a fluorescent group or a chromophore (Cantor & Tao, 1971). If an electron in a chromophore is excited to a higher energy state by the absorption of radiation, then instead of the energy being dissipated non-radiatively in the form of heat as it returns to the ground state, it loses only part of its energy as heat as it returns to the lowest vibrational level of the excited state, but then re-radiates the rest. This will necessarily be of lower energy (hence longer wavelength) than the incident radiation. This phenomenon is called fluorescence.

If the macromolecule is irradiated with polarized light, and if, in the $10^{-8}$ to $10^{-7}$ seconds it takes for the energy to be re-radiated the macromolecule has changed its orientation due to Brownian motion, there will be a net depolarization of the incident light. If the solution is continuously irradiated then a steady state depolarization will be reached depending on the ratio of the fluorescence decay time, $\tau^*$ to the harmonic mean of the three rotational relaxation times (equations 27), $\tau_h$ (Perrin, 1934):

$$\left(\frac{1}{P} - \frac{1}{3}\right) = \left(\frac{1}{P_0} - \frac{1}{3}\right) \left(1 + \frac{3\tau^*}{\tau_h}\right)$$

$$P$$ is the polarization (i.e. the ratio of the difference in intensities of light polarized parallel and perpendicular to the incident beam to their sum), $P_0$ is the intrinsic polarization of the fluorescence (the polarization that would be observed if no rotation had occurred) and $\tau_h$ is defined by
\[ \frac{1}{\tau_h} = \frac{1}{3} \left( \frac{1}{\varphi_a} + \frac{1}{\varphi_b} + \frac{1}{\varphi_c} \right) \]  

(35)

for general ellipsoids, or for ellipsoids of revolution ($\varphi_b = \varphi_c$):

\[ \frac{1}{\tau_h} = \frac{1}{3} \left[ \frac{1}{\varphi_a} + \frac{2}{\varphi_b} \right] \]

The harmonic mean relaxation time ratio $\frac{\tau_h}{\tau_0}$ can thus be plotted as a function of axial ratio (Figure 8), where $\tau_0$ is the corresponding coefficient for a sphere of the same molecular weight and volume:

\[ \tau_0 = \frac{3 \eta_0 V_e}{kT} \]  

(36a)

Thus $\frac{\tau_h}{\tau_0}$ can be related to experimental parameters by:

\[ \frac{\tau_h}{\tau_0} = \frac{kT \tau_h}{3 \eta_0 V_e} \]  

(36b)

Equation (34) is not particularly useful as it stands, since neither $P_0$ nor $\tau_h$ are known. If $\tau_h$ is approximated by $\tau_h - \tau_0$ (i.e. $= 3 \eta_0 V_e / kT$) then:

\[ \left( \frac{1}{P} - \frac{1}{3} \right) = \left( \frac{1}{P_0} - \frac{1}{3} \right) \left( 1 + \frac{kT \tau^*}{\eta_0 V_e} \right) \]  

(37)

If measurements are then made in solutions of varying viscosity (for example by adding glycerol) and/or temperature, $(1/P - 1/3)$ can be plotted against $T/\eta_0$, $1/P_0$ can be found from the intercept and hence $\tau_h$. 

27.
from the gradient, assuming $\tau^*$ can be found independently. A major disadvantage of this method is that by adding glycerol or changing the temperature the swelling due to solvation may be altered: also an independent estimate for $\tau^*$ is required.

A more accurate method in principle is nanosecond fluorescence depolarization decay (Cantor & Tao, 1971). Here the solution is irradiated with polarized light pulses of very short duration (~1ns). The anisotropy, $A$ is measured by determining the intensity of emission polarized parallel to ($I_H$) and perpendicular to ($I_L$) the incident pulse:

$$A = \frac{I_H - I_L}{I_H + 2I_L}.$$

(38)

For a rigid spherical macromolecule, the anisotropy decay is described by a single exponential term (Jablonski, 1961)

$$A(t) = A_0 e^{-t/\tau_0}$$

(39)

with $\tau_0 = \eta_0 V_e/kT$. For a rigid ellipsoid of revolution, Memming (1961) and Wahl (1966) have shown that the anisotropy is a sum of three exponential terms:

$$A(t) = \alpha_1 e^{-t/\tau_1} + \alpha_2 e^{-t/\tau_2} + \alpha_3 e^{-t/\tau_3}$$

(40)

where

$$\tau_1 = \frac{1}{6\theta_b} ; \quad \tau_2 = \frac{1}{5\theta_b + \theta_a} ; \quad \tau_3 = \frac{1}{2\theta_b + 4\theta_a}$$

(41)
The fluorescence decay time ratios are plotted in Figure 9 where \( \tau_0 \) is the corresponding coefficient for a sphere of the same molecular weight and volume:

\[
\tau_0 = \frac{\eta_0 V e}{kT} = \frac{4\pi \eta_0 ab^2}{3kT}
\]

Thus the fluorescence anisotropy decay time ratios can be related to experimental parameters by

\[
\tau_j \tau_0 \propto kT \frac{\eta_j V e}{\eta_0 V e} \quad (j=1,2,3)
\] (42)

The values of the component amplitudes \( \alpha_1, \alpha_2, \alpha_3 \) and hence the dominant relaxation time will depend on the angle between the transition moment of the chromophore to the rotation axis of symmetry of the ellipsoid of revolution. Unfortunately, resolution of a multi-term exponential decay into its components is notoriously difficult (Jost, 1978), even for relaxation times differing in orders of magnitude; this is coupled to the problem that the observed decay will be a convolution of the finite cut-off time of the incident pulse, the fluorescence decay and the anisotropy decay. There are also more serious problems:

(i) since the fluorescence itself decays within about 10ns, only molecules with very short relaxation times can be investigated,

(ii) most macromolecules do not contain a chromophoric group such as tryptophan; thus one must be introduced. This may significantly alter the true conformation of the molecule,
(iii) even if the macromolecule contains tryptophan, the decay is not perfectly exponential, due to interference between the side chain and the indole ring,
(iv) rotation of the chromophore, or of a fragment of the macromolecule to which the chromophore is attached, with respect to the rest of the macromolecule may occur: Munro et al (1979) have given evidence for internal rotation of the tryptophan residue in *Staphylococcus aureus* nuclease B ($M_r = 14,100$) and *Pseudomonas aeruginosa* azurin ($M_r = 14,000$).

1.6. Scattering

Absorption and hence fluorescence phenomena can only occur when the frequency of the exciting radiation is the same as or near to that of an allowed transition frequency of the molecule. However, at other wavelengths electro-optic interaction can still occur; the electric vector of the incident radiation polarizes the molecule by attracting the nuclear mass and repelling the electron clouds. The frequency of oscillation of the incident radiation is the same as that of the induced oscillating dipole; however, the waves emitted are by Huyghens principle spherical and hence the radiation is scattered in all directions.

The scattering by a solution of macromolecules is most rigorously analysed by considering the local concentration fluctuations of the solution; however, if we consider the particle as small compared with the wavelength of the incident light and the solution to be so dilute so that each particle can be considered independently, relations can be derived between particle shape in terms of the 'radius of gyration' (Tanford, 1961) and the scattering (van de Hulst, 1957). For small particles ($<\lambda/20$) interference effects between radiation scattered by different parts of the
macromolecule can be neglected, and the following relation between molecular weight, $M_r$ and the scattering can be derived:

$$\frac{Hc}{\tau} = \frac{1}{M_r} + 2B_c$$

where $c$ is the particle concentration, $H$ is the scattering constant ($\alpha \lambda^{-4}$, and the square of the refractive index increment, $dn/dc$), $B$ the second virial coefficient and $\tau$ is a measure of the relative scattering perpendicular to the incident beam (i.e. the fraction of light scattered (van Holde, 1971)). Hence if $Hc/\tau$ is plotted versus concentration, the molecular weight can in principle be determined from the intercept. For large particles ($d/\lambda > 20$) destructive interference occurs between light scattered from different parts of the macromolecule. Light scattered in the forward direction cannot however be subject to destructive interference. Unfortunately this cannot be viewed directly, but if the scattering is studied over a range of angles it can be extrapolated to the forward direction. This involves extrapolating to zero-angle and to zero-concentration using the so-called Zimm plot (Zimm, 1948, Stacey, 1956, Tanford, 1961). The slope of the $c=0$ line gives the radius of gyration of the particle, $R_G$, i.e. the mean extension of mass from the centre of gravity. For a sphere of radius $R$, $R_G = \sqrt{\frac{3}{5}}R$, and for a large rod of length $L$, $R_G = L/\sqrt{2}$, thus light scattering can be used to obtain information about conformation in solution, where particular models for which $R$ can be specified are applicable. Holtzer and Lowey (1956) showed by this method that $L = 1500\, \AA$ if myosin could be reasonably modelled by a long rod. Martin (1964) has shown that the radius of gyration can be related to the axial ratio of the equivalent ellipsoid of revolution provided that the swollen volume is known:
for a prolate ellipsoid and

\[ R_G^2 = \left( \frac{3V_e}{4\pi} \right)^{2/3} \left( \frac{4\beta}{p} + 4p^{-2/3} \right) \]  

(43a)

for an oblate ellipsoid.

An explicit relation relating \( R_G \) to axial ratio alone can be found by 'reducing' it

\[ (R_G)_{\text{red}} = \left( \frac{4\pi}{3V_e} \right)^{1/3} R_G = \left( \frac{5p}{4p^{-2/3}} \right)^{1/2} \]  

(44a)

for a prolate ellipsoid and

\[ (R_G)_{\text{red}} = \left( \frac{4\pi}{3V_e} \right)^{1/3} R_G = \left( \frac{p}{2p^{-2/3}} \right)^{1/2} \]  

(44b)

for an oblate ellipsoid.

This is plotted in Figure 10. Experimental determination of \( (R_G)_{\text{red}} \) requires of course a knowledge of \( V_e \).

The same analysis can be used for laser light scattering as this gives good time resolution for rapidly changing solutions (for example aggregation of macromolecules, randomly coiled macromolecules). However a major difficulty with all light scattering experiments is that all
solutions, glassware etc., must be dust free; removal, without damage to
the biological solute, poses great difficulties. Due to diffraction
effects it is also difficult to measure scattering angles less than
about five degrees, thus a clear extrapolation to zero angle may not be
possible. Another major difficulty is that, since the resolving power
depends on $(R_g/\lambda)^2$, the method fails for macromolecules below about 100 Å
(although $M_r$ may still be found). Reducing the wavelength of the
incident radiation does not help (until down to the x-ray region) since
below 200 nm most biological materials absorb very strongly. A method of
low angle x-ray scattering (LAXR) has also been developed (Beeman et al,
1957). However, due to very strong diffraction and interference effects,
the scattering is almost entirely confined to a very narrow wavelength
range. On the other hand, it is possible to collimate the x-ray beam much
better than a light beam, thus measurements can be made to a low enough
angle to a more reasonable extrapolation to zero angle.

Deductions about the size and shape of macromolecules from scattering
information is generally restricted however, since any simple interpretation
of the radius of gyration must assume that the macromolecule is homogeneous
(uniform electron density). If, therefore, the particle contains fluid
filled cavities or indentations or a monolayer of bound solvent, the
dimensions of any assumed model calculated from the $R_g$ will be incorrect.
This problem does not apply to the determination of the hydrodynamic shape
parameters considered previously since these phenomena do not depend on
interactions with or properties of the interior of the macromolecules.

1.7. The Problem of Swelling due to Solvation

In order to determine from experimental data the ellipsoid
of revolution shape functions mentioned so far, a knowledge of the swelling due to solvation (i.e. \( V_e \)) is required:

\[
\nu = \frac{[n]}{\nu_s} = \frac{[n]M_r}{N_A V_e}
\] (8)

\[
\frac{f}{f_o} = \frac{M_r(1 - \bar{\nu}_o)}{N_A 6\pi \eta_0 s} \left( \frac{4\pi}{3V_e} \right)^{1/3}
\] (20b)

\[
\frac{\theta_i}{\theta_o} = \frac{\zeta_o}{\zeta_i} = \frac{6\eta_0 V_e \theta_i}{kT}
\] (28b)

\[
\frac{\varphi_i}{\varphi_o} = \frac{kT}{3\eta_0 V_e} \varphi_i \quad (i = a, b).
\] (29b)

\[
\frac{\tau_h}{\tau_o} = \frac{kT}{3\eta_0 V_e} \tau_h
\] (36b)

\[
\frac{\tau_j}{\tau_o} = \frac{kT}{\eta_0 V_e} \tau_j \quad (j = 1, 2, 3)
\] (42b)

\[
(R_G)_{\text{red}} = \left( \frac{4\pi}{3V_e} \right)^{1/3} R_G
\] (44)

The first significant attempt at dealing with this problem was due to Oncley (1941) using a graphical analysis: If \( V_e \) is fixed then a single value of the shape parameter being considered will correspond to a single value of the axial ratio. If, however, \( V_e \) is assumed to have a range of possible values, then a single value of the shape parameter will have a
'line solution' of possible values of the axial ratio. This is shown in Figures 11a and 11b for the viscosity increment and translational frictional coefficient. However, if line solutions for two or more of the different shape parameters are compared, then in principle a unique value for the axial ratio and effective volume can be found from the intersection. On the other hand, in practice these curves could only be made to intersect by imposing large experimental errors on the data, and in one case - pepsin - the curves do not cross at all (Figure 12).

Here Oncley uses as his abscissa the 'hydration factor' \( w \), related to the effective volume, \( V_e \) by:

\[
w = \rho_o (\bar{V}_s - \bar{V}) = \rho_o \left( \frac{N_A V_e}{M_r} - \bar{V} \right)
\]

A different approach would be to eliminate \( V_e \) simultaneously by combining any two of the shape parameters together. The effective volume can then also be found by back substitution into the equations. This naturally assumes, as does the Oncley approach, that the axial ratio and the swelling are the same for both types of experiment. Scheraga and Mandelkern (1953) combined equations (8) and (20b) to produce a swelling-independent function \( \beta \) (Figure 13):

\[
\beta = \frac{N_A}{(16200\pi^2)^{1/3}} \frac{\nu^{1/3}}{\bar{f}/\bar{f}_o}
\]

or in terms of experimental parameters, from

\[
\beta = \frac{N_A s[n]^{1/3} n_o}{M_r^{1/3} (1 - \bar{V}_o) 100^{1/3}}
\]
where \([\eta]\) is in ml/gm. Scheraga and Mandelkern also combined equation (8) with equations (28b) to produce swelling independent \(\delta_a\) and \(\delta_b\) functions (Figure 14), although in their original paper, only \(\delta_b\) is given:

\[
\delta_i = \frac{\xi_o}{\xi_i} \nu = \frac{\theta_i}{\theta_o} \nu = \frac{6\eta_o \theta_i [\eta]M_i}{N_A kT}
\]  

(i=a,b)

Scheraga (1961) later combined (20b) with (28b) to produce swelling independent \(\mu_a\), \(\mu_b\) functions (Figure 15) although again only \(\mu_b\) was given:

\[
\mu_i = \left(\frac{f_o}{f_i}\right) \left(\frac{\xi_i}{\xi_o}\right) \equiv 3\frac{2\beta_s}{\theta_i} \cdot \frac{N_A(kT)^{1/3} \eta_o^{2/3}}{M_r(1 - \tilde{\nu}_o)} = 3\frac{2\beta_s}{\theta_i} \left(\frac{\eta_o^{2/3}}{kT}\right)
\]  

(i=a,b)

Squire et al (1968) combined equation (20b) with (29b) to produce swelling independent \(\gamma_a\) and \(\gamma_b\) functions:

\[
\gamma_i = \left(\frac{f_o}{f_i}\right)^3 \frac{\xi_o}{\xi_i} \equiv \frac{1}{54\pi^2 N_A kT} \cdot \frac{M_r(1 - \tilde{\nu}_o)}{s^3 \eta_o^{2/3} \theta_i}
\]  

(i=a,b)

Squire later (1970) combined (20b) with (36b) to give a swelling independent \(\psi\) function (Figure 16)

\[
\psi \left(\frac{\tau_o}{\tau_h}\right)^{1/3} \left(\frac{f_o}{f_i}\right)^{1/3} \equiv \frac{4\pi \eta_o^{1/3}}{kT} \cdot \frac{M_r(1 - \tilde{\nu}_o)}{6\pi \eta_o N_A s} \left(\frac{1}{\tau_h}\right)^{1/3}
\]  

(49)
Plots of the Squire $\gamma_a$ and $\gamma_b$ parameters as functions of axial ratio are given in Figure 16. A similar swelling independent function can be obtained by combining the viscosity increment, equation (8) instead of equation (20b) with (36b) (see Appendix II and Harding, 1980a):

$$\Lambda = \left(\frac{\tau_0}{\tau_h}\right) \equiv \frac{3\eta_o[n][\eta]}{N_A kT \tau_h}$$

(Figure 17). Also, by combining equation (8) instead of (20b) with equation (29b), swelling independent $\varepsilon_a$, $\varepsilon_b$ functions are produced (Figure 18):

$$\varepsilon_i = \frac{\varepsilon_0}{\varepsilon_i} \equiv \frac{3\eta_o[n][\eta]}{N_A kT \varepsilon_i} \quad \text{(51)}$$

(i=a, b)

By combining (8) with the fluorescence anisotropy relaxation times (42b) three new functions, $\kappa_1$, $\kappa_2$, $\kappa_3$ are produced (Figure 19):

$$\kappa_j = \frac{\tau_0}{\tau_j} = \frac{\eta_o[n][\eta]}{N_A kT \tau_j} \quad (j=1,2,3) \quad \text{(52)}$$

Alternatively, combining equation (20b) with equation (42b) (Figure 20):

$$\xi_j = \left(\frac{f}{f_0}\right)^3 \frac{\tau_0}{\tau_j} \equiv \frac{m_r^3 (1-\bar{\nu})^2}{162 N_A^2 kT n^2 \gamma_o^2 \tau_j} \quad (j=1,2,3) \quad \text{(53)}$$

As far as the author is aware, the $\Lambda$, $\varepsilon_i$, $\kappa_j$ and $\xi_j$ functions are new and have not been published before. These functions are tabulated for axial ratios between 1 and 10 (Table 1).

Martin (1964) eliminated the requirement of knowledge of the swollen volume for scattering experiments by combining (44) simultaneously with
either the translational frictional function (Figure 21):

\[ \gamma = \frac{R_G \eta_0 D}{kT M(1 - \varphi_0)} = \frac{R_G \eta_0 N_A s}{f_o f} \left( \frac{5p^{4/3} + 4p^{-2/3}}{15} \right)^{1/2} \]

(prolate ellipsoid)

\[ = \frac{1}{6\pi} f_0 f \left( \frac{p^{4/3} + 2p^{-2/3}}{5} \right)^{1/2} \]

(oblate ellipsoid)

or the viscosity increment (Figure 22):

\[ \alpha = \frac{R_G}{[\eta]^{1/3} M^{1/3}} = \frac{75}{\pi N_A} \left( \frac{5p^{4/3} + 4p^{-2/3}}{15} \right)^{1/2} \cdot \frac{1}{\nu^{1/3}} \]

(prolate ellipsoid)

\[ = \frac{75}{\pi N_A} \left( \frac{p^{4/3} + 2p^{-2/3}}{5} \right)^{1/2} \cdot \frac{1}{\nu^{1/3}} \]

(oblate ellipsoid)

where \( p \) is the axial ratio defined in section 1.4.

The molecular covolume has also been given as a function of shape and swollen volume by Nichol et al (1977) for prolate and oblate ellipsoids

\[ U = N_A V_e \left\{ 2 + \frac{3}{2} \left( \frac{1 + \sin^{-1} \frac{1}{e}}{e(1 - e^2)^{3/2}} \right) \right\} \]

\[ \left\{ 1 + \frac{1 - e^2}{2e} \ln \left( \frac{1 + e}{1 - e} \right) \right\} \]

(54)
where the ellipticity $\varepsilon$ is related to the axial ratio by

$$\varepsilon = \sqrt{1 - \frac{b^2}{a^2}}$$

for prolate ellipsoids ($b<a$), and

$$\varepsilon = \sqrt{1 - \frac{a^2}{b^2}}$$

for oblate ellipsoids ($b>a$). By 'reducing' $U$ we obtain a function $U_{\text{red}}$ in terms of shape alone:

$$U_{\text{red}} = \frac{U}{N_A V_e} = 2 + \frac{3}{2} \left( 1 + \frac{1 + \sin^{-1} \varepsilon}{\varepsilon (1 - \varepsilon^2)^{3/2}} \right)$$

$$\left( 1 + \frac{1 - \varepsilon^2}{2\varepsilon} \frac{\ln (1 + \varepsilon)}{1 - \varepsilon} \right)$$

(55)

The covolume $U$ can be found from a sedimentation equilibrium experiment in terms of the activity coefficient, as outlined by Nichol et al (1977) although in order to determine $U_{\text{red}}$, a knowledge of $V_e$ is still required. Nichol et al (1977) however eliminated $V_e$ by solving equation (55) simultaneously with the translational frictional ratio (equation 20b) to produce the swelling independent $\psi$ function (not to be confused with the Squire $\psi$ function)

$$\psi = \frac{U_{\text{red}}}{162\pi^2} \left( \frac{f_0}{f} \right)^3 = \frac{U_n^3 N_A^2 s^3}{M_r^3 (1 - \nu_0)^3}$$

(56)

As seen from Figure 23, $\psi$ has the advantage that no prior decision has to be made as to whether the macromolecule is better modelled either by a prolate or oblate ellipsoid. Unfortunately, for typical globular macromolecules (small axial ratios), the parameter is still very
sensitive to experimental error: this is clear from Nichol et al's results for ovalbumin, whose axial ratio they found to be 2.5:1 with a standard error of 3. This is largely due to the large number of terms on the right hand side of equation (56), several of them cubed.

\[ U_{\text{red}} \] can of course be combined with any of the equations (8), (20b), (28b), (29b), (36b), (42b) to eliminate \( V_e \). For example, if (55) is combined with the viscosity increment (8), a new swelling independent function is produced (Figure 24) (Harding, 1980b):

\[ \Pi = \frac{U_{\text{red}}}{v} \times \frac{U}{[\eta]_{M_r}} \]

(57)

Values of the \( \Pi \) function for various axial ratios are given in Table 1.

The results for hemoglobin are in excellent agreement with those found from x-ray crystallography (see Appendix III).

1.7.1. Hydrodynamic non-ideality: the \( R \) function

The viscosity, translational frictional and rotational parameters considered so far are normally those extrapolated to zero concentration in order to negate the effect of the net volume excluded by the particles and solute-solute interaction. However, the nature of the concentration dependence of these parameters, particularly the sedimentation coefficient "s" and the reduced specific viscosity, \( \eta_{sp}/c \), has now been shown by Rowe (1977) to give valuable information as to the conformation and swelling in solution and also an estimate of the "goodness of fit" of an ellipsoid for the macromolecule in solution.

The variation of \( s \) and \( \eta_{sp}/c \) with concentration can be represented
by regression parameters \( k_s \) and \( k_\eta \):

\[
s_c = s(1 - k_s c)
\]

(58)

\[
\frac{\eta_{sp}}{c} = [\eta] (1 + k_\eta c)
\]

(59)

where \( k_s \) and \( k_\eta \) are, respectively, the sedimentation and viscosity concentration regression coefficients. These approximate linear equations are valid only for dilute solutions. A universal equation has been derived by Rowe (see Appendix IV) for all solute concentrations up to \( \phi_p \), the critical packing fraction:

\[
\frac{s_c}{s} \frac{f_0}{f} = \frac{[\eta]}{\eta_{sp}/c} = 1 - gc
\]

(60a)

where

\[
gc = \frac{kc - (2\phi_p - 1)(c\tilde{v}_s/\phi_p)^2}{kc - 2\tilde{v}_s + 1}
\]

(60b)

where \( k = k_s \) (sedimentation) or \( k = k_\eta \) (viscosity). This provides a more accurate method for extrapolating to infinite dilution to obtain \([\eta]\) and "s", and also for finding \( k_s \) and \( k_\eta \), from a given set of data, by minimising:

\[
\{w_i[s_i - f(k_s,\tilde{v}_s,s,c_i,\phi_p)]^2
\]

(61)

\( w_i \) = weight

This procedure is unstable if \( k_s \), \( \tilde{v}_s \) and \( s \) (or the corresponding viscosity parameters) are all taken to be independent variables. However, if we assume \( \tilde{v}_s = k_s/4 \) for globular proteins, or assume \( \tilde{v}_s \) from the ratio \( \tilde{v}_s/\tilde{v} \)
\[ k'_{\eta} / k'_{s} \text{, where } k'_{\eta} \text{ and } k'_{s} \text{ are the parameters found from the approximate fit (equations 58 & 59), a stable fit may be found.} \]

Rowe (1977) has shown that the swelling, \( \bar{\nu}_s / \bar{\nu} \), can be found from:

\[
\frac{\bar{\nu}_s}{\bar{\nu}} = \frac{k_{\eta}}{k_s}
\]

(62)

Therefore

\[
\bar{\nu}_e = \frac{M}{N_A} \bar{\nu}_s = \frac{M}{N_A} \frac{k_{\eta}}{k_s} \bar{\nu}
\]

(63)

The value of \( \bar{\nu}_s / \bar{\nu} \) and hence \( \bar{\nu}_e \) thus found is independent of any assumed model for the protein. Since the determination of \( \bar{\nu}_e \) by back substitution into the equations given at the beginning of 1.7. after the axial ratio has been determined is dependent on the model chosen (i.e. an ellipsoid of revolution), an estimate for the "goodness of fit" of an ellipsoid of revolution is now available by comparing the model dependent \( \bar{\nu}_e \) with model independent \( \bar{\nu}_e \) (or, equivalently, \( \bar{\nu}_s \) or \( \bar{\nu}_s / \bar{\nu} \)).

This theory also provides a new shape function "R", which is independent of particle swelling:

\[
R = \frac{2}{\bar{\nu}} \left( 1 + \left( \frac{f}{f_0} \right)^3 \right) \equiv \frac{k_s}{[\eta]}
\]

(64)

Wales & Van Holde (1954) had previously reported that the ratio \( k_s / [\eta] \) was some unknown function of shape and equal to 1.6 for spherical particles; this agrees with that predicted by equation (44) (Figure 13). R varies rather rapidly with axial ratio for ellipsoids, even for low axial ratio, and this function provides a precise method for characterizing the axial ratio of relatively symmetrical particles.
Besides its greater sensitivity than the $\beta$ function (or the $\Psi$ function), $R$ has several other advantages:

1. unlike $\beta$ computation of $R$ does not require knowledge of the absolute solute concentration (Rowe, 1977)

2. less data is required to compute $R$ and hence the error in the final function is minimized. As rotational parameters are generally very difficult to determine, as will be evident from the earlier parts of this chapter, the $R$ function is also to be preferred over swelling independent functions involving these. The $R$ function is also to be preferred over the scattering $\gamma$ and $\alpha$ functions mainly because of the particle homogeneity problem mentioned in section 1.6. The $\beta$ function can still however be useful, precisely because of its lack of variation for oblate ellipsoids, in deciding whether the macromolecule is better modelled by either a prolate or an oblate ellipsoid. Experimental values for $\beta$ and $k_{s}/[n]$ ($\equiv R$) have been tabulated for a wide range of proteins by Creeth & Knight (1965). Values of $\beta$ below the theoretical minimum of $2.112 \times 10^6$ and above 1.6 for $R$ may indicate that some proteins cannot be modelled by an equivalent ellipsoid of revolution. This has been suggested for Bovine serum albumin (BSA). A table of values of axial ratio calculated from the $R$ function for recent data, together with a comparison of their 'model dependent' estimates for $\tilde{\nu}_{s}/\tilde{\nu}$ with their 'model independent' estimates to determine the 'goodness of fit' of an ellipsoid of revolution, is given in Table 2.

1.8. Comment

Although a hydrodynamically equivalent ellipsoid of revolution model can now be fitted with much greater precision to many rigid macromolecules with the aid of the $R$ function (and possibly the $\Pi$ function)
the distinction still has to be made as to whether the macromolecule is better modelled either by a prolate or an oblate model. It is clear from a perusal of the crystallographic models of many globular proteins such as carboxypeptidase, myoglobin and ribonuclease (Table 3) that in many cases this is quite arbitrary and indeed in some cases is impossible.

It would be a significant step forward therefore if the restriction of two equal axes on the ellipsoid were removed to allow use of the more general tri-axial ellipsoid. However, either due to the lack of the theoretical relationships linking the axial dimensions of the ellipsoid with experimental parameters, or, even if they are available, due to the lack of the necessary experimental precision, numerical inversion procedures or data analysis techniques, this model has not, to date, been available. The aim of the rest of this thesis is to show that the general tri-axial ellipsoid can now be successfully employed to model biological macromolecules in solution. We will start by deriving the tri-axial viscosity increment equation.
Table 1. Values of $A$, $e_a$, $e_b$, $\kappa_1$, $\kappa_2$, $\kappa_3$, $\xi_1$, $\xi_2$, $\xi_3$ and $\Pi$ for prolate and oblate ellipsoids of revolution

<table>
<thead>
<tr>
<th>Axial Ratio</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\Lambda_p$</td>
<td>2.500</td>
<td>2.490</td>
<td>2.692</td>
<td>3.071</td>
<td>3.575</td>
<td>4.177</td>
<td>4.862</td>
<td>5.624</td>
<td>6.457</td>
<td>7.359</td>
</tr>
<tr>
<td>$\Lambda_o$</td>
<td>2.500</td>
<td>2.356</td>
<td>2.187</td>
<td>2.070</td>
<td>1.989</td>
<td>1.931</td>
<td>1.887</td>
<td>1.854</td>
<td>1.827</td>
<td>1.805</td>
</tr>
<tr>
<td>$e_{a,p}$</td>
<td>2.500</td>
<td>1.932</td>
<td>1.574</td>
<td>1.373</td>
<td>1.251</td>
<td>1.171</td>
<td>1.115</td>
<td>1.075</td>
<td>1.044</td>
<td>1.020</td>
</tr>
<tr>
<td>$e_{a,o}$</td>
<td>2.500</td>
<td>2.522</td>
<td>2.343</td>
<td>2.202</td>
<td>2.102</td>
<td>2.029</td>
<td>1.974</td>
<td>1.931</td>
<td>1.896</td>
<td>1.868</td>
</tr>
<tr>
<td>$e_{b,o}$</td>
<td>2.500</td>
<td>2.273</td>
<td>2.110</td>
<td>2.003</td>
<td>1.932</td>
<td>1.882</td>
<td>1.844</td>
<td>1.815</td>
<td>1.792</td>
<td>1.774</td>
</tr>
<tr>
<td>$\kappa_{1,p}$</td>
<td>2.500</td>
<td>1.932</td>
<td>1.574</td>
<td>1.373</td>
<td>1.251</td>
<td>1.171</td>
<td>1.115</td>
<td>1.075</td>
<td>1.044</td>
<td>1.020</td>
</tr>
<tr>
<td>$\kappa_{1,o}$</td>
<td>2.500</td>
<td>2.522</td>
<td>2.343</td>
<td>2.202</td>
<td>2.102</td>
<td>2.029</td>
<td>1.974</td>
<td>1.931</td>
<td>1.896</td>
<td>1.868</td>
</tr>
<tr>
<td>$\kappa_{2,p}$</td>
<td>2.500</td>
<td>2.211</td>
<td>2.133</td>
<td>2.222</td>
<td>2.413</td>
<td>2.674</td>
<td>2.989</td>
<td>3.349</td>
<td>3.751</td>
<td>4.189</td>
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<tr>
<td>$\kappa_{2,o}$</td>
<td>2.500</td>
<td>2.439</td>
<td>2.265</td>
<td>2.136</td>
<td>2.045</td>
<td>1.980</td>
<td>1.930</td>
<td>1.892</td>
<td>1.862</td>
<td>1.837</td>
</tr>
<tr>
<td>$\kappa_{3,o}$</td>
<td>2.500</td>
<td>2.190</td>
<td>2.032</td>
<td>1.937</td>
<td>1.875</td>
<td>1.832</td>
<td>1.801</td>
<td>1.777</td>
<td>1.758</td>
<td>1.742</td>
</tr>
<tr>
<td>$\xi_{1,p}$</td>
<td>1.000</td>
<td>0.756</td>
<td>0.588</td>
<td>0.487</td>
<td>0.421</td>
<td>0.374</td>
<td>0.340</td>
<td>0.313</td>
<td>0.292</td>
<td>0.275</td>
</tr>
<tr>
<td>$\xi_{1,o}$</td>
<td>1.000</td>
<td>1.000</td>
<td>0.920</td>
<td>0.860</td>
<td>0.818</td>
<td>0.787</td>
<td>0.763</td>
<td>0.745</td>
<td>0.731</td>
<td>0.719</td>
</tr>
<tr>
<td>$\xi_{2,p}$</td>
<td>1.000</td>
<td>0.865</td>
<td>0.797</td>
<td>0.788</td>
<td>0.811</td>
<td>0.854</td>
<td>0.911</td>
<td>0.976</td>
<td>1.051</td>
<td>1.129</td>
</tr>
<tr>
<td>$\xi_{2,o}$</td>
<td>1.000</td>
<td>0.967</td>
<td>0.890</td>
<td>0.834</td>
<td>0.796</td>
<td>0.768</td>
<td>0.747</td>
<td>0.731</td>
<td>0.718</td>
<td>0.707</td>
</tr>
<tr>
<td>$\xi_{3,p}$</td>
<td>1.000</td>
<td>1.192</td>
<td>1.423</td>
<td>1.691</td>
<td>1.983</td>
<td>2.295</td>
<td>2.623</td>
<td>2.966</td>
<td>3.322</td>
<td>3.690</td>
</tr>
<tr>
<td>$\xi_{3,o}$</td>
<td>1.000</td>
<td>0.868</td>
<td>0.798</td>
<td>0.757</td>
<td>0.729</td>
<td>0.711</td>
<td>0.697</td>
<td>0.686</td>
<td>0.678</td>
<td>0.671</td>
</tr>
<tr>
<td>$\Pi_p$</td>
<td>3.200</td>
<td>3.122</td>
<td>2.960</td>
<td>2.778</td>
<td>2.601</td>
<td>2.438</td>
<td>2.291</td>
<td>2.159</td>
<td>2.041</td>
<td>1.935</td>
</tr>
</tbody>
</table>

subscript $p$: prolate ellipsoid

subscript $o$: oblate ellipsoid
<table>
<thead>
<tr>
<th>Protein</th>
<th>$k_s$</th>
<th>$k_\eta$</th>
<th>$[\eta]$</th>
<th>$R$</th>
<th>axial</th>
<th>model dependent ($\bar{v}_s/\bar{v}$)</th>
<th>model independent ($\bar{v}_s/\bar{v}$)</th>
<th>Conclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apoferritin</td>
<td>8</td>
<td>12</td>
<td>5.16</td>
<td>1.55</td>
<td>1.45*</td>
<td>2.6* †</td>
<td>1.5†</td>
<td>spherical</td>
</tr>
<tr>
<td>BSA</td>
<td>5.5</td>
<td>7.7</td>
<td>2.75</td>
<td>2.0</td>
<td></td>
<td>1.4</td>
<td></td>
<td>not a hydrodynamic ellipsoid (cf $\beta &lt; 2.1$)</td>
</tr>
<tr>
<td>Fibrinogen</td>
<td>7</td>
<td>14</td>
<td>7.8</td>
<td>0.9</td>
<td>6.3†</td>
<td>1.1†</td>
<td>2.0†</td>
<td>prolate ellipsoid ~6:1. Agrees with electron microscopy (Hall &amp; Slayter, 1959)</td>
</tr>
<tr>
<td>C-protein</td>
<td>11</td>
<td>15.4</td>
<td>12.6</td>
<td>0.87</td>
<td>26.0</td>
<td>0.9, 2, 12†</td>
<td>1.4†</td>
<td>oblate ellipsoid ~25:1</td>
</tr>
<tr>
<td>Myosin</td>
<td>85</td>
<td>92</td>
<td>234</td>
<td>0.38</td>
<td>30†</td>
<td>4.3†</td>
<td>1.1†</td>
<td>not hydrodynamic ellipsoids of revolution</td>
</tr>
<tr>
<td>Synthetic A-filaments</td>
<td>160.8</td>
<td>366</td>
<td>176</td>
<td>0.9</td>
<td>.19.5</td>
<td>16†</td>
<td>2.3†</td>
<td></td>
</tr>
<tr>
<td>Collagen sonicates</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(i) $M_\tau$</td>
<td>352,000</td>
<td>308</td>
<td>880</td>
<td>1252</td>
<td>0.246</td>
<td>80†</td>
<td>2.28†</td>
<td>prolate ~80:1</td>
</tr>
<tr>
<td>(ii) $M_\tau$</td>
<td>330,000</td>
<td>291</td>
<td>756</td>
<td>1078</td>
<td>0.270</td>
<td>64†</td>
<td>2.85†</td>
<td>prolate ~65:1</td>
</tr>
<tr>
<td>(iii) $M_\tau$</td>
<td>273,000</td>
<td>241</td>
<td>564</td>
<td>639</td>
<td>0.377</td>
<td>30†</td>
<td>6.12†</td>
<td>not hydrodynamic ellipsoids of revolution</td>
</tr>
<tr>
<td>(iv) $M_\tau$</td>
<td>227,000</td>
<td>193</td>
<td>428</td>
<td>400</td>
<td>0.483</td>
<td>18†</td>
<td>9.13†</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Protein</th>
<th>Dimensions (Å)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carboxypeptidase</td>
<td>50 x 42 x 38</td>
<td>Lipscomb (1971)</td>
</tr>
<tr>
<td>Myoglobin</td>
<td>43 x 35 x 23</td>
<td>Kendrew et al (1958)</td>
</tr>
<tr>
<td>Cytochrome c</td>
<td>25 x 25 x 35</td>
<td>Dickerson &amp; Geiss (1969)</td>
</tr>
<tr>
<td>Lysosome</td>
<td>45 x 30 x 30</td>
<td>Blake et al (1965)</td>
</tr>
<tr>
<td>Ribonuclease</td>
<td>38 x 28 x 22</td>
<td>Kartha et al (1967)</td>
</tr>
<tr>
<td>Pre-albumin</td>
<td>70 x 55 x 50</td>
<td>Blake et al (1978)</td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>64 x 55 x 50</td>
<td>Perutz et al (1960)</td>
</tr>
</tbody>
</table>
Figure 1. A macromolecule in solution is generally swollen due to solvent association.

Figure 2. Mathematical models for macromolecules in solution.

Figure 3. Shearing of a Newtonian fluid between parallel plates (from Van Holde, 1971).
Figure 4. Plot of the viscosity increment as a function of axial ratio for ellipsoids of revolution
Figure 5. Plot of the translational frictional ratio (the "Perrin function") as a function of axial ratio for ellipsoids of revolution.
Figure 6. Plot of the rotational diffusion coefficient ratios and rotational relaxation time ratios as a function of axial ratio for ellipsoids of revolution.
Figure 7. Dielectric dispersion curves for prolate ellipsoids of revolution. Constant dipole angle ($\theta = 45^\circ$) and varying axial ratio ($a/b$ from 1 to 50). From Oncley (1940)
Relative Contribution to Dielectric Constant

\[ \Delta f_a / \Delta f_b = 1 \]

(Dipolar Angle = 45°)
Figure 8. Plot of the harmonic mean rotational relaxation time ratio as a function of axial ratio for ellipsoids of revolution
Figure 9. Plot of the fluorescence anisotropy relaxation time ratios as a function of axial ratio for ellipsoids of revolution.
Figure 10. Plot of the 'reduced' radius of gyration as a function of axial ratio for ellipsoids of revolution
Figure 11. (a) Values of axial ratio and hydration as a function of \( v(\bar{\nu}_s/\bar{\nu}) \). Contour lines denote values of \( v(\bar{\nu}_s/\bar{\nu}) \)

(b) As above, but as a function of \( (f/f_0)(\bar{\nu}_s/\bar{\nu})^{1/3} \). Contour lines denote values of \( (f/f_0)(\bar{\nu}_s/\bar{\nu})^{1/3} \)

(from Oncley, 1941)
Figure 12. Asymmetry and hydration (i.e. solvent association) of certain protein molecules. (from Oncley, 1941)
Figure 13. Plot of the Scheraga & Mandelkern $\beta \times 10^{-6}$ and Rowe $R$ functions versus axial ratio for ellipsoids of revolution.
Figure 14. Plot of $\delta_a$ and $\delta_b$ as functions of axial ratio for ellipsoids of revolution.
Figure 15. Plot of $\mu_a$ and $\mu_b$ as functions of axial ratio for ellipsoids of revolution
Figure 16. Plot of $\gamma_a$, $\gamma_b$ and $\psi$ as functions of axial ratio for ellipsoids of revolution.
Figure 17. Plot of $\Lambda$ as a function of axial ratio for ellipsoids of revolution
Figure 18. Plot of $\varepsilon_a$ and $\varepsilon_b$ as functions of axial ratio for ellipsoids of revolution.
Figure 19. Plot of $\kappa_1$, $\kappa_2$ and $\kappa_3$ as functions of axial ratio for ellipsoids of revolution.
Figure 20. Plot of $\xi_1$, $\xi_2$ and $\xi_3$ as functions of axial ratio for ellipsoids of revolution.
Figure 21. Plot of $\gamma$ as a function of axial ratio for ellipsoids of revolution (from Martin, 1964).

Figure 22. Plot of $\alpha$ as a function of axial ratio for ellipsoids of revolution.
Figure 23. Plot of $\psi$ as a function of axial ratio for ellipsoids of revolution
Figure 24. Plot of $\Pi$ as a function of axial ratio for ellipsoids of revolution
CHAPTER 2

The Viscosity Increment for a Dilute, Newtonian Suspension of Tri-axial Ellipsoids
2.1. **Hydrodynamic Forces and Brownian Motion**

Although the forces and torques exerted upon a suspended particle by a fluid are all ultimately of molecular origin, it is convenient to distinguish those that can be explained by continuum hydrodynamics from those, due to molecular fluctuations, that give rise to Brownian motion. If we first completely neglect the Brownian motion, it is clear that, once a steady state has been attained, suspended particles free of any external imposed impressed forces or torques must move in such a way that the net hydrodynamic force and torque, $T_H$, acting upon them are zero, i.e. $T_H = 0$.

Let us consider a steady simple shearing flow (section 1.3.), as in, for example, a simple capillary or Ubbelohde viscometer experiment (Yang, 1961). The motion of the fluid in the neighbourhood of any point can be decomposed into three components: a translational velocity which varies from point to point, an angular velocity which for this type of flow is the same for all points, and a pure straining motion which again is the same for all points. If now a single, neutrally bouyant, rigid ellipsoidal particle is introduced the flow will be disturbed, although at large distances from the ellipsoid the disturbance will tend to zero. We shall assume that the motion of the ellipsoid and of the fluid is such that the Reynold's number (Batchelor, 1967) is very small. Then it is possible on the basis of work by Oberbeck (1876) and Jeffrey (1922) to say what the hydrodynamic forces and torques acting upon the particle are. In particular it is known that the force will be zero when the translational velocity of the particle is the same as the translational velocity of the point in the undisturbed flow at which the point is suspended. The situation for angular velocity is more complicated since two factors come
into play; one gives a torque if the angular velocity of the particle differs from the angular velocity defined by the undisturbed flow (or, equivalently, by the actual flow at infinity), whilst the other gives a torque if the principal axes of the ellipsoid have a different orientation from the principal axes of the straining motion defined by the undisturbed flow. Taken together, these mean that the angular motion of the particle under zero hydrodynamic torque conditions is very complicated (Chwang, 1975) and a complete solution for it is not known.

Turning to the Brownian motion which is in the nature of fluctuations the simplest question we can ask is what is the average velocity and the average angular velocity of the particle? By the average we mean in the first instance the time average, although in practice this will be assumed equal to the volume average taken over an ensemble over a very large number of particles suspended in unit volume (see Batchelor, 1970 for a detailed discussion of various methods of averaging). Ignoring for the moment the hydrodynamic forces, we can answer the question by saying that on average the particle is at rest in the local frame of reference defined by the undisturbed flow. In other words it is on average moving with the translational velocity of the point in the undisturbed flow at which it is suspended and with the angular velocity defined by the undisturbed flow (Kuhn & Kuhn, 1945, Brinkman et al, 1949, Scheraga, 1955).

When we come to consider the combined effect of the hydrodynamic forces and the Brownian motion no problem arises with the translational motion since both effects tend in the same direction - motion with the translational velocity of the flow. But for the angular motion the situation is less simple, the two effects do not have the same tendancy and we must consider a range of possibilities depending on the relative strengths of the two. This range is represented by the Peclet number
\( a = G/\theta \) (Brenner, 1972a) where \( G \) is the shear rate and \( \theta \) the mean rotational diffusion coefficient. We shall only be considering the case of overwhelming Brownian motion \((a+0)\) in which the hydrodynamic effects are completely negligible compared with the Brownian motion effects. Thus we shall take it that on average the particles are rotating with the local angular velocity of the ambient flow; and we may additionally assume that the orientation of the particles will be random. This last fact would not be so if hydrodynamic forces and torques were not negligible for they introduce systematic motions and hence preferred orientations.

2.2. The Simha Model of Overwhelming Brownian Motion

We consider a homogeneous dilute suspension of identical rigid ellipsoids randomly oriented in an incompressible Newtonian fluid in which they are neutrally buoyant. The ambient flow is taken to be a slow simple shearing flow, whilst the suspended particles are taken to be moving with the velocity and the angular velocity of the ambient flow appropriate to the point at which each is suspended. Near each particle this ambient flow is disturbed but is still taken to be a slow (low Reynolds's number) flow so that we may apply the classical results of Jeffrey (1922).

This model, which is taken to be appropriate for the case of overwhelming Brownian motion derives from Simha (1940) although in his original work doubt is left about whether or not the particles are rotating with the local angular velocity of the fluid. An attempt to clear this difficulty is made below (section 2.6.). The key simplifying feature of the model introduced by Simha is that it eliminates the complicated statistical problem presented by the Brownian motion by substituting an assembly of
particles all moving with the average motion. This, together with the assumptions of diluteness and random orientation, allows us to compute the effect of the suspended particles by simply summing their individual effects. The isotropy of the particle distribution in the model means that non-Newtonian behaviour will not appear, and also allows us to use the energy dissipation method of computing the viscosity (Batchelor, 1970, Brenner, 1972b, p93).

The simplifications of the model are achieved, however, at a price. Non-Newtonian and concentration dependent effects, which to the theoretical rheologist are of the greatest interest, have been deliberately discarded; and the model can say nothing about lesser degrees of Brownian motion. In effect we shall be calculating the first term of a series; nevertheless this is of great value to the molecular biologist who can deliberately arrange the conditions of a viscosity experiment so that the model is applicable:

(i) Giesekus (1962) has shown that non-Newtonian normal stress effects are of 2nd order, and can thus be neglected for very low shear rates as in, for example, a capillary viscometer (Yang, 1961);
(ii) Viscosity coefficients are normally extrapolated to 'infinite dilution' i.e. zero concentration-dependent effects, to give the 'intrinsic viscosity' (Van Holde, 1971), related to the viscosity increment by equation (8).

2.3. The Viscosity Increment

We let \( \eta \) be the viscosity measured in an experiment on a dilute suspension of particles in a fluid of viscosity \( \eta_0 \). If \( \phi \) is the volume concentration - the total volume of the particles in unit volume of the suspension - then the viscosity increment \( \nu \) is defined, from
equation (7), by

\[ \frac{\eta}{\eta_0} = 1 + \nu \phi \] (65)

where, when \( \nu \) is independent of \( \phi \), the linear dependence of \( \frac{\eta}{\eta_0} \) upon \( \phi \) gives the empirical characteristic of a dilute suspension.

From the theoretical point of view however, a dilute suspension is one in which there are no hydrodynamic interactions between the particles and thus one in which each particle independently contributes to the viscosity the same amount it would were it alone present. This contribution for a general ellipsoidal particle was first calculated by Jeffrey (1922) using the simple energy dissipation analysis for averaging over the particle ensemble (Batchelor, 1970) and it is a straightforward matter to extend his results to cover the case of ellipsoids rotating with the local angular velocity of the ambient flow as required by our model.

2.4. The Flow Velocity and Pressure

In order to calculate the additional dissipation of energy caused by introducing the particle into a given flow, we compare that given flow with the consequent disturbed flow within a suitable sphere, \( S \), of radius \( R \), centred on the particle position. We impose two requirements upon \( S \): first, that it is small compared with the scale of spatial variations in the given flow, and thus within it that flow is effectively given as a linear variation of velocity with position; secondly, that it is large compared with the size of the particle, and thus that the disturbed flow will not appreciably differ from the given flow by the time the surface
of $S$ is reached. Naturally, these requirements can only be met when the particle is, as we have assumed, very much smaller than the scale of spatial variations in the velocity field of the given flow.

For our purposes then, the disturbed flow may be taken to be the flow of an incompressible fluid in the region between the rotating ellipsoidal surface of the particle and the concentric spherical surface $S$. On the inner surface we impose the usual no-slip boundary condition, whilst on $S$ we require the velocity field to be equal to its value in the original flow. We give the velocity components of the two flows with respect to rectangular Cartesian axes fixed in the rotating particle so that its ellipsoidal surface will always be given by

$$\frac{x^2}{a^2} + \frac{y^2}{b^2} + \frac{z^2}{c^2} = 1$$

(66)

The undisturbed flow is given, within $S$, by

$$u_i^0 = g_{ij} x_j$$

where $g_{ij}$ are the components of the velocity gradient tensor which are by our assumptions, independent of position within $S$. In this equation and in subsequent equations, the indices range over the values 1, 2, 3 and the summation convention is used whereby when an index is repeated within a term a summation is indicated over the three values of that index.

Using ellipsoidal harmonics, Jeffrey was able to give the flow velocity and pressure in the region of $S$ for $R$ large, but finite. He gives the result under the assumption that the angular velocity is such that no net hydrodynamic torque acts on it, i.e. hydrodynamic effects alone affect the motion of the particle. In order to consider the Brownian motion we follow Simha in dropping this restriction whence the flow near $S$ is found,
to leading order, to be

\[ u_i = u_i^0 - 4\Phi x_i \left(\frac{1}{r^5} - \frac{1}{r^5}\right) + \frac{5\Phi}{\partial x_i} \left(\frac{1}{r^3} - \frac{r^2}{r^5}\right) \]  

(67)

In this equation, \( \Phi = A_{ij}x_i x_j \), whilst the \( A_{ij} \) themselves are coefficients independent of position but dependent on the \( g_{ij} \) and the components, \( \omega_i \) of the angular velocity of the particle; their explicit values are given by Jeffrey (see Table 4 for the relationship between his notation and ours).

We consider the values of the \( A_{ij} \) below.

On the assumption that terms of second order in the velocity may be neglected and that the particle spins are of the same order as the fluid velocities, the dynamical equation for the fluid reduces to

\[ \eta \nabla^2 u = \nabla p \]  

(68)

from which the pressure, \( p \), can be found. For the disturbed flow we find the pressure on \( S \) to be

\[ p = p_0 - \frac{50 \eta \Phi}{R^5} \]  

(69)

where \( p_0 \) is a constant.

2.5. The Dissipation of Energy

Assuming a steady state, we can compare the rates of dissipation of energy within \( S \) in the two flows by comparing the corresponding rates for working of the viscous stresses on the surface \( S \). This rate of working, \( dW/dt \), is given by

\[ \frac{dW}{dt} = \int_S u_i^0 \sigma_{ij} n_j \, dS \]  

(70)
where

\[ \sigma_{ij} = -p \delta_{ij} + n \left( \frac{\partial u_j}{\partial x_i} + \frac{\partial u_i}{\partial x_j} \right) \]  \hspace{1cm} (71)

are the components of the stress tensor, and

\[ n_j = \frac{x_j}{R} \]  \hspace{1cm} (72)

are the components of the unit normal to \( S \).

For the disturbed flow we find

\[ \frac{dW}{dt} = \frac{8}{3} \pi \eta a_{ij} a_{ij} R^3 + \frac{32}{3} \pi \eta A_{ij} g_{ij} \]  \hspace{1cm} (73)

where the \( a_{ij} = \frac{1}{2}(g_{ij} + g_{ji}) \) are the components of the local distortion in the undisturbed flow. On the other hand, the well-known formula of Stokes gives, for the undisturbed flow

\[ \frac{dW}{dt} = \frac{8}{3} \pi \eta a_{ij} a_{ij} R^3 \]  \hspace{1cm} (74)

We thus obtain an expression for \( \Delta \), the extra dissipation of energy when the particle is present, namely

\[ \Delta = \frac{32}{3} \pi \eta A_{ij} g_{ij} \]  \hspace{1cm} (75)

If we split \( g_{ij} \) into its symmetric and skew-symmetric parts, we have

\[ \Delta = \frac{32}{3} \pi \eta (A_{ij} a_{ij} + A_{ij} \tilde{\epsilon}_{ij}) \]  \hspace{1cm} (76)
where $\xi_{ij} = \frac{1}{2}(g_{ij} - g_{ji})$. Jeffrey, as a consequence of the dynamical assumption mentioned above, was working with symmetrical $A_{ij}$, and so naturally obtained only the first term in our expression for $A$; and it appears that Simha, although he removed the restriction on $A$, failed to find the second term. The consequence of this for his calculation will now be discussed.

2.6. The Particle Rotation

Simha takes the average angular velocity to be zero and on this basis calculates his well known formula for $v$ (equation 9), a formula which has been shown to give good agreement with observations (Mehl, Oncley & Simha, 1940, Tanford, 1961). A few years later, Saito (1951) using the assumption that the particles should rotate on average with the local undisturbed rotation of the fluid obtained precisely the same result; he suggested that Simha "has committed some errors in calculation" but does not investigate the matter further. Using Jeffrey's notation (Table 4) we have:

$$A_{ij}a_{ij} = (A\alpha + B\beta + C\gamma) + (F + F')\xi + (G + G')\eta + (H + H')\zeta$$  \hspace{1cm} (77)

$$A_{ij}\xi_{ij} = (F' - F)\xi + (G' - G)\eta + (H' - H)\zeta$$  \hspace{1cm} (78)

whilst the values of, for example, $F$ and $F'$ are

$$F = \frac{\beta_0 f - c^2 \alpha' (\xi - \omega_1)}{2\alpha' (b^2 \beta_0 + c^2 \gamma_0)}$$  \hspace{1cm} (79)
In Jeffrey's paper the \( a' \), etc. in the numerators of the above expressions are misprinted as \( a \), etc.

We can thus deduce that

\[
(F' + F')^x = \frac{2a''}{a_0} f^2 + \frac{(b^2 + c^2)f^2}{a_0} + \frac{(b^2 - c^2)(\xi - \omega_1)f}{2(b^2 \beta_0 + c^2 \gamma_0)}
\]

(81)

\[
(F' - F)^y = \frac{(b^2 - c^2)f \xi + (b^2 + c^2)(\xi - \omega_1)\xi}{2(b^2 \beta_0 + c^2 \gamma_0)}
\]

(82)

where we have utilised the various relations between \( a', \beta_0 \), etc. that are given by Jeffrey.

Now Simha apparently did not find the \( A_{ij} \xi_{ij} \) term and thus would not have had terms like \( (F' - F) \) in his calculation. We can see, however, that taking \( \omega_1 = 0 \) as he apparently did, in the \( (F + F')^x \) term gives the same final result as taking \( \omega_1 = \xi \) in the sum of the \( (F + F')^x \) and the \( (F' - F)^y \) terms. Since the same argument applies to the other terms we conclude that Simha's formula (equation 9) although incorrect for \( \omega_1 = 0 \) on account of the omission of the term \( A_{ij} \xi_{ij} \), is, by a lucky coincidence, actually correct if \( \omega_1 = \xi, \omega_2 = \eta, \omega_3 = \zeta \).

It is worth noting that if one does take \( \omega_1 = 0 \) and includes the \( A_{ij} \xi_{ij} \) term, one obtains for spherical particles \( \nu = 4 \), in contrast to Einstein's (1906, 1911) value of 2.5. The result \( \nu = 4 \) for \( \omega_1 = 0 \) agrees
with that previously found by Brenner (1970). In all that follows we take the assumption that \( \omega_1 = \xi \) etc. i.e. that the particles are on average rotating with the local angular velocity of the fluid.

2.7. The Calculation of \( \psi \)

To complete our calculation we take, as before, the given flow to be locally a simple shearing flow with shear rate \( G \). The principal axes of any particular particle will not in general coincide with the shear axes but, using the Euler angles to describe relative orientation of the two sets of axes, we can calculate the components \( g_{ij} \) relative to the particle axes in terms of \( G \) and the Euler angles \( \theta \), \( \phi \) and \( \psi \). Hence we can obtain \( \Delta \) for that particle as a function of these variables; the details can be found at least for a special case in Jeffrey's paper (1922). Since Jeffrey's calculations show that the \( A_{ij} \) are linear in the \( g_{ij} \)'s, it follows that \( \Delta \) will involve \( G^2 \) as a factor and hence that the total dissipation will be of the form \( \eta G^2 \) as originally asserted.

To find the total dissipation in unit volume we average the effects of the \( N \) particles on the assumption that they are randomly oriented, obtaining

\[
\bar{\Delta} = \frac{N}{2\pi} \int_{0}^{2\pi} \left\{ \int_{0}^{2\pi} \int_{0}^{\pi} \Delta(\theta, \phi, \psi) \sin \theta \, d\theta \, d\phi \right\} \, d\psi
\]

(83)

The integrations yield

\[
\bar{\Delta} = \frac{32}{3} \pi \eta NG^2 Z
\]

(84)
where

\[ Z = \frac{1}{30} \left( \frac{\alpha''_0 + \beta''_0 + \gamma''_0}{\beta''_0 \gamma''_0 + \gamma''_0 \alpha''_0 + \alpha''_0 \beta''_0} \right) \]

\[ + \frac{1}{40} \left( \frac{\beta'_0 + \gamma'_0}{\alpha'_0 (b^2 \beta'_0 + c^2 \gamma'_0)} + \frac{\gamma'_0 + \alpha'_0}{\beta'_0 (c^2 \gamma'_0 + a^2 \alpha'_0)} + \frac{\alpha'_0 + \beta'_0}{\gamma'_0 (a^2 \alpha'_0 + b^2 \beta'_0)} \right) \]  

(85)

Thus \( v \) is determined from

\[ \eta v V a^2 = \eta v N \frac{4}{3} \pi abc G^2 = \frac{32}{3} \pi \eta N G^2 Z \]  

(86)
as

\[ v = \frac{8Z}{abc} \]  

(87)

Hence on substituting for \( Z \) we obtain

\[ v = \frac{1}{abc} \left\{ \frac{4(\alpha''_0 + \beta''_0 + \gamma''_0)}{15(\beta''_0 \gamma''_0 + \gamma''_0 \alpha''_0 + \alpha''_0 \beta''_0)} + \frac{1}{5} \left[ \frac{\beta'_0 + \gamma'_0}{\alpha'_0 (b^2 \beta'_0 + c^2 \gamma'_0)} + \frac{\gamma'_0 + \alpha'_0}{\beta'_0 (c^2 \gamma'_0 + a^2 \alpha'_0)} + \frac{\alpha'_0 + \beta'_0}{\gamma'_0 (a^2 \alpha'_0 + b^2 \beta'_0)} \right] \right\} \]  

(88)

where \( a, b, c \) are the semi-axes, and the elliptic integrals \( \alpha_0 \) etc. now depend on \( a, b \) and \( c \) (Appendix I).
The formula reduces to the Simha-Saito formula (equation 9) when \( b = c \), and gives Einstein's value of 2.5 when \( a = b = c \). It may be of interest to note that had we followed Simha in taking \( w = 0 \) then \( Z \) would have contained the following term in addition to those given above,

\[
\frac{1}{24} \left( \frac{b^2 + c^2}{b^2 \beta_0 + c^2 \gamma_0} + \frac{c^2 + a^2}{c^2 \gamma_0 + a^2 \alpha_0} + \frac{a^2 + b^2}{a^2 \alpha_0 + b^2 \beta_0} \right)
\]

(89)

It is the presence of this added term that gives the value of \( \nu = 4 \) for spheres rather than the Einstein value \( \nu = 2.5 \) which is obtained when it is absent. The value of 2.5 has been confirmed experimentally for polystyrene latex spheres by Cheng \& Schachman (1955).

2.8. Discussion

An equation similar to (88) was given by Batchelor (1970) on the assumption that the suspended particles, although randomly oriented, moved so that zero hydrodynamic torque acted upon them. His result was

\[
\nu = \frac{1}{abc} \left\{ \frac{4(\alpha''_0 + \beta''_0 + \gamma''_0)}{15(\beta''_0 \gamma_0 + \gamma''_0 \alpha_0 + \alpha''_0 \beta_0)} + \frac{2}{5} \left( \frac{1}{\alpha'_0 (b^2 + c^2)} + \frac{1}{\beta'_0 (c^2 + a^2)} + \frac{1}{\gamma'_0 (a^2 + b^2)} \right) \right\}
\]

(90)

when written in the same notation as we have used before. It does not seem likely that (90) would be applicable to the case of overwhelming Brownian motion since one would need to include the Brownian torque \( T_B \) as well as the purely hydrodynamic torque, \( T_H \), in satisfying the
condition of zero net torque, i.e.

\[ T_B + T_H = 0 \]  \hspace{1cm} (91)

Random orientation alone is not a sufficient characterisation of overwhelming Brownian motion since one also needs to describe correctly the distribution of the angular velocity. Both (88) and (90) are obtained by methods that avoid the full statistical treatment of the angular motion but as explained earlier we consider the simplified model underlying (88) to be the appropriate one for overwhelming Brownian motion. In effect, formula (88) generalises the Simha-Saito equation for ellipsoids of revolution, whilst (90) generalises formul\(\acute{e}\) of Jeffrey for ellipsoids of revolution. In general the two formulae give quite different results as can be seen from Figure 25 and Table 5, both of which are for convenience restricted to the case of ellipsoids of revolution. Since (90) does not reduce to the classical Simha-Saito formula the classic experimental evidence on macromolecules which favours the latter (Mehl, et al, 1940, Lauffer, 1942) strengthens the view that (90) is incorrect. More recent experimental evidence is given by Tanford (1961) who allows for particle swelling due to solvation and Table 6 extends his tables to include a comparison with the Jeffrey-Batchelor equation. The table compares the axial ratio inferred from translational diffusion experiments with that inferred from viscometric experiments on the basis first of the Simha-Saito equation and secondly of the Jeffrey-Batchelor equation. Tanford (1961) says "within the accuracy of the measurements, the description of globular proteins in aqueous solution provided by the (Simha-Saito) equation is identical with that provided by (translational)
diffusion". On the other hand we see that the Jeffrey-Batchelor equation gives values of the axial ratio that are consistently too high and outside the expected experimental error bounds. We conclude that (90) is not applicable to the cases of interest to the molecular biologist.

As previously stated, we have avoided the full statistical treatment of the angular motion but have made the assumption of particles being on average at rest in the local referential frame in which they are suspended to be appropriate for the case of overwhelming Brownian motion. Although this has been rigorously proved only for axisymmetric particles (Brenner, 1972), we have made the assumption that it will be a good approximation for general tri-axial ellipsoids, at least for low axial ratios.

Since the derivation of equation (88) a general analysis using the full statistical treatment of the angular motion has been given by Rallison (1978). His results for the case of overwhelming Brownian motion show that to first-order in the shear rate the non-Newtonian stress effects vanish, which is consistent with our assumption of Newtonian behaviour for very low shear rates. He also gives an expression for \( \nu \) correct to first-order in the shear rate, although not in the form of a simple formula like equation (88), but by using numerical methods Rallison is able to give a plot of \( \nu \) for various axial ratios; the results are clearly very close to those obtained from equation (88) - compare my Figure 26 with Rallison's Figure 7. However, an exact comparison (personal communication by J.M. Rallison) shows a very slight discrepancy between values from equation (88) and Rallison's procedure, although no difference at levels likely to be experimentally significant for globular particles (i.e. \( a/b: 1.0 - 3.0, b/c: 1.0 - 3.0 \)) is observed, and the discrepancy is not
apparent within four significant figures for $a/b$: $1.0 \rightarrow 2.0$, $b/c$: $1.0 \rightarrow 2.0$. The values given in Table 7 are therefore definitive.

It has been indicated to us (J.M. Rallison, H. Brenner, private communications of unpublished work) that our formula requires the addition of a very small term related to the deviation from our assumed condition of non-axisymmetric particles rotating on average with the local angular velocity of the fluid:

$$
- \frac{1}{5abc} \left[ \frac{a^2 - b^2}{a^2 \alpha_0 + b^2 \beta_0} + \frac{b^2 - c^2}{b^2 \beta_0 + c^2 \gamma_0} + \frac{c^2 - a^2}{c^2 \gamma_0 + a^2 \alpha_0} \right]^2
$$

(88b)

The numerical results show our approximation to be extremely accurate for 'globular' particles, as noted above, but for certain particles of higher asymmetry calculations suggest that deviations of up to $1\%$ in $\nu$ can arise. It is clear though that our formula provides a good approximation over the entire molecular range. Of particular interest is the fact that the discrepancy tends asymptotically to zero for ellipsoids whose axes are all substantially different in length (i.e. $a \gg b \gg c$ - "tapes").
Table 4. The relation between the notation used in this study and that used by Jeffrey (1922)

\[
(A_{ij}) = \begin{pmatrix}
A & H & G' \\
H' & B & F \\
G & F' & C
\end{pmatrix},
\]

\[
(a_{ij}) = \begin{pmatrix}
a & h & g \\
\sim & \sim & \sim \\
h & b & f \\
\sim & \sim & \sim \\
g & f & c \\
\sim & \sim & \sim
\end{pmatrix},
\]

\[
(\xi_{ij}) = \begin{pmatrix}
0 & -\zeta & \eta \\
\zeta & 0 & -\zeta \\
-\eta & \xi & 0
\end{pmatrix}.
\]
Table 5

$v$ for an ellipsoid of revolution calculated from the Simha - Saito equation and the Batchelor - Jeffrey equation

<table>
<thead>
<tr>
<th>Axial Ratio</th>
<th>Prolate Model</th>
<th>Oblate Model</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$S - S$</td>
<td>$B - J$</td>
</tr>
<tr>
<td>1.0</td>
<td>2.500</td>
<td>2.500</td>
</tr>
<tr>
<td>2.0</td>
<td>2.908</td>
<td>2.583</td>
</tr>
<tr>
<td>3.0</td>
<td>3.685</td>
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<td>4.663</td>
<td>3.077</td>
</tr>
<tr>
<td>5.0</td>
<td>5.806</td>
<td>3.434</td>
</tr>
<tr>
<td>6.0</td>
<td>7.099</td>
<td>3.844</td>
</tr>
<tr>
<td>7.0</td>
<td>8.533</td>
<td>4.302</td>
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<tr>
<td>8.0</td>
<td>10.103</td>
<td>4.804</td>
</tr>
<tr>
<td>9.0</td>
<td>11.804</td>
<td>5.346</td>
</tr>
<tr>
<td>10.0</td>
<td>13.634</td>
<td>5.928</td>
</tr>
</tbody>
</table>
Table 6

Extension of Tanford's Tables ("Physical Chemistry of Macromolecules", 1961, Wiley & Sons, p 359 and 395) to compare the axial ratios predicted by the Simha-Saito equation and the Batchelor-Jeffrey equation, using a 0.2 grams/gram solvation for four globular proteins.

<table>
<thead>
<tr>
<th></th>
<th>Prolate</th>
<th></th>
<th>B-J</th>
<th></th>
<th>Oblate</th>
<th></th>
<th>B-J</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Diffusion</td>
<td>S-S</td>
<td></td>
<td></td>
<td>Diffusion</td>
<td>S-S</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(a/b)</td>
<td>(a/b)</td>
<td></td>
<td>(a/b)</td>
<td>(a/b)</td>
<td>(a/b)</td>
<td></td>
</tr>
<tr>
<td>Ribonuclease</td>
<td>3.6</td>
<td>2.1</td>
<td>2.9</td>
<td>5.5</td>
<td>2.2</td>
<td>3.4</td>
<td>5.3</td>
</tr>
<tr>
<td>(\beta)-lactoglobulin</td>
<td>3.6</td>
<td>3.7</td>
<td>2.9</td>
<td>5.5</td>
<td>4.0</td>
<td>3.4</td>
<td>5.3</td>
</tr>
<tr>
<td>Serum albumin</td>
<td>4.0</td>
<td>4.9</td>
<td>3.3</td>
<td>6.5</td>
<td>5.0</td>
<td>4.0</td>
<td>6.3</td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>3.8</td>
<td>2.1</td>
<td>3.1</td>
<td>6.0</td>
<td>2.2</td>
<td>3.6</td>
<td>5.8</td>
</tr>
</tbody>
</table>
Table 7. Values of $v$ as a function of $(a/b, b/c)$ for a general tri-axial ellipsoid ($a>b>c$)

<table>
<thead>
<tr>
<th>$a/b$</th>
<th>$b/c$</th>
<th>1.0</th>
<th>1.1</th>
<th>1.2</th>
<th>1.3</th>
<th>1.4</th>
<th>1.5</th>
<th>1.6</th>
<th>1.7</th>
<th>1.8</th>
<th>1.9</th>
<th>2.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oblate Ellipsoid</td>
<td>1.0</td>
<td>2.500</td>
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<td>2.524</td>
<td>2.550</td>
<td>2.503</td>
<td>2.620</td>
<td>2.661</td>
<td>2.706</td>
<td>2.753</td>
<td>2.803</td>
<td>2.854</td>
</tr>
<tr>
<td></td>
<td>1.1</td>
<td>2.507</td>
<td>2.520</td>
<td>2.544</td>
<td>2.576</td>
<td>2.614</td>
<td>2.656</td>
<td>2.702</td>
<td>2.751</td>
<td>2.803</td>
<td>2.857</td>
<td>2.913</td>
</tr>
<tr>
<td></td>
<td>1.2</td>
<td>2.525</td>
<td>2.545</td>
<td>2.575</td>
<td>2.612</td>
<td>2.655</td>
<td>2.703</td>
<td>2.754</td>
<td>2.808</td>
<td>2.865</td>
<td>2.923</td>
<td>2.983</td>
</tr>
<tr>
<td></td>
<td>1.3</td>
<td>2.553</td>
<td>2.579</td>
<td>2.615</td>
<td>2.658</td>
<td>2.706</td>
<td>2.759</td>
<td>2.815</td>
<td>2.874</td>
<td>2.935</td>
<td>2.998</td>
<td>3.063</td>
</tr>
<tr>
<td></td>
<td>1.4</td>
<td>2.588</td>
<td>2.621</td>
<td>2.662</td>
<td>2.711</td>
<td>2.764</td>
<td>2.822</td>
<td>2.883</td>
<td>2.947</td>
<td>3.013</td>
<td>3.081</td>
<td>3.151</td>
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<td></td>
<td>1.5</td>
<td>2.630</td>
<td>2.668</td>
<td>2.716</td>
<td>2.770</td>
<td>2.829</td>
<td>2.892</td>
<td>2.958</td>
<td>3.027</td>
<td>3.098</td>
<td>3.171</td>
<td>3.245</td>
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<tr>
<td></td>
<td>1.6</td>
<td>2.677</td>
<td>2.722</td>
<td>2.775</td>
<td>2.834</td>
<td>2.899</td>
<td>2.967</td>
<td>3.039</td>
<td>3.113</td>
<td>3.189</td>
<td>3.267</td>
<td>3.346</td>
</tr>
</tbody>
</table>
Figure 25. A comparison of the values of $v$ as a function of axial ratio predicted by the Simha - Saito and Batchelor - Jeffrey equations for ellipsoids of revolution.
Figure 26. Plot of $v$ as a function of $a/b$ when $b/c = 10.0$ ($a>b>c$) determined from equation (88). This plot agrees very closely with that from the numerical procedure of Rallison (Figure 7, 1978)

N.B. Rallison has $c>a>b$
CHAPTER 3

Numerical Inversion Procedures:
The Problem of the Line Solution
3.1 Solution of the Elliptic Integrals

In order to determine the viscosity increment \( \nu \) that corresponds to a particular value of the axial ratios \( a/b, b/c \), the elliptic integrals \( \alpha_0 \) etc. (Appendix I) must be solved. Analytic solutions are not possible but the integrals can be solved numerically with the aid of a high speed computer. The subroutine used for this was the United Kingdom NAG Mk. 6 routine D01AGF which evaluates a definite integral of the form

\[
B \\
I = \int_{A}^{B} f(t) \, dt
\]

where \( A=0 \), using an interval subdivision strategy developed by Oliver (1972) and based on Clenshaw–Curtis quadrature (1960). Since infinity cannot be used as the upper limit, a finite value of \( B \) must be specified. However, a satisfactory value for \( B \) can be determined by using successively higher values until the value of the integral converges to a limiting value; in this case a value for \( B \) of \( 10^6 \) was sufficient. Higher values are also suitable although evaluation of the integral takes longer. The number of interval subdivisions is also specifiable by the user; the maximum number of 50 was used. The routine also estimates the error on the integrals (O'Hara & Smith, 1968). If this error is greater than the maximum allowable error specifiable by the user the routine will stop and print an error message. The maximum allowed absolute error specified was \( 1.0 \times 10^{-8} \) (\( \approx 0.001\% \)). The subroutine for evaluating the elliptic integrals can easily be incorporated into a program for evaluating \( \nu \) for a given value of \( (a/b, b/c) \). This is given in Appendix V as Program 1.
3.2. Application to the Crystallographic Dimensions of Myoglobin; Numerical Inversion

The result can be applied to crystallographic data available for myoglobin. Kendrew et al. (1958) gave the dimensions of sperm whale myoglobin to be 43 x 35 x 23 Å (Table 3). This corresponds to a general tri-axial ellipsoid of semi-axes a = 21.5, b = 17.5, and c = 11.5 Å, and axial ratios a/b = 1.23, b/c = 1.52. Using Program 1 (Appendix V) this corresponds to a viscosity increment of 2.729. The predicted intrinsic viscosity can then be found from equation (8):

$$\eta = \frac{\nu}{V_s} = \frac{\nu}{V} \left( \frac{V_s}{V} \right)$$

(92)

where \(\frac{V_s}{V}\) is the swelling ratio (section 1.7.1). By fitting data of reduced specific viscosity against concentration (Table 8, Figure 27) I have determined the intrinsic viscosity of myoglobin to be (3.25 ± .05) ml/gm, using a weighted least squares analysis (straight line fit).

The concentrations were determined using a high precision auto density meter (Kraky et al, 1969, 1973) together with a \(\nu\) for myoglobin of .741 ml/gm (Theorell, 1934):

$$c_i = \frac{\rho_i - \rho_0}{1 - \nu \rho_o}$$

(93)

where \(\rho_0\) is the solvent density and \(\rho_i\) the solute densities. Use of the auto density meter, which is based on the time taken to perform a preset number of oscillations of a U-tube filled with the sample has the added advantage that, besides being very accurate, only small amounts of fluid are required (~1 ml). The experimental arrangement used for the viscosity and densimetric work is illustrated in Figure 28. The
platinum resistance thermometer shown was used to monitor the sample temperatures to accuracies of .005 degrees and was calibrated by myself. In order that the crystallographic dimensions gives this same value for $[\eta]$, from equation (92), a swelling ratio $(\sqrt[3]{v}/\sqrt[3]{v})$ of 1.6 is required; alternatively myoglobin is more asymmetric in solution.

In order to determine the actual dimensions of the equivalent tri-axial ellipsoid for myoglobin in solution (or any other macromolecule) from the experimental value for $[\eta]$, the situation is more complicated however. Although equation (88) defines a unique value of $v$ for a given value of $(a/b, b/c)$, an analytic inversion of (88) to produce an explicit expression for $(a/b, b/c)$ in terms of $v$ is not available. The inversion must therefore be done numerically by tabulating, or better plotting $v$ as a function of $(a/b, b/c)$. The same subroutine mentioned in section 3.1. for evaluating the elliptic integrals may be incorporated. A perusal of Table 7 (produced from Program 2) reveals however that a given value of $v$ does not correspond to a unique value of $(a/b, b/c)$ but to a 'line solution' of possible values of $(a/b, b/c)$. This is clearly illustrated in the contour plot (Figure 29) produced from Program 3 using GHOST graphical facilities where $v$ is incremented from 2.5 to 7.0 in steps of 0.5. In order to determine a unique solution for $(a/b, b/c)$ and hence the axial dimensions of a macromolecule in solution other hydrodynamic information must be used; we must therefore consider the translational and rotational frictional properties (section 1.2).
3.3. Other Tri-axial Line Solutions

3.3.1. The Translational Frictional Ratio; the $S$ and $R$ Functions

It was previously stated in section 1.4. that although Perrin (1936) had provided an explicit formula for the translational frictional ratio of a general tri-axial ellipsoid in terms of the axial ratios $(a/b, b/c)$, the elliptic integral in equation (12) could only be solved analytically for the special case of ellipsoids of revolution (i.e. two equal axes). However, since the elliptical integral is similar to those for the tri-axial viscosity increment, it too can now be solved numerically using for example the subroutine discussed in section 3.1. A higher value for the upper limit, $B$ was required: $5 \times 10^7$. A table of values of the Perrin function $f/f_0 (\equiv P)$ for values of $a/b$ and $b/c$ was thus obtained (Table 9). Again, a perusal of the table reveals that a given value of $P$ has a line solution of possible values of $(a/b, b/c)$. However, in principle at least, by combining the line solution for $P$ of a given macromolecule with the line solution for $\nu$, a unique solution for $(a/b, b/c)$ can in principle be found from their intersection. This can be illustrated by assuming a particle of $(a/b, b/c) = (1.5, 1.5)$, calculating the corresponding values for $\nu$ and $P$ using Program 1, and then plotting the line solutions using Program 4. Unfortunately Figure 30 reveals that the intersection for accuracies in $\nu$ and $P$ to four significant figures is very shallow, and allowing for $\pm 1\%$ experimental error in each there is no intersection at all in the 'globular protein' range of the figure. There is also the additional problem that in order to determine experimentally both $\nu$ and $P$, knowledge is required of the swollen volume in solution.
However, now that \( v \) and \( P \) are available for tri-axial ellipsoids, then so should the \( \beta \) and \( R \) functions which do not require a knowledge of the swollen volume (equations 45 & 64). I have thus produced tables of these also (Tables 10 & 11); all four tri-axial functions so far mentioned viz \( v \), \( P \), \( \beta \) and \( R \) are plotted in Figure 31 allowing for \( \pm 1\% \) experimental error in each. There is still no reasonable intersection; the \( \beta \) function is, as expected, seen to be of little practical use as it is very sensitive to experimental error (the \( \beta - 1\% \) line is completely off the map area). Of the 4 functions however, the \( R \) function is the most useful since it is relatively insensitive to experimental error and the experimental determination does not require a knowledge of the swollen volume (section 1.7.1.). In order to find a unique solution for \( (a/b, b/c) \) therefore, this should ideally be combined with a rotational frictional or relaxation tri-axial shape function which should satisfy the following criteria:

(i) provides a suitable intersection with \( R \)

(ii) is relatively insensitive to experimental error but sensitive to axial ratio

(iii) is experimentally measurable to a high precision with currently available apparatus and data analytic techniques and

(iv) does not require a knowledge of the swollen volume for its experimental determination.

3.3.2. The Rotational Frictional, Diffusion and Relaxation Line Solutions

For a tri-axial ellipsoid there will be three rotational frictional ratios \( \zeta_i/\zeta_o \) \((i=a,b,c)\) corresponding to rotation about each of the three axes and hence three rotational diffusion ratios \( \theta_i/\theta_o \). By analogy with the translational case in the previous section, although Perrin (1934) had
given explicit formula for the $\frac{\zeta_1}{\zeta_0}$ in terms of $(a/b, b/c)$, - eqn. (25),
the elliptic integrals could only be solved analytically for the case of ellipsoids of revolution. The integrals can now be solved numerically, again utilising the routine described in section 3.1 (Programs 1, 2 & 4). There is however no experimental technique for determining the rotational frictional or diffusion coefficients directly; rotational experiments determine rather relaxation time ratios. For example, the dielectric dispersion relaxation time ratios are related to the rotational frictional and diffusion ratios by equations (27). A plot of the rotational relaxation time ratio line solutions corresponding to $(a/b, b/c) = (1.5, 1.5)$ is given together with the R function in Figure 32. Unfortunately, because of the difficulties raised in 1.5.1. resolution of the dielectric dispersion curve into the 3 relaxation times for a homogeneous solution of tri-axial ellipsoid particles is impossible in practice.

Whereas for ellipsoids of revolution there are three fluorescence anisotropy decay times (equation 42), for general tri-axial ellipsoids, there will be five (Cantor & Tao, 1971, Small & Isenberg, 1977) related to the three rotational diffusion coefficients by:

$$
\tau_1 = \frac{1}{3(\theta + \theta_1)} \quad ; \quad \tau_2 = \frac{1}{3(\theta + \theta_2)} \quad ; \quad \tau_3 = \frac{1}{3(\theta + \theta_3)} \\
\tau_4 = \frac{1}{2(\theta - \Delta)} \quad ; \quad \tau_5 = \frac{1}{2(\theta + \Delta)}
$$

(94)

where $\theta = (\theta_1 + \theta_2 + \theta_3)/3$ is the mean rotational diffusion coefficient, and $\Delta$ is defined by

$$
\Delta = \left(\theta_1^2 + \theta_2^2 + \theta_3^2 - \theta_1\theta_2 - \theta_2\theta_3 - \theta_3\theta_1\right)^{1/2}
$$
The fluorescence anisotropy relaxation time ratios $T_j / T_0$ can thus be evaluated (equation 42, where $j$ is now = 1,2,3,4,5); these have been tabulated by Small & Isenberg (1977) and are plotted in Figure 33, for $(a/b, b/c) = (1.5, 1.5)$. Consideration of these functions however, at the moment at least, is purely academic; besides the problems cited in section 1.5.4., the necessary resolution of the decay curve into its four component exponentials (since $\tau_5 - \tau_1$ is impossible (Small & Isenberg, 1977). Furthermore, since neither the fluorescence anisotropy decay time ratios nor the dielectric dispersion relaxation time ratios for tri-axial ellipsoids are of apparent use at the moment, the same must be true of their corresponding swelling independent functions, the explicit expressions in terms of axial ratio being obtainable from:

$$\delta_i = \frac{T_0}{T_i} \nu , \quad \nu_i = (\frac{f_i}{f_0}) \left( \frac{T_i}{T_0} \right)^{\frac{i}{3}}$$

(95, 96)

$$\gamma_i = (\frac{f_i}{f_0})^3 \frac{\rho_0}{\rho_i} , \quad \epsilon_i = \nu \frac{\rho_0}{\rho_i}$$

(97, 98)

$$\kappa_j = \nu \left( \frac{T_0}{\tau_j} \right) , \quad \xi_j = (\frac{f_j}{f_0})^3 \frac{T_0}{\tau_j}$$

(99, 100)

where $i=a,b,c$ and $j=1,2,3,4,5$. The relations for these functions in terms of experimental parameters have already been given in section 1.7.

Evaluation of the harmonic mean rotational relaxation time ratio in terms of axial ratio for tri-axial ellipsoids we can similarly obtain from
(Programs 1, 2 & 4, Figure 34). The corresponding swelling independent functions \( \Psi \) and \( \Lambda \) determined by combining with the translational frictional ratio and the viscosity increment respectively we can now also obtain from

\[
\Psi = \left( \frac{\tau_0}{\tau_h} \right) \left( \frac{f}{f_0} \right)
\]

(102)

\[
\Lambda = \left( \frac{\tau_0}{\tau_h} \right) \nu
\]

(103)

(Various 1, 2 & 4, Figure 34). Unfortunately, these functions are generally very sensitive to experimental error, as Figure 35 illustrates; also the problems in determining the harmonic mean relaxation time raised in 1.5.4. still apply.

3.3.3 Electric Birefringence Decay: the \( \delta^+ \) and \( \delta^- \) Functions

In section 1.5.2. we stated that Ridgeway (1966, 1968) has shown that the decay of electric birefringence for a homogeneous suspension of asymmetric macromolecules (e.g. tri-axial ellipsoids) would consist of two exponential terms:

\[
\Delta n = \frac{N}{2n_0} \left\{ A_+ e^{-6\theta t} + A_- e^{-6\theta t} \right\}
\]

(32)
where $\Delta n$ is the birefringence, $N$ the number density of particles in suspension and $n_2$ the refractive index of the suspending medium. $A_+$ and $A_-$ are complicated functions depending on the initial orientation of the particles and their dielectric and diffusion properties. We may rewrite $NA_+ / 2n_2$ as $A_+$, the 'pre-exponential factors'. Equation (32) then becomes:

$$\Delta n = A_+ e^{-6 \theta_+ t} + A_- e^{-6 \theta_- t} \tag{104}$$

$\theta_+$ and $\theta_-$ are related to the rotational diffusion constants $\theta_i$ (and hence the rotational frictional coefficients since $\zeta_i = kT/\theta_i$) by

$$\theta_\pm = \frac{1}{3} \sum \theta_i \pm \left\{ \left( \frac{1}{3} \sum \theta_i \right)^2 - \frac{1}{3} \sum_{i>j} \theta_i \theta_j \right\}^{\frac{1}{2}} \tag{105a}$$

$$= \frac{kT}{3} \left\{ \sum \frac{1}{\zeta_i} \pm \left\{ \sum \frac{1}{\zeta_i} - \sum_{i>j} \frac{1}{\zeta_i \zeta_j} \right\}^{\frac{1}{2}} \right\} \tag{105b}$$

The dimensions of equation (105) are of energy/(volume x viscosity) we therefore 'reduce' it to a function of shape alone:

$$\theta^\text{red}_{\pm} = \left( \frac{n_0}{kT} \right) \sqrt{\theta} \pm \frac{abc}{12} \left\{ \left( \frac{1}{\zeta''_{a}} + \frac{1}{\zeta''_{b}} + \frac{1}{\zeta''_{c}} \right) \right\}$$

$$= \frac{1}{2} \left[ \left( \frac{1}{\zeta''_{a}} + \frac{1}{\zeta''_{b}} + \frac{1}{\zeta''_{c}} \right) \right]^{-\frac{1}{2}} \left( \frac{1}{\zeta''_{a} \zeta''_{b} \zeta''_{c}} \right) \tag{106}$$

where

$$\zeta''_{a} = \frac{b^2 + c^2}{b^2 \gamma_0 + c^2 \gamma_0}; \quad \zeta''_{b} = \frac{c^2 + a^2}{c^2 \gamma_0 + a^2 \alpha_0}; \quad \zeta''_{c} = \frac{a^2 + b^2}{a^2 \alpha_0 + b^2 \alpha_0} \tag{107}$$
The elliptic integrals $a_0$ etc. are those defined by Jeffrey (1922) and are given in Appendix I.

A plot of the $\theta_{\pm}^{\text{red}}$ functions, together with the R function corresponding to the point $(a/b, b/c) = (1.5, 1.5)$ allowing for $\pm 1\%$ experimental error is given in Figure 36. It is seen that the intersections are very reasonable (the $\theta_{\pm}^{\text{red}}$ - R intersection is nearly orthogonal) and the functions are relatively sensitive to axial ratio. However, experimental determination of $\theta_{\pm}^{\text{red}}$ requires of course knowledge of the swollen molecular volume in solution (equation 106). This can be conveniently eliminated however in the standard way by combining (106) either with the viscosity increment (8) or the translational frictional ratio (20b). If for example (106) is combined with the viscosity increment (8), swelling independent $\delta_{\pm}$ functions are produced (Tables 12, 13, Figure 37):

$$\delta_{\pm} = 6\theta_{\pm}^{\text{red}} \nu \equiv \frac{6}{N_A k} \left( \frac{\eta_0 \theta_{\pm}}{T} \right) [\eta] M_R$$

(108)

where $[\eta]$ is expressed in ml/gm. Alternatively, $\theta_{\pm}^{\text{red}}$ can be combined with the translational frictional ratio (20b) to give swelling independent $\gamma_{\pm}$ functions (Programs 1, 2, 4, Figure 38):

$$\gamma_{\pm} = 6\theta_{\pm}^{\text{red}} \left( \frac{f}{f_0} \right)^3 \equiv \frac{M_R^3 (1 - \sqrt{\rho_0})^3 \theta_{\pm}}{27 N_A kT \pi^2 \eta_0^2 s^3}$$

(109)

The $\delta_{\pm}$ and $\gamma_{\pm}$ functions are new. The $\delta_{\pm}$ functions are preferred over the $\gamma_{\pm}$ functions since they require fewer experimental measurements and do not involve squared or cubed terms; hence in principle can be measured more
accurately. It is seen therefore that combination of the R-function with the $\delta_\pm$ functions as a method for determining a unique solution for the axial ratios (and hence the axial dimensions, if $V_e$ is known from $k_\nu/k_s$ — section 1.7.1) of a macromolecule in solution satisfies the criteria (i), (ii) and (iv) of section 3.3.1. In order for the method to satisfy criterion (iii) however, there still remains the problem of resolving the exponential decay term into its 2 component relaxation times or decay constants (the same is true of course for the $\theta_{\text{red}}\pm$ and $\gamma_\pm$ functions). To date this has not been possible. We now show that with a new 'constrained' least squares algorithm using intersection with the R-curve as the constraint, this is now possible with currently available experimental precision.
Table 8. Values of reduced specific viscosity for various concentrations of sperm whale myoglobin (0.1M NaCl buffer, pH = 7.1)

<table>
<thead>
<tr>
<th>Concentration, c (mg/ml)</th>
<th>( \eta_{rel} )</th>
<th>( \eta_{sp}/c ) (ml/gm)</th>
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</thead>
<tbody>
<tr>
<td>90.2</td>
<td>1.450</td>
<td>4.99</td>
</tr>
<tr>
<td>66.1</td>
<td>1.298</td>
<td>4.51</td>
</tr>
<tr>
<td>53.3</td>
<td>1.224</td>
<td>4.20</td>
</tr>
<tr>
<td>50.2</td>
<td>1.215</td>
<td>4.29</td>
</tr>
<tr>
<td>40.7</td>
<td>1.163</td>
<td>4.00</td>
</tr>
<tr>
<td>34.4</td>
<td>1.138</td>
<td>4.02</td>
</tr>
<tr>
<td>30.5</td>
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<td>3.81</td>
</tr>
<tr>
<td>29.6</td>
<td>1.115</td>
<td>3.89</td>
</tr>
<tr>
<td>23.2</td>
<td>1.084</td>
<td>3.61</td>
</tr>
<tr>
<td>15.5</td>
<td>1.055</td>
<td>3.57</td>
</tr>
<tr>
<td>9.7</td>
<td>1.034</td>
<td>3.47</td>
</tr>
<tr>
<td>8.1</td>
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</tr>
</tbody>
</table>
Table 9. Values of P as a function of (a/b, b/c) for a general tri-axial ellipsoid (a>b>c)

<table>
<thead>
<tr>
<th>a/b</th>
<th>Prolate Ellipsoid</th>
<th>Oblate Ellipsoids</th>
</tr>
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<tr>
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<tr>
<td>2.0</td>
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<td>1.042</td>
</tr>
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</table>

Note: The table continues with more values for different values of b/c.
Table 10. Values of $\beta \times 10^{-6}$ as a function of $(a/b, b/c)$ for a general triaxial ellipsoid $(a>b>c)$

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<th>$a/b$</th>
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<th>1.2</th>
<th>1.3</th>
<th>1.4</th>
<th>1.5</th>
<th>1.6</th>
<th>1.7</th>
<th>1.8</th>
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<td>2.112</td>
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Table 11. Values of $R$ as a function of $(a/b, b/c)$ for a general tri-axial ellipsoid $(a>b>c)$

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<th>$b/c$</th>
<th>Oblate Ellipsoid</th>
<th>Prolate Ellipsoid</th>
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<td>1.593</td>
</tr>
<tr>
<td>1.2</td>
<td>1.592</td>
<td>1.585</td>
</tr>
<tr>
<td>1.3</td>
<td>1.582</td>
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</tr>
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<td>1.4</td>
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<td>1.509</td>
</tr>
<tr>
<td>1.8</td>
<td>1.507</td>
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<tr>
<td>2.0</td>
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<tr>
<td></td>
<td>1.471</td>
<td>1.452</td>
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</table>
Table 12. Values of $\delta$ as a function of $(a/b, b/c)$ for a general tri-axial ellipsoid $(a>b>c)$

<table>
<thead>
<tr>
<th>$\frac{b}{c}$</th>
<th>$\frac{a}{b}$</th>
<th>Prolate Ellipsoid</th>
<th>Oblate Ellipsoid</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1.0</td>
<td>1.1</td>
<td>1.2</td>
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<tr>
<td>1.0</td>
<td>2.500</td>
<td>2.541</td>
<td>2.568</td>
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<tr>
<td>1.1</td>
<td>2.549</td>
<td>2.577</td>
<td>2.596</td>
</tr>
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<td>1.2</td>
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<td>2.641</td>
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<td>1.3</td>
<td>2.648</td>
<td>2.675</td>
<td>2.692</td>
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<td>1.4</td>
<td>2.699</td>
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<td>2.748</td>
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<td>1.5</td>
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<td>1.6</td>
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<td>1.7</td>
<td>2.863</td>
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<td>1.8</td>
<td>2.922</td>
<td>2.968</td>
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</tr>
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<td>1.9</td>
<td>2.983</td>
<td>3.035</td>
<td>3.071</td>
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</table>
Table 13. Values of $\delta$ as a function of $(a/b, b/c)$ for a general tri-axial ellipsoid (a>b>c)

<table>
<thead>
<tr>
<th>a/b</th>
<th>Oblate Ellipsoid</th>
<th>Prolate Ellipsoid</th>
</tr>
</thead>
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<tr>
<td>1.0</td>
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<td>1.4</td>
<td>2.264</td>
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<td>2.160</td>
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<tr>
<td>1.6</td>
<td>2.144</td>
<td>2.100</td>
</tr>
<tr>
<td>1.7</td>
<td>2.087</td>
<td>2.042</td>
</tr>
<tr>
<td>1.8</td>
<td>2.033</td>
<td>1.987</td>
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<td>1.9</td>
<td>1.981</td>
<td>1.936</td>
</tr>
<tr>
<td>2.0</td>
<td>1.932</td>
<td>1.887</td>
</tr>
</tbody>
</table>

Values for $b/c$ range from 1.0 to 2.0, with increments of 0.1.
Figure 27. Plot of reduced specific viscosity versus concentration for sperm whale myoglobin (0.1M NaCl, buffer, pH = 7.1).

The straight line is that due to a weighted least squares fit to $\frac{\eta_{sp}}{c} = [\eta] (1 + k \eta c)$ where $[\eta] = 3.25$ ml/gm and $k = 5.9$ ml/gm.

The weight used was $\frac{1}{\text{concentration (mg/ml)}}$ (conc. < 40 mg/ml)

$\frac{1}{40}$ (conc. > 40 mg/ml)
CONCENTRATION (MG/ML)

REDUCED SP. VISCOSITY (ML/GM)
Figure 28. Photograph of the apparatus used for determining solution densities and viscosities. Temperatures were kept constant to within ±0.01° using a high precision Townson - Mercer constant temperature tank, with a pump attachment to supply the water bath in the precision density meter. These temperatures could be monitored to within ±0.005° using the platinum resistance thermometer situated directly above the density meter.
Figure 29. Contour diagram showing curves of constant $\nu$ as a function of the semi-axial ratios $a/b$, $b/c$ on the basis of equation (88).
Figure 30. Plots of constant $v$ and $P$ in the $(a/b, b/c)$ plane corresponding to $a/b = 1.5$, $b/c = 1.5$
Figure 31. Plots of constant $v$, $P$, $R$, and $\beta$, allowing for ± 1% error in their measured values, in the $a/b$, $b/c$ plane corresponding to $a/b = 1.5$, $b/c = 1.5$. 
Figure 32. Plots of constant $R$ and the rotational relaxation time ratios in the $a/b$, $b/c$ plane corresponding to $a/b = 1.5$, $b/c = 1.5$.
Figure 33. Plots of constant fluorescence anisotropy relaxation time ratios in the a/b, b/c plane corresponding to a/b = 1.5, b/c = 1.5
Figure 34. Plots of constant $R$, $\Psi$ and $\Lambda$ in the $a/b$, $b/c$ plane corresponding to $a/b = 1.5$, $b/c = 1.5$
Figure 35. Plots of constant $R$, $r$, and $\Lambda$, allowing for $\pm 1\%$ error in their measured values, in the $a/b$, $b/c$ plane corresponding to $a/b = 1.5$, $b/c = 1.5$. 
Figure 36. Plots of constant $R$, $\theta^\text{red}_+$ and $\theta^\text{red}_-$, allowing for $\pm 1\%$ error in their measured values, in the a/b, b/c plane corresponding to $a/b = 1.5$, $b/c = 1.5$.
Figure 37. Plots of constant $R$, $\delta^+$ and $\delta^-$, allowing for $\pm 1\%$ measured error in $R$ and $\pm 2\%$ measured error in $\delta^\pm$, in the $a/b$, $b/c$ plane corresponding to $a/b = 1.5$, $b/c = 1.5$. 
Figure 38. Plots of constant $R$, $\gamma_+$ and $\gamma_-$, allowing for $\pm 1\%$ measured error in $R$ and $\pm 2\%$ measured error in $\gamma_+$, in the a/b, b/c plane corresponding to $a/b = 1.5$, $b/c = 1.5$
CHAPTER 4

Determination of a Stable, Unique Solution by Combining Results from Viscosity, Sedimentation and Electric Birefringence
4.1 Methods for Analyzing the Decay Curve

Resolution of a 2-term exponential birefringence decay curve into its two component relaxation times or decay constants is notoriously difficult, even for components that differ by several orders of magnitude. The situation is especially difficult for globular macromolecules for which the decay constants will generally not differ by more than ~20% (see below). A recent review of the salient methods currently used for attempting to analyse multi-component exponential decay curves, emphasising these difficulties, has been given by Jost and O'Konski (1978). The three methods that are apparently the most useful are

(1) Graphical Peeling Analysis (O'Konski and Haltner, 1956)
(2) Non-Linear Least Squares Analysis (Wilde, 1964, Powell and Macdonald, 1972, Gill and Murray, 1976)
(3) Fourier Transform Solution of the Laplace Integral Equation (Gardner, Gardner, Laush & Meinke, 1958)

4.1.1. Graphical Peeling Analysis

In this method, the logarithm of the birefringence is plotted as a function of time. For a single term decay this should of course give a straight line. If the plot for a two-term decay can be extended to relatively long times with sufficient signal-to-noise ratio, and if the two terms are not too close, then the limiting slope will give an estimate for the longest relaxation time (or shortest decay constant). This limiting slope can be extrapolated back to zero time and then "subtracted" from the original signal; the slope of the resultant can then be determined and hence the shortest relaxation time found (Figure 39). As might be expected, this method, although rapid, is very approximate and
is of little use for resolving relaxation times of the same order of magnitude. However it is still useful for indicating the orders of magnitude of the decay constants which may be used as initial estimates in non-linear least squares iterative procedures.

4.1.2. Non-Linear Least Squares Iterative Analysis

In this method, the weighted sum of the squares of the residuals \( \chi^2 \) is calculated between a set of experimental data points and the function to be fitted. If \( x_j \) represents the value of the \( j \)'th experimental point and \( \xi_j(x_m) \) the corresponding computer point for a given estimate for the \( X \), the number of independent variables, then we define our 'goodness of fit' parameter, \( \chi^2 \), by

\[
\chi^2 = \sum_{j=1}^{n} \left( \frac{x_j - \xi_j}{\sigma_j} \right)^2
\]

where \( \sigma_j \) is the standard error in the \( j \)'th experimental point. The best values of the \( x_m \) are such that \( \frac{\partial F}{\partial x_m} = 0 \), for all the \( x_m \).

For the particular case of electric birefringence, \( \sigma_j \) is approximately constant for all the \( x_j \) (although this is not generally true for photon counting - e.g. fluorescence depolarization anisotropy - experiments) and the minimization condition becomes

\[
\frac{\partial F}{\partial x_m} = 0
\]

where

\[
F = \sum_{j=1}^{n} \{x_j - \xi_j\}^2
\]
In the case of a two-term birefringence decay, the minimization is said to be 'non-linear' in that the data are to be fitted to a function which is the sum of a product of terms consisting of an adjustable parameter (i.e. a pre-exponential factor) with another function of another adjustable parameter (i.e. a decay constant or relaxation time). In order to evaluate $\partial F/\partial X_m$ for a current estimate for the parameters $X_m$, the solution either has to be linearized using a Taylor expansion as outlined by Jost & O'Konski, or alternatively, a quadratic or quasi-Newtonian procedure can be employed (Gill & Murray, 1976). In this latter case, the parameters $X_m$ are iterated until the minimum in $F$ is found. Gill & Murray's algorithm is particularly attractive in that upper and lower limits for the variable can be specified and included as external constraints. A problem with the least squares technique however is that the method is very sensitive to subsidiary minima in $X^2$ (or $F$) leading to false 'best parameters', even for data of very high precision. The presence of these subsidiary minima can often be detected by repeating the analysis for a series of different initial guesses of the adjustable parameters.

4.1.3. Fourier Transform Solution of the Laplace Integral Equation

The birefringence $\Delta n(t) \equiv S(t)$ is written as a Stieljes integral:

$$S(t) = \sum_i A_i e^{-\theta_i t} \equiv \sum_i A_i e^{-\lambda t} = \int_0^\infty \exp(-\lambda t) dh(\lambda)$$

(112)

where $\cdot h(\lambda)$ is a step function, $i = +,-$ and $\lambda = 6\theta_i$. 

The right hand side of equation (112) can be rewritten in the form of a Laplace Integral:

\[ S(t) = \int_0^\infty \exp(-\lambda t)g(\lambda)d\lambda \]  

(113)

where \( g(\lambda) \) represents a sum of Dirac delta functions. A plot of \( g(\lambda) \) versus \( \lambda \) will give a frequency spectrum with peaks; the centre of each peak corresponds to a specific decay constant, and the height of the peak is proportional to the value of the pre-exponential factor \( A \). We transform \( \lambda = e^{-y} \) and \( t = e^{x} \). Then

\[ S(e^x) = \int_{-\infty}^{\infty} \exp[-e(x-y)]g(e^{-y})e^{-y}dy \]  

(114)

Multiplying by \( e^x \):

\[ e^x S(e^x) = \int_{-\infty}^{\infty} \exp[-e(x-y)]e(x-y)g(e^{-y})dy \]  

(115)

Taking the Fourier Transform of the left hand side of (115)

\[ F(\mu) = \frac{1}{\sqrt{2\pi}} \int_{-\infty}^{\infty} e^x S(e^x)e^{i\mu x}dx \]  

(116)

Thus

\[ F(\mu) = \frac{1}{\sqrt{2\pi}} \int_{-\infty}^{\infty} \left\{ \int_{-\infty}^{\infty} \exp[-e(x-y)]e(x-y)g(e^{-y})dy \right\} \exp[i\mu(s + y)]ds \]  

(117)
with \( s = x - y \). Rearranging

\[
F(\mu) = \sqrt{\frac{1}{2\pi}} \int_{-\infty}^{\infty} g(e^{-y}) \exp(i\mu y)\,dy \cdot \int_{-\infty}^{\infty} \exp(-e^{s})e^{s}\exp(i\mu s)\,ds
\]  

(118)

Now, if we compare equation (113) with equation (114):

\[
g(e^{-y})dy \equiv \frac{g(\lambda)}{\lambda} \, d\lambda
\]  

(119)

Thus if we obtain \( g(e^{-y}) \) as a function of \( y \), using equation (119) this will be equivalent to a plot of \( g(\lambda)/\lambda \) as a function of \( \lambda \).

The right hand side of equation (118) is the product of the Fourier Transform, \( G(\mu) \) of \( g(e^{-y}) \) and the Fourier Transform, \( K(\mu) \) of \( \exp(-e^{s}) \).

Therefore

\[
F(\mu) = \sqrt{2\pi} \, G(\mu) \, K(\mu)
\]  

(120)

i.e.

\[
G(\mu) = \sqrt{\frac{1}{2\pi}} \frac{F(\mu)}{K(\mu)}
\]  

(121)

Taking the inverse Fourier Transform of \( G(\mu) \):

\[
g(e^{-y}) = \frac{1}{2\pi} \int_{-\infty}^{\infty} \frac{F(\mu)}{K(\mu)} \, e^{-i\mu \mu} \, d\mu
\]  

(122)

\( K(\mu) \) can be evaluated analytically in terms of the complex \( \Gamma \) function:

\[
K(\mu) = \sqrt{\frac{1}{2\pi}} \, \Gamma(1 + i\mu)
\]  

(123)
The method therefore has four basic steps:

(i) Evaluate the Fourier Transform of the data (equation 116)
(ii) Divide by the complex $r$ function (equation 123)
(iii) $g(e^{-y})$ as a function of $y$ is found by using the inverse Fourier Transform
(iv) A plot of $g(\lambda)/\lambda$ is thus obtained as a function of $\lambda$

The advantage of this method is that an initial choice as to the number of exponential terms to be fitted is not required.

4.1.4. Other methods of analysis, previously used for deconvoluting

**Fluorescence decay curves**

O'Connor, Ware and Andre (1979) have recently compared methods for deconvoluting both one and two term exponential fluorescence decay curves (sections 1.5.4, 3.3.2) - methods which could be equally applicable to corresponding birefringence decays. The methods chosen were

(i) Non-Linear Least Squares
(ii) Method of Moments
(iii) Laplace Transforms
(iv) Method of Modulating Functions,
(v) Exponential series method
(vi) Fourier Transforms

They discovered that all six methods were satisfactory for analysing undistorted one-component data, but that the least squares method was most suitable when distortions are present. For resolving two closely spaced terms (9.5ns & 11.5ns) in a 2-term undistorted decay only the least squares method and the method of modulating functions proved satisfactory. They thus concluded that the non-linear least squares iterative method
was the technique of preference for the analysis of simple decay laws.

4.2. Choosing the best algorithm: computer simulation

Following the work of Jost & O'Konski (1979) and O'Connor, Ware & Andre (1979), the non-linear least squares iterative and possibly the Fourier Transform Solution of the Laplace Integral Equation methods seemed to be the best available methods for resolving a 2-term exponential birefringence decay. I attempted to test for myself these methods by assuming three proteins of known (tri-axial) dimensions and hence axial ratios (a/b, b/c), assuming a swelling ratio \( (\frac{v}{V}) = 1.3 \), and \( \bar{v} = 0.73 \) (typical for globular proteins). From these values the molecular weight, viscosity increment, R-function, \( \delta \) functions, intrinsic viscosity and hence decay constants \( \theta \) could be predicted (Table 14). We then assume pre-exponential factors \( A_1', A_2' \), of, respectively, 0.07 and 0.05 radians taken from a typical initial birefringence \( (\pm A_1' + A_2') \) of 0.12 radians (Krause & O'Konski, 1959) and hence the unperturbed decay curve for each simulated protein can be given. The actual individual values for \( A_1' \) are not significant in the analyses, except when they differ by several \( \pm \) orders of magnitude (see section 4.5). One then places simulated experimental error on each of 100 data points for the curves, using a computer normal pseudo-random number generator, and, first of all assuming no errors in the molecular weight or intrinsic viscosity, investigate how much error in the data points is tolerable, before each algorithm fails to give back the correct decay constants and hence axial ratios, within reasonable limits. The algorithms would then be tested for errors in the intrinsic viscosity and molecular weight. Figure 40 illustrates such a mock experimental decay curve with 0.1 degree standard error (about the
current available experimental precision — B. Jennings & V. Morris, private communication) on each of 1000 data points, for Protein 1 \[ \text{true } (a/b, b/c) = (1.5, 1.5) \]. In the analyses the pre-exponential factors \( A^+ \) are of course regarded as unknown variables.

4.3 Non-Linear Least Squares Iterative Method

The quasi-Newtonian quadratic method for minimizing any function (i.e. in this case, the sum of the squares of the residuals \( F \)) given by Gill & Murray (1976) and incorporated in the UK NAG Mk.VI subroutine E04JAF was used. In this algorithm the user, besides supplying the subroutine for calculating the value of \( F \) at any point \( X \), has to supply fixed upper and lower bounds on the independent variables \( x_1, x_2, \ldots, x_m \). This routine was incorporated in the FORTRAN IV program given in Appendix IV, as Program 5. This program generated its own hypothetical decay curve with normal (Gaussian) pseudo-random error generated on each data point (using NAG routine G05ADF), the amount specifiable by the user. The program attempted to retrieve the decay constants, hence the \( \delta \) functions (from the user-specified molecular weight and intrinsic viscosity) and hence the axial ratios \( (a/b, b/c) \) of the general tri-axial ellipsoid. Owing to the problem of the presence of the danger of the routine falling into subsidiary minima as mentioned by Jost & O'Konski (1978) — see section 4.1.2. — it was necessary to repeat the method for a large number (30) of initial guesses. In fact the program was written to generate its own thirty different initial guesses by using "DO" loop between user specifiable initial guess limits.

Unfortunately, even data as accurate as ,001 degree standard error on each data point (about 2 orders of magnitude greater than the current
experimental precision) failed to give back the correct \((a/b, b/c)\) within reasonable limits, and even data of machine accuracy (14 significant figures) did not generate the exact value of \((1.5, 1.5)\), as Figure 41 illustrates.

4.4. **Fourier Transform Solution Method of the Laplace Integral Equation Method**

4.4.1. **Cut-off Errors**

In order to use this method outlined in section 4.1.3., the integrals involved in taking the Fourier Transform of the data (equation 116) and in taking the inverse Fourier Transform (equation 122) have to be solved numerically. Unfortunately, the integrals extended from \(-\infty\) to \(+\infty\); with real data there exists a finite cut-off time, \(t_0\) or equivalently \(x_0\). Cut-off errors tend to increase the height of the error ripples in the final results. For equation (116), if we choose a cut-off too short for \(x_0\) there is a loss of resolution of the component peaks. On the other hand, if we choose a cut-off in \(x_0\) too long then the cut-off at \(x_0\) causes the amplitude of the error ripples to increase; \(x_0\) has to be varied therefore to obtain the optimum resolution for a given data set.

4.4.2. **Numerical Integration**

Following Gardner *et al* (1958), each value of \(S(t)\) was multiplied by the current value of \(t\) to give \(e^xS(e^x)\) (equation 115). Whereas \(t\) ranges from \(0\) to \(+\infty\), \(x\) ranges from \(-\infty\) to \(+\infty\), thus we can split the integral in equation (116) into symmetric and anti-symmetric parts:
\[
F(\mu) = \frac{1}{2\pi} \int_{0}^{x_0} [S^*(x) + S^*(-x)]e^{i\mu x} dx
\]  

(124)

Therefore

\[
F(\mu) = \frac{1}{2\pi} \int_{0}^{x_0} \{[S^*(x) + S^*(-x)]\cos \mu x
\]

\[+ i[S^*(x) - S^*(-x)]\sin \mu x \} dx
\]  

(125)

giving real and imaginary parts for \( F(\mu) \), i.e., \( F_c \) & \( F_s \). \( K(\mu) \) can be similarly split into real & imaginary parts \( K_c \) & \( K_s \). Equation (121) thus becomes:

\[
G(\mu) = \frac{1}{2\pi} \frac{F_c + iF_s}{K_c + iK_s} = \frac{(F_i + iF_s)(K_c - iK_s)}{K_c^2 + K_s^2}
\]  

(126)

and the inverse transform (122) becomes

\[
g(e^{-\gamma}) = \frac{1}{2\pi} \int_{-\mu_0}^{\mu_0} \frac{(F_c + iF_s)(K_c + iK_s)}{K_c^2 + K_s^2} \ (\cos \gamma - i\sin \gamma) d\mu
\]  

(127)

where \( \mu_0 \) and \( -\mu_0 \) are the cut-off values for \( \mu \). Since all odd values vanish,

\[
g(e^{-\gamma}) = \frac{1}{2\pi} \int_{0}^{\mu_0} \left\{ \frac{F_c K_c + F_s K_s}{K_c + K_s} \cos \mu + \frac{F_s K_c - F_c K_s}{K_c + K_s} \sin \mu \right\} d\mu
\]  

(128)
The numerical integrations (125) and (128) are solved using the NAG routine D01GAF. The value of the complex \( \Gamma \) function needed for calculating \( K_c \) and \( K_s \) was deduced using a routine given by Lucas & Terril (1970). As with the non-linear least squares iterative method, the program (Appendix IV Program 6) generated its own synthetic data using NAG normal pseudo-random number routines G05ADF & G05BBF.

4.4.3. Results

The program was firstly checked by applying it to the case first considered by Gardner et al for a single exponential decay, viz.

\[ S(t) = 100 e^{-0.02t} \]

assuming data of machine accuracy (i.e. no perturbation routine included). The retrieved \( \lambda \) from Figure 42 is 0.021, in close agreement with Gardner et al's value. The data was taken at logarithmic intervals (corresponding to equal linear intervals in \( t \)). The algorithm was then applied to the two term exponential decay curve for Protein 2. However, even with data of machine accuracy and taken at logarithmic intervals in \( t \) (impossible to obtain in practice for our particular case) the retrieved values for \( \lambda_i \) and hence the decay constants was poor and varied with the cut-off values for \( \mu_0 \) as Figure 43 and Table 15 shows. When normal pseudo-random error of 0.001 deg was applied to the data points, no resolution was possible for all values of \( \mu_0 \), as Figure 44 clearly demonstrates. We thus conclude this method to be of little use for our case of interest.
4.5. A new R-Constrained Non-Linear Least Squares Algorithm

Owing to the inadequacy of the other treatments for resolving a two-term exponential birefringence decay into its component relaxation times (or decay constants), particularly for globular proteins (close decay constants), I have now developed a new R-constrained least squares algorithm. If the R-function line solution (3.3.1), which can be found from the ratio of the sedimentation regression coefficient \( k_s \) to the intrinsic viscosity \([\eta]\), is included in the least squares algorithm (4.3) as a constraint, then the problem is effectively reduced from one of four independent variables \((a_+, a_-, A_+, A_-)\) to one of three \((a/b, A'_+, A'_-)\). The solution is constrained to lie on the R-curve, thus a given estimate for \(a/b\) will necessarily give a 'constrained' value for \(b/c\); the computer program can then calculate the values for \(\delta_+\) and \(\delta_-\) corresponding to this estimate, hence the decay constants (using also the values for \([\eta]\), \(M_r\) - equation 107), the decay curve and finally the sum of the squares of the residuals (SSR) between the computer points and the experimental curve. By iterating along this R-curve for \(a/b\) and the two pre-exponential factors \(A'_+\), the best estimate for \((a/b, b/c)\) can be found from the minimum value of the SSR.

The constraint of the R-curve was included in the algorithm (Program 7 of Appendix IV) for the three simulated proteins considered previously by use of the Leicester University Computer Library routine E01LFF, a listing of which is given towards the end of Program 7. The user specifies the coordinates of knots in the curve (see Figures 45, 46 & 47), or alternatively, the whole curve digitised, and the routine interpolates between these points using a cubic polynomial ('spline') fit (K. Brodlie,
private communication). In the main program, normal (Gaussian) random error of 0.1 degrees on each of the 100 linearly separated data points was supplied using the pseudo random number routines mentioned previously. The magnitude of this error corresponds to that expected from current experimental precision (B.R. Jennings, V. Morris, private communication). It was found in pilot runs that the danger of the algorithm falling into subsidiary minima, as present for the unconstrained case (section 4.3.) was no longer significant. The number of initial guesses was thus reduced from thirty to three to save on Computer time; the best estimates were generally the same for all three initial guesses (except those marked with an asterisk in Tables 16, 17 & 18). The values for (a/b, b/c) retrieved did however depend on the cut-off time specified for the decay curve. If there were no error in the data points then very long cut-off times would be desirable, since this region is dominated by the longest relaxation time (or shortest decay constant, \( \theta_\perp \)). However, the effect of a given absolute error is more pronounced the lower the birefringence signal.

The optimum cut-off time, and hence the best value for (a/b, b/c) was found by repeating for eight different streams of normal random data, specified by the UK NAG Mk VI routine G05BAF(0.N), where N represents the stream number of the random data; the optimum cut-off time for each decay curve was then determined by finding the best standard deviation of the a/b's from the eight streams for increments of 5ns in the cut-off times. The values for the corresponding best mean value for a/b (and hence b/c) together with the corresponding standard error for the eight streams of data could then be found (Tables 16a, 17a & 18a).

This procedure was then repeated allowing for \( \pm 1\% \) experimental error
in the R-curves (Tables 16b,c, 17b,c, 18b,c). If the points corresponding
to \(<(a/b, b/c)> + \sigma_E\) are joined together for each of the R-curves, and
then those of the \(<(a/b, b/c)> - \sigma_E\), regions of allowed values for
\((a/b, b/c)\) could then be found (Figures 48, 49 & 50). The mean values
agree very closely with the true values (Table 19). The algorithm was
then tested for the effect of experimental errors in the intrinsic
viscosity (± 1%) and molecular weight (±1.4%). These were found to be
not significant (Table 20); indeed, the molecular weight can now be found
precisely from the results of sequence analyses. Finally, the algorithm
was tested for different initially assumed values for the pre-exponential
factors \(A'\) and \(A'_-\) (Table 21). Again, these were found to have no
significant effect on the results; even for pre-exponential factors
differing by two orders of magnitude, though the retrieved \(A'_+\) was poor,
the retrieved \(a/b\) was in close agreement with the other values.

Once the value for the axial ratios \((a/b, b/c)\) has been found for a
particular protein, it can be combined with the swollen volume of the
protein, if known, to determine the axial dimensions. In Table 22 a "model
dependent" (section 1.7.1) estimate for \(V_e\) has been found for each of the
three simulated proteins we have considered by back substitution of the
mean values of \((a/b, b/c)\) determined from the analysis above into equation
(8) for the viscosity increment, and again the agreement with the initially
assumed values (Table 19) is excellent. If the model dependent values of
\(V_e\) are then combined with the values for \((a/b, b/c)\), the semi-axial
dimensions \(a, b, c\) for the three proteins considered are found to be (Å):

- **Protein 1:** 45.00, 29.98, 20.01 (45.0, 30.0, 20.0)
- **Protein 2:** 42.28, 25.59, 19.61 (42.5, 25.0, 20.0)
- **Protein 3:** 43.11, 33.58, 19.81 (42.5, 34.0, 20.0)

again, in excellent agreement with the initially assumed (bracketed) values.
Some Practical Points

In applying these equations and algorithms to real protein and other macromolecular solutions several important factors must be taken into consideration:

1. Two or more decay constants can also arise if the system is polydisperse. It is therefore essential that the solution be rendered monodisperse by, for example, gel filtration techniques.

2. It has now been well established that the single exponential decay constant previously resolvable from the birefringence decay of monodisperse protein solutions shows a concentration dependence (Riddiford & Jennings, 1967), and it was therefore necessary to determine its value at several concentrations and then extrapolate to infinite dilution. One must naturally assume therefore that the two decay constants for the decay of a monodisperse solution of asymmetric ellipsoids also show a concentration dependence, and hence must be extrapolated to infinite dilution. On the other hand, because of the constraint in our algorithm that they must correspond to \( \delta_+ \) and \( \delta_- \) line solutions that intersect with the R-curve, the values for the decay constants are such that they are not the 'true' decay constants for each particular concentration but are closer to the infinite dilution values. Since the extrapolation procedure must therefore be empirical the best estimates for \( a/b \) at particular solute concentrations rather than these 'damped' decay constants may be extrapolated to infinite dilution; once the extrapolated value for \( a/b \) has been found the corresponding value for \( b/c \) can thus also be found from the R-curve.

3. The requirement on the precision of the electric birefringence apparatus is not only in producing transient decays to a precision of 0.1 degree on each data point but also the availability of response times (i.e. the finite time it takes for the orienting electric pulse to be switched off) of about
an order of magnitude less than that of the faster relaxation time
Adequate response times are now available (Williams, Ham & Wright, 1976)
however with apparatus that uses a laser light source, cable discharge
generator and a memory oscilloscope, giving a response time of ~ 5ns.
(4) In the above analysis it has been shown that greater accuracies in
obtaining the axial ratios can be obtained if the optimum cut-off time
for the decay is found. In our simulations this was achieved by
averaging over several streams of random data; this corresponds in practice
to taking several decays of the same preparation. Different samples of
the same preparation should be used because of the danger of denaturing
the protein by continually pulsing through high electric fields (temp-
erature effects).
(5) It has also been assumed that the R function can be measured to a
precision of ~ ±1%. Since $s_c$ values in an $s_c$ versus concentration plot can
be determined to within ~ ±2% (Squire, 1978), the $k_s$ value can presumably be
measured to within ±1% (as, from equation 58, it is approximately a
function of $(s_c/s) \times$ concentration$^{-1}$). The intrinsic viscosity $[\eta]$ can
also be measured to within ~ ±1%, the limiting factor here being the accuracy
to which the flow times can be measured. The error in R will thus be of
the order of 1% after taking into consideration that any systematic errors
in measuring absolute solute concentrations will cancel in the ratio
$k_s/[\eta]$ (Rowe, 1977).
(6) Finally, it should be pointed out that because of polarisation effects
on the electrodes and also the danger of denaturation due to heating effects
mentioned in (4), solutions of low ionic strength (<0.01M) generally have
to be used. This apparently prevents the investigation of less soluble
materials. On the other hand, an interesting new method is being developed
at Brunel University by Professor B. Jennings and his co-workers in which an ultrasonic field rather than an electric field is used to initially orient the macromolecules before the decay is observed. This "acoustic birefringence" (Ballinger & Jennings, 1979) method does not suffer from the problems of electrode polarisation and denaturation associated with ionic strengths > .01M for the electric birefringence case, allowing the possibility for the investigation of less soluble materials.
Table 14. Assumed and derived characteristics of three hypothetical globular proteins

<table>
<thead>
<tr>
<th>Protein</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Characteristic</strong></td>
<td><strong>ASSUMED VALUES</strong></td>
<td><strong>DERIVED VALUES</strong></td>
<td></td>
</tr>
<tr>
<td>$a,b,c$</td>
<td>$45R,30R,20R$</td>
<td>$42.5R,25R,20R$</td>
<td>$42.5R,34R,20R$</td>
</tr>
<tr>
<td>$\bar{V}$</td>
<td>0.730 ml/gm</td>
<td>0.730 ml/gm</td>
<td>0.730 ml/gm</td>
</tr>
<tr>
<td>$\bar{V}_s/\bar{V}$</td>
<td>1.3</td>
<td>1.3</td>
<td>1.3</td>
</tr>
</tbody>
</table>

**Characteristic**
- $a/b,b/c$: $1.50,1.50$; $1.70,1.25$; $1.25,1.70$
- $\bar{V}_s$: $0.949$ ml/gm; $0.949$ ml/gm; $0.949$ ml/gm

Swollen molecular volume: $V_e = 4\pi abc / 3$

Anhydrous molecular volume: $V = (\bar{V}/\bar{V}_s) V_e$

Molecular weight: $M_r = (N_A/\bar{V}) V$

$[n] = N_A \bar{V} \omega / M_r$

R: $1.479$; $1.482$; $1.496$

$\theta^+_\text{red}, \theta^-_\text{red}$: $0.163, 0.116$; $0.171, 0.115$; $0.155, 0.125$

Decay constants: $N_A kT / 6 \eta_0 [n] M_r$

Relaxation times: $\tau^+_\pm = 1/6 \theta^+_\pm$

* $T = 293K$, $\eta_0 = 0.01$ gm cm$^{-1}$ sec$^{-1}$

$$V_e = \frac{4\pi abc}{3}$$

$$V = \frac{(\bar{V}/\bar{V}_s) V_e}{3}$$

$$M_r = \frac{(N_A/\bar{V}) V}{3}$$

$$[n] = \frac{N_A \bar{V} \omega}{M_r}$$

$$\theta^+_\text{red}, \theta^-_\text{red}$$

$$\tau^+_\pm = \frac{1}{6 \theta^+_\pm}$$

$\eta_0 = 0.01$ gm cm$^{-1}$ sec$^{-1}$
Table 15. Retrieved decay constants for varying values of $\nu_0$

<table>
<thead>
<tr>
<th>$y_1$</th>
<th>$y_2$</th>
<th>$\lambda_1 = e^{-y_1}$</th>
<th>$\lambda_2 = e^{-y_2}$</th>
<th>$\Theta_+ \times 10^{-6}$ sec$^{-1}$</th>
<th>$\Theta_- \times 10^{-6}$ sec$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>11.5</td>
<td>3.13</td>
<td>3.55</td>
<td>0.04372</td>
<td>0.02872</td>
<td>7.286</td>
</tr>
<tr>
<td>11.6</td>
<td>3.00</td>
<td>3.50</td>
<td>0.04979</td>
<td>0.03020</td>
<td>8.292</td>
</tr>
<tr>
<td>11.7</td>
<td>2.94</td>
<td>3.45</td>
<td>0.05287</td>
<td>0.03175</td>
<td>8.811</td>
</tr>
<tr>
<td>12.0</td>
<td>3.14</td>
<td>3.72</td>
<td>0.04328</td>
<td>0.02423</td>
<td>7.214</td>
</tr>
</tbody>
</table>

True value for $\Theta_+ = 7.7660465 \times 10^6$ sec$^{-1}$

True value for $\Theta_- = 5.2290121 \times 10^6$ sec$^{-1}$
Table 16. Determination of the optimum cut-off time for Protein 1.

True \((a/b, b/c) = (1.5, 1.5)\)

(a) No assumed error in \(R\)

<table>
<thead>
<tr>
<th>Cut-off time (\text{time (ns)})</th>
<th>80ns (a/b)</th>
<th>100ns (a/b)</th>
<th>110ns (a/b)</th>
<th>115ns (a/b)</th>
<th>120ns (a/b)</th>
<th>140ns (a/b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stream 1</td>
<td>1.580</td>
<td>1.534</td>
<td>1.513</td>
<td>1.503</td>
<td>1.493</td>
<td>1.454</td>
</tr>
<tr>
<td>Stream 2</td>
<td>1.946*</td>
<td>1.785</td>
<td>1.692</td>
<td>1.654</td>
<td>1.619</td>
<td>1.497</td>
</tr>
<tr>
<td>Stream 3</td>
<td>1.591</td>
<td>1.512</td>
<td>1.483</td>
<td>1.468</td>
<td>1.452</td>
<td>1.392</td>
</tr>
<tr>
<td>Stream 4</td>
<td>1.644</td>
<td>1.487</td>
<td>1.425</td>
<td>1.396</td>
<td>1.367</td>
<td>1.249</td>
</tr>
<tr>
<td>Stream 5</td>
<td>1.623</td>
<td>1.480</td>
<td>1.426</td>
<td>1.401</td>
<td>1.377</td>
<td>1.287</td>
</tr>
<tr>
<td>Stream 6</td>
<td>1.186</td>
<td>1.275</td>
<td>1.303</td>
<td>1.315</td>
<td>1.326</td>
<td>1.364</td>
</tr>
<tr>
<td>Stream 7</td>
<td>1.573</td>
<td>1.645</td>
<td>1.678</td>
<td>1.694</td>
<td>1.710</td>
<td>1.772</td>
</tr>
<tr>
<td>Stream 8</td>
<td>1.716</td>
<td>1.623</td>
<td>1.590</td>
<td>1.575</td>
<td>1.562</td>
<td>1.514</td>
</tr>
<tr>
<td>Mean</td>
<td>1.6074</td>
<td>1.5426</td>
<td>1.5138</td>
<td>1.5008</td>
<td>1.4863</td>
<td>1.4411</td>
</tr>
<tr>
<td>(\sigma (SD))</td>
<td>0.209696</td>
<td>0.148967</td>
<td>0.133899</td>
<td>0.132475</td>
<td>0.134402</td>
<td>0.163491</td>
</tr>
<tr>
<td>(\sigma (SE))</td>
<td>0.07414</td>
<td>0.05267</td>
<td>0.04734</td>
<td>0.04684</td>
<td>0.04752</td>
<td>0.05780</td>
</tr>
</tbody>
</table>

\(\sigma (SD) = \text{Standard Deviation} ; \sigma (SE) = \text{Standard Error}\)

* different answers for different initial guesses

Optimum cut-off time = 115ns

Best estimate for \(a/b = 1.501 (\pm 0.47)\)

Corresponding estimate for \(b/c = 1.498\)
(b) +1% assumed measured error in R

<table>
<thead>
<tr>
<th>Cut-off time</th>
<th>110ns</th>
<th>115ns</th>
<th>120ns</th>
<th>125ns</th>
<th>130ns</th>
<th>135ns</th>
<th>140ns</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>a/b</td>
<td>a/b</td>
<td>a/b</td>
<td>a/b</td>
<td>a/b</td>
<td>a/b</td>
<td>a/b</td>
</tr>
<tr>
<td>Stream 1</td>
<td>1.546</td>
<td>1.534</td>
<td>1.523</td>
<td>1.511</td>
<td>1.500</td>
<td>1.489</td>
<td>1.478</td>
</tr>
<tr>
<td>Stream 2</td>
<td>1.837</td>
<td>1.735</td>
<td>1.679</td>
<td>1.633</td>
<td>1.594</td>
<td>1.557</td>
<td>1.522</td>
</tr>
<tr>
<td>Stream 3</td>
<td>1.510</td>
<td>1.492</td>
<td>1.474</td>
<td>1.457</td>
<td>1.440</td>
<td>1.423</td>
<td>1.406</td>
</tr>
<tr>
<td>Stream 4</td>
<td>1.439</td>
<td>1.406</td>
<td>1.374</td>
<td>1.342</td>
<td>1.310</td>
<td>1.278</td>
<td>1.244</td>
</tr>
<tr>
<td>Stream 5</td>
<td>1.442</td>
<td>1.414</td>
<td>1.387</td>
<td>1.361</td>
<td>1.337</td>
<td>1.312</td>
<td>1.289</td>
</tr>
<tr>
<td>Stream 6</td>
<td>1.312</td>
<td>1.325</td>
<td>1.340</td>
<td>1.349</td>
<td>1.360</td>
<td>1.370</td>
<td>1.380</td>
</tr>
<tr>
<td>Stream 7</td>
<td>1.816</td>
<td>1.871</td>
<td>1.847</td>
<td>1.840</td>
<td>1.878</td>
<td>1.893</td>
<td>1.909</td>
</tr>
<tr>
<td>Stream 8</td>
<td>1.643</td>
<td>1.624</td>
<td>1.606</td>
<td>1.590</td>
<td>1.575</td>
<td>1.561</td>
<td>1.548</td>
</tr>
<tr>
<td>Mean</td>
<td>1.5681</td>
<td>1.5501</td>
<td>1.5288</td>
<td>1.5104</td>
<td>1.4992</td>
<td>1.4854</td>
<td>1.4720</td>
</tr>
<tr>
<td>( \sigma ) (SD)</td>
<td>0.185700</td>
<td>0.183700</td>
<td>0.174243</td>
<td>0.173033</td>
<td>0.186398</td>
<td>0.195406</td>
<td>0.206181</td>
</tr>
<tr>
<td>( \sigma ) (SE)</td>
<td>0.06565</td>
<td>0.06495</td>
<td>0.06160</td>
<td>0.06118</td>
<td>0.06590</td>
<td>0.06909</td>
<td>0.07290</td>
</tr>
</tbody>
</table>

Optimum cut-off time = 125ns

Best estimate for \( a/b = 1.510 \ (\pm 0.061) \)

Corresponding estimate for \( b/c = 1.400 \)
-1% assumed measured error in $R$

<table>
<thead>
<tr>
<th>Cut-off time</th>
<th>110ns</th>
<th>115ns</th>
<th>120ns</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stream 1</td>
<td>1.494</td>
<td>1.485</td>
<td>1.476</td>
</tr>
<tr>
<td>Stream 2</td>
<td>1.644</td>
<td>1.616</td>
<td>1.588</td>
</tr>
<tr>
<td>Stream 3</td>
<td>1.468</td>
<td>1.454</td>
<td>1.440</td>
</tr>
<tr>
<td>Stream 4</td>
<td>1.419</td>
<td>1.392</td>
<td>1.366</td>
</tr>
<tr>
<td>Stream 5</td>
<td>1.418</td>
<td>1.395</td>
<td>1.373</td>
</tr>
<tr>
<td>Stream 6</td>
<td>1.300</td>
<td>1.311</td>
<td>1.321</td>
</tr>
<tr>
<td>Stream 7</td>
<td>1.626</td>
<td>1.638</td>
<td>1.649</td>
</tr>
<tr>
<td>Stream 8</td>
<td>1.561</td>
<td>1.549</td>
<td>1.537</td>
</tr>
<tr>
<td>Mean</td>
<td>1.4913</td>
<td>1.4800</td>
<td>1.4688</td>
</tr>
</tbody>
</table>

$\sigma$ (SD) | 0.115922 | 0.114924 | 0.115761
$\sigma$ (SE) | 0.04098  | 0.04063  | 0.04093

Optimum cut-off time = 115ns

Best estimate for $a/b = 1.480 (\pm 0.041)$

Corresponding estimate for $b/c = 1.611$
Table 17. Determination of the optimum cut-off time for Protein 2.  

True \( (a/b, b/c) = (1.7, 1.25) \)

(a) No assumed error in \( R \)

<table>
<thead>
<tr>
<th>Cut-off time</th>
<th>85ns</th>
<th>90ns</th>
<th>95ns</th>
<th>100ns</th>
<th>105ns</th>
<th>110ns</th>
<th>120ns</th>
</tr>
</thead>
<tbody>
<tr>
<td>a/b</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stream 1</td>
<td>1.709</td>
<td>1.691</td>
<td>1.675</td>
<td>1.659</td>
<td>1.644</td>
<td>1.630</td>
<td>1.603</td>
</tr>
<tr>
<td>Stream 2</td>
<td>1.963</td>
<td>1.926</td>
<td>1.872</td>
<td>1.777</td>
<td>1.716</td>
<td>1.666</td>
<td>1.579</td>
</tr>
<tr>
<td>Stream 3</td>
<td>1.670</td>
<td>1.645</td>
<td>1.622</td>
<td>1.600</td>
<td>1.578</td>
<td>1.558</td>
<td>1.520</td>
</tr>
<tr>
<td>Stream 4</td>
<td>1.602</td>
<td>1.561</td>
<td>1.523</td>
<td>1.486</td>
<td>1.452</td>
<td>1.418</td>
<td>1.351</td>
</tr>
<tr>
<td>Stream 5</td>
<td>1.600</td>
<td>1.566</td>
<td>1.534</td>
<td>1.505</td>
<td>1.478</td>
<td>1.453</td>
<td>1.408</td>
</tr>
<tr>
<td>Stream 6</td>
<td>1.482</td>
<td>1.496</td>
<td>1.509</td>
<td>1.521</td>
<td>1.533</td>
<td>1.544</td>
<td>1.566</td>
</tr>
<tr>
<td>Stream 7</td>
<td>1.924</td>
<td>1.924</td>
<td>1.923</td>
<td>1.923</td>
<td>1.923</td>
<td>1.923</td>
<td>1.922</td>
</tr>
<tr>
<td>Stream 8</td>
<td>1.847</td>
<td>1.803</td>
<td>1.771</td>
<td>1.745</td>
<td>1.723</td>
<td>1.703</td>
<td>1.669</td>
</tr>
<tr>
<td>Mean</td>
<td>1.7246</td>
<td>1.7015</td>
<td>1.6786</td>
<td>1.6520</td>
<td>1.6309</td>
<td>1.6119</td>
<td>1.5773</td>
</tr>
</tbody>
</table>

\( \sigma (SD) \)  
|      | 0.170801 | 0.166408 | 0.161586 | 0.154362 | 0.155373 | 0.159776 | 0.173689 |

\( \sigma (SE) \)  
|      | 0.06039 | 0.05883 | 0.05713 | 0.05458 | 0.05493 | 0.05649 | 0.06141 |

Optimum cut-off time = 100ns

Best estimate for \( a/b = 1.652 (\pm 0.055) \)

Corresponding estimate for \( b/c = 1.305 \)
(b) +1% assumed measured error in $R$

<table>
<thead>
<tr>
<th>Cut-off time</th>
<th>75ns</th>
<th>80ns</th>
<th>85ns</th>
<th>90ns</th>
<th>95ns</th>
<th>100ns</th>
<th>105ns</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stream 1</td>
<td>1.856</td>
<td>1.856</td>
<td>1.856</td>
<td>1.820</td>
<td>1.821</td>
<td>1.767</td>
<td>1.834</td>
</tr>
<tr>
<td>Stream 2</td>
<td>1.856</td>
<td>1.856</td>
<td>1.856</td>
<td>1.856</td>
<td>1.856</td>
<td>1.856</td>
<td>1.856</td>
</tr>
<tr>
<td>Stream 3</td>
<td>1.856</td>
<td>1.821</td>
<td>1.791</td>
<td>1.732</td>
<td>1.691</td>
<td>1.658</td>
<td>1.628</td>
</tr>
<tr>
<td>Stream 4</td>
<td>1.843</td>
<td>1.728</td>
<td>1.655</td>
<td>1.599</td>
<td>1.551</td>
<td>1.508</td>
<td>1.467</td>
</tr>
<tr>
<td>Stream 5</td>
<td>1.834</td>
<td>1.716</td>
<td>1.655</td>
<td>1.608</td>
<td>1.568</td>
<td>1.532</td>
<td>1.501</td>
</tr>
<tr>
<td>Stream 6</td>
<td>1.471</td>
<td>1.492</td>
<td>1.511</td>
<td>1.528</td>
<td>1.544</td>
<td>1.560</td>
<td>1.575</td>
</tr>
<tr>
<td>Stream 7</td>
<td>1.856</td>
<td>1.856</td>
<td>1.856</td>
<td>1.856</td>
<td>1.856</td>
<td>1.856</td>
<td>1.856</td>
</tr>
<tr>
<td>Stream 8</td>
<td>1.856</td>
<td>1.856</td>
<td>1.856</td>
<td>1.856</td>
<td>1.856</td>
<td>1.856</td>
<td>1.856</td>
</tr>
<tr>
<td>Mean</td>
<td>1.8035</td>
<td>1.7726</td>
<td>1.7545</td>
<td>1.7319</td>
<td>1.7179</td>
<td>1.6991</td>
<td>1.6841</td>
</tr>
<tr>
<td>$\sigma$ (SD)</td>
<td>0.134604</td>
<td>0.127668</td>
<td>0.131819</td>
<td>0.135561</td>
<td>0.145962</td>
<td>0.153037</td>
<td>0.163378</td>
</tr>
<tr>
<td>$\sigma$ (SE)</td>
<td>0.04759</td>
<td>0.04514</td>
<td>0.04661</td>
<td>0.04791</td>
<td>0.05161</td>
<td>0.05411</td>
<td>0.05776</td>
</tr>
</tbody>
</table>

Optimum cut-off time = 80ns

Best estimate for $a/b = 1.773 (\pm 0.045)$

Corresponding estimate for $b/c = 1.0875$
(c) -1% assumed measured error in R

<table>
<thead>
<tr>
<th>Cut-off time</th>
<th>80ns</th>
<th>85ns</th>
<th>90ns</th>
<th>95ns</th>
<th>100ns</th>
<th>105ns</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>a/b</td>
<td>a/b</td>
<td>a/b</td>
<td>a/b</td>
<td>a/b</td>
<td>a/b</td>
</tr>
<tr>
<td>Stream 1</td>
<td>1.670</td>
<td>1.656</td>
<td>1.643</td>
<td>1.630</td>
<td>1.617</td>
<td>1.605</td>
</tr>
<tr>
<td>Stream 2</td>
<td>1.936</td>
<td>1.861</td>
<td>1.799</td>
<td>1.749</td>
<td>1.705</td>
<td>1.665</td>
</tr>
<tr>
<td>Stream 3</td>
<td>1.648</td>
<td>1.628</td>
<td>1.608</td>
<td>1.589</td>
<td>1.570</td>
<td>1.552</td>
</tr>
<tr>
<td>Stream 4</td>
<td>1.614</td>
<td>1.576</td>
<td>1.541</td>
<td>1.507</td>
<td>1.475</td>
<td>1.444</td>
</tr>
<tr>
<td>Stream 5</td>
<td>1.606</td>
<td>1.573</td>
<td>1.543</td>
<td>1.516</td>
<td>1.490</td>
<td>1.466</td>
</tr>
<tr>
<td>Stream 6</td>
<td>1.452</td>
<td>1.466</td>
<td>1.478</td>
<td>1.489</td>
<td>1.499</td>
<td>1.509</td>
</tr>
<tr>
<td>Stream 7</td>
<td>1.815</td>
<td>1.842</td>
<td>1.870</td>
<td>1.902</td>
<td>1.936</td>
<td>1.936</td>
</tr>
<tr>
<td>Stream 8</td>
<td>1.759</td>
<td>1.737</td>
<td>1.716</td>
<td>1.698</td>
<td>1.681</td>
<td>1.666</td>
</tr>
<tr>
<td>Mean</td>
<td>1.6875</td>
<td>1.6674</td>
<td>1.6498</td>
<td>1.6350</td>
<td>1.6216</td>
<td>1.6054</td>
</tr>
<tr>
<td>σ (SD)</td>
<td>0.147596</td>
<td>0.137488</td>
<td>0.136111</td>
<td>0.142556</td>
<td>0.153928</td>
<td>0.157728</td>
</tr>
<tr>
<td>σ (SE)</td>
<td>0.05218</td>
<td>0.04861</td>
<td>0.04812</td>
<td>0.05040</td>
<td>0.05442</td>
<td>0.05577</td>
</tr>
</tbody>
</table>

† Upper limit ( \( b/c = 1.0 \) )

Optimum cut-off time = 90ns

Best estimate for a/b = 1.650 (± 0.048)

Corresponding estimate for b/c = 1.3905
Table 18. Determination of the optimum cut-off time for Protein 3.

True \((a/b, b/c) = (1.25, 1.7)\)

(a) No assumed error in \(R\)

<table>
<thead>
<tr>
<th>Cut-off time</th>
<th>80ns</th>
<th>100ns</th>
<th>105ns</th>
<th>110ns</th>
<th>115ns</th>
<th>120ns</th>
<th>140ns</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stream 1</td>
<td>1.367</td>
<td>1.315</td>
<td>1.303</td>
<td>1.291</td>
<td>1.278</td>
<td>1.266</td>
<td>1.215</td>
</tr>
<tr>
<td>Stream 2</td>
<td>1.881</td>
<td>1.587</td>
<td>1.547</td>
<td>1.510</td>
<td>1.476</td>
<td>1.442</td>
<td>1.313</td>
</tr>
<tr>
<td>Stream 3</td>
<td>1.388</td>
<td>1.301</td>
<td>1.281</td>
<td>1.263</td>
<td>1.240</td>
<td>1.219</td>
<td>1.119</td>
</tr>
<tr>
<td>Stream 4</td>
<td>1.464</td>
<td>1.285</td>
<td>1.244</td>
<td>1.200</td>
<td>1.151</td>
<td>1.089</td>
<td>1.001</td>
</tr>
<tr>
<td>Stream 5</td>
<td>1.448</td>
<td>1.278</td>
<td>1.239</td>
<td>1.200</td>
<td>1.157</td>
<td>1.108</td>
<td>1.000</td>
</tr>
<tr>
<td>Stream 6</td>
<td>1.000</td>
<td>1.000</td>
<td>1.000</td>
<td>1.000</td>
<td>1.000</td>
<td>1.000</td>
<td>1.000</td>
</tr>
<tr>
<td>Stream 7</td>
<td>1.314</td>
<td>1.393</td>
<td>1.409</td>
<td>1.424</td>
<td>1.439</td>
<td>1.453</td>
<td>1.505</td>
</tr>
<tr>
<td>Stream 8</td>
<td>1.514</td>
<td>1.421</td>
<td>1.402</td>
<td>1.385</td>
<td>1.370</td>
<td>1.354</td>
<td>1.297</td>
</tr>
<tr>
<td>Mean</td>
<td>1.4220</td>
<td>1.3225</td>
<td>1.3031</td>
<td>1.2841</td>
<td>1.2639</td>
<td>1.2414</td>
<td>1.1813</td>
</tr>
<tr>
<td>(\sigma (SD))</td>
<td>0.243637</td>
<td>0.165850</td>
<td>0.160554</td>
<td>0.158626</td>
<td>0.161019</td>
<td>0.168224</td>
<td>0.184692</td>
</tr>
<tr>
<td>(\sigma (SE))</td>
<td>0.08614</td>
<td>0.05864</td>
<td>0.05676</td>
<td>0.05608</td>
<td>0.05693</td>
<td>0.05948</td>
<td>0.06530</td>
</tr>
</tbody>
</table>

Optimum cut-off time = 110ns

Best estimate for \(a/b = 1.284 (\pm 0.056)\)

Corresponding estimate for \(b/c = 1.695\)
(b) \( \pm 1\% \) assumed measured error in R

\[ \text{Optimum cut-off time} = 110\text{ns} \]

Best estimate for a/b = 1.302 (\( \pm .062 \))

Corresponding estimate for b/c = 1.5395

<table>
<thead>
<tr>
<th>Cut-off time</th>
<th>105ns</th>
<th>110ns</th>
<th>115ns</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>a/b</td>
<td>a/b</td>
<td>a/b</td>
</tr>
<tr>
<td>Stream 1</td>
<td>1.319</td>
<td>1.306</td>
<td>1.293</td>
</tr>
<tr>
<td>Stream 2</td>
<td>1.618</td>
<td>1.562</td>
<td>1.516</td>
</tr>
<tr>
<td>Stream 3</td>
<td>1.294</td>
<td>1.272</td>
<td>1.249</td>
</tr>
<tr>
<td>Stream 4</td>
<td>1.247</td>
<td>1.199</td>
<td>1.145</td>
</tr>
<tr>
<td>Stream 5</td>
<td>1.245</td>
<td>1.202</td>
<td>1.155</td>
</tr>
<tr>
<td>Stream 6</td>
<td>1.000</td>
<td>1.000</td>
<td>1.000</td>
</tr>
<tr>
<td>Stream 7</td>
<td>1.444</td>
<td>1.462</td>
<td>1.481</td>
</tr>
<tr>
<td>Stream 8</td>
<td>1.431</td>
<td>1.412</td>
<td>1.394</td>
</tr>
<tr>
<td>Mean</td>
<td>1.3248</td>
<td>1.3019</td>
<td>1.2791</td>
</tr>
</tbody>
</table>

\( \sigma \text{(SD)} \) 0.181404 0.176485 0.178272

\( \sigma \text{(SE)} \) 0.06414 0.06240 0.06303
(c) -1% assumed measured error in $R$

Optimum cut-off time = 110ns

Best estimate for $a/b = 1.275 (± 0.052)$

Corresponding estimate for $b/c = 1.764$
Table 19. Mean values for the retrieved axial ratios compared with the real values

<table>
<thead>
<tr>
<th>Protein</th>
<th>Retrieved $\left( \frac{a}{b}, \frac{b}{c} \right)$</th>
<th>Real $\left( \frac{a}{b}, \frac{b}{c} \right)$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein 1</td>
<td>(1.501, 1.498)</td>
<td>(1.50, 1.50)</td>
</tr>
<tr>
<td>Protein 2</td>
<td>(1.652, 1.305)</td>
<td>(1.70, 1.25)</td>
</tr>
<tr>
<td>Protein 3</td>
<td>(1.284, 1.695)</td>
<td>(1.25, 1.70)</td>
</tr>
</tbody>
</table>
Table 20. Effect of experimental errors in the intrinsic viscosity and molecular weight

(and hence the product [n].M, used in calculating the decay constants - cf Table 14 and equation 108)

Assumed error in [n] = ±1% \( \Rightarrow \) total error = ±1.7% (calculated from formula given in Paradine & Rivett (1960))

Results are for Protein 1, cut-off time = 115ns, ± 0.1° standard error on each of the 100 data points

<table>
<thead>
<tr>
<th>Stream no. of random data</th>
<th>- 1.7%</th>
<th>No error a/b</th>
<th>+ 1.7%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.493</td>
<td>1.503</td>
<td>1.520</td>
</tr>
<tr>
<td>2</td>
<td>1.638</td>
<td>1.654</td>
<td>1.679</td>
</tr>
<tr>
<td>3</td>
<td>1.455</td>
<td>1.468</td>
<td>1.487</td>
</tr>
<tr>
<td>4</td>
<td>1.374</td>
<td>1.396</td>
<td>1.424</td>
</tr>
<tr>
<td>5</td>
<td>1.383</td>
<td>1.401</td>
<td>1.425</td>
</tr>
<tr>
<td>6</td>
<td>1.305</td>
<td>1.315</td>
<td>1.333</td>
</tr>
<tr>
<td>7</td>
<td>1.695</td>
<td>1.694</td>
<td>1.704</td>
</tr>
<tr>
<td>8</td>
<td>1.566</td>
<td>1.575</td>
<td>1.593</td>
</tr>
</tbody>
</table>

| mean a/b                  | 1.4886 | 1.5008       | 1.5206 |
| \( \sigma_{SD} \)         | 0.136305 | 0.132475   | 0.130253 |
| \( \sigma_{SE} \)         | 0.04819  | 0.04684     | 0.04668  |

\( \sigma_{SD} \) = standard deviation
\( \sigma_{SE} \) = standard error
Table 21. Effect of using different initially assumed values for the pre-exponential factors $A'_+$

Protein 1, Cut off time = 100 ns, 0.1 s.e. on each of the 100 data points*

<table>
<thead>
<tr>
<th>Assumed</th>
<th>Retrieved</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A' / A'</td>
</tr>
<tr>
<td>0.06</td>
<td>0.06</td>
</tr>
<tr>
<td>0.07</td>
<td>0.05</td>
</tr>
<tr>
<td>0.09</td>
<td>0.03</td>
</tr>
<tr>
<td>0.11</td>
<td>0.01</td>
</tr>
<tr>
<td>0.119</td>
<td>0.001</td>
</tr>
</tbody>
</table>

*The data for this table were obtained after the UK NAG Mk VI routines had been updated to Mk VII; the new random number routines corresponding to G05ADF & G05BAF in Mk VI are G05CAF & G05CBF
Table 22. Comparison of model dependent estimates for $V_e$ with the real values

<table>
<thead>
<tr>
<th></th>
<th>Retrieved $(a, b, c)$</th>
<th>Model-dependent $V_e$ (cm$^3$)</th>
<th>Real $V_e$ (cm$^3$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein 1</td>
<td>(1.501, 1.498)</td>
<td>$1.131 \times 10^{-19}$</td>
<td>$1.131 \times 10^{-19}$</td>
</tr>
<tr>
<td>Protein 2</td>
<td>(1.652, 1.305)</td>
<td>$0.889 \times 10^{-19}$</td>
<td>$0.890 \times 10^{-19}$</td>
</tr>
<tr>
<td>Protein 3</td>
<td>(1.284, 1.695)</td>
<td>$1.202 \times 10^{-19}$</td>
<td>$1.211 \times 10^{-19}$</td>
</tr>
</tbody>
</table>

*calculated by determining the value of $v$ corresponding to $(a/b, c)$ and then back substituting into the equation $v = [n]M_r/N_A V_e$ where $[n]$ is in ml/gm
Figure 39. Birefringence decay (expressed in radians) in Helix Pomatia hemocyanin solutions. The triangles represent the difference between the tangential curve (long relaxation time) and the experimental points. (From Pytkowicz & O'Konski, 1959)
Figure 40. Synthetic two-term exponential electric birefringence decay curve assuming a standard error of ± 0.1° on each data point.
Relaxation times assumed: 28.66ns, 40.10ns
Figure 41. Plot of the $R$, $\delta_+$ and $\delta_-$ values obtained from the non-linear least squares analysis assuming birefringence data of machine accuracy.
Figure 42. Frequency spectrum of $\lambda$ ($\equiv$/relaxation time) for a single exponential decay and $\mu_0 = 6.0$, assuming decay data of machine accuracy (14 figures). The position of the highest peak corresponds to a value of $\lambda$ of 0.021, in agreement with the initially assumed value of 0.02
Figure 43 (I - VI)

Effect of increasing $\mu_0$ to determine best resolution of the frequency spectrum corresponding to the decay for Protein 2, for 140 logarithmically increasing data points of machine accuracy (14 figures)
\[ \mu_0 = 6.0 \]

\[ \mu_0 = 8.0 \]
$\mu_0 = 11.5$

$\mu_0 = 11.6$
$\mu_0 = 11.7$

$\mu_0 = 12.0$
Figures 44 (I - VI)

As for Figure 43 but for data of 0.001° standard error on each of the 140 logarithmically increasing data points.
\( \mu_0 = 6.0 \)

\( \mu_0 = 6.5 \)
Figure 45. Knots in the R curves for specification in the R - constrained least squares analysis (Program 7) for Protein 1
Figure 46. As for Figure 45 but for Protein 2
Figure 47. As for Figure 45 but for Protein 3
Figure 48. The area marked by dots represents the allowed band of retrieved axial ratios determined using the new R-constrained least squares algorithm for Protein 1. Simulated experimental error of ± 0.1° standard error on each data point for the electric birefringence decay curve was assumed.
Figure 49. As for Figure 48 but for Protein 2
Figure 50. As for Figure 48 but for Protein 3
CHAPTER 5

Concluding Remarks
In this study an extensive review of all the possible shape functions available for modelling a biological macromolecule in solution in terms of an ellipsoid model with the restriction of two equal axes has been given, thus updating the classical reviews of Edsall (1953) and Tanford (1961). It was concluded that the most suitable shape parameter (particularly for axial ratios less than 20:1) was the \( R \) parameter which can be determined from the ratio of the sedimentation regression coefficient, \( k_s \), to the intrinsic viscosity, \([\eta]\). A word of warning should perhaps be given out here in that the \( k_s \) value found from fitting sedimentation coefficient versus concentration data either to the general equation (60) or to the approximate linear equation (58), is the value based on particle migration relative to the solvent, whereas the \([\eta]\) values are normally measured to solution density (Tanford, 1955). The value of \( k_s \) must therefore be corrected to solution density, and this can be achieved simply by subtracting the value of the partial specific volume, \( \bar{\nu} \) (Rowe, 1977) since this latter can be equated to the reciprocal density of the solute, an assumption reasonably accurate for proteins and possibly for nucleic acids (Pearce et al, 1975). It is also now possible to estimate a value for \( k_s \) direct from a knowledge of the sedimentation coefficient, the molecular weight and \( \bar{\nu} \) (Appendix VI).

Despite the availability of the \( R \) function for determining the 'equivalent hydrodynamic ellipsoid of revolution' for a structure in solution to a reasonable precision (and also the \( \Pi \) function for prolate ellipsoids - Appendix III), it was clear from a perusal of the crystallographic dimensions given in Table 3 and a comparison of model dependent with model independent estimates for \( \bar{\nu}_s/\bar{\nu} \) in Table 2, that for many macromolecules the assumption of two equal axes on the ellipsoid model is a poor
approximation to the real structure in solution. This stimulated my attempts to develop the necessary theoretical and data analysis techniques so that the restriction of two equal axes could be dispensed with and the subsequent research has shown that the more general tri-axial ellipsoid can now, in principle at least, be successfully employed for modelling biological macromolecules in solution.

The first step was to derive an explicit expression for the viscosity increment $\nu$ for a dilute suspension of general tri-axial ellipsoids in overwhelming Brownian motion, based on a model first given by Simha (1940) and improved by Saito (1951) for ellipsoids of revolution. Although the assumption of the particles rotating on average with the same local angular velocity of the fluid has only been rigorously proved so far for ellipsoids of revolution (Brenner, 1972a), it was assumed that this would be a very close approximation for tri-axial ellipsoids, particularly for low axial ratios ($<3.0$, i.e. the globular particle range). After the derivation of equation (88) a numerical procedure (involving complicated numerical matrix inversions), but based on a full statistical analysis of the angular motion was made available by Rallison (1978). It was explained in section 2.8. how the difference in the results predicted by equation (88) and Rallison's approach was negligible ($<0.01\%$) for the globular particle range mentioned above, and for some particles of higher asymmetry discrepancies of not more than $1\%$ arose. Rallison has also given a numerical procedure for calculating the normal stress coefficients in terms of axial ratio; normal stress effects are however second order in the shear rate, thus in order to measure these coefficients it is necessary to use high shear rates. However, the assumption of overwhelming Brownian motion with respect to the shear rate ceases to be valid, and hence, unfortunately, the normal stress coefficients cannot be applied.
It was described how the problem of the line solution (i.e. how a
given value for \( v \) does not uniquely fix a value for the axial ratios
(a/b, b/c)) could be dealt with by combining it graphically with
translational frictional or rotational relaxation line solutions. I
was able to give the \( R \) function for tri-axial ellipsoids and also many
other tri-axial functions whose experimental determination did not require
a knowledge of the swollen molecular volume in solution. After a careful
consideration of all these line solutions with regard to giving suitable
intersections, experimental measurability, insensitivity to experimental
error and sensitivity to axial ratio, it was decided that the best approach
for determining a unique solution would be to combine the \( R \) line solution
graphically with the \( \delta_+ \) and \( \delta_- \) line solutions, the latter to be determined
from the two electric birefringence decay constants and the intrinsic
viscosity.

Unfortunately, this still requires having to resolve the two decay
constants or relaxation times from a two-term exponential birefringence
decay for a homogeneous solution of asymmetric particles. This problem
is notoriously difficult, as reported by Jost & O'Konski (1978) and
O'Connor, Ware & Andre (1979), particularly for close relaxation times (as
applies to globular proteins). The currently best available methods evident
from these studies, viz. the non-linear least squares iterative method and
possibly the Fourier Transform solution of the Laplace Integral equation
method of Gardner et al (1959) were tested by exhaustive computer
simulation to see how much error on the data points each could tolerate
before failing to resolve the decay constants within reasonable limits. The
Fourier method failed, even for data of machine accuracy (14 figures). The
non-linear least squares method was found to be unstable due to the problem
of subsidiary minima located in the iteration procedure, even for data of two orders of magnitude more precise than that currently available from the best instrumentation.

The idea of applying the R function line solution as a constraint in the least squares analysis was then applied to the three simulated decays thus effectively reducing the problem from one of four independent variables (the two pre-exponential factors and the two decay constants) to one of three (two pre-exponential factors and one axial ratio, a/b). The algorithm was then shown to be very successful for synthetic data corresponding to that available from current experimental precision. The problem of the concentration dependence of the decay constants (or equivalently the relaxation times) was then mentioned, and the necessity for extrapolating the values for the axial ratios determined at various concentrations to infinite dilution. The need for extrapolating axial ratios is somewhat conceptually difficult to envisage at first sight, since one would more naturally extrapolate the decay constants and then calculate the axial ratios from them. In the algorithm however, I have included the R value as the constraint — the R function line solution of possible values of \((a/b, b/c)\) is the value applicable at infinite dilution, thus the decay constants in the algorithm are constrained to lie on the 'infinite dilution' curve; hence none of these values are the true values for the decay constants at each particular solute concentration. Any extrapolation procedure is therefore empirical, whether it be for the decay constants or for the values of the axial ratio a/b.

Investigation of the theoretical reasons for the concentration dependence of the decay constants provides however both an interesting and important field for further work. It has been described (section 1.7.1. &
Appendix IV) how several important results have arisen from consideration of the concentration dependence of the 'translational' (i.e. viscosity, sedimentation and diffusion) transport coefficients: for example, in producing the $R$ function and making available an estimate of the swollen volume of a macromolecule in solution independent of any model assumed for the macromolecule. The analysis of the concentration dependence of the decay constants is however much more complicated: Rowe's (1977) theory for the translational coefficients was derived assuming only hydrodynamic (i.e. volume flux) concentration effects, viz. solutions of high ionic strength (>0.1M) and such that electric charge effects (solute-solute interactions) were not present. The situation is apparently the reverse when we come to consider the decay constants: since we are dealing with a rotary macromolecular property, there should be no solute volume flux effects on average giving rise to the hydrodynamic concentration effects considered by Rowe. On the other hand, the current practical restriction of low ionic strengths for the electric birefringence probably results in some solute-solute electric charge effects; the double layer thickness of charge around a macromolecule in solution is inversely proportional to the square root of the ionic strength (Guoy, 1910, Chapman, 1913). For example, for a macromolecule suspended in a 0.1M NaCl buffer the thickness of the double layer is ~1nm, whereas in a 0.001M NaCl buffer, the thickness is as high as 10nm (Shaw, 1970). There is therefore a greater likelihood of interference between the relaxations of individual macromolecules, the degree of which one would expect to increase with concentration.

In section 1.6, the techniques of light and low-angle x-ray scattering were discussed as an alternative to the hydrodynamic techniques, and stated how Martin (1964) had given formulas relating the radius of gyration to
axial ratio for ellipsoids of revolution. Mendelson and Hartt (1980) have applied results from low angle X-ray scattering in terms of a general triaxial ellipsoid model to the regulatory light chains of scallop myosin, and determined axial dimensions of 16nm x 4.16nm x 1.26nm.

We also mentioned however that the major disadvantage of the scattering approach was that it is necessary to assume the macromolecule to be of uniform electron density; this can lead to errors of the order of 3%, notwithstanding other errors in measurement as the simple calculation given in Appendix VII for a hypothetical spherical macromolecule with a cavity (based on the electron microscopy and X-ray diffraction results for apoferritin – Harrison, 1959) shows.

It is hoped however that the results of the research described here have now made it possible to determine the gross conformation of biological macromolecules in solution in terms of a general ellipsoid – independent of any assumptions concerning the internal homogeneity of the macromolecules – by combining the results of viscosity, sedimentation and electric (or acoustic) birefringence. There are some macromolecules however that apparently will never be modelled by an ellipsoid, even tri-axial.

Bovine serum albumin (BSA) is a typical example; McCammon et al (1975) have attempted to account for a value for δ below the theoretical minimum of $2.112 \times 10^6$ (and above the theoretical maximum for R of 1.6 – see Table 2) by assuming its structure to be porous with respect to the solvent, but found the discrepancy was still far too large. With the availability of the tri-axial ellipsoid model and a comparison with model independent estimates for the swollen molecular volume, a classification of proteins into those which do and those which do not behave as hydrodynamic tri-axial ellipsoids in solution can now be made.
APPENDICES
Appendix I  Elliptic Integrals used in this study

\[ \alpha_0 = \int_0^\infty \frac{d\lambda}{(a^2 + \lambda)\Delta} \quad ; \quad \beta_0 = \int_0^\infty \frac{d\lambda}{(b^2 + \lambda)\Delta} \quad ; \quad \gamma_0 = \int_0^\infty \frac{d\lambda}{(c^2 + \lambda)\Delta} \]

where \( \lambda \) is the positive root of

\[ \frac{x^2}{a^2 + \lambda} + \frac{y^2}{b^2 + \lambda} + \frac{z^2}{c^2 + \lambda} = 1 \]

and \( \Delta = \{(a^2 + \lambda)(b^2 + \lambda)(c^2 + \lambda)\}^{\frac{1}{2}} \).

Also

\[ \alpha_0' = \int_0^\infty \frac{d\lambda}{(b^2 + \lambda)(c^2 + \lambda)\Delta} \quad ; \quad \alpha_0'' = \int_0^\infty \frac{\lambda d\lambda}{(b^2 + \lambda)(c^2 + \lambda)\Delta} \]

\[ \beta_0' = \int_0^\infty \frac{d\lambda}{(c^2 + \lambda)(a^2 + \lambda)\Delta} \quad ; \quad \beta_0'' = \int_0^\infty \frac{\lambda d\lambda}{(c^2 + \lambda)(a^2 + \lambda)\Delta} \]

\[ \gamma_0' = \int_0^\infty \frac{d\lambda}{(a^2 + \lambda)(b^2 + \lambda)\Delta} \quad ; \quad \gamma_0'' = \int_0^\infty \frac{\lambda d\lambda}{(a^2 + \lambda)(b^2 + \lambda)\Delta} \]
Appendix II  Illustration of use of the $\Lambda$ function (equation 50) by application to data available for the tryptic subfragments of fibrinogen

It is apparent from Figure 17 that, until the harmonic mean relaxation time $\tau_h$ can be measured to a precision greater than that currently available ($\pm 3\%$ at best, assuming no significant internal rotations of the chromophore or segmental rotations of parts of a macromolecule relative to other parts), use of $\Lambda$ will generally be restricted to prolate ellipsoidal particles above an axial ratio of about three.

Unfortunately, there is at present a lack of reliable steady state fluorescence depolarization data for macromolecules in this axial range. Use of the function may however be illustrated by application to data available for the tryptic fragment of bovine fibrinogen. By using a steady-state fluorescence - depolarization technique, Johnson & Mihalyi (1965) reported a harmonic mean relaxation time for fibrinogen of 195±5 ns, a value lower than the corresponding value for a sphere of the same volume (299 ns); the value for $\tau_h$ of the tryptic subfragment was 178 ns, strongly suggesting that the tryptic subfragments had rotational freedom within the fibrinogen molecule. Assuming there is still no further internal rotation within the subfragment itself, one can combine this result with viscosity and molecular-weight data obtained previously by Mihalyi & Godfrey (1963).

Taking $M_c$ as 95,000±2,000, [$\eta$] as (7.18±0.07) ml g$^{-1}$ and assuming a ±5 ns standard error in $\tau_h$, $\Lambda$ is calculated to be 4.74±0.17 where the method for calculating the standard error in $\Lambda$ is given by Paradine & Rivett (1960). This corresponds from Figure 17 to a prolate ellipsoid of axial ratio 6.8±0.3 consistent with the estimates of the axial ratio
derived from four other hydrodynamic parameters, three of which assume no particle swelling due to solvent association (Table 23). The results from electron microscopy studies suggest however that the subfragments are nearly spherical (Hall & Slayter, 1959); as Mihalyi & Godfrey (1963) have previously stated, this difference is probably too large to be explained by drying effects alone. At least part of this difference can, however, be possibly ascribed to an apparent discrepancy between the viscosity data of their Figure 4 with the sedimentation data of their equation 2; the latter suggests a sedimentation regression coefficient, $k_s$, of -3.6 (after correction to solution density; Rowe, 1977), whereas the viscosity regression coefficient, $k_n$, is only -2.5. Rowe (1977) has shown that the ratio $k_n/k_s$ is equal to the swelling ratio $V_s/V$, where $V_s$ is the swollen specific volume in solution. Mihalyi & Godfrey's (1963) data apparently gives a value for the swelling of less than 1, indicating the particle to contract in solution, an unlikely event. Unfortunately, although the pH values of the solutions used for the sedimentation and harmonic mean relaxation time measurements are given and are near (6.5 and 7.1 respectively), that for the viscosity is not given, so this is a possible source of error.

It is hoped that the availability of the new $A$ function will encourage the production of more reliable data in order to resolve these difficulties, and also accelerate improvement in the methodology so that $\tau_h/\tau_0$ can be measured with much greater precision, enabling application of the $A$ function to prolate ellipsoids of axial ratio less than three and also to oblate ellipsoids.
Table 23. Hydrodynamic parameters and axial ratios for the tryptic subfragments of fibrinogen

<table>
<thead>
<tr>
<th>Hydrodynamic Parameter</th>
<th>Derived Axial Ratio</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>$v^*$</td>
<td>7.8</td>
<td>Mihalyi &amp; Godfrey (1963)</td>
</tr>
<tr>
<td>$f / f_0^*$</td>
<td>7.1</td>
<td>&quot;</td>
</tr>
<tr>
<td>$\beta^*$</td>
<td>9.3</td>
<td>&quot;</td>
</tr>
<tr>
<td>$\tau / \tau_0^*$</td>
<td>5.0</td>
<td>Johnson &amp; Mihalyi (1965)</td>
</tr>
<tr>
<td>$\lambda$</td>
<td>6.8</td>
<td>This study</td>
</tr>
</tbody>
</table>

* Assuming no particle swelling due to solvent association
Appendix III  Illustration of use of the $\Pi$ function (equation 57) by application to data for hemoglobin

The $\Pi$ function obtained in section 1.7. can be applied to molecular covolume and viscosity data available for hemoglobin. The molecular covolume, $U$, is related to the 2nd virial coefficient, $B$ employed in osmometry by $U = 2BM_\tau^2$. Baghurst et al (1975), using a molecular weight of 64,500 found the value of the product $BM_\tau$ to be 4.8 ml/gm. Using instead the exact value for the molecular weight found by sequence analysis to be 64,793, this product becomes 4.78 ml/gm; this gives the ratio $U/M_\tau$ to be 9.56 ml/gm. From the plot of reduced specific viscosity against concentration (Figure 51) an intrinsic viscosity of 2.99 ml/gm has been determined by a least squares fit to the new universal equation for transport coefficients at all solute concentrations (see section 1.7.1. and Appendix IV). The value of $\Pi$ is calculated to be 3.20, corresponding to a spherical particle (Figure 24). This is consistent with the findings of x-ray crystallography (Perutz et al, 1960). The value of $v$ for a spherical particle is the Einstein value of 2.5. By back substitution into equation (8) and using a value for $M_\tau$ of 64,793 one obtains a value for $V_e$ of $1.286 \times 10^{-19}$ cm$^3$. This corresponds to a Stokes radius of 31.3 Å, in excellent agreement with the result of 32.3 Å calculated by Alpert & Banks (1976) from the diffusion coefficient determined by laser correlation spectroscopy and agrees exactly with the result of 31.3 Å calculated by Laurent & Killander (1964) from the diffusion coefficient determined by gel filtration, both groups assuming a hard sphere model. The Stokes radius can also be found directly from the molecular covolume and molecular weight assuming a hard sphere model: Baghurst et al (1975)
determined a value of $31.3 \, \text{Å}$, again in exact agreement. The corresponding radius of the sphere calculated from the crystallographic dimensions of $64 \times 55 \times 50 \, \text{Å}$ of Perutz et al. (1960) is $28.0 \, \text{Å}$, indicating hemoglobin to be swollen in solution by approximately $40\% \,(v/v)$.

If one uses standard errors of $\pm 0.3 \,(\equiv 1\%)$ and $\pm 0.096 \,(\equiv 1\%)$ in $\frac{U}{M}$ respectively, the calculated standard error (Paradine & Rivett, 1960) in $\pi$ is $\pm 0.045$. The maximum error corresponds to an axial ratio of 1.8 for a prolate model but as high as 6.8 for an oblate model, indicating the difficulty in applying $\pi$ to macromolecules that are oblatoid.
Figure 51.

Plot of reduced specific viscosity versus concentration for human oxy-hemoglobin (0.1M KCl, buffer, pH = 6.0).

The curve fitted is that due to a weighted least squares fit to the new universal equation for the concentration dependence of transport coefficients (equation 60). The weighting factor used was (1/concentration). 

\[ [\eta] = 2.99 \text{ ml/gm}, \ k_\eta = 7.8 \text{ ml/gm}. \]
Appendix IV  Viscous Flow and Sedimentation of Concentrated Dispersions of Particles (quoted from A.J. Rowe, mss. in preparation)

The hydrodynamic properties of dispersions of particles in fluids are quite well described at very high particle dilution, both for simple models (spheres, ellipsoids, rods) and for more complex models which may be represented as assemblies of simple models and the appropriate interaction tensors computed. Restricting ourselves to the case of small particles (Brownian motion dominant) suspended in liquids, the work of Stokes, Einstein, Perrin, Simha, Broersma and others (for simple models) and of Kirkwood, Bloomfield and others (for assemblies) enables a reasonably accurate description to be given of the sedimentation and viscous flow properties of such suspensions to be given at 'infinite dilution'.

At real particle concentrations however, no theory has proved adequate, even for the simplest particle model – the sphere. The need for such a theory is evident in many fields: in my own field of Biochemistry it would be useful both for methodological purposes in characterising macromolecular properties and for the description of 'in vivo' systems, which are generally rather concentrated dispersions of macromolecular particles. I have been concerned to derive such a theory, relating the properties of suspensions of particles at real concentration to their 'infinite dilution' behaviour. In a recent paper (Rowe, 1977) a first part of such a theory was described: the extension of this theory to cover the case of high concentrations is now described.

The State of the Problem

It has long been noted that the concentration dependence of
sedimentation and of reduced specific viscosity is finite even at high
dilution, and remains nearly linear over moderate ranges of concentration:

\[
(sedimentation) \quad s_c = s(1 - k_s c) \sim s(1 + k_s c)^{-1}
\]

\[
(viscous \ flow) \quad \frac{\eta_{sp}}{c} = \left(\frac{\eta_{sp}}{c}\right)_{c=0} \cdot (1 + k_s c)
\]

A recently derived theory (Rowe, 1977) shows that

\[
k = k_s = 2\bar{v} \left( \frac{\bar{v}_s}{\bar{v}} + \left(f/f_0^s\right)^3 \right)
\]

for compact particles, where \(\bar{v}\) is the partial specific volume of the
particle and \(f/f_0^s\), the frictional ratio, is a parameter computable for
simple models and for assemblies of sub-units. This theory is thus
applicable to particles of any conformation. The values predicted for
both spheres and other particles agree well with experimental evidence
and with earlier theoretical predictions for spheres (Figure 52, Table 24).

At higher concentrations two further effects must be considered:

(i) mutually proximity of the particles affects the rate of energy
dissipation at constant shear (the 'cloud effect' of Burgers).
In general this poses a many-body problem which is not amenable
to solution by classical techniques.

(ii) the critical packing fraction (\(\phi_p\)) will be approached. Semi-
empirical equations due to Mooney (1951), Dougherty and
Kreiger (1972) and others describe the viscosity of suspensions
of spheres in terms of \(\phi_p\).

A New General Approach

The theory applicable to high dilutions (Rowe, 1977) was based on
the supposition that only a 'frame-of-reference' effect need be considered
in this case. Derived in terms of sedimentation, it is shown that the
latter must be unchanged with concentration in a frame of reference
defined by the solvent in return flux (i.e. solvent not transported
or convected with the particles). The equation above then follows from
the relation between the defined frame of reference and the cell-fixed
frame of reference in which measurements are normally made.

To extend this approach to higher concentrations we re-define the
problem by considering the system to consist of a large but finite
number of volume elements, each element small in comparison to a
particle. These volume elements can be classified as elements of
disjoint sets $V_1 \ldots V_4$, shown in a Venn diagram for two particles
(Figure 53).

Among interesting properties which may be noticed are that

(i) Sets $V_1$, $V_3$ can be classified into sub-sets

$v_1^1 \ldots v_1^n, v_3^1 \ldots v_3^n$, for $n$ particles in the system

(ii) $v_1^i \cap v_1^j = \emptyset$; but $v_3^i \cap v_3^j \neq \emptyset$

(iii) In Newtonian flow, the magnitude of the flow vector of the
solvent at any point in the system is defined by the fraction
of the volume elements in the vicinity of that point classified
as in $V_2 \cup V_3$ in relation to those in $V_4 \cup V_2 \cup V_3$

(iv) $v_1^i \cap V_2 \neq \emptyset$: more completely $V_2$ is partitioned into the
disjoint subsets $V_{2a}$ and $V_{2b}$, where $v_1^i \cap V_{2a} = \emptyset$
$v_1^i \cap V_{2b} \neq \emptyset$: and $\phi_p$, the critical packing volume of the
particles, determines the relative number of elements in
$V_{2a}$ and $V_{2b}$ \{ $\phi = 1$; $V_{2a} = \emptyset$ \}.

On the assumptions that $n$ is large, and that the elements in $V_3$ are
located randomly in $V_3 \cup V_4$, then a simple finite probability space
can be constructed, enabling us to calculate the number of elements in $V_1 \ldots V_4$, and hence the quantity $g_c$ in

$$\frac{g_c}{s} = \frac{\eta_{sp}/c}{(\eta_{sp}/c)_c + 0} = (1 - g_c)$$

since $V_1 \cup V_2 \cup V_3 = g_c$.

The result is given by

$$\frac{g_c - z}{1 - z} = \Phi = (kc - 2c\bar{v}_s) - \sum_{i=2}^{i=\infty} (i - 1)(\Phi - \Phi^{i+1})$$

where $z = 2c\bar{v}_s - \frac{1}{\phi_p} (c\bar{v}_s)^2 (1 - Q)_+ ; \ Q = (1 - \phi_p)/\phi_p$

which for almost all cases simplifies to

$$g_c = \frac{k c - \frac{2\phi_p - 1}{\phi_p^2} (c\bar{v}_s)^2}{k c - 2c\bar{v}_s + 1}$$

where $k = k_s$ or $k_n$ ; $\bar{v}_s$ = specific volume of the hydrodynamic particle.

This equation predicts rather accurately the high-shear viscosity of latex spheres over the entire concentration range (figures 54 - 58). It is applicable only to Newtonian flow, but is free of arbitrary or empirical constants. The treatment used has some affinity with the widely used approach involving transient doublets, triplets, etc. ($i = 2$ in the above summation refers to 'doublet' interaction, etc.), but as no particle model is employed, the results should be general for all particles. The $\phi_p$ term would often be difficult to estimate, but computer simulation shows that an exact knowledge of $\phi_p$ is unimportant except at the highest concentrations.
Figures 54 - 58 demonstrate the success of the theory in predicting known properties of sedimentation and viscous flow at real concentrations.
Table 24. Various theoretical estimates and a practical estimate for $k_2$, the second coefficient in the expansion for $n_{rel}$ in terms of $\phi$ (volume fraction)

$$n_{rel} = 1 + k_1\phi + k_2\phi^2 + \ldots$$

<table>
<thead>
<tr>
<th>Estimate for $k_2$</th>
<th>Author</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.5</td>
<td>Vand (1948)</td>
</tr>
<tr>
<td>9.15</td>
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</tr>
<tr>
<td>7.5</td>
<td>Kynch (1956)</td>
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<tr>
<td>14.1</td>
<td>Gold (1937)</td>
</tr>
<tr>
<td>12.6</td>
<td>Simha (modification of Gold) (1952)</td>
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<td>7.6</td>
<td>Batchelor &amp; Green (1972)</td>
</tr>
<tr>
<td>10.0</td>
<td>Rowe (1977)</td>
</tr>
<tr>
<td>'about 10'</td>
<td>Cheng &amp; Schachman data on PSL spheres (1955)</td>
</tr>
</tbody>
</table>
Empirical data and the equation for transport-concentration dependence (Rowe 1977), at high solute dilution. The equation enables $M_r$ (molecular weight) values to be calculated from $s$ and $k_s$ only. The agreement found between values for $M_r$ computed thus and $M_r$ values from the literature (various methods) is good evidence for the applicability of the equation to a wide range of systems.

<table>
<thead>
<tr>
<th>Solute</th>
<th>$\frac{M_r(s+k_s)}{M_r(lit)}$</th>
<th>Standard error</th>
<th>Symbol</th>
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<tbody>
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<td>Proteins, nucleic acids,</td>
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<td>0.01</td>
<td>□</td>
</tr>
<tr>
<td>viruses</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cellulose derivatives</td>
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<td>0.09</td>
<td>○</td>
</tr>
<tr>
<td>in CUAM</td>
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<td></td>
<td></td>
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<tr>
<td>Cellulose derivatives</td>
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<td>△</td>
</tr>
<tr>
<td>in ACETONE</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Levans (aqueous)</td>
<td>0.99</td>
<td>0.04</td>
<td>□</td>
</tr>
<tr>
<td>Poly(methylylacrylate)</td>
<td>1.05</td>
<td>0.08</td>
<td>△</td>
</tr>
<tr>
<td>in ETHYL ACETATE</td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>
Figure 53. Venn diagram showing the volume elements for two particles in solution

V1 - particle volume
V2 - particle co-volume
V3 - solvent frictional forward flux
V4 - solvent return volume flux
Figure 54. Relative viscosity of spheres as a function of volume fraction.

Predicted line, for $k = 4, \bar{V}_s = 1, \phi_p = 0.64$. Experimental data points are also shown.
Figure 55. For highly asymmetric particles, the sedimentation coefficient falls very steeply with concentration, to reach a relatively constant 'plateau' value. Computed curves.
Figure 56. Viscosity/sedimentation coefficients as a function of concentration for $k_s(k_\eta) = 1000, \bar{v}_s = 1$. The $1/s$ plot is linear, whilst the direct plot is markedly curved. Computed curves.
Figure 57. Viscosity/sedimentation coefficients as a function of concentration for $k_s(k_\eta) = 6$. Both plots are reasonably linear over this concentration range. Computed curves.
Figure 58. Hydrodynamic data for Bovine serum albumin fitted using the new general equation for transport at all solute concentrations.
Appendix V  FORTRAN IV computer programs

Program 1:  Evaluates values of the various hydrodynamic shape functions for tri-axial ellipsoids for a user specifiable value of the axial ratios (a/b, b/c)

Program 2:  Produces tables of these functions for axial ratios between 1.0 and 2.0 in steps of 0.1

Program 3:  Produces a contour map of v in the (a/b, b/c) plane for axial ratios between 1.0 and 3.0

Program 4:  Produces plots of the various tri-axial functions in the (a/b, b/c) plane corresponding to the point (1.5, 1.5). Several plots allow for arbitrary errors in measurement

Program 5:  Non-linear least squares iterative method for resolving a 2-term exponential birefringence decay. This program (and the following two) produces its own synthetic data

Program 6:  Fourier Transform solution of the Laplace Integral Equation method

Program 7:  R-constrained non-linear least squares iterative method
<table>
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<tr>
<th>Function</th>
<th>Computer Symbol</th>
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<td>a/b</td>
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</tr>
<tr>
<td>b/c</td>
<td>B/C</td>
</tr>
<tr>
<td>v</td>
<td>NU,S</td>
</tr>
<tr>
<td>f/fₐ (ξp)</td>
<td>F</td>
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<td>ζₐ/ζₒ</td>
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<td>Λ</td>
<td>LAMBDAD</td>
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<tr>
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<td>TPLS, THETA⁺⁺⁺, Z</td>
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<tr>
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</tr>
<tr>
<td>$\tau_4/\tau_0$</td>
<td>T4</td>
</tr>
<tr>
<td>$\tau_5/\tau_0$</td>
<td>T5</td>
</tr>
<tr>
<td>$\theta_+$</td>
<td>THPLUS</td>
</tr>
<tr>
<td>$\theta_-$</td>
<td>THMNUS</td>
</tr>
</tbody>
</table>
** This program generates values of the various hydrodynamic shape functions for a user specifiable value of the axial ratios A/B (=N1) & B/C (=N2)

Program MAIN (INPUT, OUTPUT, TAPE2=INPUT, TAPE3=OUTPUT)
COMMON/PARAM/A,C,NN
EXTERNAL FUN
DIMENSION ALPHA(10)
REAL NU,M,0,P
REAL MUA,MUB,MUC,PSI,N1,N2,RHOA,RHOB,RHOC
C ENTER AS DATA AT THE FOOT OF THE PROGRAM (A/B, B/C)
READ (2,*) N1,N2
NN=0
C COMPUTE THE ELLIPTIC INTEGRALS
DO 45 K=1,10
A=N1
B=1.0
C=1.0/N2
NN=NN+1
C SET LIMITS FOR NUMERICAL INTEGRATION (THESE LIMITS BELOW HAVE BEEN PREVIOUSLY TESTED FOR CONVERGENCE)
AA=0.0
BB=1000000
IF(NN.EQ.10)88=500000000
MAXDIV=50
EPS=1.0E-08
ACC=0.0
IFAIL=0
C CALL U.K. "NAG" LIBRARY ROUTINE FOR NUMERICAL EVALUATION
C OF THE INTEGRALS "ALPHA" GIVEN IN THE SUBROUTINE BELOW
CALL D01AGF (AA, BB, FUN, MAXDIV, EPS, ACC, ANS, ERR, NOFUN, IFAIL)
ALPHA(NN)=ANS
45 CONTINUE
C NOW COMPUTE THE FUNCTION VALUES USING THE STORED INTEGRAL VALUES
NU=(.0/(A*B*C))(.0/15.0)+((ALPHA(7)+ALPHA(8))
+(7)+ALPHA(7)+ALPHA(8))+(1.0/5.0)+((ALPHA(2)
+(8)+ALPHA(3)/ALPHA(1))/ALPHA(5)/(C*A*ALPHA(3)
+(1)+ALPHA(1)+ALPHA(3)+ALPHA(2)+ALPHA(1)+B*B*ALPHA(2))
TPLS=(A*C)/(12.0)+(((1.0/M)+(1.0/0)+(1.0/P)
+)+(1.0/M)**2.0)+(1.0/0)**2.0)+(1.0/P**2.0)
+((1.0/M0)+(1.0/0**P)+(1.0/P**M)
+))**(0.5)
TMNS=(A*B*C)/(12.0)+(((1.0/M)+(1.0/0)+(1.0/P)
+)+(1.0/M)**2.0)+(1.0/0**2.0)+(1.0/P**2.0)
+((1.0/M0)+(1.0/0**P)+(1.0/P**M)
+))**(0.5)
DPLS=6.0*TPLS*NU
DMNS=6.0*TMNS*NU
F=2.0/((A*B*C)**(1.0/3.0))*ALPHA(10)
0=1.0*11.0*(F**3.0)/NU
BETA=(.0/1000000.0)+((6.0249**23.0/0.03)
+)((1620.0131.0/151926543.014192654)**(1.0/3.0))
+(1.0/3.0))
CA=1.0/3.0*A*B*C+M
CC=1.0/3.0*A*B*C+P
RHOA=2.0/((1.0/CC)+(1.0/CC))
RHOB=2.0/((1.0/CC)+(1.0/CC))
RHOC=2.0/((1.0/CC)+(1.0/CC))
DELA=NU/CA
DELB=NU/CC
DELU=NU/CC
TAU=3.0/((1.0/CA)+(1.0/CC))
PSI=F/**(1.0/TAU)**(1.0/3.0))
V=NU/TAU
ZT=1.0/2.0*(F**3.0)
GAMMAA=ZT**2/((1.0/CC)+(1.0/CC))
GAMMAP=ZT**2/((1.0/CC)+(1.0/CC))
GAMMAC=ZT**2/((1.0/CC)+(1.0/CC))
MUA=CA**2/((1.0/CC)+(1.0/CC))
MUB=CB**2/((1.0/CC)+(1.0/CC))
MUC=CC**2/((1.0/CC)+(1.0/CC))
GAMM=6.0*TPLS*(F**3.0)
GMNS = 6.0 * TMNS * (F**3,0)
X1 = 0.5 * ((1.0/RHOB) + (1.0/RHOC) - (1.0/RHOA))
X2 = 0.5 * ((1.0/RHOC) + (1.0/RHOA) - (1.0/RHOB))
X3 = 0.5 * ((1.0/RHOA) + (1.0/RHOB) - (1.0/RHOC))
X4 = (X1 + X2 + X3) / 3.0
X5 = ((X1**2.0) + (X2**2.0) + (X3**2.0) - (X1*X2) - (X2*X3) - (X3*X1)) / 2.0

T1 = 1.0 / (X4 + X1)
T2 = 1.0 / (X4 + X2)
T3 = 1.0 / (X4 + X3)
T4 = (6.0 * X4) / (2.0 * X5)
T5 = (6.0 * X4) / (2.0 * X5)

WRITE (3, 97)

97 FORMAT ("")

WRITE (3, 98)

98 FORMAT ("")

WRITE (3, 99) A, 1/C

WRITE (3, 100) NU

100 FORMAT (5X, "NU", F10.5)

WRITE (3, 101) TPLS

101 FORMAT (5X, "THE 3 ROTATIONAL FRICTIONAL RATIO:", F15.5)

WRITE (3, 102) TMNS

102 FORMAT (5X, "THETA+", F15.5)

WRITE (3, 103) DPLS

103 FORMAT (5X, "DELTA+", F15.5)

WRITE (3, 104) GMNS

104 FORMAT (5X, "DELTA-", F15.5)

WRITE (3, 105) R

105 FORMAT (5X, "R", F15.5)

WRITE (3, 106) F

106 FORMAT (5X, F", F10.5)

WRITE (3, 107) BETA

107 FORMAT (5X, "BETA", F10.5)

WRITE (3, 108) CA

108 FORMAT (5X, "CA", F10.5)

WRITE (3, 109) CB

109 FORMAT (5X, "CB", F10.5)

WRITE (3, 110) CC

110 FORMAT (5X, "CC", F10.5)

WRITE (3, 111) RHOA

111 FORMAT (5X, "RHOA", F10.5)

WRITE (3, 112) RHOB

112 FORMAT (5X, "RHOB", F10.5)

WRITE (3, 113) RHOC

113 FORMAT (5X, "RHOC", F10.5)

WRITE (3, 114) DELA

114 FORMAT (5X, "DELTAA", F10.5)

WRITE (3, 115) DELB

115 FORMAT (5X, "DELTAB", F10.5)

WRITE (3, 116) DELC

116 FORMAT (5X, "DELC", F10.5)

WRITE (3, 117) TAU

117 FORMAT (5X, "TAU", F10.5)

WRITE (3, 118) PSI

118 FORMAT (5X, "PSI", F10.5)

WRITE (3, 119) V

119 FORMAT (5X, "LAMDA", F10.5)

WRITE (3, 120) GAMMAA

120 FORMAT (5X, "GAMMAA", F10.5)

WRITE (3, 121) GAMMAB

121 FORMAT (5X, "GAMMAB", F10.5)

WRITE (3, 122) GAMMAC

122 FORMAT (5X, "GAMMAB", F10.5)

WRITE (3, 123) MUA

123 FORMAT (5X, "MUA", F10.5)

WRITE (3, 124) MUB

124 FORMAT (5X, "MUB", F10.5)

WRITE (3, 125) MUC

125 FORMAT (5X, "MUC", F10.5)

WRITE (3, 126) 3PLS

126 FORMAT (5X, "GAMMA+", F10.5)

WRITE (3, 127) GMNS

127 FORMAT (5X, "GAMMA-", F10.5)

WRITE (3, 201)
201 FORMAT( 5X,"FLUORESCENCE ANISOTROPY RELAXATION TIME RATIOS:")
WRITE(3,130)T1
130 FORMAT( 5X,"T1 \(F15.5\)")
WRITE(3,131)T2
131 FORMAT( 5X,"T2 \(F15.5\)")
WRITE(3,132)T3
132 FORMAT( 5X,"T3 \(F15.5\)")
WRITE(3,133)T4
133 FORMAT( 5X,"T4 \(F15.5\)")
WRITE(3,134)T5
134 FORMAT( 5X,"T5 \(F15.5\)")
STOP
END

REAL FUNCTION FUN(X)
COMMON/PARAM/A,C,NN
B=1.0
GOTO(10,20,30,40,50,60,70,80,90,100),NN
10 FUN=1/((A*A+X)**1.5*(B*B+X)**0.5*(C*C+X)**0.5)
RETURN
20 FUN=1/((A*A+X)**0.5*(B*B+X)**1.5*(C*C+X)**0.5)
RETURN
30 FUN=1/((A*A+X)**0.5*(B*B+X)**0.5*(C*C+X)**1.5)
RETURN
40 FUN=1/((A*A+X)**0.5*(B*B+X)**1.5*(C*C+X)**1.5)
RETURN
50 FUN=1/((A*A+X)**1.5*(B*B+X)**0.5*(C*C+X)**1.5)
RETURN
60 FUN=1/((A*A+X)**1.5*(B*B+X)**1.5*(C*C+X)**0.5)
RETURN
70 FUN=X/((A*A+X)**0.5*(B*B+X)**1.5*(C*C+X)**1.5)
RETURN
80 FUN=X/((A*A+X)**1.5*(B*B+X)**0.5*(C*C+X)**1.5)
RETURN
90 FUN=X/((A*A+X)**1.5*(B*B+X)**1.5*(C*C+X)**0.5)
RETURN
100 FUN=1.0/(((A*A+X)*(B*B+X)*(C*C+X))**0.5)
RETURN
END
Program 1 Results

<table>
<thead>
<tr>
<th></th>
<th>A/B</th>
<th>B/C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1.50</td>
<td>1.50</td>
</tr>
<tr>
<td>NU</td>
<td>2.89170</td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>1.04410</td>
<td></td>
</tr>
<tr>
<td>THETA+</td>
<td>.16261</td>
<td></td>
</tr>
<tr>
<td>THETA-</td>
<td>.11622</td>
<td></td>
</tr>
<tr>
<td>DELTA+</td>
<td>2.82127</td>
<td></td>
</tr>
<tr>
<td>DELTA-</td>
<td>2.01645</td>
<td></td>
</tr>
<tr>
<td>R</td>
<td>1.47887</td>
<td></td>
</tr>
<tr>
<td>BETA</td>
<td>2.12334</td>
<td></td>
</tr>
<tr>
<td>THETA+</td>
<td>.16261</td>
<td></td>
</tr>
<tr>
<td>THETA-</td>
<td>.11622</td>
<td></td>
</tr>
<tr>
<td>DELTA+</td>
<td>2.82127</td>
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<tr>
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</tr>
<tr>
<td>R</td>
<td>1.47887</td>
<td></td>
</tr>
<tr>
<td>BETA</td>
<td>2.12334</td>
<td></td>
</tr>
</tbody>
</table>

**The 3 Rotational Frictional Ratios:**
- CA: .89762
- CB: 1.39800
- CC: 1.47041

**The 3 Rotational Relaxation Time Ratios:**
- RHOA: 1.43329
- RHOB: 1.11474
- RHOC: 1.09327
- DELTAA: 3.22152
- DELTAB: 2.06846
- DELTAC: 1.96660

**The Harmonic Mean Relaxation Time Ratio:**
- TAU: 1.19548
- PSI: 1.98378

**LAMBDA**
- 2.41886

**GAMMA**
- .79414
- 1.02107
- 1.04112
- 1.02107
- 1.04112
- 1.02107
- 1.04112
- 1.02107
- 1.04112

**MUB**
- 1.07092

**MUC**
- 1.08910

**GAMMA+**
- 1.11051

**GAMMA-**
- .79372

**Fluorescence Anisotropy Relaxation Time Ratios:**
- T1: 1.02536
- T2: 1.28883
- T3: 1.31877
- T4: 1.43405
- T5: 1.02497
Program 2

·······~··················~··············~·················~••+•••••
THIS PROGRAM COMPUTES TABLES OF THE VISCOSITY INCREME~T NU,
THE TRANSLATIONAL FRICTIJNAL RATIO (f/FQ), THE ROTATIONAL
FRICTIONAL RATIOS< CA/CO, CB/CO, CC/CO ), THE ROTATIONAL
RELAXATION TIME RATIOSCRnOA/RHOQ, RH08/~HOO, RHOC/RHOO>, THE
HARMONIC HEAN RELAXATION TIME RATIO( TAU/TAUQ ), THE RIDGEWAY
EL~CTRIC BIREFRINGENCE DeCAY CONSTANTS( THETA+, THETA-), AND
THEIR CORRESPONDING SWELLING - INDEPENDENT FUNCTIONS:
R, BETA, DELTAA, DELTAS, DELTAC, GAHMAA, GAMMAB, GAMMAC,
MUA, MUB, MUG, PSI, DELTA+, DELTA-, GAMMA+ AND GAMMAAS FUNCTIONS OF THE AXIAL RATIOS A/8, 8/C, IN THE RANGE 1.0 TQ
2.0 IN STEPS OF 0.1, FOR A GENERAL TRIAXIAL ELLIPSOID PARTICLE
MODEL. { THE SUBSCRIPT ••o•• REFERS TO THE CORRESPONDING COEFFICIENT
FOR A SPHERE OF EQUAL VOLUME )
·············~··················~········~·~························
PROGRAM MAINCINPUT,OUTPUT,TAPE2=INPUT,TAPE3=0UTPUT)
COMMON/PARAM/A,C,NN
EXTERNAL FUN
DIMENSION ALPHAC10,11,11l,NUC11,11),f(11,11),R(11,11),H(1)
+ , BET A C11, 11 ) , CA ( 11, 11) , CB ( 11 , 11) , CC C11, 11 l , M( 11 , 11 ) , 0 C11, 11 )
+ ,PC11,11) ,TPLS(11,11) ,TMNS (11,11) ,RHOAC11,11) ,RH08(11,11)
+ ,RHOCC11,11l,TAUC11,11),PSIC11,11l,OELA(11,11),0ELBC11,11)
+ ,DELC(11,11),GAMMAA(11,11),GAMMABC11,11),GAMMACC11,11»,MUA(11,11)
+ ,HUBC11,11) 9 MUC(11,11l,OPLSC11,11l,OHNS(11,11),GPLS(11,11)
+ ,GMNSC11,11) ,Y(11) ,Zl11) ,ZZl11,11>
REAL NU,M,O,P,HUA,MUB,MUC,PSI
COMPUTE THE ELLIPTIC INTE~RALS
NN=O
0045 K=1,10
A= 0.9
NN=NN+1
004 0 I=1,11
A=A+ 0.1
C=1.01 0.9
0030 N=1,11
C=C/t1.0+0.1•Cl
SET LIMITS FO R NUMERICAL INTEGRATION (THESE LIMITS
BELOW HAVE BEEN PREVIOUSLY TESTED FOR CONVERGENCE)
AA=O.O
88=1000000
IF<NN.EQ.10)88=500000000
MAXOIV=50
EPS=i.OE-08
ACC=O.O
IFAIL=O
CALL U.K. "NAG" LIBRARY ROUTINE FOR NUMERICAL EVALUATION
OF THE INTEGRALS ••ALPHA"• GIVEN IN THE SUBROUTINE BELOW
CALL 001AGFCAA,BS,FUN,MAXDIV,EPS,ACC,ANS,ERROR,NOFUN,IF4IL>
ALPHACNN,!,N)=ANS
30 CONTINUE
ftO CONTINUE
45 CONTINUE
8= 1. 0
A=0.9
0080 J=1,11
A=A+0.1
'f(J)=A
C=i.0/0.9
0070 N=1,11
C=C/C1.0+0.1•Cl
ZCN>=1/C
NOW COMPUTE THE FUNCTION VALUES USING THE STORED INTEGRAL VALUES
NU(J~N)=(1.0/(A•8~C)l•((4.0/15.0l•CCALPHAC7,J~N)+ALPHAl8,J,Nl
+ +ALPHA(9 9 J,N))/(ALPHAC8,J,N)•ALPHAC9,J,Nl+ALPHAC9,J,N)~ALPHA
+ ( 7, J, N) +A LP HA ( 7, J, N) • ALPHA ( 8, J, N) ) ) + ( 1. 0 I 5. 0) • ( ( (ALPHA {2, J, N)
+ +ALPHA(3,J,N))/{ALPHA(4,J,N)•(B•B•ALPHAt2,J,Nl+C•C•ALPHA(3,J,N)
+ )))+((ALPHA(3,J,N)+ALPHA(1 9 J,N))/(ALPHA(5,J,N)•(C•C•ALPHA(3~J,N)
+ +A•A•ALPHA(1,J,N))))+((ALPHA(1,J,N)+ALPHAC2,J,N))/(ALPHA(5,J,N)
+ ~cA•A•ALPHAt1,J,N)+B•B•ALPHA(2,J, N ))) )))
F(J,N)=2.0/(((A•B•C»••t1.0/3.0))•ALPHAC1 0,J,N))
R<J,Nl=2.0•C1.0+CFCJ,N)••3.Q))/NUCJ,N)
BET A( J , N) = ( 1 • 0/1 0 0 C0 0 0 • 0 ) ~ < ( ( 6 • J 2 4- g•"'" ( 1. 0I 3. J 1 ) • ( 1 0 • 0 • • ( 23 • 0I 3 • 0
+ )))/((16200.0•J.141592654•3.141592654)•~(1.013.Q)))•(NU(J,N)
+ ••(1.013.0))/F(J,N)
M(J,N)=CB•B+C•C)/(B•B•ALPHAC2,J,N}+C•C•ALPHAC3,J,N))
Q(J,N>=CC•C•A•A)/(C•C~AL?HA(3,J,N)+A•A•ALPHAC1,J,N))
P(J,N)=(A•A+8•8)/(A•A•ALPHA(1,J,N)+S•B•ALPHA(2,J,N))
TPLS(J,N)=(<A•B•C)/(12. 0))•{(((1.0/M(J,N))+(1.0/0(J,N))+(1.0/P(J,
+ N)))+(((1.0/M(J,Nt••2.0)+(1.0/0(J,N)••2.u)+(1.0/P{J,N)••z.O))-


+ ((1.0/(H(J,N)*O(J,N)))+(1.0/(O(J,N)*P(J,N)))+(1.0/(P(J,N)*M(J,N)))))**(0.5))

TNS(J,N)=(1.0/(B*C)^(12.0))(((1.0/(M(J,N)))+(1.0/(O(J,N)))+(1.0/(P(J,N))))-
((1.0/(M(J,N)**2.0))+(1.0/(O(J,N)**2.0))+(1.0/(P(J,N)**2.0)))-
((1.0/(M(J,N)*O(J,N)))+(1.0/(O(J,N)*P(J,N)))+(1.0/(P(J,N)*M(J,N)))))**(0.5))

CA(J,N)=(2.0/(3.0*A*B*C))*M(J,N)
CB(J,N)=(2.0/(3.0*A*B*C))*O(J,N)
CC(J,N)=(2.0/(3.0*A*B*C))*P(J,N)
RHOA(J,N)=2.0/(1.0/(CA(J,N))+(1.0/(CC(J,N))))
RHOB(J,N)=2.0/(1.0/(CA(J,N))+(1.0/(CB(J,N))))
RHO(C(J,N)=2.0/(1.0/(CA(J,N))+(1.0/(CB(J,N))))
DEL(CA(J,N)=NU(J,N)/CA(J,N)
DEL(CB(J,N)=NU(J,N)/CB(J,N)
DEL(CCC(J,N)=NU(J,N)/CC(J,N)
TAU(J,N)=3.0/(1.0/(CA(J,N))+1.0/(CB(J,N))+1.0/(CC(J,N)))
PSI(J,N)=F(J,N)*((1.0/TAU(J,N))**2.0)
ZZ(J,N)=(1.0/(RHOA(J,N)))*F(J,N)
GMMAA(J,N)=ZZ(J,N)**2.0/(O(J,N))*F(J,N)
GMMAX(J,N)=ZZ(J,N)**2.0/(O(J,N))*F(J,N)
MU(J,N)=CA(J,N)**2.0/F(J,N)
MUB(J,N)=CB(J,N)**2.0/F(J,N)
MUC(J,N)=CC(J,N)**2.0/F(J,N)
DPLS(J,N)=6.0*TPLS(J,N)*NU(J,N)
GMNS(J,N)=6.0*TMNS(J,N)*CA(J,N)
GMNS(J,N)=6.0*TMNS(J,N)*F(J,N)**2.0
CONTINUE
THE PROGRAM NOW WRITES OUT THE COMPUTED VALUES IN TABLE FORM
WRITE(3,600)
CONTINUE
PRINT(3,600)
WRITE(3,600)
CONTINUE
WRITE(3,610)
CONTINUE
WRITE(3,620)
CONTINUE
WRITE(3,630)
CONTINUE
WRITE(3,640)
CONTINUE
PRINT(3,640)
PRINT(3,640)
CONTINUE
PRINT(3,630)
CONTINUE
PRINT(3,620)
CONTINUE
PRINT(3,610)
CONTINUE
PRINT(3,600)
CONTINUE
PRINT(3,600)
CONTINUE
PRINT(3,600)
CONTINUE
WRITE(3,600)
CONTINUE
WRITE(3,600)
CONTINUE
WRITE(3,600)
CONTINUE
WRITE(3,600)
WRITE(3, 650)
FORMAT("
")
PRINT(3, 655)
FORMAT("1 A/B B/C MU(A) MU(B) MU(C)"")
DO 94 J=1,11
DO 95 N=1,11
WRITE(3, 96) Y(J), Z(N), MUA(J, N), MUB(J, N), MUC(J, N)
96 CONTINUE
94 CONTINUE
WRITE(3, 660)
FORMAT(""")
PRINT(3, 665)
FORMAT("1 A/B B/C THETA+ THETA- TAU/TAU0 PSI"")
DO 101 J=1,11
DO 102 N=1,11
WRITE(3, 103) Y(J), Z(N), TPLS(J, N), TMNS(J, N), TAU(J, N), PSI(J, N)
103 CONTINUE
102 CONTINUE
WRITE(3, 670)
FORMAT(""")
PRINT(3, 675)
FORMAT("1 A/B B/C DELTA+ DELTA-"")
DO 104 J=1,11
DO 105 N=1,11
WRITE(3, 106) Y(J), Z(N), OPLS(J, N), OMLS(J, N)
106 CONTINUE
105 CONTINUE
WRITE(3, 680)
FORMAT(""")
PRINT(3, 685)
FORMAT("1 A/B B/C GAMMA+ GAMMA-"")
DO 108 J=1,11
DO 109 N=1,11
WRITE(3, 110) Y(J), Z(N), GPLS(J, N), GMNS(J, N)
110 CONTINUE
109 CONTINUE
STOP
END

REAL FUNCTION FUN(X)
COMMON/PARAM/A, G, NN
B=1.0
GOTO (10, 20, 30, 40, 50, 60, 70, 80, 90, 100), NN
10 FUN=1/(A*A+X)**1.5*(B*B+X)**0.5*(C*C+X)**0.5
RETURN
20 FUN=1/(A*A+X)**0.5*(B*B+X)**1.5*(C*C+X)**0.5
RETURN
30 FUN=1/(A*A+X)**0.5*(B*B+X)**0.5*(C*C+X)**1.5
RETURN
40 FUN=1/(A*A+X)**1.5*(B*B+X)**0.5*(C*C+X)**1.5
RETURN
50 FUN=1/(A*A+X)**1.5*(B*B+X)**0.5*(C*C+X)**0.5
RETURN
60 FUN=1/(A*A+X)**1.5*(B*B+X)**0.5*(C*C+X)**0.5
RETURN
70 FUN=X*(A*A+X)**0.5*(B*B+X)**0.5*(C*C+X)**0.5
RETURN
80 FUN=X*(A*A+X)**1.5*(B*B+X)**0.5*(C*C+X)**0.5
RETURN
90 FUN=X*(A*A+X)**1.5*(B*B+X)**0.5*(C*C+X)**0.5
RETURN
100 FUN=1.0/((A*A+X)*(B*B+X)*(C*C+X))**0.5
RETURN
END
<table>
<thead>
<tr>
<th>A/B</th>
<th>B/C</th>
<th>NU</th>
<th>F/O0</th>
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Program 3

**************************************************
THIS PROGRAM PRODUCES A CONTOUR MAP OF THE VISCOSITY
INCREMENT OVER THE AXIAL RANGE A/B, B/C 1.0 TO 3.0, IN
STEPS OF 0.5 OF THE FUNCTION FROM NU=2.5 TO NU=7.0
**************************************************

PROGRAM MAIN(INPUT,OUTPUT,TAPE2=INPUT,TAPE3=OUTPUT)
COMMON/PARAM/A,C,NN
EXTERNAL FUN
DIMENSION ALPHA(10,21,21),NU(21,21),H(10)
REAL NU
NN=2
COMPUTE THE ELLIPTIC INTEGRALS
DO45 K=1,10
A=0.9
NN=NN+1
DO40 I=1,21
A=A+0.1
C=1.0/0.9
DO30 J=1,21
C=C/(1.0+0.1*C)
SET LIMITS FOR NUMERICAL INTEGRATION (THESE LIMITS
BELOW HAVE BEEN PREVIOUSLY TESTED FOR CONVERGENCE)
AA=0.0
BB=100.0
IF(NN.EQ.10)BB=500000000
MAXDIV=50
EPS=1.0E-08
ACC=0.0
IFAIL=0
CALL U.K. "NAG" LIBRARY ROUTINE FOR NUMERICAL EVALUATION
OF THE INTEGRALS "ALPHA" GIVEN IN THE SUBROUTINE BELOW
CALL D01AGF(AA,BB,FUN,MAXDIV,EPS,ACC,ANS,ERROR,NOFUN,IFAIL)
ALPHA(NN,I,N)=ANS
30 CONTINUE
40 CONTINUE
45 CONTINUE
B=1.0
A=0.9
DO83 J=1,21
A=A+0.1
C=1.0/0.9
DO70 N=1,21
C=C/(1.0+0.1*C)
NOW COMPUTE THE FUNCTION VALUES USING THE STORED INTEGRAL VALUES
NU(J,N)=(1.0/(A*B*C))*(4.0/15.0)*(ALPHA(7,J,N)+ALPHA(8,J,N)+ALPHA(9,J,N)+ALPHA(3,J,N)+ALPHA(2,J,N)+ALPHA(1,J,N)+ALPHA(6,J,N)+ALPHA(5,J,N)+ALPHA(4,J,N)+ALPHA(3,J,N)+ALPHA(2,J,N)+ALPHA(1,J,N))
70 CONTINUE
80 CONTINUE
CALL CONTOUR PLOTTING ROUTINE
CALL PAPER(1)
H(1)=2.5
H(2)=3.0
H(3)=3.5
H(4)=4.0
H(5)=4.5
H(6)=5.0
H(7)=5.5
H(8)=6.0
H(9)=6.5
H(10)=7.0
CALL MAP(1.0,3.0,1.0,3.0)
CALL CAL
CALL BORDER
CALL CONTR(NU,1,21,21,1,21,21,1,1,15)
CALL GREN
STOP
END
REAL FUNCTION FUN(X)
COMMON/PARAM/A, C, NN
B = 1.0
GOTO(10, 20, 30, 40, 50, 60, 70, 80, 90, 100), NN
10 FUN=1/(A*A+X)**1.5*(B*B+X)**0.5*(C*C+X)**0.5)
RETURN
20 FUN=1/(A*A+X)**0.5*(B*B+X)**1.5*(C*C+X)**0.5)
RETURN
30 FUN=1/(A*A+X)**-0.5*(B*B+X)**0.5*(C*C+X)**1.5)
RETURN
40 FUN=1/(A*A+X)**0.5*(B*B+X)**1.5*(C*C+X)**1.5)
RETURN
50 FUN=1/(A*A+X)**1.5*(B*B+X)**0.5*(C*C+X)**1.5)
RETURN
60 FUN=1/(A*A+X)**1.5*(B*B+X)**1.5*(C*C+X)**0.5)
RETURN
70 FUN=X/(A*A+X)**0.5*(B*B+X)**1.5*(C*C+X)**1.5)
RETURN
80 FUN=X/(A*A+X)**1.5*(B*B+X)**0.5*(C*C+X)**1.5)
RETURN
90 FUN=X/(A*A+X)**1.5*(B*B+X)**1.5*(C*C+X)**0.5)
RETURN
100 FUN=1.0/((A*A+X)*(B*B+X)*(C*C+X))**0.5)
RETURN
END
*** THIS PROGRAM GIVES PLOTS OF THE LINE SOLUTIONS OF THE VARIOUS TRIAXIAL SHAPE FUNCTIONS (AS TABULATED IN THE RESULTS PROGRAM) FOR AXIAL RATIOS A/B = B/C = 1.5. IN ORDER TO DETERMINE THE BEST COMBINATION FOR PRODUCING A UNIQUE SOLUTION.

PROGRAM MAIN(INPUT, OUTPUT, TAPE2=INPUT, TAPE3=OUTPUT)
COMMON/PARAM/A, C, NN
EXTERNAL FUN
DIMENSION ALPHA(10, 21, 21), NU(21, 21), F(21, 21), R(21, 21), H1(21)
    + BETA(21, 21), CA(21, 21), CB(21, 21), CC(21, 21), M(21, 21), O(21, 21),
    + P(21, 21), TPLS(21, 21), TMNS(21, 21), RHOA(21, 21), RHOB(21, 21),
    + MNC(21, 21), NU1(21, 21), PS1(21, 21), DCA(21, 21), DELB(21, 21),
    + DELC(21, 21), GAMMA(21, 21), GAMMAB(21, 21), GAMMAC(21, 21), MUA(21, 21),
    + MUB(21, 21), MUC(21, 21), DPLS(21, 21), DMNS(21, 21), GPLS(21, 21),
    + GMNS(21, 21), V(21, 21),
    + X1(21, 21), X2(21, 21), X3(21, 21), X4(21, 21), X5(21, 21),
    + T1(21, 21), T2(21, 21), T3(21, 21), T4(21, 21), T5(21, 21),
REAL NU, H, O, P, MUA, MUB, MUC, PSI
COMPUTE THE ELLIPTIC INTEGRALS
NN=0
DO 45 K=1, 10
   A=0.95
   NN=NN+1
   DO 40 I=1, 21
      A=A+0.05
      C=I/0.95
      DO 30 N=1, 21
         C=C*(I+N)**0.35
         SET LIMITS FOR NUMERICAL INTEGRATION (THESE LIMITS
         BELOW HAVE BEEN PREVIOUSLY TESTED FOR CONVERGENCE)
         AA=0.0
         BB=1000000
         IF (NN.EQ.10) BB=5000000000
         MAXDIV=50
         EPS=1.E-08
         ACC=0.0
         IFAIL=0
         CALL U.K. "NAG" LIBRARY ROUTINE FOR NUMERICAL EVALUATION
         OF THE INTEGRALS ALPHA GIVEN IN THE SUBROUTINE BELOW
         CALL D01AGF(AA, BB, FUN, MAXDIV, EPS, ACC, IFAIL, IFAIL)
         ALPHA(NN, I, N)=ANS
      30 CONTINUE
   40 CONTINUE
   CONTINUE
   CONTINUE
   B=1.0
   A=1.0
   C=1.0/0.95
   DO 70 N=1, 21
      C=C*(I+N)**0.35
      COMPUTE THE FUNCTION VALUES USING THE STORED INTEGRAL VALUES
      NU(J,N)=((1.0/(I*J*C))**((4.0/I**5.0)*((ALPHA(7, J, N)+ALPHA(8, J, N))
      +ALPHA(9, J, N))/((ALPHA(8, J, N)+ALPHA(9, J, N)+ALPHA(9, J, N)*ALPHA
      +ALPHA(7, J, N)+ALPHA(7, J, N)+ALPHA(7, J, N))+1.0/5.0)*((ALPHA(2, J, N)
      +ALPHA(3, J, N))/ALPHA(2, J, N))+(ALPHA(4, J, N)+ALPHA(4, J, N))
      +ALPHA(5, J, N))/((ALPHA(5, J, N)+ALPHA(5, J, N)+ALPHA(5, J, N))
      +ALPHA(4, J, N)+ALPHA(4, J, N)))*ALPHA(1, J, N))
      ALFA(J,N)=((1.0/((I*J*C)**((1.0/3.0J)*ALPHA(10, J, N)))
      R(J,N)=2.0*(1.0+((F(J,N)*3.0))/NU(J,N))
      BETA(J,N)=((1.0/100000000.0)**((6.0/243)**((1.0/3.0J))**10.0)**((23.0/3.0J)
      M(J,N)=(B**C)/(B**C)+C/ALPHA(3, J, N))
      P(J,N)=(C**C)/(C**C)+C/ALPHA(1, J, N))
      TPLS(N,J)=((4.0**C)/(12.0)**(((1.0/M(J,N))+1.0/O(J,N))**1.0/(P(J,
      N)+1.0/M(J,N)**2.0)+(1.0/O(J,N)**2.0)+(1.0/O(J,N)**2.0)+1.0/(P(J,
      N)**2.0)-((1.0/(O(J,N)**2.0)+(1.0/O(J,N)**2.0)+(1.0/O(J,N)**2.0)+1.0/(P(J,
      N)**2.0)-((1.0/O(J,N)**2.0)+(1.0/O(J,N)**2.0)+(1.0/(P(J,N)**2.0)+1.0/(P(J,
      N)**2.0)+1.0/O(J,N)**2.0)+1.0/(P(J,N)**2.0)+1.0/O(J,N)**2.0)+1.0/(P(J,N)**2.0)
      TMNS(J,N)=(((1.0/M(J,N))**2.0)+1.0/O(J,N)**2.0)+(1.0/O(J,N)**2.0)+1.0/(P(J,
      N)**2.0)-((1.0/O(J,N)**2.0)+(1.0/O(J,N)**2.0)+(1.0/O(J,N)**2.0)+1.0/(P(J,
      N)**2.0)+1.0/O(J,N)**2.0)+1.0/(P(J,N)**2.0)+1.0/O(J,N)**2.0)+1.0/(P(J,N)**2.0)
      CA(J,N)=((2.0/3.0)**C)/(M(J,N)))
      CB(J,N)=(((2.0/3.0)**C)/(M(J,N)))*D(J,N)

222.
CC(J,N) = (2.0/(3.0*A*B*C)) * P(J,N) 
RHOA(J,N) = 2.0/(1.0/CA(J,N) + 1.0/CC(J,N)) 
RHOB(J,N) = 2.0/(1.0/CA(J,N) + 1.0/CC(J,N)) 
RHOC(J,N) = 2.0/(1.0/CA(J,N) + 1.0/CC(J,N)) 
DELN(J,N) = N(J,N)/CA(J,N) 
DELB(J,N) = N(J,N)/CC(J,N) 
DELC(J,N) = N(J,N)/CC(J,N) 
TAU(J,N) = 3.0/(1.0/CA(J,N) + 1.0/CC(J,N)) 
PSI(J,N) = F(J,N) * (1.0/TAU(J,N)**(1.0/3.0)) 
RHOB(J,N) = 2.0/((1.0/CA(J,N)) + (1.0/CC(J,N))) 
RHOCC(J,N) = 2.0/((1.0/CA(J,N)) + (1.0/CC(J,N))) 
DELN(J,N) = N(J,N)/CA(J,N) 
DELB(J,N) = N(J,N)/CC(J,N) 
DELC(J,N) = N(J,N)/CC(J,N) 
PSI(J,N) = F(J,N) * (1.0/TAU(J,N)**(1.0/3.0)) 

(TAU(J,N) = 
3.0/(1.0/CA(J,N)) + (1.0/CC(J,N)) + (1.0/CC(J,N))) 

RHOB(J,N) = 2.0/((1.0/CA(J,N)) + (1.0/CC(J,N))) 
RHOCC(J,N) = 2.0/((1.0/CA(J,N)) + (1.0/CC(J,N))) 
DELN(J,N) = N(J,N)/CA(J,N) 
DELB(J,N) = N(J,N)/CC(J,N) 
DELC(J,N) = N(J,N)/CC(J,N) 
PSI(J,N) = F(J,N) * (1.0/TAU(J,N)**(1.0/3.0)) 

(TAU(J,N) = 
3.0/(1.0/CA(J,N)) + (1.0/CC(J,N)) + (1.0/CC(J,N))) 

RHOB(J,N) = 2.0/((1.0/CA(J,N)) + (1.0/CC(J,N))) 
RHOCC(J,N) = 2.0/((1.0/CA(J,N)) + (1.0/CC(J,N))) 
DELN(J,N) = N(J,N)/CA(J,N) 
DELB(J,N) = N(J,N)/CC(J,N) 
DELC(J,N) = N(J,N)/CC(J,N) 
PSI(J,N) = F(J,N) * (1.0/TAU(J,N)**(1.0/3.0)) 

(TAU(J,N) = 
3.0/(1.0/CA(J,N)) + (1.0/CC(J,N)) + (1.0/CC(J,N))) 

RHOB(J,N) = 2.0/((1.0/CA(J,N)) + (1.0/CC(J,N))) 
RHOCC(J,N) = 2.0/((1.0/CA(J,N)) + (1.0/CC(J,N))) 
DELN(J,N) = N(J,N)/CA(J,N) 
DELB(J,N) = N(J,N)/CC(J,N) 
DELC(J,N) = N(J,N)/CC(J,N) 
PSI(J,N) = F(J,N) * (1.0/TAU(J,N)**(1.0/3.0)) 

(TAU(J,N) = 
3.0/(1.0/CA(J,N)) + (1.0/CC(J,N)) + (1.0/CC(J,N))) 

RHOB(J,N) = 2.0/((1.0/CA(J,N)) + (1.0/CC(J,N))) 
RHOCC(J,N) = 2.0/((1.0/CA(J,N)) + (1.0/CC(J,N))) 
DELN(J,N) = N(J,N)/CA(J,N) 
DELB(J,N) = N(J,N)/CC(J,N) 
DELC(J,N) = N(J,N)/CC(J,N) 
PSI(J,N) = F(J,N) * (1.0/TAU(J,N)**(1.0/3.0)) 

(TAU(J,N) = 
3.0/(1.0/CA(J,N)) + (1.0/CC(J,N)) + (1.0/CC(J,N)))
CALL FRAME
CALL FULL
CALL BLKPEN
CALL BORDER
CALL CSPACE(0.0, 1.0, 0.0, 1.0)
CALL SCALSI(0.1, 0.1)
CALL CSPACE(0.0, 0.0001, 0.0, 0.0001)
H(1) = 1.479
CALL CONTRL(R, 1, 21, 1, 21, 1, 21, H, 1, 1)
H(1) = 1.433
CALL CONTRL(RHOA, 1, 21, 1, 21, 1, 21, H, 1, 1)
H(1) = 1.115
CALL REDPEN
CALL CONTRL(RHOB, 1, 21, 1, 21, 1, 21, H, 1, 1)
H(1) = 1.093
CALL GRNPEN
CALL CONTRL(RHOC, 1, 21, 1, 21, 1, 21, H, 1, 1)

(IV) PLOT OF THE R FUNCTION WITH +/- 1% ASSUMED ERROR AND THE ROTATIONAL RELAXATION TIME RATIOS WITH +/- 2% ASSUMED ERROR

(V) PLOT OF THE R FUNCTION, HARMONIC MEAN RELAXATION TIME RATIO (TH/TO) THE PSI & LAMBDA FUNCTIONS

(VI) PLOT OF R (+/- 1%), DELTA1 (+/- 1%), DELTA2 (+/- 1%) AND DELTA3 (+/- 1%)
CALL CONTRL(R, 1, 21, 21, 1, 21, 21, H, 1, 1)
H(1) = 1.46421
CALL CONTRL(R, 1, 21, 21, 1, 21, 21, H, 1, 1)
H(1) = 3.2849
CALL CONTRL(DELA, 1, 21, 21, 1, 21, 21, H, 1, 1)
H(1) = 3.15608
CALL CONTRL(REDEL, 1, 21, 21, 1, 21, 21, H, 1, 1)
H(1) = 2.110
CALL REDPEN
CALL CONTRL(REDEL, 1, 21, 21, 1, 21, 21, H, 1, 1)
H(1) = 2.072
CALL CONTRL(REDEL, 1, 21, 21, 1, 21, 21, H, 1, 1)
H(1) = 2.006
CALL GRNPEN
CALL CONTRL(REDEL, 1, 21, 21, 1, 21, 21, H, 1, 1)
H(1) = 1.928
CALL CONTRL(REDEL, 1, 21, 21, 1, 21, 21, H, 1, 1)
H(1) = 1.879
CALL FRAME
CALL BLKPEN
CALL BORDR
CALL CSPACE(0.0, 0.0, 0.0, 1.0)
CALL SCALSI(0.1, 0.1)
CALL CSPACE(0.0, 0.0, 0.001, 0.0, 0.0001)
H(1) = 1.479
CALL CONTRL(R, 1, 21, 21, 1, 21, 21, H, 1, 1)
H(1) = 3.795
CALL CONTRL(GAMMAA, 1, 21, 21, 1, 21, 21, H, 1, 1)
H(1) = 1.022
CALL REDPEN
CALL CONTRL(GAMMAA, 1, 21, 21, 1, 21, 21, H, 1, 1)
H(1) = 1.042
CALL GRNPEN
CALL CONTRL(GAMMAC, 1, 21, 21, 1, 21, 21, H, 1, 1)
H(1) = 1.02116
CALL FRAME
CALL BLKPEN
CALL BORDR
CALL CSPACE(0.0, 0.0, 0.0, 1.0)
CALL SCALSI(0.1, 0.1)
CALL CSPACE(0.0, 0.0, 0.001, 0.0, 0.0001)
H(1) = 1.49379
CALL CONTRL(R, 1, 21, 21, 1, 21, 21, H, 1, 1)
H(1) = 3.814938
CALL CONTRL(GAMMAA, 1, 21, 21, 1, 21, 21, H, 1, 1)
H(1) = 7.8302
CALL CONTRL(GAMMAA, 1, 21, 21, 1, 21, 21, H, 1, 1)
H(1) = 1.04244
CALL REDPEN
CALL CONTRL(GAMMAA, 1, 21, 21, 1, 21, 21, H, 1, 1)
H(1) = 1.00156
CALL CONTRL(GAMMAA, 1, 21, 21, 1, 21, 21, H, 1, 1)
H(1) = 1.06284
CALL CONTRL(GAMMAC, 1, 21, 21, 1, 21, 21, H, 1, 1)
H(1) = 1.02116
CALL CONTRL(GAMMAC, 1, 21, 21, 1, 21, 21, H, 1, 1)
H(1) = 1.071
CALL FRAME
CALL BLKPEN
CALL BORDR
CALL CSPACE(0.0, 0.0, 0.0, 1.0)
CALL SCALSI(0.1, 0.1)
CALL CSPACE(0.0, 0.0, 0.001, 0.0, 0.0001)
H(1) = 1.479
CALL CONTRL(R, 1, 21, 21, 1, 21, 21, H, 1, 1)
H(1) = .924
CALL CONTRL(MUA, 1, 21, 21, 1, 21, 21, H, 1, 1)
CALL REDPEN
H(1) = 1.071
CALL CONTRL(MUB, 1, 21, 21, 1, 21, 21, H, 1, 1)
CALL GRNPEN
H(1)=1.089
CALL CONTRL(MUC,1,21,21,1,21,21,H,1,1)

(XI) PLOT OF R (+/-1%), MUA (+/-1%), MUB (+/-1%) AND MUC (+/-1%)

CALL FRAME
CALL BLKPEN
CALL BORDER
CALL CSPACE(0.0,1.0,0.0,1.0)
CALL SCALSI(0.1,0.1)
CALL CSPACE(0.0,0.0001,0.0,0.0001)
H(1)=1.49379
CALL CONTRL(R,1,21,21,1,21,21,H,1,1)
H(1)=1.46421
CALL CONTRL(R,1,21,21,1,21,21,H,1,1)
H(1)=1.94248
CALL CONTRL(MUA,1,21,21,1,21,21,H,1,1)
H(1)=1.0552
CALL CONTRL(MUA,1,21,21,1,21,21,H,1,1)
H(1)=1.46421
CALL REOPEN
CALL CONTRLCR,1,21,21,1,21,21,H,1,1)
CALL REOPEN
H(1)=1.163
CALL CONTRLCR,TPLS,1,21,21,1,21,21,H,1,1)
H(1)=1.116
CALL CONTRLTMNS,1,21,21,1,21,21,H,1,1)

(XII) PLOT OF THE R, REDUCED THETA+ AND REDUCED
THETA- FUNCTIONS

CALL FRAME
CALL BLKPEN
CALL BORDER
CALL CSPACE(0.0,1.0,0.0,1.0)
CALL SCALSI(0.1,0.1)
CALL CSPACE(0.0,0.0001,0.0,0.0001)
H(1)=1.49379
CALL CONTRL(R,1,21,21,1,21,21,H,1,1)
H(1)=1.46421
CALL CONTRL(R,1,21,21,1,21,21,H,1,1)
H(1)=1.16463
CALL CONTRLCR,TPLS,1,21,21,1,21,21,H,1,1)
H(1)=1.16137
CALL CONTRLTMNS,1,21,21,1,21,21,H,1,1)
H(1)=1.11716
CALL CONTRLTMNS,1,21,21,1,21,21,H,1,1)
H(1)=1.14854
CALL CONTRLTMNS,1,21,21,1,21,21,H,1,1)

(XIII) PLOT OF THE R, DELTA+ AND DELTA- FUNCTIONS
CALL FRAME
CALL BLKPEN
CALL BORDER
CALL CSPACE(0.0,1.0,0.0,1.0)
CALL SCALSI(0.1,0.1)
CALL CSPACE(0.0,0.0001,0.0,0.0001)
H(1)=1.479
CALL CONTRL(R,1,21,21,1,21,21,H,1,1)
CALL REDPEN
H(1)=2.821
CALL CONTRL(DPLS,1,21,21,1,21,21,H,1,1)
CALL GRNPEN
H(1)=2.016
CALL CONTRL(DMNS,1,21,21,1,21,21,H,1,1)

(XIV) PLOT OF R (+/-1%), DELTA+ (+/-2%) AND DELTA- (+/-2%)

CALL FRAME
CALL BLKPEN
CALL BORDER
CALL CSPACE(0.0,1.0,0.0,1.0)
CALL SCALSI(0.1,0.1)
CALL CSPACE(0.0,0.0001,0.0,0.0001)
H(1)=1.49379
CALL CONTRL(R,1,21,21,1,21,21,H,1,1)
H(1)=1.46421
CALL CONTRL(R,1,21,21,1,21,21,H,1,1)
CALL REDPEN
H(1)=2.87742
CALL CONTRL(DPLS,1,21,21,1,21,21,H,1,1)
H(1)=2.79453
CALL CONTRL(DPLS,1,21,21,1,21,21,H,1,1)
CALL GRNPEN
H(1)=2.09632
CALL CONTRL(DMNS,1,21,21,1,21,21,H,1,1)
H(1)=1.97568
CALL CONTRL(DMNS,1,21,21,1,21,21,H,1,1)

(XV) PLOT OF THE R, GAMMA+ AND GAMMA- FUNCTIONS

CALL FRAME
CALL BLKPEN
CALL BORDER
CALL CSPACE(0.0,1.0,0.0,1.0)
CALL SCALSI(0.1,0.1)
CALL CSPACE(0.0,0.0001,0.0,0.0001)
H(1)=1.479
CALL CONTRL(R,1,21,21,1,21,21,H,1,1)
CALL REDPEN
H(1)=1.111
CALL CONTRL(GPLS,1,21,21,1,21,21,H,1,1)
CALL GRNPEN
CALL CONTRL(GMNS,1,21,21,1,21,21,H,1,1)

(XVI) PLOT OF R (+/-1%), GAMMA+ (+/-2%) AND GAMMA- (+/-2%)

CALL FRAME
CALL BLKPEN
CALL BORDER
CALL CSPACE(0.0,1.0,0.0,1.0)
CALL SCALSI(0.1,0.1)
CALL CSPACE(0.0,0.0001,0.0,0.0001)
H(1)=1.49379
CALL CONTRL(R,1,21,21,1,21,21,H,1,1)
H(1)=1.46421
CALL CONTRL(R,1,21,21,1,21,21,H,1,1)
CALL REDPEN
H(1)=1.13322
CALL CONTRL(GPLS,1,21,21,1,21,21,H,1,1)
H(1)=1.08378
CALL CONTRL(GPLS,1,21,21,1,21,21,H,1,1)
CALL GRNPEN
H(1)=.80988
CALL CONTRL(GMNS,1,21,21,1,21,21,H,1,1)
H(1)=.77812
CALL CONTRL(GMNS,1,21,21,1,21,21,H,1,1)

(XVII) PLOT OF THE 5 FLUORESCENCE ANISOTROPY RELAXATION TIME RATIOS

CALL FRAME
CALL BLKPEN
CALL BORDER
CALL CSPACE(0.0,1.0,0.0,1.0)
CALL SCALSI(0.1,0.1)
CALL CSPACE(0.0,0.0001,0.0,0.0001)
H(1)=1.02536
CALL CONTRL(T1,1,21,21,1,21,21,H,1,1)
H(1)=1.28883
CALL REDPEN
CALL CONTRL(T2,1,21,21,1,21,21,H,1,1)
CALL GRNPEN
H(1)=1.31877
CALL CONTRL(T3,1,21,21,1,21,21,H,1,1)
CALL BROKEN(4,0,8,8)
H(1)=1.43405
CALL CONTRL(T4,1,21,21,1,21,21,H,1,1)
CALL BLKPEN
H(1)=1.02497
CALL CONTRL(T5,1,21,21,1,21,21,H,1,1)
STOP
END

REAL FUNCTION FUN(X)
COMMON/PARAM/A,C,NN
B=1.0
GOTO(10,20,30,40,50,60,70,80,90,100),NN
10 FUN=1/((A*A*X)**1.5*(B*B*X)**0.5*(C*C*X)**0.5)
RETURN
20 FUN=1/((A*A*X)**0.5*(B*B*X)**1.5*(C*C*X)**0.5)
RETURN
30 FUN=1/((A*A*X)**0.5*(B*B*X)**0.5*(C*C*X)**1.5)
RETURN
40 FUN=1/((A*A*X)**0.5*(B*B*X)**1.5*(C*C*X)**1.5)
RETURN
50 FUN=1/((A*A*X)**1.5*(B*B*X)**0.5*(C*C*X)**1.5)
RETURN
60 FUN=1/((A*A*X)**1.5*(B*B*X)**1.5*(C*C*X)**0.5)
RETURN
70 FUN=X/((A*A*X)**0.5*(B*B*X)**1.5*(C*C*X)**1.5)
RETURN
80 FUN=X/((A*A*X)**1.5*(B*B*X)**0.5*(C*C*X)**1.5)
RETURN
90 FUN=X/((A*A*X)**1.5*(B*B*X)**1.5*(C*C*X)**0.5)
RETURN
100 FUN=1.0/((A*A*X)*(B*B*X)*(C*C*X))**0.5
RETURN
END
NON-LINEAR LEAST SQUARES METHOD USING AN ALGORITHM OF GILL AND MURRAY (1976)

GAMMA = BIREFRINGENCE (RADS), F = SUM OF SQUARES OF THE RESIDUALS

DPLS = DELTA+, DMNS = DELTA-

X(1) = CURRENT INITIAL GUESS FOR THETA+
X(2) = " " " " A'+
X(3) = " " " " A'-
X(4) = " " " " A''

X1 = CURRENT ESTIMATE FOR THETA+
X2 = " " " " A''
X3 = " " " " A''
X4 = " " " " A''

************
LINEAR TIME INCREASE, 100PTS.
.001 DEGREES S.E.

RANDOM NUMBER RUN 50

************

PROTEIN 3

************

PROGRAM MAINCINPUT,OUTPUT,TAPE2=INPUT,TAPE3=OUTPUT
COMMON/PARAM/GAMMA(101),T(101),R(101)
REAL BL(4),BU(4),X(4),F,W(54)
INTEGER IQ(0)
P=-1.0
DO 21 I=1,101
P=P+1.0
      R(I)=P
      T(I)=R(I)*1.0E-09
21 CONTINUE
WRITE(3,32)
32 FORMAT( " TIME(NS) GAMMA")
C G05BBF REINITIALIZES THE STREAM OF THE RANDOM NUMBERS
CALL G05BBF
C CALCULATE THE UNPERTURBED DECAY CURVE
DO 50 I=1,101
GAMMA(I)=0.07*EXP(-T(I)*5.0*5.187243E06)+
          +0.05*EXP(-T(I)*6.0*4.167486E06)
50 CONTINUE
WRITE(3,33)
33 FORMAT( F10.5,F8.5) 50 CONTINUE
N=4
IBOUND=0
Q1=1.0
C DO THE MINIMIZATION FOR 30 INITIAL GUESSES TO ATTEMPT TO AVOID
C SUBSIDIARY MINIMA
DO 10 I=1,30
C CALL RECTANGULAR PSEUDO RANDOM NUMBER ROUTINE G05AAF
Z=G05AAF(X)
X(1)=4.0+2.0*Z
X(2)=3.0+2.0*Z
X(3)=6.0+2.0*Z
X(4)=4.0+2.0*Z
BL(1)=4.0
BL(2)=3.0
BL(3)=6.0
BL(4)=4.0
BU(1)=6.0
BU(2)=5.0
BU(3)=8.0
BU(4)=8.0
LIW=6
LW=54
IFAIL=1
CALL E04JAF(N,IBOUND,BL,BU,X,F,IW,LIW,LW,IFAIL)
IF(IFAIL.NE.0)WRITE(3,100)IFAIL
IF(IFAIL.EQ.1)GOTO 20
100 FORMAT("ERROR EXIT TYPE",I3,"SEE ROUTINE MANUAL")
WRITE(3,1101)
110 FORMAT( " FUNCTION VALUE ON EXIT IS",F15.12)
120 FORMAT( ' "THETA+","F10.7," THETA-","F10.7,"A ",F7.4,
+A-","F7.4) IF(Q1.LE.F)GOTO 30
Q1=F Q2=X(1) Q3=X(2) Q4=X(3) Q5=X(4)
30 CONTINUE WRITE (3,130)
130 FORMAT( ' "") WRITE(3,140)
140 FORMAT( ' "")
10 CONTINUE
150 FORMAT( ' BEST LEAST SQUARES VALUE = ",F15.12)
WRITE(3,140)
140 FORMAT( ' "")
141 FORMAT( ' "") WRITE(3,142)
142 FORMAT( ' "") WRITE(3,143)
143 FORMAT( ' "") WRITE(3,145)
145 FORMAT( ' "") WRITE(3,150)
150 FORMAT( ' "")
WRITE(3,160)
160 FORMAT( ' "") WRITE(3,170)
170 FORMAT( ' "") WRITE(3,180)
180 FORMAT( ' "") WRITE(3,190)
190 FORMAT( ' "")
WRITE(3,200)
200 FORMAT( ' "")
WRITE(3,210)
210 FORMAT( ' "")
20 STOP
END

C SUBROUTINE FOR CALCULATING THE SUM OF SQUARES OF THE RESIDUALS FOR THE
C CURRENT ESTIMATES OF THE ADJUSTABLE PARAMETERS
SUBROUTINE FUNCT1(N,XC,FC) COMMON/PARAM/GAMMA(101),T(101),R(101)
EXTERNAL FUN REAL Q,FC INTEGER N REAL XC(N) REAL X1,X2,X3,X4
X1=XC(1) X2=XC(2) X3=XC(3) X4=XC(4)
FC=0.0 DO75 I=1,101
Q=(GAMMA(I)-(0.01*X3*EXP(-6.0E06*X1*T(I))+0.01*X4
+EXP(-6.0E06*X2*T(I))))**2.0
FC=FC+Q
75 CONTINUE RETURN
END
Program 6

FOURIER TRANSFORM SOLUTION OF THE LAPLACE INTEGRAL EQUATION METHOD OF GARDNER, GARDNER, LAUSH AND MEINKE (1959)

S = BIREFRINGENCE (RADS), GM = INTEGRAND OF EQUATION , G = G(EXP(-Y))
T = TIME (SEC), R = TIME (NS), EC AND ES ARE THE ERROR ESTIMATES IN FC
AND FS RESPECTIVELY DUE TO THE NUMERICAL INTEGRATION IN EQN. 125
AND ER IS THE ERROR ESTIMATE DUE TO THE NUMERICAL INTEGRATION IN
EQN. 128

LOGARITHMIC TIME INCREASE, 140 DATA PTS.
*001 DEGREES S.E.

RANDOM NUMBER RUN 2

PROTEIN 2

PROGRAM MAIN(INPUT,OUTPUT,TAPE2=INPUT,TAPE3=OUTPUT)
EXTERNAL FUNXN
REAL MMU(66),KC(66),KS(66),FC(66),FS(66),GM(66),MT
REAL YYY,G(690),Y(690),EC(66),ES(66)
INTEGER N,I,IFAIL INTEGER L
REAL X(141),S(141),R(141),T(141),YC(141),YS(141)
COMPLEX Z,C,K,CGAMMA
P=-7.1
DO21 I=1,141
P=P+.1
WRITE(3,500)
500 FORMAT(3,50)
X(I)=P
T(I)=(1.0E-09)*EXP(X(I))
R(I)=T(I)*1.0E09
21 CONTINUE
WRITE(3,32)
32 FORMAT( TIME(NS) X S(T) = S(EXP(-X))")
WRITE(3,501)
501 FORMAT( ""
WRITE(3,502)
502 FORMAT( ""
WRITE(3,503)
503 FORMAT( ""
WRITE(3,504)
504 FORMAT( ""
CALL G05BBF
G05BBF REINITIALIZES THE STREAM OF THE RANDOM NUMBERS
C G05BBF REINITIALIZES THE STREAM OF THE RANDOM NUMBERS
C ALLOCATE THE UNPERTURBED DECAY CURVE
S(I)=0.07*EXP(-T(I)*4.6596278E07)+
+0.05*EXP(-T(I)*3.137407E07)
C NOW PERTURB EACH OF THE 140 DATA PTS USING A NORMAL PSEUDO-RANDOM
F NUMBER GENERATOR
S(I)=S(I)+(0.1*1.74555E-04)*G05ADF(Y))
33 FORMAT(3,33)
WRITE(3,33)
WRITE(3,505)
505 FORMAT( ""
WRITE(3,506)
506 FORMAT( ""
WRITE(3,507)
507 FORMAT( ""
DO47 L=1,20
WRITE(3,504)
47 CONTINUE
C THE NEXT PART OF THE PROGRAM EVALUATES THE FOURIER TRANSFORM OF THE DATA
MU=0.1
DO99 J=1,66
99 FORMAT(3,99)
WRITE(3,99)
WRITE(3,505)
505 FORMAT( ""
WRITE(3,506)
506 FORMAT( ""
WRITE(3,507)
507 FORMAT( ""
DO10 J=1,66
MU=MU+.1
MMU(J)=MU
DO51 I=1,141
YC(I)=CEXP(X(I)))*S(I)*COS(MU*X(I))
YS(I)=CEXP(X(I)))*S(I)*SIN(MU*X(I))
51 CONTINUE
N=141
IFAIL=1
CALL NUMERICAL INTEGRATION ROUTINE FROM THE UK NAG LIBRARY (MK 6)
CALL D01GA(F, YC, N, ANS, ER, IFAIL)
FC(J) = (1.0 / (2.0 * 3.141593) ** 0.5) * ANS
EC(J) = (1.0 / (2.0 * 3.141593) ** 0.5) * ER
FS(J) = (1.0 / (2.0 * 3.141593) ** 0.5) * ANS
ES(J) = (1.0 / (2.0 * 3.141593) ** 0.5) * ER

C EVALUATE THE COMPLEX GAMMA FUNCTION FOR THE CURRENT VALUE OF MU USING
C THE SUBROUTINE BELOW
Z = CMPLX(1.0, MU)
C = GAMMA(Z)

C DETERMINE THE EULER INTEGRAL OF THE COMPLEX GAMMA FUNCTION (EQN. 123)
K = ((1.0 / (2.0 * 3.141593)) ** 0.5) * C
KC(J) = REAL(K)
KS(J) = AIMAG(K)
C = GAMMA(Z)
WRITE(3, 101) MU, K
101 FORMAT(7X, 1F8.2, 2E15.7)
10 CONTINUE
D049 L = 1, 20
WRITE(3, 509)
509 FORMAT("
  ""
+ (ERR)"
"
")
WRITE(3, 510)
510 FORMAT("
  ""
")
WRITE(3, 511)
511 FORMAT("***************
***************
")
WRITE(3, 512)
512 FORMAT("
  ""
")
D039 J = 1, 66
WRITE(3, 91) MU(J), FC(J), EC(J), FS(J), ES(J)
91 FORMAT(1X, 1F8.2, 2E14.7)
38 CONTINUE
D052 L = 1, 20
WRITE(3, 513)
513 FORMAT("
  ""
")
WRITE(3, 514)
514 FORMAT("
  ""
")
WRITE(3, 515)
515 FORMAT("
  ""
")
WRITE(3, 516)
516 FORMAT("
  ""
")
YY = -.01
M = 1, 690
YYY = YYY + .01
Y(M) = YYY
D029 J = 1, 66

C DETERMINE THE FOURIER TRANSFORM OF THE DATA BY THE EULER INTEGRAL FOR THE
C COMPLEX GAMMA FUNCTION
GM(J) = (((FC(J) * KC(J)) + (FS(J) * KS(J))) * COS(YYY * MU(J))) / ((KC(J) +
* KC(J)) + ((KS(J) * KS(J)) + ((FS(J) * KC(J)) - (FC(J) * KS(J))) * SIN(YYY * MU(J))) +
+ (KC(J) * KC(J)) + (KS(J) * KS(J)))
29 CONTINUE
NN = 66

C NOW G(EXP(-Y)) AS A FUNCTION OF Y IS FOUND FROM THE INVERSE FOURIER
C TRANSFORM USING THE SAME NUMERICAL INTEGRATION ROUTINE
CALL D01GA(GM(Y), X, 25, Y, G, NN, ANS, ER, IFAIL)
G(M) = (1.0 / 3.141593) * ANS
ER = (1.0 / 3.141593) * ER
WRITE(3, 290) YYY, G(M), ER
290 FORMAT(1X, 1F8.2, 1E14.7)
241 WRITE(3, 289)
261 CONTINUE
290 FORMAT(1X, 3F8.2, 1E14.7)
289 FORMAT(1X, 28H LESS THAN 4 POINTS SUPPLIED)
31 CONTINUE

C HENCE A PLOT OF G(EXP(-Y)) AGAINST Y IS OBTAINED
CALL PAPER(1)
CALL MAPCO(Q, Q, Q, Q)
CALL CURVEO(Y, G, 0.0, 1.0, 0.0, 0.0, 0.0, 0.0, 0.0)
CALL SCALES
CALL BORDER
CALL GRAPH(FUNXN)
SUBROUTINE FOR CALCULATING THE COMPLEX GAMMA FUNCTION C = CGAMMA(Z) FOR THE CURRENT VALUE OF MU. THIS SUBROUTINE IS THAT GIVEN BY LUCAS AND TERRILL (1970).

C COMPLEX FUNCTION CGAMMA(Z)
C
C DIMENSION C(2)
C LOGICAL REFLEX C SET OUT FOR PROPER OUTPUT CHANNEL OF COMPUTER SYSTEM FOR ERROR MESSAGES
C C
C DETERMINE WHETHER Z IS TOO CLOSE TO A POLE
C IF (X .GE. TOL) GO TO 20
C IF Z IS TOO CLOSE TO A POLE, PRINT ERROR MESSAGE AND RETURN
C C WITH CGAMMA = (1.E7, 0.F0)
C WRITE (IOUT, 90012)
C CGAMMA = (1.E7, 0.F0)
C RETURN
C C FOR REAL(Z) NEGATIVE EMPLOY THE REFLECTION FORMULA
C GAMMA(Z) = PI (SINPI*Z) * GAMMA(1-Z)
C AND COMPUTE GAMMA(1-Z). NOTE REFLEX IS A TAG TO INDICATE THAT THIS RELATION MUST BE USED LATER.
C
C IF Z IS NOT TOO CLOSE TO A POLE, MAKE REAL(Z) > 10 AND ARG(Z) < PI/4
C COEFFICIENTS IN STIRLING'S APPROXIMATION FOR LN(GAMMA(T))
C(1)=1./12.  C(2)=-1./24.  C(3)=1./720.  C(4)=-1./2520.
C(5)=1./1260.  C(6)=-1./7560.  C(7)=1./5040.  C(8)=1./20160.
C(9)=-691./6720.  C(10)=7./6720.  C(11)=3617./510600.
C(12)=-43867./33993600.

SUM=(T-(*.5,.0,.0))*CLOG(T)-T*CMPLX(.5*ALOG(2,.3,14159),0.0)
J=1
70 TERM=C(J)/DEN
C TEST REAL AND IMAGINARY PARTS OF LN(GAMMA(Z)) SEPARATELY FOR CONVERGENCE. IF Z IS REAL SKIP IMAGINARY PART OF CHECK.
IF(ABS(REAL(TERM))/REAL(SUM)).GE.TOL GOTO 80
IF(ABS(REAL(TERM)).GT.TOL)GOTO 100
C CHECK TO SEE IF REFLECTION FORMULA SHOULD BE USED
100 IF(REFLEX)GOTO 130
C STIRLING SERIES DID NOT CONVERGE. PRINT ERROR MESSAGE AND PROCEED.
90 WRITE(IOUT,910)Z
910 FORMAT(1X,2F14.7,10X,49HARGUMENT OF GAMMA FUNCTION IS TOO CLOSE TO + A POLE, 14X,4H7,2F14.7)
END
R-CONSTRAINED NON-LINEAR LEAST SQUARES METHOD (HARDING)

\[ \text{GAMMA} = \text{BIREFRINGENCE (RADS)}, \quad \text{FC} = \text{SUM OF SQUARES OF THE RESIDUALS} \]
\[ \text{X(1)} = \text{CURRENT INITIAL GUESS FOR A/B} \]
\[ \text{X(2)} = \ldots \quad \ldots \quad \ldots \quad \ldots \quad \text{A+} \]
\[ \text{ON EXIT, X(1), X(2), X(3) CONTAIN THE BEST ESTIMATES FOR THESE PARAMETERS} \]
\[ \text{XC(1)} = \text{A = CURRENT ESTIMATE FOR A/B} \]
\[ \text{XC(2)} = \text{CURRENT ESTIMATE FOR A+} \]
\[ \text{XC(3)} = \ldots \quad \ldots \quad \ldots \quad \ldots \quad \text{A-} \]

\[ \text{D} = 1/C = \text{VALUE OF B/C} \]
\[ \text{CORRESPONDING TO THIS ESTIMATE} \]
\[ \text{S = VISCOSITY INCREMENT} \]
\[ \text{V = \ldots \quad \ldots \quad \ldots \quad \ldots \quad \text{DELTA+}} \]
\[ \text{W = \ldots \quad \ldots \quad \ldots \quad \ldots \quad \text{DELTA-}} \]
\[ \text{THPLUS = \ldots \quad \ldots \quad \ldots \quad \ldots \quad \text{THETA+}} \]
\[ \text{THMNUMS = \ldots \quad \ldots \quad \ldots \quad \ldots \quad \text{THETA-}} \]
\[ \text{XC(2)} = \text{CURRENT ESTIMATE FOR A+} \]
\[ \text{XC(3)} = \ldots \quad \ldots \quad \ldots \quad \ldots \quad \text{A-} \]
\[ \text{AA(1), AA(2), \ldots \quad \ldots \quad \ldots \quad \ldots \quad \text{AA6 ARE THE A/B COORDINATES FOR 6 PTS ON THE R-CURVE}} \]
\[ \text{AD1, AD2, \ldots \quad \ldots \quad \ldots \quad \ldots \quad \text{AD6}} \]
\[ \text{THE VALUE FOR B/C CORRESPONDING TO THE CURRENT ESTIMATE FOR A/B CAN THEN BE FOUND USING A CUBIC POLYNOMIAL INTERPOLATION PROCEDURE BETWEEN THE SIX GIVEN POINTS, THIS IS DONE USING A LIBRARY ROUTINE E01LFI} \]

************
PROTEIN 1
************

LINEAR TIME INCREASE, 100PTS.

CUT OFF TIME: 100NS
STREAM OF RANDOM NUMBERS: 3
0.1 DEG STANDARD ERROR ON EACH DATA Pt.

PROGRAM MAINC (INPUT, OUTPUT, TAPE2=INPUT, TAPE3=OUTPUT)
COMMON/PARAM/GAMMA(101), T(101), A,C,D,NN,AA(6), AD(6)
REAL R(101)
REAL AA(1), AA(2), AA(3), AA(4), AA(5), AA(6), A1, AD2, AD3, AD4, AD5, AD6
REAL BL(3), BU(3), X(3), F, W(39)
INTEGER IBOUND, IFAIL, J, L, IW, LW, N
INTEGER IWC(3)
REAL Q1, Q2, Q3, Q4
WRITE (3, 37)
37 FORMAT ("PROTEIN 1, 0.1 DEG. STANDARD ERROR")
WRITE (3, 38)
38 FORMAT (" + 100NS, 100PTS")
WRITE (3, 39)
39 FORMAT (" + STREAM 3")
P = -1.1
DO 21 I = 1, 101
P = P + 1.1
R(I) = R(I) * 1.0E09
R(I) = T(I) * 1.0E09
21 CONTINUE
C G05BAF(0.X) SPECIFIES THE XTH STREAM OF THE RANDOM NUMBERS
CALL G05BAF (0.3)
DO 50 I = 1, 101
C CALCULATE THE UNPERTURBED DECAY CURVE
GAMMA(I) = 0.07 * EXP(-T(I) * 5.0 * 8.15383E06) +
0.05 * EXP(-T(I) * 6.0 * 4.154612E06)
NOW PERTURB EACH OF THE 100 DATA POINTS USING A NORMAL PSEUDO RANDOM
NUMBER GENERATOR G05ADF
GAMMA(I) = GAMMA(I) + ((1.74555E-03) * G05ADF (Y))
50 CONTINUE
C READ IN THE VALUES FOR THE LOWER LIMITS FOR THE INITIAL GUESSES OF THE
A/B, A+, A- (X100), THE LOWER AND UPPER LIMITS FOR THE COMPUTER ESTIMATES
THE COORDINATES OF THE A/B VALUES AND THEN THE COORDINATES OF THE B/C
FOR THE R-CURVE TO WHICH THESE ESTIMATES ARE CONSTRAINED
AA(1) = AA1
AA(2) = AA2
AA(3) = AA3
AA(4) = AA4
AA(5) = AA5
AA(6) = AA6
AD1 = AD1
AD2 = AD2
AD3 = AD3
AD4 = AD4
AD5 = AD5
AD(6) = AD6
N = 3
IBOUND = 0
Q1 = 1., 0
DO 10 I = 1, 3
Z = G05A AF(X)
X(1) = X(1) + 0.2 • Z
X(2) = X(2) + 2.0 • Z
X(3) = X(3) + 2.0 • Z
BL (1) = BBL1
BL (2) = BBL2
BL (3) = BBL3
BU (1) = BBU1
BU (2) = BBU2
BU (3) = BBU3
LIW = 5
LW = 39
IFAIL = 1
CALL E04JAF(N, IBOUND, BL, BU, X, F, IW, LIW, LW, IFAIL)
IF (IFAIL .NE. 0) WRITE (3, 100) IFAIL
IF (IFAIL .EQ. 1) GOTO 20
100 FORMAT ("ERROR EXIT TYPE", I3, "SEE ROUTINE MANUAL")
X(1) = X(1) • 0.1
X(2) = X(2) • 0.1
WRITE (3, 120) (X(I), I = 1, 3)
120 FORMAT ("FUNCTION VALUE ON EXIT IS", F15.12)
WRITE (3, 130) F
Q1 = F
Q2 = X(1)
Q3 = X(2)
Q4 = X(3)
30 CONTINUE
WRITE (3, 130)
130 FORMAT (" ")
WRITE (3, 140)
140 FORMAT ("")
10 CONTINUE
WRITE (3, 150) Q1
150 FORMAT (" BEST LEAST SQUARES VALUE = ", F15.12)
WRITE (3, 160) Q2
160 FORMAT (" A / B = ", F8.5)
WRITE (3, 170) Q3
170 FORMAT (" B / C = ", F8.5)
WRITE (3, 180) Q4
180 FORMAT (" A+ = ", F7.4)
WRITE (3, 190) Q4
190 FORMAT (" A- = ", F7.4)
20 STOP
END
C SUBROUTINE FOR CALCULATING THE SUM OF THE SQUARES OF THE RESIDUALS FOR C CURRENT ESTIMATES OF A/B, A+ AND A- C
KB = BOLTZMANN'S CONSTANT, NA = AVOGADROS NUMBER C

SUBROUTINE FUNCT1(N,XC,FC)
COMMON/PARAM/GAMMA(101),T(101),A,C,D,NN,AA(6),AD(6)
EXTERNAL FUN
REAL Q,FC
INTEGER N
DIMENSION ALPHA(9)
REAL X2,X3
REAL THPLUS,THMNUS,TEMP,ETA,M,O,P,KB,NA,MW

NN=O
A=XC(1)

45 CONTINUE
S=(1.0/(A*X2*C))*(4.0/15.0)*((ALPHA(7)+ALPHA(8)
+ALPHA(9))/((ALPHA(8)*ALPHA(9)+ALPHA(9)*ALPHA
+(7)+ALPHA(7)*ALPHA(8)))+((1.0/5.0)*((ALPHA(2)
+ALPHA(3))/((ALPHA(4)*((B*C*ALPHA(2))+C*C*ALPHA(3))
+3))))+/((ALPHA(3)+ALPHA(1))/((ALPHA(5)*((C*C*ALPHA(3))
+2)))+(ALPHA(1))/((ALPHA(4)*ALPHA(2))/((ALPHA(6)
+2))+(((A+A*ALPHA(1))+(B*B*ALPHA(2))))))

M=((B*C)/((B*B*ALPHA(2))+C*C*ALPHA(3)))
O=((C+C+A)/((C*C*ALPHA(3))+A*A*ALPHA(1)))
P=((A+B+B)/((A*A*ALPHA(1))+B*B*ALPHA(2)))
Z=((A*B*C))/((12.0)*)((1.0/(M**2.0))+((1.0/0**2.0)+((1.0/0**2.0))
+((1.0/0/(M**2.0))+(1.0/0/(P**2.0))))

45 CONTINUE
S=(1.0/(A*X2*C))*(4.0/15.0)*((ALPHA(7)+ALPHA(8)
+ALPHA(9))/((ALPHA(8)*ALPHA(9)+ALPHA(9)*ALPHA
+(7)+ALPHA(7)*ALPHA(8)))+((1.0/5.0)*((ALPHA(2)
+ALPHA(3))/((ALPHA(4)*((B*C*ALPHA(2))+C*C*ALPHA(3))
+3))))+/((ALPHA(3)+ALPHA(1))/((ALPHA(5)*((C*C*ALPHA(3))
+2)))+(ALPHA(1))/((ALPHA(4)*ALPHA(2))/((ALPHA(6)
+2))+(((A+A*ALPHA(1))+(B*B*ALPHA(2))))))

M=((B*C)/((B*B*ALPHA(2))+C*C*ALPHA(3)))
O=((C+C+A)/((C*C*ALPHA(3))+A*A*ALPHA(1)))
P=((A+B+B)/((A*A*ALPHA(1))+B*B*ALPHA(2)))
Z=((A*B*C))/((12.0)*)((1.0/(M**2.0))+((1.0/0**2.0)+((1.0/0**2.0))
+((1.0/0/(M**2.0))+(1.0/0/(P**2.0))))

V=6.0*U*S
W=6.0*Z*S

C ENTER INTRINSIC VISCOSITY
ETA=2.746
C ENTER TEMPERATURE
TEMP=293.0
C ENTER MOLECULAR WEIGHT
MW=71744.0
KB=1.38046E-16
NA=6.0248E23
THPLUS=NA*KB*100.0/6.0)*((TEMP/(ETA*MW)))*V
THMNUS=NA*KB*100.0/6.0)*((TEMP/(ETA*MW)))*W
X2=XC(2)
X3=XC(3)
FC=0.0
D075 I=1,110
1=GAMMA(II)-(0.01*X2*EXP(-6.0*THPLUS*T(I))+0.01*X3*EXP
+(-(6.0*THMNUS*T(I))))**2.0
FC=FC+Q
75 CONTINUE
RETURN
END
C LEICESTER UNIVERSITY COMPUTER LIBRARY SUBROUTINE FOR A CUBIC POLYNOMIAL FIT TO A SET OF POINTS (K. BROOLIE)

SUBROUTINE E01LF1(N,AX,AY,X,Y,IEXIT)
DIMENSION AX(N),AY(N)
C CHECK DATA POINTS ARE MONOTONICALLY INCREASING
IEXIT=1
DO 5 I=2,N
IF (AX(I) .LE. AX(I-1)) RETURN
5 CONTINUE
C CHECK THAT X IS A VALID POINT
IEXIT=2
IF ((AX(1)-X) .GT. 0) RETURN
C LOCATE INTERVAL IN WHICH X LIES -
AX(J-1) .LT. X .LE. AX(J)
IEXIT=0
Y=AY(1)
IF (X.EQ.AX(1)) RETURN
DO 10 I=2,N
10 CONTINUE
Y=AY(J)
IF (X.EQ.AX(J)) RETURN
C ESTIMATE SLOPE AT AX(J-1)
CALL E011A(N,AX,AY,J-1,G1)
C ESTIMATE SLOPE AT AX(J)
CALL E011A(N,AX,AY,J,G2)
C CONSTRUCT INTERPOLATING CUBIC POLYNOMIAL IN INTERVAL
D=AY(J-1)
G1=H1=AX(J)-AX(J-1)
S1=2.0*(AY(J)-AY(J-1))/H1-(AY(J)-AY(J-1))/H1
B1=(3.0*S1-2.0*G1-G2)/H2
A1=G1+G2-2.0*S1/H2/H2
C EVALUATE POLYNOMIAL AT POINT
H=X-AX(J-1)
Y=(CA1*H+B1*H+C1*H+D)
RETURN
END

SUBROUTINE E011A(N,AX,AY,J,G)
DIMENSION AX(N),AY(N)
C CALCULATE SLOPES ON EITHER SIDE OF DATA POINT
JM1=J-1
JP1=J+1
IF (J.NE.1) GO TO 20
C END-POINT IS DIFFERENT
H1=AX(2)-AX(1)
S1=2.0*(AY(2)-AY(1))/H1-(AY(3)-AY(2))/(AX(3)-AX(2))
GO TO 30
20 CONTINUE
H1=AX(J)-AX(J-1)
S1=AY(J)-AY(J-1)/H1
30 CONTINUE
IF (J.NE.N) GO TO 40
C END-POINT IS DIFFERENT
H2=AX(N)-AX(N-1)
S2=2.0*(AY(N)-AY(N-1))/H2-(AY(N-1)-AY(N-2))/(AX(N-1)-AX(N-2))
GO TO 50
40 CONTINUE
H2=AX(JP1)-AX(J)
S2=AY(JP1)-AY(J)/H2
50 CONTINUE
S12=S1*S2
C IF DATA IS NOT MONOTONIC SET SLOPE TO ZERO
IF (S12.GT.0.0) GO TO 10
G=0.0
RETURN
10 CONTINUE
C WHEN DATA IS MONOTONIC USE WEIGHTED AVERAGE OF NORMAL SLOPES
T1=2.0*H1-H2
T2=H1+2.0*H2
T=(T1*S2+T2*S1)/(3.0*(H1+H2))
G=S12/T
RETURN
END
SUBROUTINE FOR CALCULATING THE ELLIPTIC INTEGRALS USED FOR DETERMINING THE S VALUE FROM THE CURRENT GUESS FOR A/B

REAL FUNCTION FUN(Y)
COMMON/PARAM/GAMMA(101),T(101),A,C,D,NN,AA(6),AD(6)
B=1.0
GOTO(10,20,30,40,50,60,70,80,90),NN
10 FUN=1.0/((A*A+Y)**1.5*(B*B+Y)**0.5*(C*C+Y)**0.5)
   RETURN
20 FUN=1.0/((A*A+Y)**0.5*(B*B+Y)**1.5*(C*C+Y)**0.5)
   RETURN
30 FUN=1.0/((A*A+Y)**0.5*(B*B+Y)**0.5*(C*C+Y)**1.5)
   RETURN
40 FUN=1.0/((A*A+Y)**0.5*(B*B+Y)**1.5*(C*C+Y)**1.5)
   RETURN
50 FUN=1.0/((A*A+Y)**1.5*(B*B+Y)**0.5*(C*C+Y)**1.5)
   RETURN
60 FUN=1.0/((A*A+Y)**1.5*(B*B+Y)**1.5*(C*C+Y)**0.5)
   RETURN
70 FUN=Y/((A*A+Y)**0.5*(B*B+Y)**1.5*(C*C+Y)**1.5)
   RETURN
80 FUN=Y/((A*A+Y)**1.5*(B*B+Y)**0.5*(C*C+Y)**1.5)
   RETURN
90 FUN=Y/((A*A+Y)**1.5*(B*B+Y)**1.5*(C*C+Y)**0.5)
   RETURN
END

An estimate for $k/k'$ is required, thus this equation would normally be used as a check for internal consistency between values for sedimentation and viscosity parameters, since $k/k' = k'/k$.

Results:

PROTEIN 1, 0.1 DEG. ABSOLUTE ERROR

FUNCTION VALUE ON EXIT IS 0.00399962998
A/B = 1.48309 A+ = 0.070458728408 A- = 0.048831535604

FUNCTION VALUE ON EXIT IS 0.00399962998
A/B = 1.48309 A+ = 0.070458845275 A- = 0.048831534073

FUNCTION VALUE ON EXIT IS 0.00399962998
A/B = 1.48309 A+ = 0.070458881237 A- = 0.048831534081

BEST LEAST SQUARES VALUE = 0.00399962998
A/B = 1.48309
A+ = 0.0707
A- = 0.0488

110NS, 100PTS STREAM3
Appendix VI Use of $M_r$, $\bar{v}$ and $s$ to determine $k_s$

Rowe (1977) has shown that

$$M_r = N_A \left[ \frac{6\pi n_0 s}{(1-\bar{v}\rho_o)} \right]^{3/2} \cdot \left[ \frac{(3\bar{v})}{4\pi} \left( \frac{k_s - \bar{v}_s}{2\bar{v}} \right) \right]^{1/2}$$

This can be rearranged to give

$$k_s = 2\bar{v} \left[ \frac{4\pi}{3\bar{v}} \frac{M_r}{N_A^{\frac{2}{3}}} \left( \frac{1-\bar{v}\rho_o}{6\pi n_0 s} \right)^3 + \frac{\bar{v}_s}{\bar{v}} \right]$$

An estimate for $\bar{v}_s/\bar{v}$ is required, thus this equation would normally be used as a check for internal consistency between values for sedimentation and viscosity parameters, since $\bar{v}_s/\bar{v} = k_s/k_s$. 

Appendix VII  Comparison of the radius of gyration for a sphere of uniform mass with that for a sphere of the same mass but with a spherical cavity

The radius of gyration, $R_G$ of a sphere of uniform mass and radius $R$ is given by:

$$R_G^2 = \frac{\int_0^R 4\pi r^4 \, dr}{\int_0^R 4\pi r^2 \, dr} = \frac{3}{5} R^2$$  \hspace{1cm} (i)

(Tanford, p 306, 1961). The radius of gyration of a spherical shell of uniform mass with radius $R_2$ and with a centrally placed spherical cavity of radius $R_1$ is given by:

$$R_G^2 = \frac{\int_{R_1}^{R_2} 4\pi r^4 \, dr}{\int_{R_1}^{R_2} 4\pi r^2 \, dr} = \frac{3}{5} \left( \frac{R_2^5 - R_1^5}{R_2^3 - R_1^3} \right)$$  \hspace{1cm} (ii)

The results of electron microscopy and x-ray diffraction (Harrison, 1959, Farrant, 1954, Kuff & Dalton, 1957, Labaw & Wycoff, 1957) suggest that apoferritin consists of twenty four sub-units, each of molecular weight 20,000, arranged in the form of a spherical shell of diameter 109 Å. If we take the radius of the hollow to be 18.5 Å, and the outer radius of the shell to be 54.5 Å, $R_G$ is calculated using formula (ii) to be 43.0 Å. The radius of gyration, had the same mass been concentrated into a uniform sphere of density identical to the shell would have been 41.6 Å, using formula (i); i.e. a discrepancy of $\sim 3.4\%$
BIBLIOGRAPHY
Alexander, A.E. and Johnson, P. (1949)  
*Colloid Science*, Volume 2, Oxford University Press

Alpert, S.S. and Banks, G. (1976)  
*Biophysical Chem.* 4, 287

*Biochem. J.* 147, 575

Ballinger, K.W.A. and Jennings, B.R. (1979)  
*Nature* 282, 699

Batchelor, G.K. (1967)  
*An Introduction to Fluid Mechanics*, Cambridge University Press

Batchelor, G.K. (1970)  
*J. Fluid Mech.* 41, 545

Batchelor, G.K. and Green, J.T. (1972)  
*J. Fluid Mech.* 56, 375

in *Handbuck der Physik*, (Flugge, S. ed.), 32, 321, Springer-Verlag (Berlin)

Benoit, H. (1951)  
*Ann. Physik.* 6, 561

Blake, C.C.F. (1975)
Essays in Biochem. II, 37

J. Mol. Biol. 121, 339

Nature 206, 757

Biopolymers 5, 135

Bresler and Talmud (1944)
CR Acad. Sci. URSS, 43, 310


J. Coll. Int. Sci. 32, 141
Brenner, H. (1972a)
Chem. Eng. Sci. 27, 1069

Brenner, H. (1972b)
Progr. Heat and Mass Transfer 5, 93

Proc. Nucl. Acid Res. 2, 31

Cerf, R. and Scheraga, H.A. (1952)
Chem. Revs. 51, 185

Chapman, P.F. (1913)
Phil. Mag. 25, 475

Cheng, P.Y. and Schachman, H.K. (1955)
J. Polymer Sci. 16, 19

Chu, B. (1974)
'Laser Light Scattering', Academic, New York

Chwang, A.T. (1975)
J. Fluid Mech. 72, 17

Clenshaw, C.W. and Curtis, A.R. (1960)
Num. Math. 2, 197
Creeth, J.M. and Knight, C.G. (1965)

Biochim. Biophys. Acta 102, 549


(eds.) 'Photon Correlation and Light Beating Spectroscopy', Plenum, New York

Dickerson, R.E. and Geiss, I. (1969)

'The Structure and Action of Proteins', Benjamin, California

Dougherty, J. and Kreiger, I.M. (1972)

in Kreiger, Adv. Colloid Sci. 3, 111

Edsall, J.T. (1953)

in 'The Proteins' (Neurath, H. and Bailey, K. eds) 18, Chapter 7, Academic, New York

Edwardes, D. (1892)

Quart. J. Math. 26, 70

Einstein, A. (1905)

Ann. Physik 17, 549

Einstein, A. (1906)

Ann. Physik 34, 591

Einstein, A. (1911)

Ann. Physik 34, 591
Ernes, C.H. (1977)
Ph. D. thesis, University of Leicester

Emes, C.H. and Rowe, A.J. (1978a)
Biochim. Biophys. Acta 537, 110

Emes, C.H. and Rowe, A.J. (1978b)
Biochim. Biophys. Acta 537, 125

Farrant, J.L. (1954)
Biochim. Biophys. Acta 13, 569

Feldman, R.J. (1976)
'Atlas of Macromolecular Structure on Microfiche', Traco Jitco Int., Rockville, Md. USA

Gans, R. (1928)
Ann. Physik 86, 628

Biopolymers 19, 628

Garcia de la Torre, J. and Bloomfield, V.A. (1977a)
Biopolymers 16, 1747

Garcia de la Torre, J. and Bloomfield, V.A. (1977b)
Biopolymers 16, 1765
Garcia de la Torre, J. and Bloomfield, V.A. (1977c)
Biopolymers 16, 1779

Garcia de la Torre, J. and Bloomfield, V.A. (1978)
Biopolymers 17, 1605

J. Chem. Phys. 31, 978

Giesekus, H. (1962)
Rheol. Acta 2, 50

Nat. Phys. Lab. report NAC 72

Gold, O. (1937)
Ph. D. thesis, University of Vienna

Goodwin, J.W. (1975)
Colloid Science 2 (Chemical Society), 246

Guoy, L.G. (1910)
J. Phys. Chem. 9, 457

Hall, C.E. and Slayter, H.S. (1959)
J. Biophys. Cytol. 5, 11
Harding, S.E. (1980a)

Biochem. J. 189 (in press)

Harding, S.E. (1980b)

mss. submitted to J. Phys. Chem.

Harrison, P.M. (1959)

J. Mol. Biol. 1, 69


Z. Phys. Chem. A167, 329


J. Am. Chem. Soc. 78, 5954

Jablonski, A. (1961)

Naturforsch 169, 1

Jeffrey, G.B. (1922)


Johnson, P. and Mihalyi, E. (1965)

Biochim. Biophys. Acta 102, 476


in 'Molecular Electro Optics' (O'Konski, C.T. ed.) Volume 2, 529
Nature 213, 862

Nature 181, 665

in 'Biochemistry' (Stryer, L.) 653, Freeman, San Francisco

Kirkwood, J.G. (1967)
'Macromolecules' (Auer, P.L. ed.), Gordon and Breach

Koenig, S.H. (1975)
Biopolymers 14, 2421

Z. Angew. Phys. 27, 273

27, 98, Academic, London

J. Am. Chem. Soc. 81, 5082

J. Ultrastructure Res. 1, 62
Kuhn, W. and Kuhn, H. (1945)
*Helvetica Chimica Acta* 38, 97

Kynch, G.J. (1956)

Labau, L.W. and Wycoff, R.W.G. (1957)
*Biochim. Biophys. Acta* 25, 263

Lauffer, M.A. (1942)
*Chem. Rev.* 31, 561

Laurent, T.C. and Killander, J. (1964)
*J. Chromatog.* 14, 317

Lipscomb, W.N. (1971)


*Collected Algorithms from CACM*, No. 404

*Canad. J. Chem.* 32, 763
Martin, R.B. (1964)
'Introduction to Biophysical Chemistry' Chap. 11, McGraw-Hill, New York

Biopolymers 14, 2479

Mehl, J.W., Oncley, J.L. and Simha, R. (1940)
Science 92, 132

EMBO Workshop on Muscle Contraction, Alpbach Conf. Austria

Mihalyi, E. and Godfrey, J. (1963)
Biochim. Biophys. Acta 67, 90

Memminger, R. (1961)
Z. Physik. Chem. (Frankfurt) 28, 169

Mooney, M. (1951)
J. Coll. Sci. 6, 162

Mooney, M. (1957)
J. Coll. Sci. 12, 575

Munro, I., Pecht, I. and Stryer, L. (1979)
Proc. Nat. Acad. Sci. (USA) 76, 56
Nichol, L.W., Jeffrey, P.D., Turner, D.R. and Winzor, D.J. (1977)
J. Phys. Chem. 81, 776

Nisihara, T. and Doty, P. (1958)
Proc. Nat. Acad. Sci. (USA) 44, 411

Oberbeck, A. (1876)
J. reine. angew. Math. 81, 62

J. Phys. Chem. 83, 1333

Offer, G., Moos, C. and Starr, R. (1973)
J. Mol. Biol. 74, 653

O'Hara and Smith (1968)
Comp. Journal 11, 213

O'Konski, C.T. and Haltner, A.J. (1956)
J. Am. Chem. Soc. 78, 3604

Oliver, J. (1972)
Comp. Journal 15, 141

Oncley, J.L. (1940)
J. Phys. Chem. 44, 1103
Oncley, J.L. (1941)
Ann. New York Acad. Sci. 41, 121

Paradine, C.G. and Rivett, B.H.P. (1960)
'Statistical Methods for Technologists', English Universities Press, London

Pearce, T.C., Rowe, A.J. and Turnock, G. (1975)
J. Mol. Biol. 97, 193

Perrin, F. (1934)
J. Phys. Radium 5, 497

Perrin, F. (1936)
J. Phys. Radium 7, 1

Nature 185, 416

Powell, D.R. and MacDonald, J.R. (1972)
Comp. Journal 15, 148

J. Am. Chem. Soc. 81, 5082

Rallison, J.M. (1978)
J. Fluid Mech. 84, 237
Riddiford, C.L. and Jennings, B.R. (1967)
*Biopolymers* 5, 757

*J. Am. Chem. Soc.* 88, 1104

Ridgeway, D. (1968)
*J. Am. Chem. Soc.* 90, 18

Rowe, A.J. (1977)
*Biopolymers* 16, 2595

Rowe, A.J. (1978)
*Techniques in Protein and Enzyme Biochem.* 8105a, 1

Saito, N. (1951)
*J. Phys. Soc. (Japan)* 6, 297

Scheraga, H.A. (1955)
*J. Chem. Phys.* 23, 1526

Scheraga, H.A. (1961)
*Protein Structure*, Academic, New York

Scheraga, H.A. and Mandelkern, L. (1953)
*J. Am. Chem. Soc.* 79, 179
Simha, R. (1940)
J. Phys. Chem. 44, 25

Simha, R. (1952)
J. Appl. Phys. 23, 1020

Shaw, D.J. (1970)
'Introduction to Colloid and Surface Chemistry' (2nd edn.), Butterworths

Small, E.W. and Isenberg, I. (1977)
Biopolymers 16, 1907

Sorensen, N.A. (1930)
CR Lab. Carlsberg 18, No. 5

Squire, P.G. (1970)
Biochim. Biophys. Acta 221, 425

Squire, P.G. (1978)
in 'Molecular Electro Optics' (O'Konski, C.T. ed.) Volume 2, 565

Squire, P.G., Moser, L. and O'Konski, C.T. (1968)
Biochemistry 7, 4261

Stacey, K.A. (1956)
'Light Scattering in Physical Chemistry', Butterworths, London
Stokes, Sir G. (1851)
Trans. Cambridge Phil. Soc. 9, 8

Stokes, Sir G. (1880)
'Mathematical and Physical Papers', Cambridge University Press

Svedberg, T. and Pedersen, K.O. (1940)
'The Ultracentrifuge', Oxford University Press

Tanford, C. (1955)
J. Phys. Chem. 59, 798

Tanford, C. (1961)
'Physical Chemistry of Macromolecules', Wiley, New York

Theorell, H. (1934)
Biochem. Z. 268, 46

Vand, V. (1948)
J. Phys. Coll. Chem. 52, 277

Van de Hulst, H.C. (1957)
'Light Scattering by Small Particles', Wiley, New York

Van Holde, K.E. (1971)
'Physical Biochemistry', Prentice Hall, New Jersey
Wahl, P. (1966)
Compt. Rend. Acad. Sci. (Paris) 2630 1525

Wales, M. and Van Holde, K.E. (1954)
J. Polymer Sci. 14, 81

Wilde, D.J. (1964)
'Optimum Seeking Methods', Prentice Hall, New Jersey

Williams, R.C., Ham, W.T. and Wright, A.K. (1976)
Anal. Biochem. 73, 52

Wilson, R.W. and Bloomfield, V.A. (1979a)
Biopolymers 18, 1205

Wilson, R.W. and Bloomfield, V.A. (1979b)
Biopolymers 18, 1543

Yang, J.T. (1961)
Adv. Protein Chem. 16, 323

Zimm, B.H. (1948)
J. Chem. Phys. 16, 1093

Zimm, B.H. (1956)
J. Chem. Phys. 24, 269
Hydrodynamic shape functions for modelling biological macromolecules in solution in terms of an ellipsoid of revolution model are reviewed. Several new, hitherto unpublished shape functions whose experimental determination does not require knowledge of the swollen molecular volume in solution, are given. The limitations and inadequacies of this model are explained. The viscosity increment $\nu$ for a dilute dispersion of tri-axial ellipsoids of semi-axes $a > b > c$, under dominant Brownian motion is derived and an explicit expression in terms of $a$, $b$ and $c$ is given. Knowledge of the viscosity increment alone is not sufficient to uniquely determine the axial ratios $(a/b, b/c)$ because (i) in order to determine $\nu$, knowledge of the swollen volume in solution is required and (ii) a particular value for $\nu$ has a line solution of possible values for $(a/b, b/c)$. (i) is dealt with by combining $\nu$ with the tri-axial frictional ratio function $P$ to give the tri-axial R function and (ii) by combining graphically the R line solution with $\delta_+$ and $\delta_-$ swelling independent line solutions. The experimental determination of $\delta_+$ and $\delta_-$ requires the resolution of a 2-term electric birefringence decay into its component relaxation times; current data analysis techniques are however not satisfactory for resolving close relaxation times (as for globular proteins) with the current experimental precision. It is however shown by exhaustive computer simulation that using a new R-constrained non-linear least squares iterative analysis this is now possible. It is thus concluded that the general tri-axial ellipsoid as a model for the gross conformation of biological macromolecules in solution can now be employed.