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Modelling the Gross Conformation of Assemblies using Hydrodynamics: The Whole Body Approach

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1. INTRODUCTION

There are two basic approaches to modelling macromolecular conformation using hydrodynamic techniques. One, pioneered by Bloomfield, Garcia de la Torre and co-workers involves modelling the particles as arrays of spheres that interact in a way described by the Burgers-Oseen tensor: such advances have been described by Garcia de la Torre[1] earlier in this volume. In this Chapter I will describe the progress made over the last few years using the alternative 'whole body approach' in terms of general triaxial ellipsoids: viz. ellipsoids with three unequal axes. This extends the classical 'ellipsoid of revolution' approach of Perrin, Simha, Scheraga, Mandelkern and others to a model which allows a much greater variety of gross conformations. The experimental options available include various combinations of viscosity, sedimentation and rotational diffusional parameters together with measurements of radii of gyrations and molecular covolumes. I will discuss the problems of macromolecular solvation or "hydration" and the general approximation of a macromolecule to an ellipsoid.

2. WHY THE 'WHOLE BODY' APPROACH?

It is possible now to predict a number of hydrodynamic shape parameters for many complex structures - including flexible ones - by representing such structures as arrays of spheres that interact in a way described by the Burgers-Oseen (or modifications thereof) tensor. It is possible to predict from a model for such structures the sedimentation coefficient, intrinsic viscosity and rotational diffusional parameters, and by successively refining the model satisfactory agreement with experimental data can in general be achieved[1]. This type of modelling has had many interesting applications, and its major

use has been in facilitating the choice between possible models based on prior information about the molecule (from e.g. x-ray crystallography). Specific examples have been given elsewhere in this volume by J. Garcia de la Torre[1], D. Porschke & J. Antosiewicz[2] (DNA-protein assemblies) and S. Perkins[3] (the immunological complement system).

With the undoubted power of the 'multiple sphere' or 'bead model' approach it could be questioned whether the alternative 'whole body' approach was now of value. By 'whole body' approach I mean starting off with just assuming a single general model - namely an ellipsoid - and then calculating the form of this 'equivalent' ellipsoid directly from one, two or three types of hydrodynamic measurement.

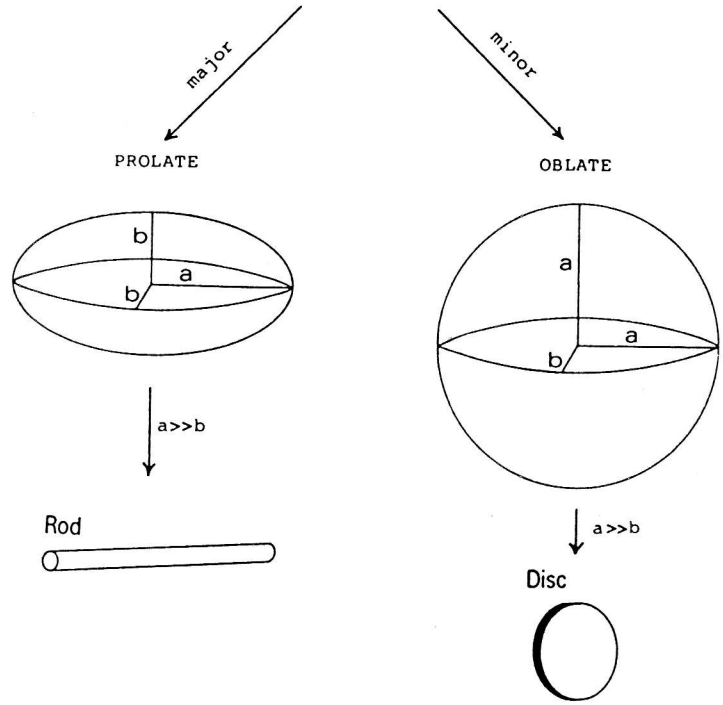
The usefulness of this latter 'whole body approach' lies in the following: Firstly there are two inherent limitations of the bead model approach. One is the 'uniqueness' problem where, although by successive refinement, an accurate fit to the observed experimental data can in general be obtained for a given hydrodynamic model, then depending on the complexity of the assumed model, there could be a large number of other models of comparable complexity which give an equally good fit to the data. The other limitation of the bead model approach is that important assumptions have to be made about macromolecular solvation or "hydration". This is normally an elusive parameter to measure (see, e.g. ref. 4) with the result that somewhat unsatisfactory - or at best very difficult - estimates have to be made: indeed, some hydrodynamic shape functions are a more sensitive function to "hydration" than to shape.

The second reason supporting the utility of the whole-body approach derives from the so-called 'biotech boom'. That is with the large amount of newly engineered macromolecules now being produced, a relatively quick estimate of their properties in solution compared to non-engineered macromolecules is highly desirable - and obviously this includes the ability to model the gross conformation of the macromolecule without any prior clues as to what this shape could be. Moreover the 'whole-body' approach - using general triaxial ellipsoids - can be employed without any need to 'assume' a hydration - by using hydration independent functions - other than it is assumed similar for two to three types of measurement.

I want to now consider the recent advances in the whole-body approach involving ellipsoidal shapes with 3 degrees of freedom - the general tri-axial ellipsoid - but before I do so it would be useful to briefly review the earlier 'ellipsoid of revolution' approach (Fig. 1).

Figure 1 The Ellipsoid of Revolution

Formed by rotating ellipse about either the major or the minor axis



3. EARLY MODELS: THE SPHERE AND ELLIPSOID OF REVOLUTION

For over 80 years hydrodynamic (Greek: 'water moving') measurements have provided a valuable and relatively rapid way of estimating the dimensions of macromolecules - both synthetic and natural - in solution. The earlier calculations were based on spherical particles in terms of their frictional flow properties (sedimentation or diffusion) *through* a solution[5] and on their effect on the bulk viscous flow properties *of* the solution[6,7,8]. The advent of the analytical ultracentrifuge in the 1920's allowed the measurement of particle frictional ratios and the use of Perrin's[9] solutions in terms of ellipsoids, the latter being an extension of Stokes's solution[5] for spheres. Unfortunately, because of the complexity of the elliptic integral involved[9], macromolecules could only be modelled in terms of prolate or oblate 'ellipsoids of revolution'

Table 1 Hydrodynamic Shape Parameters for Ellipsoids of Revolution [semi-axes a,b,c and a>b=c (prolate) or a=b>c (oblate)]

Shape Parameter (as function of a/b)	Related Experimental Parameter	Ref
	1. Bulk Solution Properties	
Viscosity increment, ν	Intrinsic viscosity, $[\eta]$	10,12,14
Reduced excluded volume, u_{red}	Thermodynamic 2nd virial coefficient, B (from light scattering, sedimentation equilibrium, etc.)	18,19
	2. Translational Frictional Property	
Perrin function, P	Sedimentation coefficient, s; Translational diffusion coefficient, D	9,14
	3. Rotational Frictional Property[a]	
'Reduced' birefringence decay constant, θ^{red}	[b]Electric birefringence decay constant, θ	21
Harmonic mean rotational relaxation time ratio, τ_h/τ_o	Harmonic mean rotational relaxation times (from steady state fluorescence depolarisation studies), τ_h	22
Fluorescence anisotropy depolarisation rotational relaxation time ratios, τ_i/τ_o (i = 1-3)	[c]Fluorescence anisotropy depolarisation relaxation times, τ_i	23

a. All use Perrin's[20] solutions for the rotational frictional ratios for ellipsoids.

b. There are two if optical axis does not coincide with geometric axis of ellipsoid of revolution. There are also two ($\theta_\pm$) for general ellipsoids (see below).

c. Three for ellipsoids of revolution, five for general ellipsoids.

Table 2 Hydration of Proteins Calculated from the Frictional Ratio, f/f_o. Axial ratios estimated from crystallographic dimensions of the proteins. From Squire & Himmel[15] & references cited therein.

Protein	Dimensions (Å)	a/b[a]	P(a/b)	f/f_o^b	Hydration[c]
Basic trypsin inhibitor	29x19x19	1.53	1.016	1.447	0.86
Cytochrome C	25x25x37	1.48	1.014	1.116	0.24
Ribonuclease-A	38x28x22	1.52	1.016	1.290	0.73
Lysosyme	45x30x30	1.50	1.015	1.240	0.57
Myoglobin	44x44x25	1.76	1.028	1.170	0.35
Adenylate kinase	40x40x30	1.33	1.007	1.167	0.41
Trypsin	50x40x40	1.25	1.004	1.187	0.47
Bence-Jones protein REI	40x43x28	1.48	1.013	1.156	0.35
Chymotrypsinogen A	50x40x40	1.25	1.004	1.262	0.71
Elastase	55x40x38	1.41	1.010	1.214	0.53
Subtilisin	48x44x40	1.14	1.002	1.181	0.47
Carbonic anhydrase B	47x41x41	1.15	1.002	1.053	0.12
Superoxide dismutase	72x40x38	1.85	1.034	1.132	0.23
Carboxypeptidase A	50x42x38	1.25	1.004	1.063	0.14
Phosphoglycerate kinase	70x45x35	1.75	1.028	1.377	1.04
Concanavalin A	80x45x30	2.13	1.053	1.299	0.64
Hemoglobin, oxy	70x55x55	1.22	1.005	1.263	0.74
Bovine serum albumin	140x40x40	3.5	1.147	1.308	0.35
Malate dehydrogenase	64x64x45	1.42	1.011	1.344	1.00
Alcohol dehydrogenase	45x55x110	2.2	1.058	1.208	0.37
Lactate dehydrogenase	84x74x74	1.20	1.003	1.273	0.77

a. For the equivalent prolate or oblate ellipsoid. b. f_o in its use here corresponds to the frictional coefficient of an ***anhydrous*** spherical particle having the same mass and partial specific volume ($\bar{v}$) as the protein under consideration. In this definition $f/f_o = P.(f/f_h)$, where P is the Perrin function or 'frictional ratio due to shape'[15] and (f/f_h) a term due to hydration. c. Hydration, w (g solvent/g protein) $= [(f/f_h)^3]\bar{v}\rho_o$ where ρ_o is the solvent density.

and not general ellipsoids (3 unequal axes). Simha[10] extended Jeffrey's[11] earlier treatment for the viscous flow of solutions of ellipsoids of revolution to include the case of Brownian motion and gave an explicit relationship for the viscosity increment ν in terms of the semi-axes a, b of these ellipsoids. Saito[12] independently obtained the same result, suggesting that Simha had made an incorrect assumption (particles rotating with zero angular velocity) but had arrived at the correct result by making an 'error in calculation', a discrepancy resolved some 30 years later[13].

4. THE "HYDRATION" OR SOLVATION PROBLEM

An important problem in using the viscosity increment ν or the Perrin frictional ratio, P, is the experimental requirement of a value for the volume, V, of the macromolecule in solution, or equivalently protein "hydration", w[14]. [Strictly speaking the term "solvation" should be used instead because other solvent species as well as water molecules can be trapped or bound to the macromolecule. However, since the term "hydration" has been used almost ubiquitously for several decades[14] we will hitherto follow the convention of using it to represent "associated solvent"]. Both functions are still commonly used direct, by using "assumed" hydration values. For example a hydration level, of 0.2-0.35 g water/g protein could be taken as typical for many globular proteins, although this value is still very arbitrary (see for example refs. 14, 15 for a discussion on this). Use of the other shape functions summarised in Table 1 also requires 'assumed hydrations'.

Unfortunately as we have mentioned above, hydration is a notoriously difficult parameter to measure with any meaningful precision. On the other hand if a reasonable estimate for the axial ratio of the molecule were known (from e.g. x-ray crystallography) then a hydration level could be calculated: Table 2 gives a summary of calculations performed by Squire & Himmel[15] to estimate protein hydration levels by using measured frictional ratios and estimated axial ratios from x-ray crystallography with the assumption that the protein has the same shape in solution as in crystallized form.

5. HYDRATION INDEPENDENT HYDRODYNAMIC SHAPE FUNCTIONS

The idea of combining analytically hydrodynamic shape functions dates back as long ago as 1953, with Scheraga & Mandelkern[16] who combined the relations of Perrin & Simha to give the well-used "β-function" (Table 2), extending the original 'graphical' approach of Oncley[17]. The β- function has proved however very insensitive to shape and has had most use as a quasi-constant shape parameter for determining molecular weights from intrinsic viscosity & sedimentation data.

Rotational diffusional phenomena provide in general parameters which

Table 3 'Compound' Hydration Independent Hydrodynamic Shape Parameters

Shape Parameter[a] (as function of a/b)	Comment	Ref
$\beta(\nu, P)$	Very poor sensitivity to axial ratio and high sensitivity to experimental error	16
$\Psi(\tau_h/\tau_o, P)$	Very poor sensitivity to axial ratio and high sensitivity to experimental error	34
$\psi(u_{red}, P)$	Very poor sensitivity to axial ratio and high sensitivity to experimental error	27
$R(\nu, P)$	Sensitive function at low axial ratio	30
$\Lambda(\tau_h/\tau_o, \nu)$	Very sensitive function, except at very low axial ratio (a/b $\leq$ 2.0)	35
$\Pi(u_{red}, \nu)$	Very sensitive function, except at very low axial ratio (a/b $\leq$ 2.0)	28

a: Source hydration dependent parameters are shown in parentheses.

are more sensitive functions of shape than the corresponding translational ones (Table 1): the hydration problem can also be accounted for by combination with appropriate translational parameters (either ν, or, P). This extra sensitivity comes however at a price: the two techniques commonly used, electric birefringence and fluorescence anisotropy depolarisation decay have some important practical limitations. In the case of electric birefringence, this is principally the requirement of having to use very low ionic strength solvents (due to conductivity problems)[24]; in the case of fluorescence depolarisation, the principal limitation is of internal rotation of chromophores or domains of the macromolecule relative to other parts[25]. Both techniques have difficulties of deconvolution of light source functions[24,26], and perhaps more seriously for asymmetric scatterers, both suffer from difficulties of resolution of multi-exponential decay terms and we will discuss this further below. A more recent development has been the use of a compound hydration-independent function (Λ) involving the *harmonic mean* rotational relaxation time[35] which

largely avoids these problems and appears a sensitive function of axial ratio (Fig. 2).

Another recent development has been the use of compound shape functions involving molecular excluded volumes[27,28,29]. The molar covolume, U ($ml.mol^{-1}$) for a system of macromolecules can be obtained from the thermodynamic second virial coefficient, B (after correction for - or suppression of - charge effects). This covolume function is both a function of shape and hydration, but can be 'reduced' to give a function (u_{red}) of shape alone[28]. To experimentally determine it, a value for the hydration is still required, but again, the latter can be eliminated by combination with either the Perrin frictional ratio to give the hydration independent parameter ψ (ref. 27) or with the viscosity increment ν to give the hydration independent Π function[28]. Although the ψ function is very insensitive to shape - and rather disappointingly so, - the Π - function is on the other hand quite sensitive, and appears to be the most useful of the 'hydration independent' ellipsoid of revolution shape functions available.

Another 'hydration independent' parameter is the ratio $R = k_s/[\eta]$ where k_s is the sedimentation concentration dependence regression parameter and $[\eta]$ the intrinsic viscosity. It is known empirically[30,31,32] that $R \sim 1.6$ for spheroidal particles (nb. after correction of sedimentation coefficients for solution density[31] - a higher value is obtained for coefficients corrected for solvent density) and < 1.6 for more asymmetric particles; after a number of assumptions and approximations a simple relation between R, ν and P has also been provided[33].

6. LIMITATIONS OF ELLIPSOIDS OF REVOLUTION. THE GENERAL ELLIPSOID

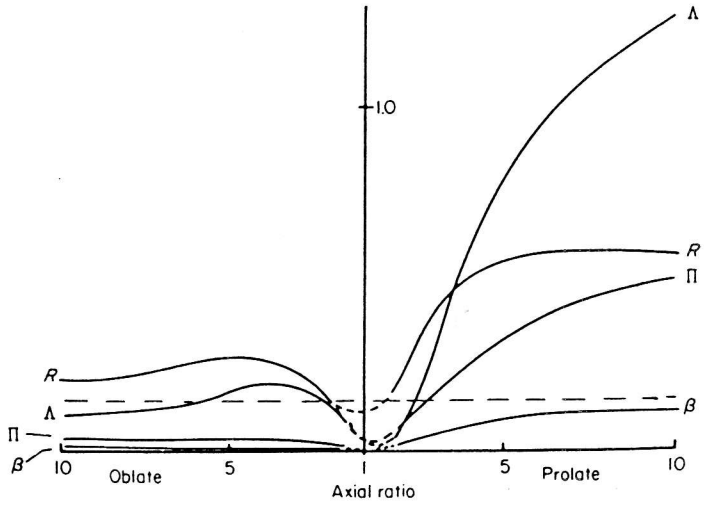
For many macromolecules the ellipsoid of revolution model can apparently give a reasonable representation of the gross conformation of macromolecules in solution. Indeed further examination of Table 2 will reveal that for many proteins, two of the three axial dimensions (derived from x-ray crystallographic data) are approximately equal. The disadvantages however of having to use a model with two axes equal are clear:

1. A decision has to be made *a priori* between the two types of ellipsoid of revolution (viz. prolate and oblate): virtually all of the usable hydration independent shape functions do not distinguish between the two (viz. they are not single-valued).

2. There are many classes of macromolecule which lie intermediate between a prolate shape (one long axis, two short) and an oblate (two long axes, one short).

As a result, hydrodynamicists have for a long time recognised the advantages of having a 'whole-body' model which does not have this restriction of two equal axes: the general triaxial ellipsoid (semi-axes $a \geq b \geq c$). This caters

Figure 2 Relative sensitivities of hydration independent shape functions
Broken line indicates minimum value this sensitivity must have if an axial ratio precise to ± 20% is to be retrieved from the measured function, assumed precise to ± 3%. ψ and Ψ not shown (very insensitive - close to baseline).



for a much wider range of shapes, from discs (a=b>>c), rods (a >>b=c), tapes (a>>b>>c) and all intermediary shapes (Fig. 3).

The difficulty has been that the relation between the shape functions and the *two* axial ratios which characterise a general ellipsoid had either not been worked out (e.g. ν, u_{red}) or where computationally unavailable (satisfactory numerical routines for the evaluation of the elliptic integrals involved with many of the shape functions - notably the Perrin translational and rotational frictional ratio functions - and associated convergence problems). Over the last 15 years both of these problems have largely been addressed. Small & Isenberg[36] demonstrated that the Perrin elliptic integrals could be solved numerically using fast computers to evaluate the rotational and translational frictional ratio functions. The subsequent availability of the viscosity increment ν both numerically[37] and analytically[38] together with the reduced excluded volume[39] u_{red} for general 'tri-axial' ellipsoids, has now meant that a virtually complete set of hydration independent triaxial shape parameters are now available. A FORTRAN routine is available[40] for evaluating the set of hydrodynamic parameters for a particle for any given value of its axial

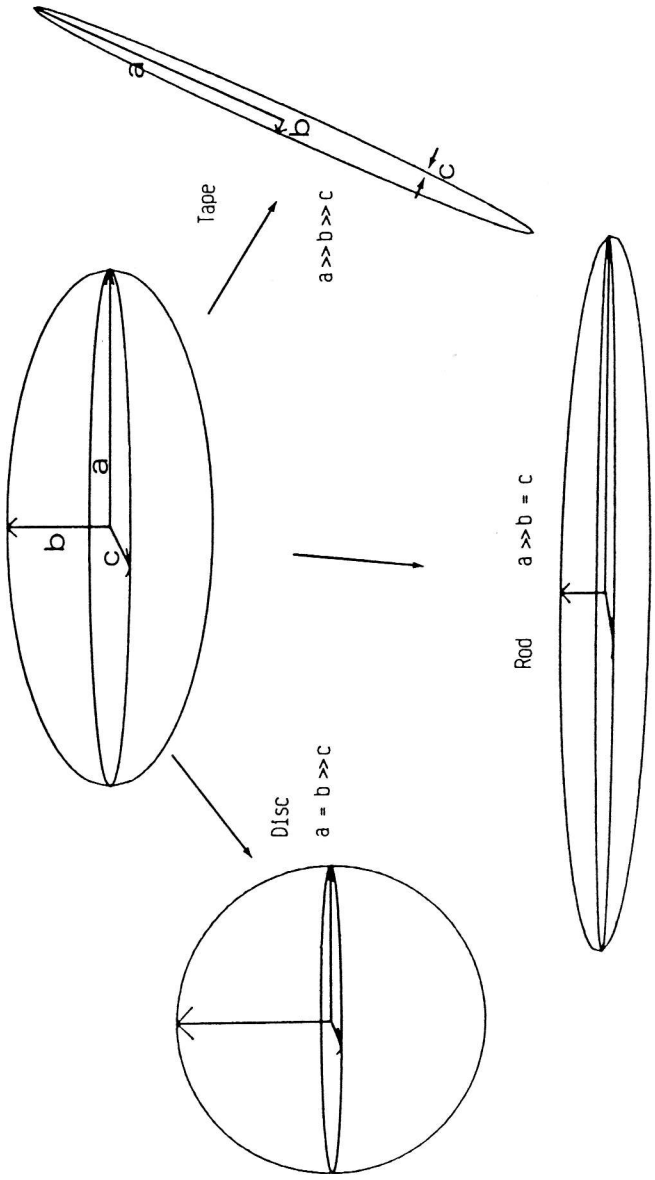


Figure 3 The General Triaxial Ellipsoid (semi-axes $a \geq b \geq c$) and its extremes

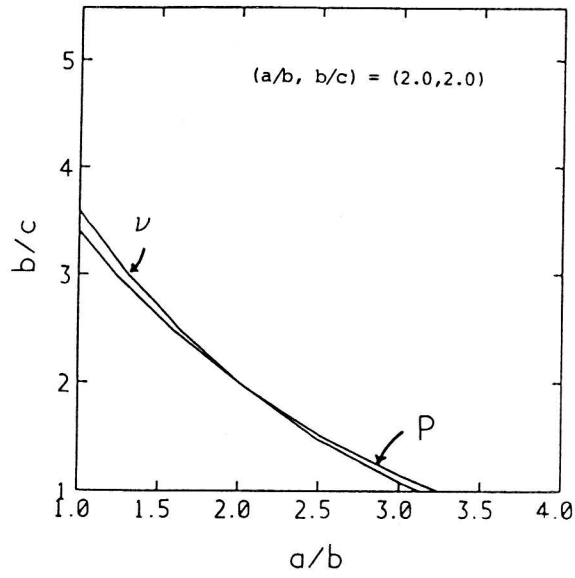


Figure 4 Plots of constant values for ν and P in the (a/b, b/c) plane corresponding to an (a/b, b/c) = (2.0, 2.0)
From ref. 41

dimensions.

7. LINE SOLUTIONS: THE GRAPHICAL INTERSECTION METHOD

All the triaxial ellipsoid shape functions share the common property of having a line solution of possible values for the axial ratios (a/b, b/c) for any given value of the hydrodynamic function. A unique solution for these two axial ratios may be found from the intersection of two or more of these "line solutions" (Figs 4-10). Fig 4 illustrates two line solutions for ν and P for a hypothetical ellipsoid particle of (a/b, b/c) = (2.0, 2.0). Evidently this represents a very poor combination of functions, because of the shallowness of the intersection and their dependence on assumed values for hydration. To use the triaxial ellipsoid we have to find two suitable shape functions that are

1. hydration independent

Table 4 Hydration Independent Hydrodynamic Shape Parameters for Triaxial Ellipsoids: Nature of Graphical Intersection

	Λ^a	G^b	$\delta_{\pm}^c$	R^d
Π	Poor intersection	Good intersection at high axial ratios	Good intersection at high axial ratios	NE
Λ		Good intersection at all axial ratios	NE	Good intersection at all axial ratios
G			Good intersection at low axial ratios	NE
$\delta_{\pm}$				Good intersection at low ratios

a: assumes no internal rotation of chromophore or segmental rotation
b: from radius of gyration measurements (x-ray scattering or light scattering)
c: involves resolution of a two-term exponential decay
d: some approximations concerning concentration dependence of sedimentation coefficient
NE: not examined

2. experimentally measurable to a reasonable precision
3. are sensitive to shape (and insensitive to experimental error) and
4. give a reasonable intersection (i.e. as orthogonal as possible)

These criteria are quite restrictive, and in Table 4 we have summarised the intersection properties between the most useful functions. Formal definitions of these are given in the Appendix in the form of explicit relations in terms of the semi-axes a,b,c via the source parameters (ν, P, τ_h, $\theta_{\pm}^{red}$ and u_{red}), and also the corresponding experimental parameters.

Choice of shape function

In choosing two suitable hydration independent functions we try to avoid where possible those involving measurement of rotational diffusional or relaxational parameters. This is because almost always (except for those involving

the measurement of the harmonic mean relaxation time from steady state fluorescence depolarisation)[22], a step involving resolution of multi-exponential decay data is required. For example for general homogeneous ellipsoidal particles without an axis of symmetry there are two electric birefringence decay constants[42–44] and five fluorescence anisotropy depolarisation relaxation times[23,35]. Further, if solutions of macromolecules are not monodisperse, or if there is some self-association, there will be further exponential components. For example, two component electric birefringence relaxation data for haemocyanin solutions have been interpreted either as a polydisperse system of ellipsoids of revolution or as a monodisperse system of ellipsoids[43].

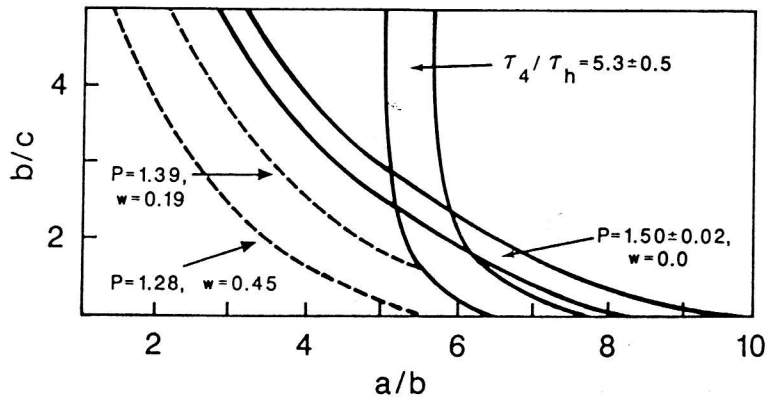
With electric birefringence there is the added complication of designing an instrument to have an adequate response time, deconvoluting the finite time to switch off the orienting field and problems of having to work at very low ionic strengths to avoid serious heating effects caused by the high electric fields used[24]. With fluorescence anisotropy depolarisation decay, besides having a formidable number of decay times to contend with (although in practice two-three are very similar) there is also a problem of deconvoluting the light source function from the decay data[26], together with the assumption of no significant internal rotation of the chromophore(s) or segmental rotation of parts of a given macromolecule relative to other regions of the same. A good demonstration of the segmental rotation problem has been given by Johnson & Mihalyi[25] for fibrinogen. The problems of multi-exponential resolution are not unique to macromolecular modelling and considerable attention has been paid and progress made as described elsewhere in this volume[47,48].

Despite the difficulties of rotational measurements an early attempt at modelling the triaxial conformation of (scallop) myosin light chains was made by Stafford & Szent-Gyorgi[49]: these workers made the approximation that of the five fluorescence anisotropy decay times, four similar 'fast' ones could be represented by a single harmonic mean, τ_h, which could be resolved from the 'slower' decay time τ_4. Although the ratio τ_h/τ_4 is hydration independent, at that time other hydration independent functions were not available, and so it was only possible to give limits for the axial ratios using a graphical combination of τ_h/τ_4 with the Perrin translational frictional function P, values for the latter evaluated using assumed values for the hydration. Although the intersection given for the case of 'no hydration' (Fig. 5) is rather meaningless, for more realistic values the intersection would appear to suggest an extended prolate shape of axial ratio between 5 and 6 to 1.

Use of hydration independent functions

Fortunately, shape function combinations are now available which largely avoid the difficulties referred to above, and a good example is the combination of the Π and G functions[50]. The availability of explicit relations between

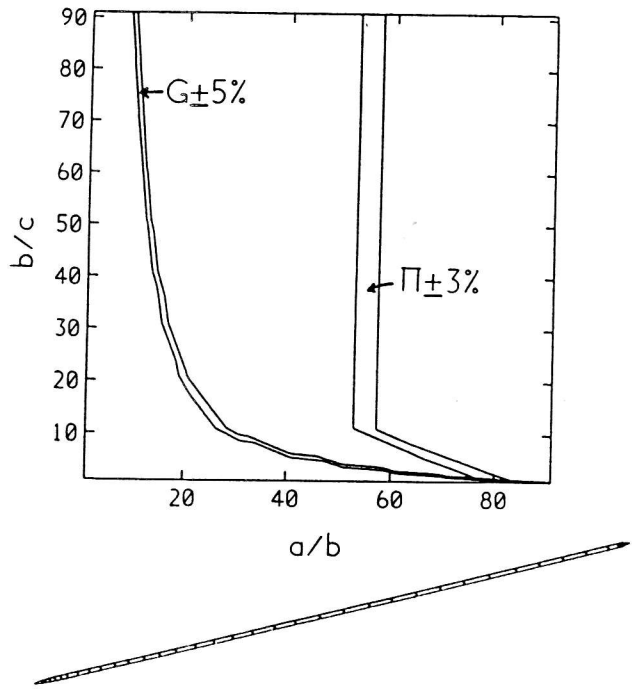
Figure 5 Plots of constant values for τ_4/τ_h and P in the (a/b, b/c) plane for scallop myosin light chains.
P is given for 3 assumed values for the hydration, w(g H_2O/g protein). Redrawn from ref. 49.



axial ratio (a/b, b/c) with the viscosity increment ν and reduced excluded volume, u_{red} for triaxial ellipsoids has enabled the Π function to be defined also[50]. As stated above Π can be obtained experimentally from measurements of intrinsic viscosity, and the thermodynamic 2nd virial coefficient (from e.g. light scattering, or sedimentation equilibrium - see, for example, the procedure described by Jeffrey *et al*[27]) after correction for Donnan effects.

The G-function has also been defined for triaxial ellipsoids[50] and can be obtained from the radius of gyration, again for example from light scattering or from low-angle x-ray scattering measurements. The G function is a very sensitive function to axial ratio, and an illustration of its use in conjunction with the Π function for a macromolecule of high axial ratio is given in Fig. 6 for myosin. In this particular example an attempt to model the gross conformation of the myosin molecule without prior assumptions about molecular hydration or using prior information from electron microscopy was made. Despite the extra degree of freedom the general ellipsoid gives, the myosin molecule appears as a prolate ellipsoid of (a/b, b/c) $\simeq$ (80, 1). Nonewithstanding the difficulties of modelling an ellipsoid to a particle that in reality has a "lop- sided" end, this result is in good agreement with predicted results from electron microscopy, and would appear to suggest that local variations in particle shape (principally in the case of myosin the S1 heads) or flexibility

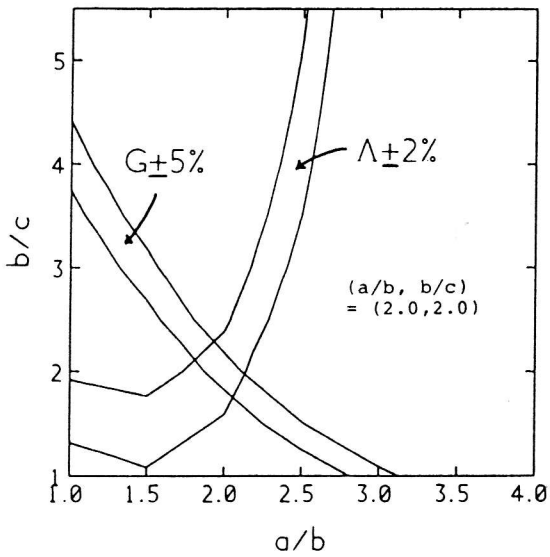
Figure 6 (a) Plots of constant values for Π and G in the (a/b, b/c) plane for myosin. From ref. 50
(b) Gross conformation predicted



(the HMM/LMM interface) do not seriously distort estimates for the gross conformation of the molecule using the triaxial ellipsoid in this way.

For the modelling of globular particles of low axial ratio (one axial ratio $\lesssim 5$) the intersection of G with Π is poor (largely through insensitivity of the Π function in this region) and it is necessary to consider the use of other combinations of hydration independent shape functions. A combination not hitherto suggested, and which appears useful (Fig. 7) is the G - Λ combination. Λ[35,52] requires the measurement of intrinsic viscosity and the harmonic mean rotation relaxation time, τ_h, a parameter which can be obtained from steady state fluorescence depolarisation measurements without the need for multi-exponential resolution. The G-Λ combination has, as yet not had any practical application. The Λ function has however been combined[53] with the R function, obtained from the ratio of the concentration dependence of the

Figure 7 Plots of constant values for G and Λ in the (a/b, b/c) plane corresponding to an (a/b, b/c) = (2.0, 2.0)



sedimentation coefficient, k_s, to the intrinsic viscosity, $[\eta]$. This provides a similarly sensitive intersection at low axial ratio and this combination of line solutions has been used to provide us with an indication of the likely mode of association of monomers of the neural protein neurophysin into dimers (Fig. 8)[53]. Again, as for myosin, despite the extra degree of freedom the general ellipsoid allows, the monomer still appears as a prolate model with two axes approximately equal (a/b, b/c) $\simeq$ (4.0, 1.0). For the dimer this reduces to an overall (a/b, b/c) of $\simeq$ (2.8, 2.5), and the data therefore supports observations made earlier using ellipsoid of revolution models[54] that the association process is of a side-by-side rather than an end-to-end type.

In some applications the use of steady state fluorescence depolarisation functions can be limited, with the result that recourse to a function involving rotational diffusion is required. The $\delta_{\pm}$ are two such hydration independent functions obtained experimentally from the ratio of the (reduced) electric birefringence decay constants $\theta_{\pm}^{red}$ to the intrinsic viscosity, $[\eta]$. These functions also provide useful intersections with the G, Π and R functions [41,55] and Fig. 9 gives an example.

These 'useful intersections' however come at a price, namely the require-

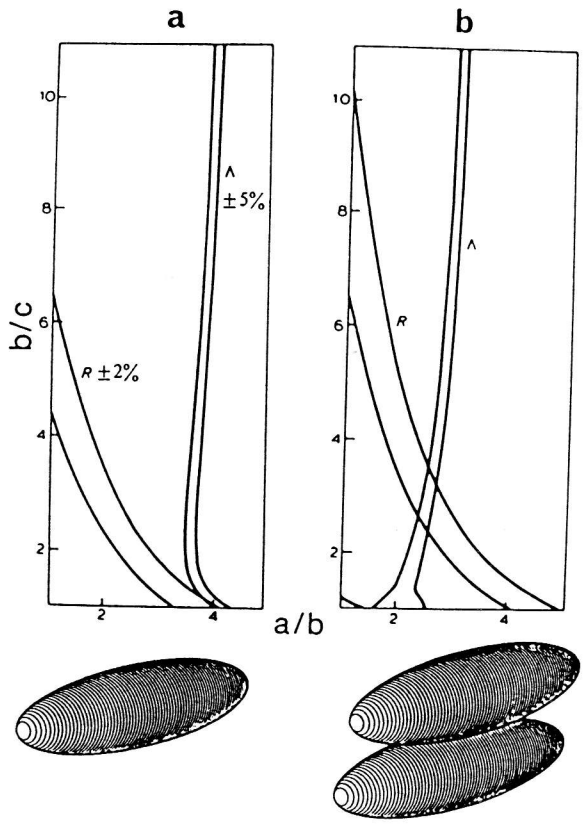
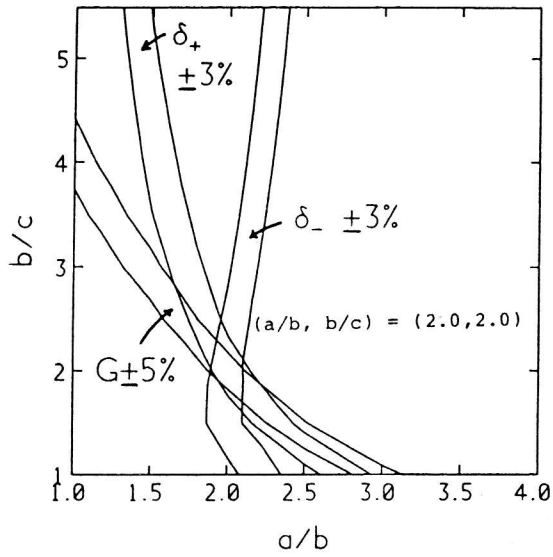


Figure 8 Plots of constant values for R and Λ in the (a/b, b/c) plane for neurophysin monomers (a) and dimers (b). From ref. 53.

Figure 9 Plots of constant values of G, δ_+ and δ_- in the (a/b, b/c) plane corresponding to an (a/b, b/c) = (2.0, 2.0)



ment of extraction of the two exponential decay constants characterising general ellipsoids. We tried a whole series of procedures (non-linear least squares, Laplace transforms, method of moments etc.) on synthetic data with random error[52] but found the only reliable method for capturing the constants for data of "real" experimental precision were constrained least squares procedures, whereby estimates for the decay constants $\theta_\pm$ during an iteration process are constrained so that their corresponding values for $\delta_\pm$ in the (a/b, b/c) plane lie on a curve defined by another line solution (for example, G, Π or R). Fig. 10 gives an example of the "band" of allowed axial ratios (a/b, b/c) satisfactorily obtained in this way[41] for synthetic birefringence data with random expected 'experimental' noise. The limits of the band depend on the experimental precision of the constraining line solution and the birefringence data.

8. BEAD MODELS OR WHOLE-BODY MODELS?

Amongst the number of hydration independent shape functions now available, summarised in Table 4 and defined explicitly in the Appendix below it is hoped that there is at least one combination of functions suitable for mod-

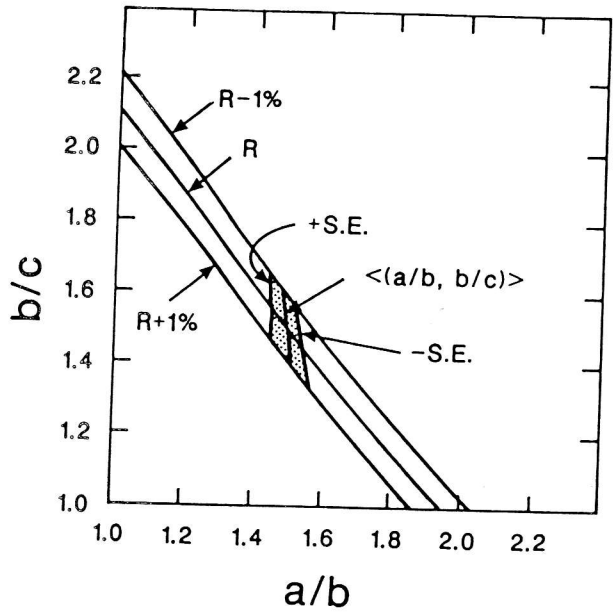


Figure 10 Constrained non-linear least squares fit of electric birefringence decay data. The plots are of constant R in the (a/b, b/c) plane corresponding to an (a/b, b/c) of (1.5, 1.5). Shaded area corresponds to allowed band of axial ratios obtained by constraining the estimates for the δ_+ and δ_- functions to lie on the R curves. Simulated data, true (a/b, b/c) = (1.5, 1.5); 0.1 deg. random standard error on the birefringence decay data.

elling the gross conformation of a given macromolecular system. It is also hoped that the 'bead model approach' for macromolecular modelling described by Garcia de la Torre elsewhere in this volume and the 'whole body' approach described here using triaxial ellipsoid shape functions prove complementary methods; the first, when close starting estimates for the structure are available from other sources and where the 'hydration' is known reasonably accurately, thereby facilitating a complex model; the second, when no prior shape information is available, and only the gross dimensions of the macromolecule in solution are required.

APPENDIX

Table 5 gives the formulation and relation to experimental parameters of the principal hydration dependent (i.e. requiring knowledge of the volume V of a particle, including associated solvent or 'hydration') and hydration independent shape functions for general triaxial ellipsoids of semi-axes ($a \geq b \geq c$). In these formulae:

1. In the equation for ν (the viscosity increment), δ is a small term ($\lesssim$ 1% of the other term) given by

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

2. In the equation for the reduced excluded volume, u_{red}, the R and S are double integrals given by

$$R = \frac{2}{3\pi}\int_0^{\pi/2}\int_0^{\pi/2} cos\ u\ du\ dv \left\{\left(\frac{a}{bc}+\frac{b}{ac}+\frac{c}{ab}\right)\triangle^2\right.$$

$$-sin^2\ v\ cos^2\ v\ cos^2 u\ \triangle^4\left(\frac{1}{a^2}-\frac{1}{b^2}\right)\frac{1}{c}\left(\frac{b}{a}-\frac{a}{b}\right)$$

$$-sin^2\ u\ cos^2\ u\ \triangle^4\left(\frac{cos^2\ v}{a^2}+\frac{sin^2\ v}{b^2}-\frac{1}{c^2}\right)$$

$$\left.\cdot\left[\frac{1}{c}\left(\frac{b\ cos^2\ v}{a}+\frac{a\ sin^2\ v}{b}\right)+\frac{c}{ab}-\frac{b}{ac}-\frac{a}{bc}\right]\right\}$$

Table 5 Shape functions for General Triaxial Ellipsoids

Function	Formulation	Relation to Experimental Parameters	Ref.
$*\nu$	$\frac{1}{abc}\left\{\frac{4(\alpha_7+\alpha_8+\alpha_9)}{15(\alpha_8\alpha_9+\alpha_9\alpha_7+\alpha_7\alpha_8)}\right.$ $+\frac{1}{5}\left[\frac{\alpha_2+\alpha_3}{\alpha_4(b^2\alpha_2+c^2\alpha_3)}\right.$ $+\frac{\alpha_3+\alpha_1}{\alpha_5(c^2\alpha_3+a^2\alpha_1)}$ $\left.\left.+\frac{\alpha_1+\alpha_2}{\alpha_6(a^2\alpha_1+b^2\alpha_2)}\right]\right\}+\delta,$	$[\eta].M_r/(V.N_A)$	38,42 46,52
$*P$	$2/\{(abc)^{\frac{1}{3}}/\alpha_{10}\}$	$[(\bar{\nu}M_r)/(VN_A)](f/f_o)$	9,35,52
$*u_{red}$	$2+(\frac{3}{2\pi abc})R.S,$	$\frac{U}{N_AV}\equiv\frac{1}{N_AV}.$ $[2BM_r^2-f(Z,I)]$	39
$*\tau_h/\tau_o$	$1/[a^2\alpha_1+b^2\alpha_2+c^2\alpha_3]$	$(kT\tau_h)/(3\eta_oV)$	52,53
$*\theta_{\pm}^{red}$	$\frac{abc}{12}\left\{(\frac{1}{Q_a}+\frac{1}{Q_b}+\frac{1}{Q_c})\right.$ $\pm\left[(\frac{1}{Q_a^2}+\frac{1}{Q_b^2}+\frac{1}{Q_c^2})\right.$ $-(\frac{1}{Q_aQ_b}+\frac{1}{Q_bQ_c}$ $\left.\left.+\frac{1}{Q_cQ_a})\right]^{\frac{1}{2}}\right\}$	$(\eta_o/kT).V.\theta_{\pm}$	43,44, 52,40
β	$(N_A^{1/3}/16200\pi^2)^{1/3}.$ $(\nu^{1/3}/P)$	$N_As[\eta]^{1/3}\eta_o/$ $[M_r^{2/3}(1-\bar{\nu}\rho_o)100^{\frac{1}{3}}]$	41,51,52
Π	u_{red}/ν	$U/([\eta].M_r)$ $\equiv[2BM/[\eta]]-$ $f(Z,I)/([\eta]M_r)$	50
G	$(1/5)[(a^2+b^2+c^2)$ $/(abc)^{2/3}]$	$[(4\pi N_A)/(3\bar{\nu}M_r)]^{2/3}.$ R_g^2	50
Λ	$\nu/(\tau_h/\tau_o)$	$(3\eta_o[\eta]M_r)/(N_AkT\tau_h)$	53
R	$2[1+P^3]/\nu$	$k_s/[\eta]$	41
$\delta_{\pm}$	$6\theta_{\pm}^{red}{}_{\nu}$	$(6\eta_o/N_AkT).[\eta].M_r.\theta_{\pm}$	41

$*$: hydration dependent

and

$$S = \frac{8}{3}\int_0^{\pi/2}\int_0^{\pi/2} cos\ u\ du\ dv \left\{ \left(\frac{bc}{a} + \frac{ca}{b} + \frac{ab}{c} \right) \triangle \right.$$

$$-sin^2\ v\ cos^2\ v\ cos^2\ u\ \triangle^3\ c \left(\frac{b}{a} - \frac{a}{b} \right) \left(\frac{1}{a^2} - \frac{1}{b^2} \right)$$

$$-sin^2\ u\ cos^2\ u\ \triangle^3 \left(\frac{cos^2\ v}{a^2} + \frac{sin^2\ v}{b^2} - \frac{1}{c^2} \right)$$

$$\left. \cdot \left[c \left(\frac{b\ cos^2\ v}{a} + \frac{a\ sin^2\ v}{b} \right) - \frac{ab}{c} \right] \right\}$$

where

$$\triangle^{-2} = \frac{cos^2\ u\ cos^2 v}{a^2} + \frac{cos^2\ u\ sin^2 v}{b^2} + \frac{sin^2\ u}{c^2}$$

3. In the equation for the reduced decay constants $\theta_{\pm}^{red}$, the terms Q_a, Q_b and Q_c are given by

$$Q_a = \frac{b^2 + c^2}{b^2\alpha_2 + c^2\alpha_3}, Q_b = \frac{c^2 + a^2}{c^2\alpha_3 + a^2\alpha_1}, Q_c = \frac{a^2 + b^2}{a^2\alpha_1 + b^2\alpha_2}$$

4. The elliptic integrals $\alpha_1 - \alpha_{10}$ are given by

$$\alpha_1 = \int_0^\infty \frac{d\lambda}{(a^2 + \lambda)\triangle}; \alpha_2 = \int_0^\infty \frac{d\lambda}{(b^2 + \lambda)\triangle}; \alpha_3 = \int_0^\infty \frac{d\lambda}{(c^2 + \lambda)\triangle}$$

$$\alpha_4 = \int_0^\infty \frac{d\lambda}{(b^2 + \lambda)(c^2 + \lambda)\triangle}; \alpha_7 = \int_0^\infty \frac{\lambda d\lambda}{(b^2 + \lambda)(c^2 + \lambda)\triangle}$$

$$\alpha_5 = \int_0^\infty \frac{d\lambda}{(c^2 + \lambda)(a^2 + \lambda)\triangle}; \alpha_8 \int_0^\infty \frac{\lambda d\lambda}{(c^2 + \lambda)(a^2 + \lambda)\triangle}$$

$$\alpha_6 = \int_0^\infty \frac{d\lambda}{(a^2 + \lambda)(b^2 + \lambda)\triangle}; \alpha_9 = \int_0^\infty \frac{\lambda d\lambda}{(a^2 + \lambda)(b^2 + \lambda)\triangle}$$

$$\alpha_{10} = \int_0^\infty \frac{d\lambda}{\triangle} \quad where\ \triangle = [(a^2 + \lambda)(b^2 + \lambda)(c^2 + \lambda)]^{1/2}$$

and λ is a dummy variable

These integrals can be solved numerically using standard computational packages without convergence problems - see e.g. ref 40 for a simple FORTRAN program illustrating their use.

5. The following experimental parameters are:

$[\eta]$ Intrinsic viscosity (ml/g)

M_r Molecular weight (g/mol)

V Particle volume (including associated solvent or 'hydration') (ml)

N_A Avogadro's number (mol^{-1})

$\bar{v}$ Partial specific volume (ml/g)

(f/f_o) Frictional ratio. Following the most popular convention (ref 14), f_o refers to the frictional coefficient of a spherical particle of the same mass and *anhydrous* volume as the macromolecule whose frictional coefficient is f. This differs from our previous usage, which is that of Scheraga and Mandelkern[16] where f_o refers to a sphere of the same *hydrated* volume.

U Molar covolume (ml/mol)

B Thermodynamic second virial coefficient ($ml.mol.g^{-2}$)

f(Z,I) Function of macromolecular charge Z and solution ionic strength, I; f= 0 at the isoelectric pH for proteins, and $\rightarrow$ 0 as I is increased

k Boltzmann constant (erg. K^{-1})

T Absolute temperature (K)

τ_h Harmonic mean rotational relaxation time (sec)

η_o Solvent viscosity (Poise)

θ_+, θ_- Electric birefringence decay constants (2 for monodisperse solution of triaxial ellipsoids) (sec^{-1})

s Sedimentation coefficient (sec)

R_g Radius of Gyration (cm)

k_s Concentration dependence sedimentation regression coefficient (ml/g)

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