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ARTICLE

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The *ELLIPS* suite of macromolecular conformation algorithms

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Abstract This paper describes a series of four programmes for the PC based on ellipsoidal representations of macromolecular shape in solution using Universal shape functions. *ELLIPS1* is based on simple ellipsoid of revolution models (where two of the three axes of the ellipsoid are fixed equal to each other). If the user types in a value for a shape function from sedimentation or other types of hydrodynamic measurement, it will return a value for the axial ratio of the ellipsoid. *ELLIPS2* is based on the more general triaxial ellipsoid with the removal of the restriction of two equal axes. The user enters the three semi-axial dimensions of the molecule or the equivalent two axial ratios and *ELLIPS2* returns the value of all the hydrodynamic shape functions. It also works of course for ellipsoids of revolution. *ELLIPS3* and *ELLIPS4* do the reverse of *ELLIPS2*, that is they both provide a method for the unique evaluation of the triaxial dimensions or axial ratios of a macromolecule (and without having to guess a value for the so-called "hydration") after entering at least three pieces of hydrodynamic information: *ELLIPS3* requires EITHER the intrinsic viscosity with the second virial coefficient (from sedimentation equilibrium, light scattering or osmometry) and the radius of gyration (from light or x-ray scattering) OR the intrinsic viscosity with the concentration dependence term for the sedimentation coefficient and the (harmonic mean) rotational relaxation time from fluorescence depolarisation measurements. *ELLIPS4* evaluates the tri-axial shape of a macromolecule from electro-optic decay based Universal shape functions using another Universal shape function as a constraint in the extraction of the decay constants.

Key words Macromolecular shape · Ellipsoids · Hydrodynamics

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Introduction

There are two approaches to representing the conformation of fairly rigid macromolecules in solution. The first approach is the so-called "Bead" or "Multiple sphere" approach pioneered by V. A. Bloomfield at the University of Minnesota and J. Garcia de la Torre at the University of Murcia (see, e.g. Garcia de la Torre and Bloomfield 1977; 1981) whereby a macromolecule or macromolecular assembly is approximated as an array of spherical beads. Using computer programmes that are currently available (based on how these spheres interact) such as HYDRO (Garcia de la Torre et al. 1994) it is possible for a given Bead Model to predict its hydrodynamic properties. One can model quite sophisticated structures by this approach but it suffers from uniqueness problems: for example, one can predict the sedimentation coefficient for a particular complicated model, but there will be many many other equally complicated models which give the same sedimentation coefficient. This type of modelling is therefore best for choosing between plausible models for a structure, or for refining a close starting estimate for a structure from, say, x-ray crystallography. Another problem which is often overlooked is the so-called hydration problem, whereby a guess as to the amount of water binding has to be made. This is particularly serious insofar as the sedimentation coefficient is concerned, in that it is not the most sensitive of hydrodynamic functions of conformation (Eisenberg 1976) and is in fact a more sensitive function of molecular weight and molecular hydration. A significant step forward has been the launch of a new routine SOLPRO (Garcia de la Torre et al. 1997) which provides for the prediction of hydration-independent shape functions for a given model.

A complementary approach to bead modelling is to make no assumptions concerning starting estimates and to calculate the shape directly from hydrodynamic measurements. This is called the "ellipsoid" or "whole body" approach (Harding 1989) so called because the investigator instead of approximating the macromolecule as an array of

spheres approximates the macromolecule instead as a smooth whole regular structure – an ellipsoid, or "three dimensional ellipse" characterised by three perpendicular semi-axes $a \geq b \geq c$ (Fig. 1). Of course only simple representations are possible but by combining shape parameters together there are no hydration or uniqueness problems. This approach is best for giving a relatively quick idea of the overall dimensions or shape of a macromolecule in solution.

There are two types of ellipsoid approach: The *ellipsoid of revolution* and the *general triaxial ellipsoid.* The simplest of these (which has been used in one form or another for over half a century, Tanford 1961) is the ellipsoid of revolution in which two of the three semi-axes are equal ($c = b$). Ellipsoids of revolution (Fig. 2) are so-called because they are the shapes formed by rotating an ellipse of semi-axes, *a, b* either about the major (*a*) axis to give a prolate ellipsoid (semi-axes *a, b, b*) or about the minor (*b*) axis to give an oblate ellipsoid (*a, a, b*), both defined by the axial ratio (*a/b*) (where $a \geq b$). One hydrodynamic measurement can uniquely define (*a/b*), after assuming a value for the hydration of the molecule; two hydrodynamic measurements are normally sufficient to define (*a/b*) without assumptions concerning hydration; a third is occasionally necessary to distinguish whether an oblate ellipsoid or prolate ellipsoid is the more appropriate (usually the latter for proteins). In the extremes $a \gg b$ the prolate $\rightarrow$ rod and the oblate $\rightarrow$ disc and the other extreme of $a = b$ is of course a sphere.

The most sophisticated of the whole body approaches is the general triaxial ellipsoid where the restriction of two equal axes $b = c$ is removed. This allows a much greater variety of conformations ranging from rods ($a \gg b = c$), discs ($a = b \gg c$) and tapes ($a \gg b \gg c$) as well as the prolate ($a > b = c$), oblate ($a = b > c$) ellipsoids of revolution and the sphere ($a = b = c$).

All the necessary theoretical developments for applying either of these "ellipsoid" strategies are in place. What has been lacking is a coherent set of easy-to-use algorithms – available on PC as opposed to computer mainframes – which the general user has access to. The launch of the suite of four ELLIPS algorithms in this paper here should address this. All these algorithms along with a related algorithm COVOL – for the accurate prediction of the thermodynamic non-ideality of a macromolecule from its triaxial shape – are available free of charge as indicated at the end of this paper. Table 1 summarises the purpose of each of the four ELLIPS routines.

All four are also available on floppy disk in compiled form. Although ELLIPS2, 3, 4 all use a FORTRAN 77 compiler – the SALFORD (1991) FTN77/486 system – and the NAG (1991, 1992) Graphics and numerical integration routines, these are all inbuilt into the programme and the user does not need his own FORTRAN or NAG compiler. ELLIPS1 (written in QUICK BASIC) is also supplied in compiled form.

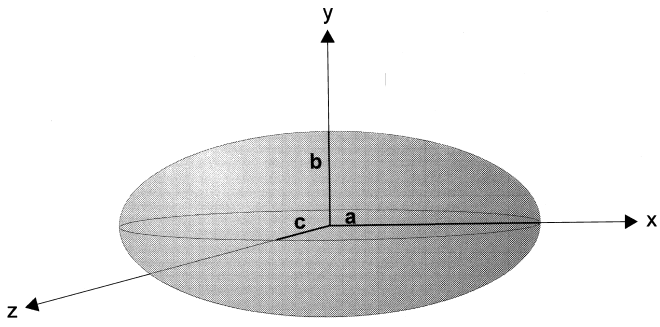

Fig. 1 The general ellipsoid. It has three semi-axes ($a \geq b \geq c$) and its shape is represented by two axial ratios (*a/b, b/c*)

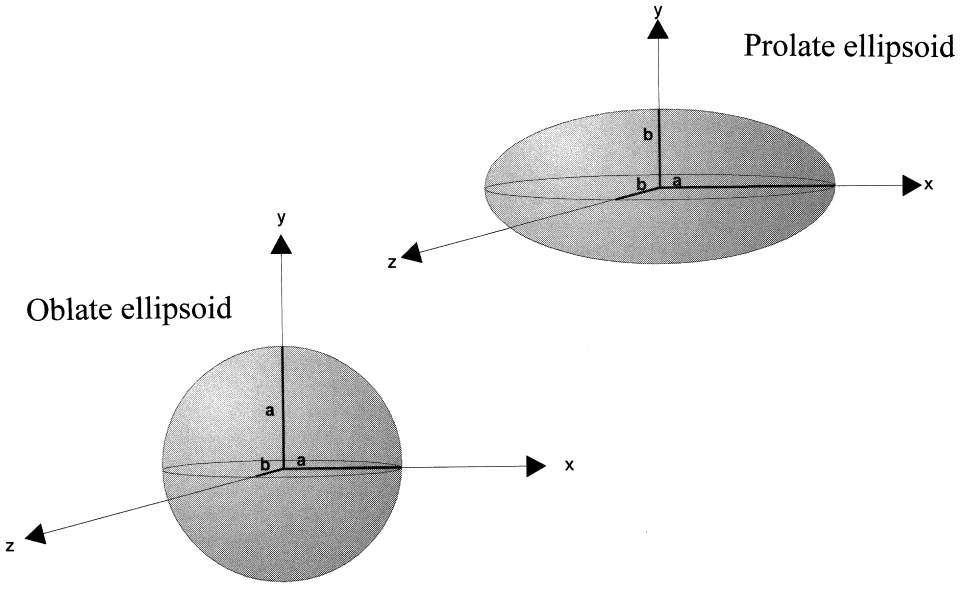

Fig. 2 Ellipsoids of revolution. A prolate ellipsoid has semi-axes (*a, b, b*). An oblate ellipsoid has semi-axes (*a, a, b*). The shapes of both are represented by a single axial ratio (*a/b*), with $a \geq b$

Table 1 The ELLIPS routines

Routine	Language	Model	Purpose
ELLIPS1	QUICKBASIC	Ellipsoid of Revolution	Prediction of axial ratio (*a/b*) (equivalent prolate or oblate ellipsoid of revolution) from user specified shape function
ELLIPS2	FORTRAN 77	General Triaxial Ellipsoid	Evaluates the values of all the hydrodynamic shape functions from user specified (*a*, *b*, *c*) or (*a/b*, *b/c*)[a]
ELLIPS3	FORTRAN 77	General Triaxial Ellipsoid	Evaluates (*a/b*, *b/c*) from combinations of hydration independent shape functions
ELLIPS4	FORTRAN 77	General Triaxial Ellipsoid	Evaluates (*a/b*, *b/c*) from electro-optic decay combined with other hydrodynamic data

[a] Equivalent to SOLPRO (Garcia de la Torre et al. 1997) for bead models

Universal shape functions: hydration dependent and hydration independent

Before we consider each routine in detail we will summarise the hydrodynamic shape functions involved. To be consistent with the bead modelling programme SOLPRO we call these *Universal Shape Functions*. By this we mean each is specifically a function of shape alone (and not volume): it makes no odds what the size is: a Universal shape function will have the same value, it will only depend on the shape. All these universal shape functions have been worked out in terms of the axial ratio (*a/b*) for ellipsoids of revolution and now the two axial ratios (*a/b*, *b/c*) for general ellipsoids. All of these (with the exception of the exclusion volume based shape functions u_{red} and Π) are also available for bead models from SOLPRO (Garcia de la Torre et al. 1997). For all the ellipsoid formulae the user is referred to Harding (1995) and references therein and for all the bead formulae the user is referred to Garcia de la Torre et al. (1997) and references therein. However, the investigator need not concern himself with these: all of these formulae are inbuilt into the ELLIPS routines in the case of ellipsoid modelling and SOLPRO in terms of bead modelling. In terms of the experimental measurement of these Universal shape functions, some require knowledge of the hydration δ (mass in g of H_2O bound per g of dry macromolecule) or hydrated volume V (ml) of the particle, the others do not. The particle volume V is often presented in two equivalent forms:

$$V = v_{\mathrm{s}} \cdot M/N_A \tag{1}$$

where M is the molecular weight or molar mass (g/mol) and N_A is Avogadro's number (6.02205×10^{23} mol^{-1}), and v_{s} is the specific volume (ml/g) of the hydrated macromolecule (volume occupied by the hydrated macromolecule per unit mass of dry macromolecule) or

$$V = (\bar{v} + \delta/\rho_0) \cdot M/N_A \tag{2}$$

where $\bar{v}$ is the partial specific volume (ml/g).

Hydration dependent universal shape functions

Those Universal shape functions requiring knowledge of δ or V for their experimental measurement are:

– Viscosity increment, ν (Simha 1940; Saito 1951)

$$\nu = [\eta]\, M/(N_A V) \tag{3}$$

$\nu = 2.5$ for a sphere (Einstein 1906, 1911)

– Perrin function, P (Perrin 1936)

$$P = (f/f_0)/\{1 + \delta/(\bar{v}\rho_0)\}^{-1/3} \tag{4}$$

where (f/f_0), the frictional ratio (Tanford 1961) is related to the sedimentation coefficient $s^0_{20,\mathrm{w}}$ by

$$(f/f_0) = M(1-\bar{v}\rho_0)/(N_A \cdot 6\pi\eta_0 s^0_{20,\mathrm{w}})\,(4\pi N_A/3\bar{v}M)^{1/3} \tag{5}$$

or the translational diffusion coefficient $D^0_{20,\mathrm{w}}$ by

$$(f/f_0) = \frac{k_B T}{6\pi\eta_0}\left(\frac{4\pi N_A}{3\bar{v}M}\right)^{1/3} \cdot \frac{1}{D^0_{20,\mathrm{w}}} \tag{6}$$

where $T = 293.15$ K, η_0 is the viscosity of water at 293.15 K (0.010 Poise), ρ_0 is the density of water at 293.15 K (0.99823 g/ml) and k_B is Boltzmann's constant (1.3807×10^{-16} erg. K^{-1}). $P = 1$ for a sphere (Perrin 1936)

– Reduced excluded volume, u_{red}

$$u_{\mathrm{red}} = u/V = \{2BM^2 - Z^2/2I\}/(N_A V) \tag{7}$$

u is the excluded volume (ml), B is the second thermodynamic (or "osmotic pressure") virial coefficient, from osmotic pressure, light scattering or sedimentation equilibrium measurements, Z is the valency of the macromolecule, measurable by titration (Jeffrey et al. 1977) and I is the ionic strength of electrolyte in the solvent (mol/ml). At sufficient ionic strengths, the $Z^2/2I$ term becomes negligible compared with $2BM^2$. Of course for uncharged macromolecules and proteins at the isoelectric point $Z = 0$. $u_{\mathrm{red}} = 8$ for a sphere (Tanford 1961)

– Harmonic mean rotation relaxation time ratio:

$$\tau_h/\tau_0 = \{k_B T/\eta_0 V\} \cdot \tau_h \tag{8}$$

where τ_h (sec) is the harmonic mean rotational relaxation time, traditionally measured using steady state fluores-

cence depolarisation methods (Van Holde 1971, 1985), and τ_0 the corresponding value for a spherical particle of the same volume:

$$\tau_0 = \eta_0 V/k_B T \tag{9}$$

In earlier representations a factor of 3 was introduced because the rotational relaxation time was referred to on a dielectric dispersion basis (compensated for in the equations for steady state anisotropy depolarisation) although this is no longer necessary – compare van Holde (1971) with van Holde (1985). This is further discussed in Garcia de la Torre et al. (1997). $\tau_h/\tau_0 = 1$ for a sphere (Perrin 1934).

– Time-resolved rotational (fluorescence depolarisation anisotropy) relaxation time ratios

$$\tau_k/\tau_0 = \{k_B T/\eta_0 V) \cdot \tau_k \tag{10}$$

For ellipsoids of revolution $k = 1–3$; for general ellipsoids and general particles, $k = 1–5$. $\tau_k/\tau_0 = 1$ for a sphere (Small and Isenberg 1977).

– Reduced electro-optic decay constants

$$\theta_i^{\text{red}} = (\eta_0 V/k_B T) \cdot \theta_i \tag{11}$$

where θ_i are the electric birefringence or electric dichroism decay constants. For ellipsoids of revolution that are homogeneous i.e. where the geometric axis of symmetry coincides with the electrical axis, $i = 1$. For general ellipsoids that are homogeneous i.e. where the geometric axes coincide with the electrical axes, $i = 2$, termed "+" and "–" (Ridgeway 1966, 1968); for general particles $i = 1–5$ (Wegener et al. 1979). For a sphere, $\theta_i^{\text{red}} = 0.66667$.

Hydration independent universal shape functions

Those Universal shape functions *NOT* requiring knowledge of δ or V for their experimental measurement are:

– Scheraga-Mandelkern (1953) parameter

$$\beta \equiv \frac{[\eta]^{1/3}\eta_0}{M^{2/3}(1-\bar{v}\rho_0)100^{1/3}} = \frac{N_A^{1/3}}{(16200\pi^2)^{1/3}}\frac{v^{1/3}}{P}. \tag{12}$$

The β parameter is unfortunately very insensitive to shape, and Eq. (12) is used more as an equation of consistency, or for measuring M from sedimentation velocity and viscosity measurements. $\beta = 2.1115 \times 10^6$ for a sphere

– Pi function (Harding 1981a)

$$\Pi = \{2BM/[\eta]\} - \{Z^2/2IM[\eta]\} = u_{\text{red}}/v \tag{13}$$

with the 2nd term (a good approximation of the charge contribution for polyelectrolytes) $\rightarrow 0$ at sufficient values of I, and of course $= 0$ for uncharged macromolecules or proteins at the isoelectric point ($Z = 0$). $\Pi = 3.2$ for a sphere

– Wales-van Holde (1954; Rowe 1977) parameter

$$R = k_s/[\eta] = 2(1+P^3)/v \tag{14}$$

where k_s (ml/g) is the concentration dependence parameter of the sedimentation coefficient in the limiting relation $s_{20,w} = s^0_{20,w}(1-k_s c)$ or $1/s_{20,w} = \{1/s^0_{20,w}\}(1+k_s c)$. Although the theory behind Eq. (14) is less rigorous than that for Π (because of the greater complexity of "hydrodynamic" as opposed to "thermodynamic equilibrium" based non-ideality), it does have a strong experimental basis (Creeth and Knight 1965; Rowe 1977, 1992). To apply k_s in this way it is important that charge contributions to k_s are absent or if the macromolecule is a polyelectrolyte, charge contributions are suppressed by working in a solvent of sufficient ionic strength. $R = 1.6$ for a sphere.

– Reduced radius of gyration function G (Harding 1987)

$$G = R_g^2 \cdot \{4\pi N_A/(3vM)\}^{2/3} \tag{15}$$

where R_g is the radius of gyration (cm), from light scattering, x-ray scattering or neutron scattering measurements. Provided that there is no difference in scattering density of the surface bound solvent compared with free solvent, and there is no significant internal swelling of the macromolecule, we can take, to a good approximation, $v \sim \bar{v}$. Otherwise G must also be treated as another hydration dependent parameter. $G = 0.6$ for a sphere.

– Psi-function (Squire 1970)

$$\Psi = \left(\frac{4\pi\eta_0}{3kT}\right)^{1/3}\frac{M(1-\bar{v}\rho_0)}{6\pi\eta_0 N_A s^0_{20,w}}\left(\frac{1}{\tau_h}\right)^{1/3} = P/(\tau_h/\tau_0)^{1/3} \tag{16}$$

For spheres, $\Psi = 1$. It should be stressed that Ψ, like β is very insensitive to shape and is more use as an equation of consistency. $\Psi = 1$ for a sphere.

– Lambda function (Harding 1980a)

$$\Lambda = (\eta_0 \cdot [\eta] \cdot M)/(N_A \cdot k_B T \tau_h) = v/(\tau_h/\tau_0) \tag{17}$$

For spheres, $\Lambda = 2.5$.

– Lambda and psi functions for each relaxation time (Garcia de la Torre et al. 1997)

$$\Lambda_k = (\eta_0 \cdot [\eta] \cdot M)/(N_A \cdot k_B T \tau_k) = v/(\tau_k/\tau_0) \tag{18}$$

$$\Psi_k = \left(\frac{4\pi\eta_0}{3kT}\right)^{1/3}\frac{M(1-\bar{v}\rho_0)}{6\pi\eta_0 N_A s^0_{20,w}}\left(\frac{1}{\tau_k}\right)^{1/3} = P/(\tau_k/\tau_0)^{1/3} \tag{19}$$

($k = 1–3$ for ellipsoids of revolution; 1–5 for general triaxial ellipsoids). For spheres, $\Psi_k = 1$ and $\Lambda_k = 2.5$.

– Electro-optic delta and gamma (Harding and Rowe 1983) functions

$$\delta_i = (6\eta_0/N_A k_B T)[\eta] M \cdot \theta_i = 6\theta_i^{\text{red}} v \tag{20}$$

$$\gamma_i = (M^3(1-\bar{v}\rho_0)^3/(27N_A^3 k_B T \pi^2 \eta_0^2 s^{0\,3}_{20,w})) \cdot \theta_i = 6\theta_i^{\text{red}} P^3 \tag{21}$$

(for homogeneous ellipsoids of revolution $i = 1$ and for homogeneous triaxial ellipsoids, $i =$ "+" and "–"). For spheres, $\gamma_i = 1.0$ and (the more sensitive) $\delta_i = 2.5$.

ELLIPS1

Aim. Prediction of axial ratio (*a/b*) (equivalent prolate or oblate ellipsoid of revolution) from a user specified value for a shape function.

Description. ELLIPS1 is based on simple ellipsoid of revolution models (where two of the three axes of the ellipsoid are fixed equal to each other); if the user types in a value for a shape function from sedimentation or other types of hydrodynamic measurement, it will return a value for the axial ratio of the ellipsoid. The question an experimenter wishes to address usually is not "what is the shape function for a specified value of the axial ratio *a/b*?" but rather "what is the axial ratio *a/b* for my macromolecule specified by my (Universal) shape function which I have experimentally measured?". Unfortunately, although there are exact analytical formulae linking each shape function with *a/b* (Harding and Cölfen 1995), the reverse is not true: inversion is analytically impossible. In the past, the investigator has had to interpolate from tables of data (Harding and Cölfen 1995) or from graphs to obtain his *a/b* from *P* (obtained from the sedimentation or diffusion coefficient), ν (from the intrinsic viscosity), *R*, Π, Λ etc. Harding and Cölfen (1995) have provided a simple polynomial based inversion procedure giving *a/b* versus the various Universal shape functions to an acceptable degree of accuracy (i.e. to better than the precision of the measurement, which is normally no better than a few percent) and within the limits of the assumption that an ellipsoid of revolution shape is a reasonable approximation of a macromolecule. The polynomial formulae used is simply

$$(a/b) = a_0 + \sum_i a_i x^i \tag{22}$$

with $x = P, \nu, \beta, R, \Pi$ or Λ and for both prolate and oblate ellipsoids in each case.

The fits are split into 3 ranges: Range 1 ($1.1 < a/b < 2.0$); Range 2 ($2 < a/b < 10$) and Range 3 ($10 < a/b < 100$) and a polynomial of degree 7 or less is necessary to give a good to excellent fit (by this we mean the fit is at least as good as the precision to which the function can be measured – usually to no better than a few percent). The only exceptions are the relatively uninteresting cases of prolate β range 1, oblate β range 2, oblate β range 3 and oblate Π range 1: in these cases the ELLIPS routine returns the warning "POOR FIT: USE GRAPHICAL INTERPOLATION". Some functions do not distinguish between prolate and oblate ellipsoids: in this case the *a/b* values for both cases are returned and the user has to choose between the two. For proteins the prolate usually gives the closest representation. All the coefficients in Eq. (22) for each function for each range and for both prolate and oblate ellipsoids are given in Harding and Cölfen (1995), although again, the user need not concern himself with these since they are inbuilt into the compiled program.

Output. Figure 3 gives the user screens for an example run on the protein egg albumin using two of the hydration independent universal shape functions (a) the Pi function and (b) the *R*-function. In the case of (a), $\Pi = 3.18$ {from $BM = 5.55$ ml/g and $[\eta] = 3.49$ ml/g ($Z^2/2I \sim 0$)}: this gives an $a/b \sim 1.5$ but after experimental error it could be anything between 1 and 3. However, use of the *R* function (b), which is very sensitive for particles of low asymmetry confirms this value for the axial ratio for ovalbumin (egg albumin). Interestingly this finding of 1981 (Harding 1981b) was confirmed from the crystal structure (Fig. 3c) published 10 years later (Stein et al. 1991). More interestingly the axial ratio of a size 3 U.K. standard egg (Fig. 3d) is also ~1.5.

Although the MSDOS (Microsoft, Redmond, Washington, USA) routine can only draw a crude 2D representation of the structure, CORELDRAW (Corel Co., Ontario) will give a 3D presentation (Fig. 3c).

ELLIPS2

Aim. Evaluates the values of *all* the Universal hydrodynamic shape functions from user specified axial dimensions (*a*, *b*, *c*) or axial ratios (*a/b*, *b/c*) for the macromolecule as modelled by a general triaxial ellipsoid.

Description. ELLIPS2 is essentially analagous to SOLPRO (Garcia de la Torre et al. 1997) in that from a given structure (as represented by an array of beads in SOLPRO or as a general triaxial ellipsoid in ELLIPS2) the *complete set* of Universal shape functions is returned. ELLIPS2 also evaluates the excluded volume shape functions u_{red} and Π, unavailable on SOLPRO. It is based on an earlier version of the program written in FORTRAN for mainframe computer (Harding 1982). The earlier version also lacked u_{red} and Π for the simple reason these hadn't been worked out for triaxial allipsoids until 1985 (Rallison and Harding 1985).

Most of the universal shape functions involve one or more of 10 different elliptic integrals (called alpha 1 … alpha 10 – see Harding 1995) of the form

$$\int_0^\infty f(x)\,\mathrm{d}x \tag{23}$$

There is still no packaged or published numerical routine available for integrals of this type; only routines of the form

$$\int_A^B f(x)\,\mathrm{d}x \tag{24}$$

where *A* can be zero but *B* must have a finite value, specifiable by the user. ELLIPS2, like its mainframe forerunner uses a NAG (1991) quadrature routine, in this case D01AJF. *B* can be set as high as the programmer wishes: higher values take more computer time though, and in practice, satisfactory convergence of the integral is obtained if *B* is set to 10^6 for 9 of the intergrals (alpha1 – alpha9) and 10^8 for the remaining alpha10. It is also found that each integration is best split into 2 parts: part 1 (between $A = 0$ and $B = 100$) and part 2 ($A = 100$, $B = 10^6$ or 10^8). D01AJF

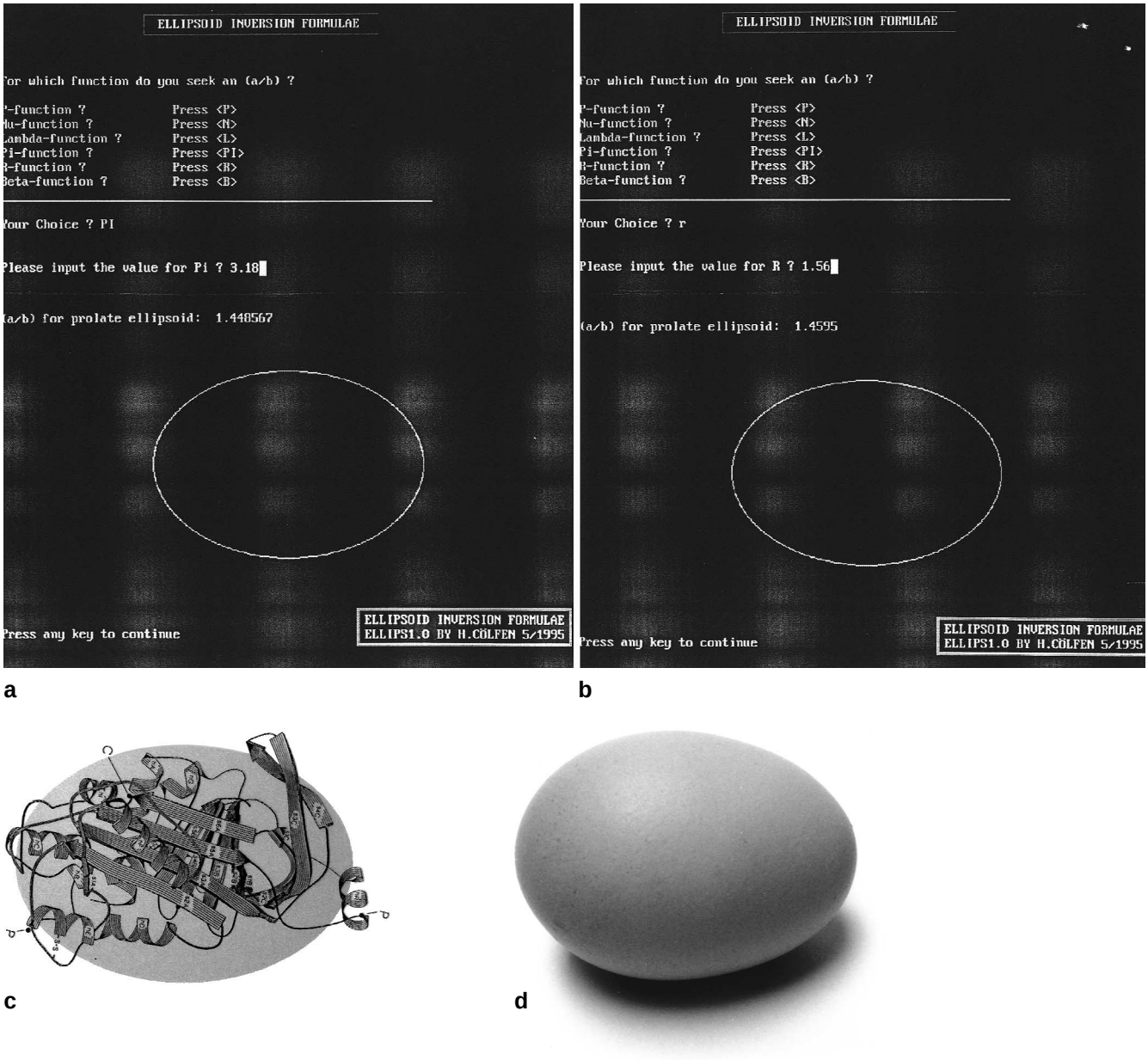

Fig. 3 ELLIPS1 output screens for the determination of the axial ratio (a/b) for ovalbumin (egg albumin) using the Universal shape functions (**a**) Π or (**b**) R. (**c**) Prolate ellipsoid of $a/b = 1.5$ drawn by the WINDOWS based CORELDRAW enclosing the crystal structure line-drawing of Stein et al. (1991). (**d**) A standard egg of $a/b \sim 1.5$

also requires the following settings: epsabs = 0.0; epsrel $= 0.5 \times 10^{-4}$. u_{red} and Π also require numerical integrations, this time for two double integrals of the form

$$\int_0^{\pi/2} \int_0^{\pi/2} f(x_1, x_2)\, dx_2\, dx_1 \qquad (25)$$

where $f(x_1, x_2)$ is a complicated transcandental function in both cases. Analytical solutions are not possible, so the NAG routine D01DAF is employed. This quadrature routine performs a two-dimensional integral and has the setting absacc $= 1.0 \times 10^{-5}$.

Output. Fig. 4(a) gives the output data for an (a/b, b/c) = (1.23, 1.52), based on the crystallographic axial dimensions of $43 \times 35 \times 23$ Å for myoglobin (Kendrew et al. 1958) (Fig. 4b). Besides giving the excluded volume based u_{red} and Π, the Λ_k and Ψ_k ($k = 1 \rightarrow 5$) shape parameters are given, instead of the less useful "rotational frictional ratios".

A spin-off of this routine is another called COVOL (which will be described elsewhere): this uses the predicted value of u_{red} to evaluate the second theromodynamic virial coefficient B, as an aid to the prediction of the non-ideality terms which appears in analyses of molecular interaction phenomena using light scattering or sedimentation equilibrium.

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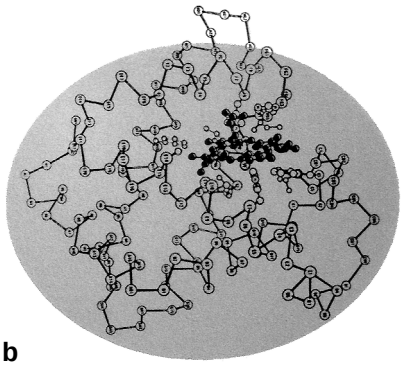
*************************************************
              ELLIPS2 Output Data
*************************************************

(a/b, b/c) =     1.2300    1.5200
Viscosity increment, nu =     2.7290
Perrin function, P =     1.0270
Corresponding hydration independent functions:
Wales-van Holde, R =     1.5268
Scheraga-Mandelkern, 10**-6xbeta =     2.1171
Functions based on the 2nd virial coefficient:
Reduced excluded volume, u_red     8.6713
Corresponding hydration independent function:
Pi function =     3.1775
G function (from radius of gyration) =     0.6784
Reduced electro-optic decay constants:
     Theta+ =     0.1622
     Theta- =     0.1336
Corresponding hydration independent functions:
Delta+ =     2.6554
Delta- =     2.1883
Gamma+ =     1.3921
Gamma- =     1.1473
Harmonic mean rotational relaxational time ratio:
tau_h/tau_0 =     1.1268
Corresponding hydration-independent functions:
PSI (Squire-Himmel) function =     0.9869
LAMBDA =     2.4219
Fluorescence anisotropy relaxation time ratios:
tau_1/tau_0 =     1.0316
tau_2/tau_0 =     1.1523
tau_3/tau_0 =     1.2119
tau_4/tau_0 =     1.2471
tau_5/tau_0 =     1.0277
Corresponding hydration independent functions:
lambda_1 =     2.6455
lambda_2 =     2.3684
lambda_3 =     2.2517
lambda_4 =     2.1883
lambda_5 =     2.6554
psi_1 =     1.0164
psi_2 =     0.9796
psi_3 =     0.9633
psi_4 =     0.9541
psi_5 =     1.0177
*************************************************
```

a



b

Fig. 4 **a** ELLIPS2 output for a macromolecule of axial ratios (*a/b*, *b/c*) = (1.23, 1.52) (myoglobin). **b** Ellipsoidal representation of the crystal structure of myoglobin (*a/b*, *b/c*) = (1.23, 1.52) {based on axial dimensions of 45×35×23 Å (Kendrew et al. 1958) and line drawing of Dickerson and Geis (1969)}

ELLIPS3

Aim. Evaluates the tri-axial shape of a macromolecule (*a/b*, *b/c*) using two possible combinations of Universal *hydration independent* shape functions:

(a) Π (from the second virial coefficient and intrinsic viscosity measurements) with G (from radius of gyration measurements), or
(b) Λ (from the harmonic mean rotation relaxation time τ_h and intrinsic viscosity $[\eta]$ measurements) with R (from the concentration dependence sedimentation term k_s and intrinsic viscosity measurements).

Description. Whereas an (*a/b*, *b/c*) specifies uniquely values for all the hydrodynamic shape functions, the reverse is unfortunately not true: measurement of P, R, Λ etc. does not uniquely fix (*a/b*, *b/c*) but rather gives a line solution of possible values: Fig. 5 shows the all the possible values of (*a/b*, *b/c*) for $\nu = 3.8016$ and all the possible values of (*a/b*, *b/c*) for $P = 1.1302$. However, it can be seen immediately from Fig. 5 that a unique solution for (*a/b*, *b/c*) could be fixed from the intersection of the "ν-line solution" with the "P-line solution": … at least in principle … from the graphical intersection of these two lines {in this case (*a/b*, *b/c*) = (2.0, 2.0)}. This particular combination is however useless in practical terms since the intersection is so shallow (after allowance for experimental error there is indeed no intersection) and also both P and ν require for their measurement knowledge or a guess of the hydration parameter δ. One of us (Harding 1995) has explored the variety of possible combinations of those Universal shape parameters which do not need δ for their experimental measurement. Based on this latter criterion, along with orthogonality of the graphical intersection and sensitivity to shape (and insensitivity to experimental error), ELLIPS3

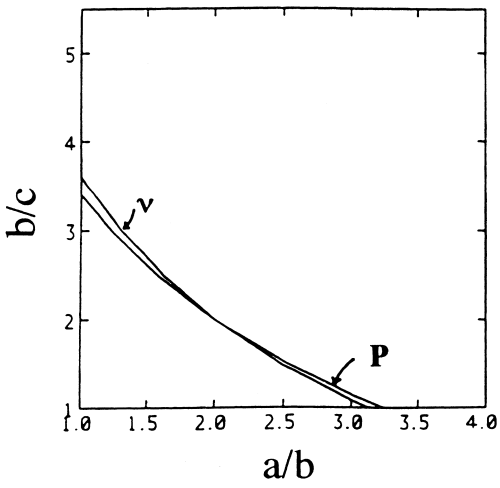


Fig. 5 Triaxial (*a/b*, *b/c*) plot showing that a single value of a Universal shape function corresponds to a range (specified by a line) of possible values of (*a/b*, *b/c*). Two examples shown: $\nu = 3.8016$ and $P = 1.1302$

provides for 2 of the most promising combinations. The first is a combination of Π (from the second thermodynamic virial coefficient and intrinsic viscosity) with G (from x-ray, neutron or light scattering), the second is a combination of Λ (from steady state fluorescence depolarization measurements and the intrinsic viscosity) with R (from the concentration dependence sedimentation term k_s and intrinsic viscosity measurements).

ELLIPS3 uses as its basis the function calculation routine of ELLIPS2 except that a whole array of such values are evaluated in the (*a/b*, *b/c*) plane (a matrix of 40×40 values). A Contour plotting routine (J06GAF in the NAG FORTRAN Library) interpolates between these matrix points and can plot the Π, G, Λ and R functions (or any other of the universal shape functions if the programmer so decides) in the (*a/b*, *b/c*) plane. In the pre-compiled version available for ELLIPS3, the user does not have to concern himself with the details behind the program if he is happy with either the $\Pi - G$ or $\Lambda - R$ combinations.

Output. Figure 6a shows an example of running ELLIPS3 for the $\Pi - G$ combination: the user selects the "Pi and G plot" option and has a choice of entering Π and G directly, or if these have not been evaluated from the raw data, has the option of entering BM, and $[\eta]$ (and, if necessary, a charge correction) for Π, and/or R_g and the specific volume, v for G. The user specifies the experimental uncertainty in both functions and then the program gives the user a choice of axial ratio ranges for his plot. If no prior information about macromolecular shape is known the user is recommended to choose a wide range (such as $1 \rightarrow 100$) in

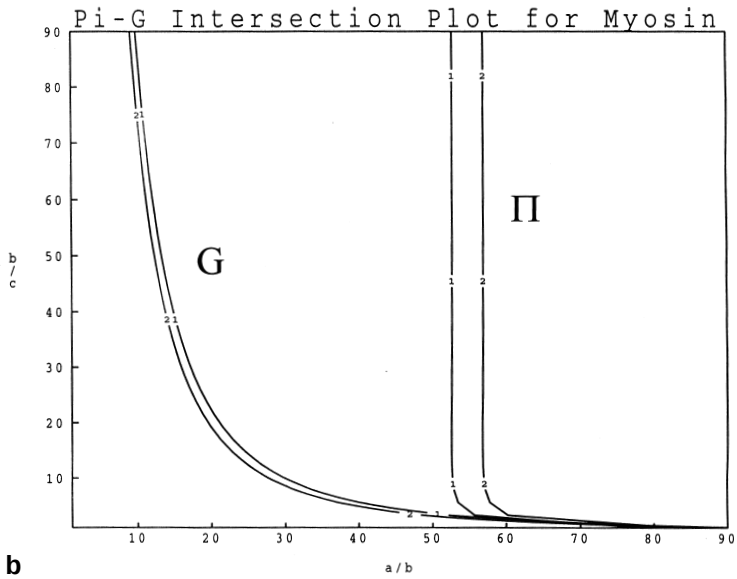
Fig. 6a–c ELLIPS3 output. Determining the triaxial shape (*a/b*, *b/c*) of a macromolecule using the Pi-G intersection method. The example shown is for myosin (Harding 1987 and references therein) (**a**) running the programme (**b**) Pi-G output plot (**c**) CORELDRAW triaxial shape of (*a/b*, *b/c*) = (80, 1)

```
C:\FORTRAN>run77 ellips3
 Choose type of plot:-
    1. Pi and G plot;
    2. Wales-van Holde and lambda plot.
 Choose option:
1
 Enter Pi directly (1) or via formula (2)?
 Method of entry:
1
 Pi
0.47
 Uncertainty in pi
0.141

 Enter G directly (1) or via formula (2)?
 Method of entry:
1
 G
82
 Uncertainty in G
4.1
 Title: Pi-G Intersection Plot for ...
'Myosin'

 Four range options available:-
    1. Range 1 to 2 in steps of 0.025;
    2. Range 1 to 10 in steps of 0.25;
    3. Range 1 to 100 in steps of 2.5;
    4. Own range
 Choose option:
4
 a/b start
1
 a/b end
90
 b/c start
1
 b/c end
90
```

a



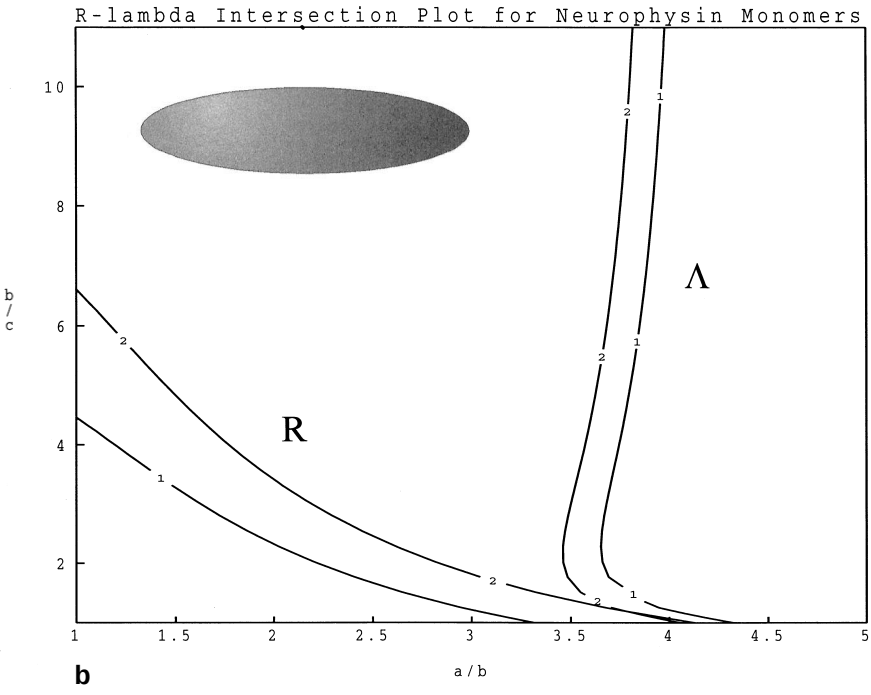
b

c

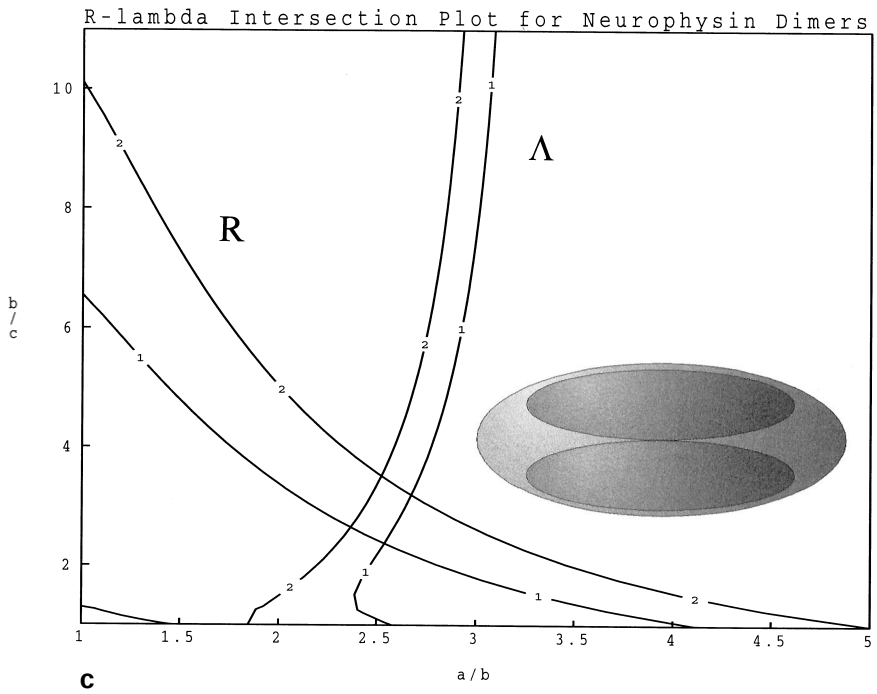
```
FILE ellips3.EXE
Creating run file C:\FORTRAN\ELLIPS3.EXE
C:\FORTRAN>run77 ellips3
 Choose type of plot:-
    1. Pi and G plot;
    2. Wales-van Holde and lambda plot.
 Choose option:
2
 Enter R directly (1) or via formula (2)?
 Method of entry:
1
 R
1.18
 Uncertainty in R
0.059

 Enter lambda directly (1) or via formula (2)?
 Method of entry:
1
 lambda
3.16
 Uncertainty in lambda
0.0632
 Title: R-lambda Intersection Plot for ...
'Neurophysin Monomers'
 Four range options available:-
    1. Range 1 to 2 in steps of 0.025;
    2. Range 1 to 10 in steps of 0.25;
    3. Range 1 to 100 in steps of 2.5;
    4. Own range
 Choose option:
4
 a/b start
1
 a/b end
5
 b/c start
1
 b/c end
11
```

a



b



c

Fig. 7a–c ELLIPS3 output. Determining the triaxial shape (*a/b*, *b/c*) of a macromolecule using the *Λ*-R intersection method. The example shown is for neurophysin (Harding and Rowe 1982 and references therein) (**a**) running the programme (**b**) *Λ*-R plot for neurophysin monomers (inset – CORELDRAW ellipsoid of (*a/b*, *b/c*) = (4,1)) (**c**) *Λ*-R plot for neurophysin dimers yielding (*a/b*, *b/c*) = (2.5, 2.85) {inset shows likely mode of assembly of the monomers}

the first instance, and then replot at higher resolution in the range of interest. The myosin example is given to show that, without any prior assumptions about the conformation (rod, sphere disc, etc.) and despite hinge regions of limited flexibility in the molecule and the presence of the S1 protrusions at one end, the overall gross conformation of a rod shape of axial ratio ~80 : 1 is returned.

The second example of ELLIPS3 (Fig. 7) is for the $\Lambda - R$ plot applied to the neural protein neurophysin: details of how $[\eta]$, τ_h and k_s was extracted for both monomers and dimers is described in Nicolas et al. (1981) and also Harding and Rowe (1982). Figure 7a shows the running of ELLIPS3 for $\Lambda - R$ and Fig. 7b and c the output for the monomers and dimerised form of the protein, with the latter clearly indicating that when the 4 : 1 prolate ellipsoidal monomers dimerise they must do so in a side-by-side as opposed to end-on process.

ELLIPS4

Aim. Evaluates the tri-axial shape of a macromolecule (*a/b, b/c*) from electro-optic decay based Universal shape functions combined with other hydrodynamic data.

Description. Rotational hydrodynamic shape functions, based around rotational diffusion measurements are attractive for determining the shapes of macromolecules in solution since they are generally more sensitive functions of shape compared to other shape functions. This sensitivity comes however at a price because they are generally more difficult to measure. A lot of the difficulty centres around resolution of multi-exponential decay functions. Electro-optic measurements are more attractive than time-resolved fluorescence depolarization anisotropy measurements in the sense that for homogeneous triaxial ellipsoids at least, there are only two exponential terms to resolve (the decay constants or reciprocal relaxation times θ_+ and θ_-) as opposed to five ($\tau_1 - \tau_5$):

$$\Delta n = A'_+ \exp(-6\theta_+ t) + A'_- \exp(-6\theta_- t)$$

(Ridgeway 1968, Harding and Rowe 1983) where Δn is the birefringence or dichroism (often expressed as "optical retardation" in degrees) at time t after the aligning electric field has been switched off. A practical problem with electro-optic decay methods is the potential local heating effects from the high electric fields used, especially if the experiments are conducted in solutions of high ionic strength: the investigator is advised to consult an article by Porschke and Obst (1991) describing how these effects can be minimised.

After eliminating hydration (via e.g. combination with $[\eta]$) to give the Universal hydration independent shape functions δ_+ and δ_- and graphical combination with another Universal hydration independent shape function such as *R* (Harding and Rowe 1983) or Π (Harding 1986) the triaxial shape as represented by the two axial ratios (*a/b, b/c*) can be evaluated. Resolution however of even two exponential terms is not easy, particular for globular macromolecules where θ_+ and θ_- are similar (Small and Isenberg 1977), and no-matter what form of mathematical deconvolution is applied, whether it be non-linear least squares or more refined types of analysis (Harding 1980b; Jost and O'Konski 1978; O'Connor et al. 1979; see Johnsen and Brown (1992) for the analagous problem in dynamic light scattering analysis of polydisperse systems). In our hands (Harding 1980b; Harding and Rowe 1983) we have found a more reliable method of extraction is to use another hydrodynamic function as a constraining parameter in the analysis of the electro-optic decay data: in this way the problem is reduced to one of four variables (A'_+, θ_+, A'_-, θ_-) to one of three (A'_+, A'_-, *a/b*) since *a/b* will specify, by the constraining function a unique value for *b/c* (and hence θ_+, θ_-). ELLIPS4 has been written to facilitate this procedure for PC based on an earlier non-interactive version of the programme written for mainframe computer (Harding 1980b, 1983). Its use is best illustrated by application to synthetic data (with error) generated for a macromolecule "Protein 1" (Harding and Rowe 1983) which includes the following characteristics: (*a/b, b/c*) = (1.5, 1.5); M = 71744 Da; $[\eta]$ = 2.74 ml/g, and the following electro-optic decay parameters: = A'_+ = 0.07, A' = 0.05, $\theta_+ = 5.81538 \times 10^6$ s^{-1}, $\theta_- = 4.15646 \times 10^6$ s^{-1}, T = 293.15 K, η_0 = 0.01 Poise. Figure 8 shows the electro-optic decay for this based on expected error (standard deviation) of ±0.1 degrees (optical retardation) or ±0.0017 rads random normal error on the decay data.

With ELLIPS4 the user puts his electro-optic decay data (Δn versus t) into a data file which is read in. The user also has to specify values for $[\eta]$ (ml/g), the molecular weight M (Da), the solvent viscosity (Poise) and temperature (K) at which the electro-optic measurements were made. The user also needs to specify the coordinates of a line of (*a/b, b/c*) values (based on measurement of *R*, Π or some other hydration independent Universal shape function) in a second data file: Fig. 9 shows such a constraining line of allowed (*a/b, b/c*) values for "Protein 1" which has an *R*-function value of 1.479. This constrains each iteration of (*a/b, b/c*) and hence θ_+, θ_-, to work along the line specified by the constraining function, since each value of (*a/b, b/c*) specifies a value for δ_+ and δ_- – (worked out using the same procedure involving the NAG routine D01AJF used in ELLIPS2 and 3) which, combined with the user entered values for T, η_0, $[\eta]$ and M gives the θ_+, θ_- for each iteration. This reduces the risk of the fitting routine falling into subsidiary minima. When the minimum of the least squares procedure has been formed ELLIPS4 successfully returns *a/b*, its corresponding value of *b/c* and the pre-exponential factors A'_+ and A'_-. The programme runs automatically 4 times using successively the 4 different values of *a/b* entered from the constraining function (excluding the first and last data points) as starting estimates for *a/b*: this provides a further check against the dangers of subsidiary minima. For the starting estimates for A'_+ and A'_- the routine automatically takes these as $\Delta n_{max}/2$. The routine has inbuilt various error warnings concerning the reliability of each estimation. If no error warning is returned the result

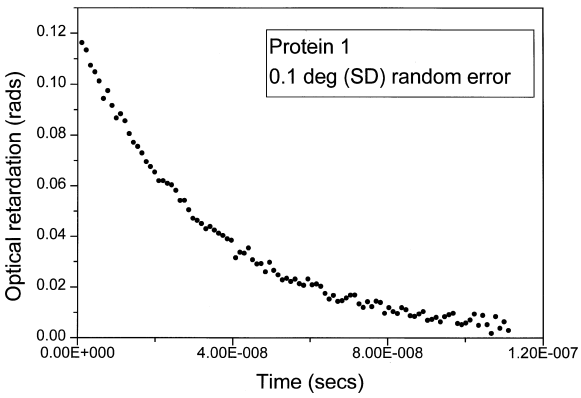


Fig. 8 Electro-optic decay (expressed as the decay of optical retardation with time, t [s]). Synthetic data shown corresponding to a protein of true (*a/b, b/c*) = (1.5, 1.5) {"Protein 1" of Harding and Rowe 1983}. The electro-optic data is fed in as a date-file into ELLIPS4

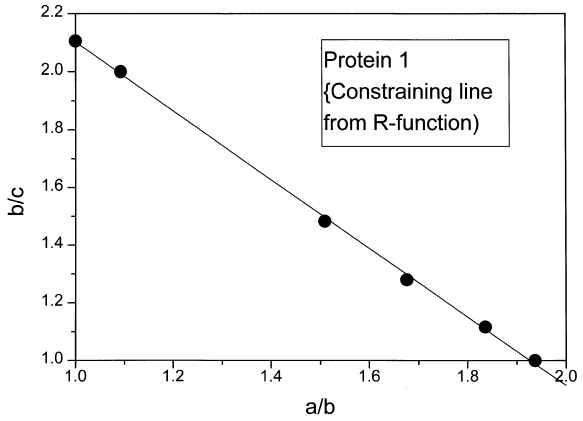


Fig. 9 ELLIPS4 constraining data extraction. The user takes his determined value of R or other suitable constraining function (Π, G, Λ etc.) and plots the line of corresponding values of (a/b, b/c) using ELLIPS3 (ELLIPS3 gives 2 lines which allow for experimental error, both of which can be used in successive runs using ELLIPS4. It also plots 2 lines for a 2nd function which should be entered = 0). The user then reads off 6 (a/b, b/c) coordinates from this line which can be either entered as a 2nd data file into ELLIPS4, or instead entered at run-time

for the evaluation from a particular starting point should be reliable.

Output. Figure 10 shows (a) the running of the programme and (b) the output for a run on the data of Fig. 8 for Protein 1, which returns a value for (a/b, b/c) ~ (1.62, 1.34) – i.e. to within two tenths of an axial ratio unit of the true axial ratios. In practical terms however, the user is advised to (i) repeat the whole operation several times with various cut-off times for the decay data (at longer times the signal/noise data gets progressively worse, on the other hand more information concerning the slower relaxation time, or larger decay constant, θ_+ is contained in this region) to be certain of no subsidiary-minima problems (ii) repeat the operation allowing for experimental error in the constraining function (iii) check for any concentration dependence of the returned parameters: and extrapolate if necessary to zero concentration (Riddiford and Jennings 1967). These and other features have been extensively explored with the earlier mainframe version of the programme (Harding 1980b; Harding 1983; Harding and Rowe 1983). A possible area of further improvement includes the additional constraint that $A'_+ + A'_- = \Delta n_{max}$ although this may cause problems if the data is noisy and the $t = 0$ position is not precisely defined.

Concluding comment

All the routines ELLIPS1, 2, 3 and 4 are available on disk or via email/ftp as explained below and all should be simple to use. The user ultimately however has to decide just how far he wants to take his hydrodynamic conformation determinations on a macromolecule, assuming it is fairly rigid (if the molecule fails this criterion then there are other

a

```
C:\FORTRAN>run77 ellips4
 Name of output file -
'prot1.out'
 Name of macromolecule -
'Protein 1  0.1 deg (SD) random error on electro-optic data'
 Name of decay data (t(i), opt. retardation (i)) file -
'rough.txt'
 Enter temperature (in K) of the electro-optic measurements -
293
 Enter solvent viscosity (in Poise) e.g. ~ 0.01
0.01
 Enter intrinsic viscosity (in ml/g) of macromolecule -
2.7
 Enter molecular weight (in Da) of macromolecule -
72000
 Input SIX constraining co-ordinate points (a/b, b/c) from keyboard (1) or fil
(2)?
2
 Name of input file -
'prot1.in'
```

b

```
*******************************************
          ELLIPS4 Output Data
*******************************************

Protein 1  0.1 deg (SD) random error on electro-optic data

Constraining co-ordinates:
   a/b       b/c
( 1.000,    2.106)
( 1.092,    2.000)
( 1.509,    1.483)
( 1.676,    1.280)
( 1.836,    1.116)
( 1.937,    1.000)

Temperature  293.000 K
Solvent viscosity  0.01000 Poise
Intrinsic viscosity    2.700 ml/g
Molecular weight  0.72000E+05 Da

From a starting estimate of a/b =  1.09200
Best least squares value =  0.000258733747
a/b =  1.62197 A+ =  0.058319855861 A- =  0.061743343049


From a starting estimate of a/b =  1.50900
Best least squares value =  0.000258733747
a/b =  1.62199 A+ =  0.058319708923 A- =  0.061743502221


From a starting estimate of a/b =  1.67600
Best least squares value =  0.000258733747
a/b =  1.62198 A+ =  0.058319756193 A- =  0.061743450515


From a starting estimate of a/b =  1.83600
Best least squares value =  0.000258733747
a/b =  1.62199 A+ =  0.058319656529 A- =  0.061743559225


*************************************************
Optimum best least squares value =  0.000258733747
          (a/b, b/c) =  1.622,  1.343
*************************************************



In case of error output:
TYPE 2 or 3: No convergence - discard this result
TYPE 4: (unlikely) Overflow - discard this result
TYPE 5-8: some doubt about this result.  The higher the error no. the
greater the doubt.  TYPE 5 means this value is almost certainly reliable;
TYPE 8 means this result is very doubtful
TYPE 9: There is probably a mistake in your constraining data or your
experimental values for mol. wt, temperature, intrinsic viscosity
```

Fig. 10a, b ELLIPS4: Determining the triaxial shape (a/b, b/c) of a macromolecule using electro-optic decay data. Illustrated by application to "Protein 1" of true (a/b, b/c) = (1.5, 1.5) and based on the electro-optic decay data of Fig. 8 and constraining line solution of Fig. 9. **a** Running the programe. The user needs to enter values for the temperature, solvent viscosity, intrinsic viscosity [η] of the macromolecule and the molecular weight M. The programme runs 4 times with the constraining values (a/b, b/c) {excluding the first and last point) as the initial estimates. **b** Output giving the returned values for (a/b, b/c) and the pre-exponential factors for each of the four starting estimates for (a/b, b/c) and the final "best" result. A list of potential error warning estimates is also given

procedures available, as reviewed by Harding 1995). Ellipsoids of revolution – via the routine ELLIPS1 give a relatively quick impression of the overall form of the molecule (providing a distinction as to whether its best modelled by a prolate or oblate can be made). Although general ellipsoid modelling, using ELLIPS2, like bead modelling using SOLPRO (Garcia de la Torre et al. 1997) can now easily predict the hydrodynamic properties of a macromolecule of given shape: the reverse is more difficult. With SOLPRO the problems are one of uniqueness (i.e. the multiplicity of models which yield the same set of hydrodynamic parameters); ELLIPS3 and 4 circumvent these uniqueness problems but only by forgoing molecular detail and using a graphical extraction strategy, with undoubtedly ELLIPS3 the easiest to perform.

Whatever he does the investigator needs to moderate his desire for high precision with a sense of realism: whether it be ellipsoids or beads, these are only approximations to the true conformation of a macromolecule (the hydrodynamic theory behind the latter is also only an approximation); even though the so-called hydration problem can be countered with the use of those Universal shape functions which are hydration-independent, there is still a further assumption (usually reasonable) that the hydration of a macromolecule is ~the same for different measurements. Because of these reasons, hydrodynamics will always be a so called "low" or "fairly-low" resolution approach to conformation analysis.

Nonetheless, the relative speed with which the measurements can be performed, coupled with the limitations of the so-called "high-resolution" techniques (which in many instances cannot be applied and can never be applied anyway to a molecule in dilute solution) make modern hydrodynamic conformation algorithms highly attractive as "overall solution structure" (ellipsoids) or "solution molecular refinement" (beads) algorithms.

Program availability The ELLIPS programs are available. Free at charge either directly from the authors (email: steve.harding@nottingham.ac.uk or coelfen@mpikg-teltow.mpg.de) or from the Internet. Log in as anonymous ftp on BBRI.HARVARD.EDU and then change to /RASMB/SPIN/MS_DOS/ELLIPS-HARDING.

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