Eur Biophys J (1997) 25: 361–372

ARTICLE

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SOLPRO: theory and computer program for the prediction of SOLution PROperties of rigid macromolecules and bioparticles

Accepted: 1 November 1996

Abstract Single-valued hydrodynamic coefficients of a rigid particle can be calculated from existing theories and computer programs for either bead models or ellipsoids. Starting from these coefficients, we review the procedures for the calculation of complex solution properties depending on rotational diffusion, such as the decays of electric birefringence and fluorescence anisotropy. We also describe the calculation of the scattering form factor of bead models. The hydrodynamic coefficients and solution properties can be combined to give universal, shape-dependent functions, which were initially intended for ellipsoidal particles, and are extended here for the most general case. We have implemented all these developments in a new computer program, SOLPRO, for calculation of SOLution PROperties, which can be linked to existing software for bead models or ellipsoids.

Key words Hydrodynamics programs · Bead modelling · Fluorescence · Birefringence · Scattering

1 Introduction

The solution properties of macromolecules have traditionally been valuable sources of information on the size and shape of biological macromolecules, and interest in them has been increasing over the years (Harding 1995). The 1970’s and 80’s saw the development of optical and electro-optical techniques that yield properties quite sensitive to macromolecular size and conformation, and more recently in the 90’s, the classical technique of ultracentrifugation is showing a renaissance owing to emerging, new instrumentation.

Theories and computational tools are required for the analysis or prediction of solution properties. For the large family of hydrodynamic properties, bead modelling was proposed as an approach enabling the representation of rigid shapes of arbitrary complexity (Bloomfield et al. 1967). Then, rigorous theories and computational procedures for the hydrodynamics of bead models were developed (García de la Torre and Bloomfield 1977, 1981; García de la Torre 1989, 1992) and recently the computer software HYDRO for the calculation of the hydrodynamic coefficients has been published (García de la Torre et al. 1994a).

An alternative theoretical and computational approach for rigid particles is based on the representation of the macromolecular shape by a triaxial ellipsoid with three distinct semiaxes (Harding and Row 1982, 1983; Harding 1989, 1994). Although the models that can be represented by this “whole body” approach are much simpler compared with the bead approach, unique structures could be predicted by combination of three measurements together to (i) avoid the problems of assignment of a value for the “molecular hydration” (i.e. the proportion of associated solvent) (ii) to provide a unique pair of semi-axial ratios which define an ellipsoid. A suite of four FORTRAN based software algorithms (ELLIPS1, 2, 3 & 4) has also been developed for this type of modelling (Harding et al. 1996).

In connection mainly with the hydrodynamic theory of ellipsoids, it has been shown that some combinations of hydrodynamic properties can thus be formulated in such a way that they are explicit functions only of the shape of the particle: we now call these “Universal shape functions”. These include the viscosity increment ν (Simha 1940), the Perrin “frictional ratio due to shape” function P (Perrin 1936; Rowe 1977; Squire and Himmel 1979), fluorescence depolarization anisotropy relaxation time ratios (Small and Isenberg 1977), the so-called harmonic mean rotational relaxation time ratio (see e.g., Squire 1970) and the “reduced” radius of gyration function G (Harding

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1987). For their experimental measurement these parameters still require knowledge of the volume (or equivalent parameters) of the macromolecule (V) and hence the degree of solvent association or “hydration” of the macromolecule. However by combining two hydrodynamic properties together, additional Universal shape functions can be generated in which the volume (and hence hydration) requirement is removed. A well know example is the Scheraga-Mandelkern (1953) parameter, and various other combined which are more sensitive to shape have subsequently been formulated, the most sensitive of which generally involve combination of intrinsic viscosity measurements with rotational hydrodynamic parameters, although the latter can be more difficult to measure.

Some modern optical or electro-optical techniques, such as transient electric birefringence or fluorescence polarization anisotropy decay, monitor the rotational hydrodynamics or Brownian dynamics of the macromolecular particle in a somehow complex way. The rotational coefficients or relaxation times provided by bead or ellipsoidal models are necessary but not sufficient for the prediction of the time-dependence of properties observed by those techniques. The theory relating those techniques and the rotational quantities was first available for ellipsoids (Ridgeway 1966, 1968; Small and Isenberg 1977), and also for the most general case of an arbitrary shape (Wegener et al. 1979; Belford et al. 1972). In our opinion, however, the various theories are scattered in the literature and are not well connected to each other. Furthermore, computer programs such as HYDRO calculate rotational diffusion coefficients and relaxation times, but not the full decays.

Thus, one of the main purposes of this paper is to summarize in a comparative review the theories for rotational diffusion as observed in time-dependent birefringence or anisotropy, and also to provide the experimentalist or practically-minded user with a new computer program, SOLPRO (named after ‘SOLution PROperties’). This program allows the prediction of the time-dependent properties. The rotation-containing shape-dependent functions have also been generalized for the various types of rotational coefficients and relaxation times. By the way, we include in SOLPRO a most important non-hydrodynamic solution property, namely the form factor in radiation scattering, which gives the angular dependence of light or x-rays scattered by the macromolecule in solution.

As the second purpose of this paper, we have expressed and programmed “Universal” functions which are explicit functions of shape alone (as opposed to size and shape), which can be obtained for bead models in the same way as done for ellipsoids. In this way the two different approaches for modelling the conformations of rigid bodies in solution – beads and ellipsoids – become completely complementary and compatible.

Actually SOLPRO is a FORTRAN subroutine that accepts output from HYDRO and can be linked with it and with user-supplied subprograms, but is has been programmed independently from HYDRO, and is distributed separately.

2 Theory

2.1 Relaxation times of a general particle

The rotational Brownian dynamics of a rigid body is governed by its rotational diffusion tensor, $\mathbf{D_r}$, (García de la Torre 1981), which is a 3×3 symmetric matrix whose components can be calculated using bead modelling (García de la Torre and Bloomfield 1981).

From the fundamental work of Favro (1960), it is known that the observable properties related to rotational motion will involve up to five relaxation times, given by

$$\tau_1 = (6D - 2\Delta)^{-1} \tag{1}$$

$$\tau_2 = (3(D + D_1))^{-1} \tag{2}$$

$$\tau_3 = (3(D + D_2))^{-1} \tag{3}$$

$$\tau_4 = (3(D + D_3))^{-1} \tag{4}$$

$$\tau_5 = (6D + 2\Delta)^{-1} \tag{5}$$

where D_1, D_2, D_3 are the eigenvalues of $\mathbf{D}_r$ in ascending order, and

$$D = (1/3)\, Tr\,(\mathbf{D}_r) \tag{6}$$

$$\Delta = (D_1^2 + D_2^2 + D_2^3 - D_1 D_2 - D_1 D_3 - D_2 D_3)^{1/2} \tag{7}$$

Tr indicates the trace of a matrix, i.e., the sum of its diagonal components. Thus, τ_5 is the shortest relaxation time, and τ_1 is the longest one.

As specified later for particular cases, the time-dependencies, $p(t)$, of observable properties are multiexponential functions containing up to five components:

$$p(t) = \sum_{k=1}^{5} c_k \exp(-t/\tau_k) \tag{8}$$

It is common to normalize properties to the zero-time value, expressing

$$p^*(t) = p(t)/p(0) = \sum_{k=1}^{5} a_k \exp(-t/\tau_k) \tag{9}$$

where

$$a_k = c_k \Big/ \sum_{k=1}^{5} c_k \tag{10}$$

with $\sum_k a_k = 1$. The extraction of the five relaxation times, τ_k, and their corresponding amplitudes a_k or c_k from an experimentally measured $p(t)$ or $p^*(t)$ decay is nearly impossible owing to the well-known ill-conditioned nature of multiexponential fitting. However, it is possible to characterize the decay in terms of other relaxation times that can be more precisely determined (Carrasco et al. 1996). The most significant one is the mean relaxation time, defined in general as:

$$\tau_{\text{mean}} = \int_0^\infty p^*(t)\, dt \tag{11}$$

and for the present case of a multiexponential function,

$$\tau_{\text{mean}} = \sum_{k=1}^{5} a_k \tau_k \tag{12}$$

Note, that τ_{mean} is actually a weighted arithmetic mean of the five relaxation times with weights a_k. Another characteristic time is the initial relaxation time, defined as

$$(\tau_{\text{ini}})^{-1} = -\left[\frac{d\ln p^*(t)}{dt}\right]_{t=0} \tag{13}$$

which gives the initial slope of the $p^*(t)$ curve. In the terminology of light scattering, τ_{ini} is the reciprocal of the first cumulant. For multiexponential decays,

$$\frac{1}{\tau_{\text{ini}}} = \sum_{k=1}^{5} a_k \frac{1}{\tau_k} \tag{14}$$

Thus, τ_{ini} is a weighted harmonic mean of the relaxation times. It should be noted that, although the five relaxation times τ_k are fixed for a given particle, τ_{ini} and τ_{mean} depend on the amplitudes, which in a turn are specific for each property and depend on electric or optical properties. Thus, in general, τ_{ini} and τ_{mean} will be different for the various observable properties; the mean relaxation time will not necessarily be the same for say, electric birefringence and fluorescence anistropy or polarization.

For a sphere with radius a and volume V, all the τ_k relaxation times (including τ_{ini} and τ_{mean}) reduce to a single value, denoted as τ_0, and given by

$$\tau_0 = \frac{1}{6D_{r,0}} \tag{15}$$

For the sphere, $D_{r,0} = kT/8\pi\eta_0 a^3$, where kT is Boltzmann's factor, and η_0 is the solvent viscosity, so that τ_0 is proportional to the volume,

$$\tau_0 = \frac{\eta_0 V}{kT} \tag{16}$$

The rotational relaxation times described so far are applicable to the relaxation of properties related to second-rank tensors or second Legendre Polynomials. These include transient birefringence or dichroism, fluorescence anisotropy decay and depolarized dynamic light scattering. Some years ago, the technique of dielectric dispersion was used for characterization of rotational motion. This technique is related to the orientational relaxation of the first Legendre polynomial of the angle. Brownianly travelled by the dipole moment. Thus, it involves only three relaxation times, ρ_l, which differ from the τ_i's. They are given by

$$\rho_l = (3D_r - D_l)^{-1} \qquad l = 1, 2, 3 \tag{17}$$

As the dielectric dispersion technique is not commonly used nowadays, we shall make no further developments on the ρ_i's. Now we just mention that in the case of a spherical particle the three values coincide and are equal to

$$\rho_0 = \frac{1}{2D_{r,0}} = \frac{3\eta_0 V}{kT} \tag{18}$$

so that

$$\rho_0 = 3\tau_0 \tag{19}$$

(Small and Isenberg 1977). It is worth noting that historically workers have referred to these relaxation times (ρ_l and ρ_0) as just "rotational relaxation times") (see Small and Isenberg 1977, Van Holde 1985 and Harding 1995 and references cited herein) although the definitions according to Eqs. (1)–(5) and (15) are actually more general.

2.2 Harmonic mean relaxation time

Owing to convention or historical reasons, rotational dynamics have often been characterized in terms of a single value, the "harmonic relaxation mean time". For a triaxial ellipsoid (in general, for a triaxial body) there are three main axes (a, b, c) which coincide with the principal axes of rotational diffusion. The corresponding rotational diffusion coefficients D_a, D_b, D_c coincide with the eigenvalues D_1, D_2, D_3. Relaxation times for each of these axes may be defined by analogy with the $\tau - D$ relationship for spheres or axysymmetric particles:

$$\tau_a = \frac{1}{6D_a} \tag{20}$$

and the same holds for b and c. Then, the harmonic mean relaxation time, τ_h, is defined as

$$\frac{1}{\tau_h} \equiv \frac{1}{3}\left[\frac{1}{\tau_a} + \frac{1}{\tau_b} + \frac{1}{\tau_c}\right] \tag{21}$$

These definitions can be elaborated further in terms of the quantities presented in the preceding section. It is evident that

$$\frac{1}{\tau_h} = 6\left(\frac{D_a + D_b + D_c}{3}\right) \tag{22}$$

or, more compactly,

$$\tau_h = \frac{1}{6D_r} \tag{23}$$

where D_r is, simply speaking, the mean rotational diffusion coefficients or, more precisely, the mean value of the eigenvalues of $\mathbf{D}_r$, as defined in Eq. (1). It is interesting to note that as the trace of a tensor is an invariant, τ_h can be obtained from the diagonal components of $\mathbf{D}_r$ in any system of axes, without previous calculation of eigenvalues.

Owing to the peculiar symmetry shown by the five τ_k's, it is easily seen that

$$\frac{1}{\tau_h} = \frac{1}{5}\sum_{k=1}^{5} \frac{1}{\tau_k} \tag{24}$$

so that τ_h is actually an unweighted harmonic mean of the τ_k's. It will be different in general from the τ_{ini} (Eq. (14)) relaxation time which is experimentally accessible for the various time-dependent properties: this is because the latter is actually weighted by the component amplitudes (a_k or c_k). Nonetheless, τ_h can be used as an approximation for τ_{ini} or even it might coincide with it in some special cases.

Looking again at the expressions for the τ_k's, we further find an alternative and even simpler relationship: τ_h is the harmonic mean of the longest and shortest relaxation times:

$$\frac{1}{\tau_h} = \frac{1}{2}\left[\frac{1}{\tau_1} + \frac{1}{\tau_5}\right] \tag{25}$$

2.3 Electric birefringence and dichroism decays

For many years, the decay of electric birefringence or dichroism had been interpreted in terms of the theoretical results for revolution or asymmetric, triaxial ellipsoids (Ridgeway 1966, 1968). The problem for a general rigid body of arbitrary shape was solved by Wegener et al. (1979) who obtained for the birefringence decay, $\Delta n(t)$, an expression of the type

$$\Delta n(t) \propto \sum_{k=1}^{5} c_k \exp(-t/\tau_k) \tag{26}$$

with amplitudes

$$\begin{aligned} c_1 = {} & (3/4)\,(c/M)^2\,(\alpha_{11}-\alpha_{22})\,(\chi_{11}-\chi_{22}) - (\sqrt{3}/4)\,(bc/M^2) \\ & \cdot [(\alpha_{11}-\alpha_{22})\,(3\chi_{33}-Tr\,\chi) + (\chi_{11}-\chi_{22})\,(3\alpha_{33}-Tr\,\alpha)] \\ & + (1/4)\,(b/M)^2\,(3\chi_{33}-Tr\,\chi)\,(3\alpha_{33}-Tr\,\alpha) \end{aligned} \tag{27}$$

$$c_2 = 3\alpha_{23}\,\chi_{23} \tag{28}$$

$$c_3 = 3\alpha_{13}\,\chi_{13} \tag{29}$$

$$c_4 = 3\alpha_{12}\,\chi_{12} \tag{30}$$

$$\begin{aligned} c_5 = {} & (3/4)\,(c/M)^2\,(\alpha_{11}-\alpha_{22})\,(\chi_{11}-\chi_{22}) + (\sqrt{3}/4)\,(bc/M^2) \\ & \cdot [(\alpha_{11}-\alpha_{22})\,(3\chi_{33}-Tr\,\chi) + (\chi_{11}-\chi_{22})\,(3\alpha_{33}-Tr\,\alpha)] \\ & + (1/4)\,(b/M)^2\,(3\chi_{33}-Tr\,\chi)\,(3\alpha_{33}-Tr\,\alpha) \end{aligned} \tag{31}$$

where

$$\chi_{lm} = \mu_l\,\mu_m/(kT)^2 + \varepsilon_{lm}/kT \tag{32}$$

ε_{lm} and α_{lm} being, respectively, the l, m component ($l, m = 1, 2, 3$) of the electrical and optical polarizability tensors, ε and α, respectively, and μ_k are the component of the electric dipole moment, μ. In Eqs. (27)–(31), 3 is the direction in which the electric field is applied. The components of ε and α must be expressed in the system of axes defined by the eigenvectors of the rotational diffusion tensor, $\mathbf{D_r}$. In the above equations,

$$b = 3\,(D_3 - D) + 2\,\Delta \tag{33}$$

$$c = \sqrt{3}\,(D_1 - D_2) \tag{34}$$

$$M = 2\,(\Delta b)^{1/2} \tag{35}$$

For the case of electric dichroism, the theoretical result is exactly the same, with the only particularity that α denotes the intrinsic optical absorption tensor.

We particularize now for the case when the particle is (or is approximately assumed to be) a triaxial body. Then the main axes of rotational diffusion ($\mathbf{D}_r$), optical polarizability (α), electric polarizability and electric alignment (χ) should coincide. In the axes of D_r, all the other tensors have no off-diagonal components. As a consequence

$$c_2 = c_4 = c_4 = 0 \qquad (\alpha_{lm} = \chi_{lm} = 0 \quad \text{for} \quad l = m) \tag{36}$$

and thus only two relaxation terms are involved in the birefringence decays

$$\frac{\Delta n(t)}{\Delta n(0)} = a_1 \exp(-t/\tau_1) + a_5 \exp(-t/\tau_5) \tag{37}$$

with $a_1 = c_1/(c_1 + c_5)$, $a_5 = c_5/(c_1 + c_5)$, c_1 and c_5 being given by Eqs. (27) and (31). This biexponential result was predicted in the work by Ridgeway (1968) although its rigorous validity was not rigorously stated in that work.

Finally we note that, as a computational trick, it is valid to replace α_{lm} and χ_{lm} in Eqs. (27)–(31) by the relative traceless quantities $\hat{\alpha}_{lm}$ and $\hat{\chi}_{lm}$ defined by

$$\hat{\alpha}_{lm} = (\alpha_{lm} - \delta_{lm}\,Tr\,\alpha)/Tr\,\alpha \tag{38}$$

$$\hat{\chi}_{lm} = (\chi_{lm} - \delta_{lm}\,Tr\,\chi)/Tr\,\chi \tag{39}$$

2.4 Time-dependent fluorescence polarization anistropy

The historic development of the theory for this property is similar to that of electric birefringence. While theoretical results were available for a long time for the particular case of ellipsoids (Weber 1952, 1953) it was not until the work of Belford et al. (1972) that the problem was solved for the general, arbitrary particle. According to these authors, the decay of polarization anisotropy is again a sum of five exponentials

$$r(t) = \sum_{k=1}^{5} c_k \exp(-t/\tau_k) \tag{40}$$

where

$$c_1 = (6/5)\,(F + G)/4 \tag{41}$$

$$c_2 = (6/5)\,\phi_2\,\phi_3\,\omega_2\,\omega_3 \tag{42}$$

$$c_3 = (6/5)\,\phi_3\,\phi_1\,\omega_3\,\omega_1 \tag{43}$$

$$c_4 = (6/5)\,\phi_1\,\phi_2\,\omega_1\,\omega_2 \tag{44}$$

$$c_5 = (6/5)\,(F - G)/4 \tag{45}$$

In the above equations, ϕ_l and ω_l are the direction cosines of the absorbing and emitting dipoles, respectively, with respect to the principal axes of rotational diffusion, and

$$F = \sum_{l=1}^{3} \phi_l^2\,\omega_l^2 - (1/3) \tag{46}$$

$$G = \left[\sum_{\substack{l=1 \\ l \neq m \neq n \neq m}}^{3} D_l\,(\phi_l^2\,\omega_l^2 + \phi_m^2\,\omega_n^2 + \phi_n^2\,\omega_m^2) - D\right]\Big/\Delta \tag{47}$$

These results are valid for a chromophore or fluorescent probe rigidly attached to the macromolecular particle and with the specific orientation given by the vectors ω and ϕ. If the attachment is not rigid, but instead the probe experiences local motion, the above results are not valid. One case of some interest is that when the chromophore is rigidly attached, but with an orientation that varies at random from molecule to molecule. Then the amplitudes should be averaged over all the possible orientations of the dipoles ω and ϕ, keeping the condition that the angle between them, δ, must be constant. The result (Chuang and Eisenthal 1972; Ehrenberg and Rigler 1972) is that the $5\,c_k$'s are identical, so that

$$r(t) = \frac{r_0}{5} \sum_{k=1}^{5} \exp(-t/\tau_k) \tag{48}$$

with $a_k = 1/5$, and therefore

$$\tau_{\mathrm{mean}} = (1/5) \sum_{k=1}^{5} \tau_k \tag{49}$$

$$\frac{1}{\tau_{\mathrm{ini}}} = (1/5) \sum_{k=1}^{5} \frac{1}{\tau_k} \equiv \frac{1}{\tau_h} \tag{50}$$

Thus, for random orientation of the probe, the initial rate in the decay gives the harmonic mean relaxation time, ($\tau_{\mathrm{ini}} = \tau_h$ for this case) while the area under the decay curve gives the arithmetic mean, τ_{mean}.

2.5 Steady-state fluorescence depolarization

The steady-state anisotropy, $\bar{r}$, is calculated from the anisotropy decay function, $r(t)$, as (Yguerabide 1972)

$$\bar{r} = \frac{\int_0^\infty i(t)\, r(t)\, dt}{\int_0^\infty i(t)\, dt} \tag{51}$$

where $i(t)$ is the time-dependent total fluorescence intensity. In the case of a fluorophore with an homogeneous environment, this intensity decays monoexponentially,

$$i(t) = i_0 \exp(-t/\tau_F) \tag{52}$$

τ_F being the fluorescence life-time. Now, if $r(t)$ is, in general, a sum of exponential as in Eq. (8), then we have

$$\bar{r} = r_0 \sum_{k=1}^{5} a_k \left(1 + \frac{\tau_F}{\tau_k}\right)^{-1} \tag{53}$$

with $a_k = c_k / \sum c_k$. We define a reduced relaxation time, $\tau_k^* = (T/\eta_0)\, \tau_k$, which depend only on the size and shape of the molecule but not on the solvent viscosity, η_0, nor the temperature, T. In the Perrin-Weber plot, the reciprocal of $\bar{r}$ is plotted vs. T/η_0. This function is:

$$\frac{1}{\bar{r}} = \frac{1}{r_0} \left[\sum_{k=1}^{5} a_k \left(1 + \frac{\tau_F}{\tau_k^*} \frac{T}{\eta_0}\right)^{-1} \right]^{-1} \tag{54}$$

For low values of the combined quantity T/η_0 we express $1/\bar{r}$ as a Taylor expansion at $T/\eta_0 \to 0$

$$\frac{1}{\bar{r}} = \frac{1}{r_0} \left(1 + \left[\frac{d(r_0/\bar{r})}{d(T/\eta_0)}\right]_{(T/\eta_0)=0} \frac{T}{\eta_0}\right) \tag{55}$$

One easily finds

$$\left[\frac{d(r_0/\bar{r})}{d(T/\eta_0)}\right]_{(T/\eta_0)=0} = \tau_F \sum_{k=1}^{5} \frac{a_k}{\tau_k^*} = \frac{\tau_F}{\tau_{\mathrm{ini}}^*} \tag{56}$$

where $\tau_{\mathrm{ini}}^* = (T/\eta_0)\, \tau_{\mathrm{ini}}$ is the reduced initial relaxation time (see Eq. (14)). Then, substitution of Eq. (56) into Eq. (55) leads to

$$\frac{1}{\bar{r}} = \frac{1}{r_0} \left[1 + \frac{\tau_F}{\tau_{\mathrm{ini}}^*} \frac{T}{\eta_0}\right] \tag{57}$$

In the plot of $1/\bar{r}$ (or $1/\bar{p} - 1/3$, where $\bar{p}$ is the steady-state polarization) vs. T/η_0, the initial slope, followed at sufficiently low T/η_0, gives $\tau_F/\tau_{\mathrm{ini}}^*$. Alternatively, Eq. (57) can be rewritten as

$$\tau_{\mathrm{ini}} = \tau_F \left[\frac{r_0}{\bar{r}} - 1\right]^{-1} \tag{58}$$

Thus, an extrapolation of the experimental value of the quantity in the right hand side of Eq. (58) to $T/\eta_0 \to 0$ yields τ_{ini}. It is interesting to note that the steady-state measurement gives essentially the same information as the initial decay in the dynamic, time-dependent experiment. As indicated above, for a random distribution of the probe chromophore, τ_{ini} is the same as the harmonic mean relaxation time, τ_h, which is the result of the steady-state measurement in such case.

Some care however needs to be used in terms of the experimental evaluation of τ_{ini} or τ_h as noted above. For historical reasons, representations for steady state fluorescence depolarization often refer to the "rotational relaxation time" (see end of section 2.1) i.e. the traditional equivalent dielectric dispersion relaxation time,

$$\rho_h = 3\, \tau_h \tag{59}$$

Owing to this, the equation for anisotropy decay (with random orientation) was written years ago as

$$r(t) = r_0 \exp(-3t/\rho_h) \tag{60}$$

as we read for instance in Van Holde (1971). Authors concerned with properties other than dielectric relaxation formulated

$$r(t) = r_0 \exp(-t/\tau_h) \tag{61}$$

(see, for instance Jablonsky 1961). It is interesting to note that in the 1985 edition of the Van Holde's book, the form given in Eq. (61) has been adopted.

2.6 Static radiation scattering

In addition to the above quantities, we have included in the bead model calculation a non-dynamic property, namely, the form factor for scattering of light, X-rays or other electromagnetic radiation.

The angular dependence of radiation scattered by a system of N scattering elements is given by the Debye equation

$$I(h) \propto \sum_{i=1}^{N} \sum_{j=1}^{N} F_i(h)\, F_j^*(h) \sin(h r_{ij})/h r_{ij} \tag{62}$$

where h is the angular variable,

$$h = (4\pi/\lambda)\sin(\theta/2) \tag{63}$$

θ is the scattering angle, λ is the wavelength of the radiation in the scattering medium. $F_i(h)$ is the amplitude of the radiation emitted by element i, and r_{ij} is the distance between the elements i and j.

For a model composed of spherical scatterers, with radii σ_i, $i=1, \ldots, N$, it has been shown that the form factor, defined as $P(h)=I(h)/I(0)$ is given by (Diaz et al. 1993)

$$P(h) = \left(\sum_{i=1}^{N} \sigma_j^6 P_i(h) + 2 \sum_{i<}^{N} \sum_{j}^{N} \sigma_i^3 \sigma_j^3 Q_{ij}(h) \right) \Bigg/ \sum_{i=1}^{N} \sum_{j=1}^{N} \sigma_i^3 \sigma_j^3 \tag{64}$$

where

$$P_i(h) = [3(\sin(h\sigma_i) - h\sigma_i \cos(h\sigma_i))/(h\sigma_i)^3]^2 \tag{65}$$

and

$$Q_{ij}(h) = [P_i(h)\,P_j(h)]^{1/2}\,(\sin(hr_{ij})/hr_{ij}) \tag{66}$$

σ_i being the radius of the i-th bead. Equations (64)–(66) can be readily evaluated from the coordinates and radii of the beads.

2.7 Universal shape functions

Physico-chemical parameters such as the intrinsic viscosity $[\eta]$ (ml/g), the sedimentation coefficient, s (s), the translational diffusion coefficient, D (cm^2/s), the radius of gyration, R_g (cm) or rotational relaxation times τ_k (s) from electro-optical properties or fluorescence depolarization anisotropy (time dependent or steady state) are by *themselves* ambiguous functions of shape – that is they also depend on the volume V of a macromolecule, and as noted in Section 1 above this has led to the formulation of a number of Universal shape functions such as ν, P, G and the rotational relaxation time ratios, τ_k/τ_0 which are explicit functions of shape only. For experimentally measuring these, one still needs an estimate of V. Although for the G function from radius of gyration measurements, V can be approximated by the anhydrous volume under certain conditions (depending on the macromolecule, the density of bound compared to free solvent and the nature of the scattering radiation), there will be a considerable contribution to V from molecular hydration or amount of solvent associated with the macromolecule (chemically bound or physically entrained). The volume V is actually more conveniently expressed by experimentalists as a specific volume v_s (ml/g), where $v_s=\bar{v}+\delta\rho_0$. $\bar{v}$ is the partial specific volume (which refers to the anhydrous particle), δ is the so-called "hydration" parameter expressed as "grams of water (solvent) bound per gram of dry macromolecule" (Tanford 1961). The symbol "w" is often used for δ (Squire and Himmel 1979). ρ_0 is the density of the hydrating solvent. $\bar{v}$ can generally be measured accurately using densimetry (the limiting factor now being concentration measurement) (Kratky et al. 1973), or can be estimated from the chemical composition (Perkins 1986). Typically $\bar{v} \sim 0.73$ ml/g for a protein and ~ between 0.5–0.6 ml/g for a saccharide. As is well known, δ is notoriously difficult to measure with any precision at all and seems to be a strong function of the measurement technique applied (Kuntz and Kauzmann 1974). Values of between 0.2–0.35 have been used as "typical" for proteins, although ~ 0.5 has been suggested as more representative (Squire and Himmel 1979). This spectrum of possible values severely limits the precision with which a hydrodynamic model can be applied, and has inspired the generation of compound Universal shape functions (principally β, R, Ψ_k and Λ_k) where the experimental requirement for V (or δ) has been removed by combining two hydrodynamic parameters together. The assumption is made of course that V is the same for the particle in both types of measurement (sedimentation, viscosity, fluorescence depolarization etc.).

With the Universal shape functions, we can therefore distinguish two or three categories, whose main characteristic is whether they depend or not (for their calculation from experimental data) on the degree of hydration.

Firstly, there are some shape functions depending on one solution property and the particle's volume (ambiguous; hydration-dependent).

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

$$\nu = \frac{[\eta]\,M}{N_A\,V} \tag{67}$$

- Perrin function, P (Perrin 1936; Rowe 1977; Squire and Himmel 1979)

$$P = \frac{f}{6\pi\,\eta_0\,(3V/4\pi)^{1/3}} \tag{68}$$

- Rotational relaxation time ratios (Small and Isenberg 1977)

$$\frac{\tau_k}{\tau_0} = \frac{kT}{\eta_0\,V}\,\tau_k \qquad k = 1, 2, 3, 4, 5, h, ini, mean \tag{69}$$

- G ratio for the square radius of gyration

$$G = \frac{R_g^2}{a^2} = \left(\frac{4\pi}{3V}\right)^{2/3} R_g^2 \tag{70}$$

Actually P in Eq. (68) is equal to the ratio of the friction coefficient of the bead model to the friction coefficient of a sphere having the same volume, given by $6\pi\,\eta_0\,a$ where the radius of the equivalent sphere is $a=(3V/4\pi)^{1/3}$. The relation of P with the frictional ratio is discussed elsewhere in this volume (Harding et al. 1996). In Eq. (69) τ_0 is the rotational relaxation time for birefringence or anisotropy decay of a sphere with the same volume given by Eq. (16). This definition can be applied also to the initial and mean relaxation times specific of anisotropy or birefringence decay (Eqs. (11) and (14)), and to the general, harmonic-mean relaxation time (Eq. (24) or (25)).

Then, there are other shape functions involving two hydrodynamics properties (unambiguous, hydration-independent)

- Translation + viscosity
  - Scheraga-Mandelkern (1953) parameter

$$\beta \equiv \frac{N_A\, s[\eta]^{1/3}\,\eta_0}{M_r^{2/3}\,(1-\bar{v}\,\rho_0)\,100^{1/3}} = \frac{N_A^{1/3}}{(16\,200\,\pi^2)^{1/3}} \cdot \frac{\nu^{1/3}}{P} \tag{71}$$

  - Wales-Van Holde (1954; Rowe 1977) parameter

$$R = \frac{k_s}{[\eta]} = \frac{2}{\nu}\,[1+P^3] \tag{72}$$

- Translation + rotation

$$\Psi_k \equiv \left(\frac{4\pi\,\eta_0}{3kT}\right)^{1/3} \frac{M_r\,(1-\bar{v}\,\rho_0)}{6\pi\,\eta_0\,N_A\,S} \left(\frac{1}{\tau_k}\right)^{1/3} = \left(\frac{\tau_0}{\tau_k}\right)^{1/3} P$$
$$k = 1, 2, 3, 4, 5, h, mean, ini \tag{73}$$

- Rotation + viscosity

$$\Lambda_k \equiv \frac{\eta_0\,[\eta]\,M_r}{N_A\,kT\,\tau_k} \left[\frac{\tau_0}{\tau_k}\right] \nu$$
$$k = 1, 2, 3, 4, 5, h, mean, ini \tag{74}$$

In previous work, Eq. (73) for Ψ (Squire 1970) and Eq. (74) for Λ (Harding 1980 a; Harding and Rowe 1982) were defined specifically for τ_h. A formally identical combination has been formulated for the τ_k's $k=1-5$, and denoted as κ_i (Harding 1980 b; Harding 1995). Now we include *all* the cases under the common notation Λ_k, including the *h*, *mean* and *ini* values. Similarly, Ψ was previously defined for τ_h exclusively. Later, functions $\xi_k=(\tau_0/\tau_k)P^3$ were introduced (Harding 1980 b; Harding 1995). Using the common notation Ψ_k, we see that $\xi_k=\Psi_k^3$.

Note there is a slight difference between the formulations in terms of experimental parameters for the Ψ_k and Λ_k given here and those from previous work referring specifically to Ψ_h and Λ_h (see e.g., Harding and Rowe 1982) in that a factor of 3 in earlier representations is no longer necessary. As noted in the above description of relaxation times, for purely historical reasons previous work used "traditional" rotational relaxation times (i.e. dielectric-dispersion relaxation times), given in the case of a sphere by Eq. (18), while to achieve compatibility with time resolved representations τ_h along with the other τ_k in Eqs. (73) and (74), now refers to birefringence or fluorescence relaxation times decays directly which for a sphere is 3 time smaller (Eq. (19)). The values of Ψ_h and Λ_h are of course unaffected by the adoption of this new convention (e.g. for a sphere $\Psi_h=1$, $\Lambda_h=2.5$, as before). We propose that this convention and convenient form of notation be now adopted universally.

There is also a third, or intermediate, class of Universal shape function which are particularly useful for the representation of *linear* classes of macromolecule (e.g., nucleic acids, polysaccharides, synthetic polymers) of varying degrees of flexibility. These shape functions depend on one hydrodynamic property and the radius of gyration. Therefore, for rigid, hydrated particles their use can be ambiguous, depending on whether or not hydration is included in the radius of gyration. We formulate these functions here for the sake of completeness.

- Flory (1953) P_0 function (P in Flory's notation)

$$P_0 = \frac{f}{\sqrt{6}\,\eta_0\,R_g} = \frac{kT}{\sqrt{6}\,\eta_0\,R_g\,D} \tag{75}$$

- Flory (1953) Φ function

$$\Phi = \frac{[\eta]\,M}{6^{3/2}\,R_g^3} \tag{76}$$

- $K_{\tau r}$ function, previously used by Navarro et al. (1995) for birefringence decay

$$K_{\tau r,k} = \frac{RT}{\eta_0}\frac{\tau_k}{R_g^3} \tag{77}$$

For flexible, linear chain macromolecules, the best estimates for these Universal functions are $P_0=6.0$, $\Phi=2.5\times10^{23}$ (García de la Torre et al. 1984, and reference cited herein for experimental results), and $K_{\tau r}=1.51\times10^{24}$. For rigid macromolecules, these functions can be used as shape indicators with the mentioned caution about hydration. For the simple case of a sphere, with $R_g=(\sqrt{3/5})\,a$, we easily find $P_0=9.93$, $\Phi=9.23\times10^{23}$ and $K_{\tau r}=5.42\times10^{24}$.

3 Methods

3.1 Calling HYDRO: bead model, solution data and hydrodynamic coefficients

As a preliminary step, the user has to build a bead model (Bloomfield et al. 1967; García de la Torre and Bloomfield 1977, 1981; García de la Torre 1989; García de la Torre et al. 1994 a) of the rigid structure of the particle. In principle, the number of beads can be as large as desired. Actually, a large number of beads can be convenient to model every detail in the particle shape; however it must be recalled the CPU time grows roughly as N^3. The bead model will be described simply as a list of Cartesian coordinates of the beads and their radii. The conventional working of HYDRO predicts hydrodynamic parameters depending on the shape AND size of the model, and so the bead model has to reflect the shape AND size of the macromolecule: absolute coordinates and radii are required. If however the user is simply interested in the simpler case of the shape of the molecule, and hence just the evaluation of the Universal shape functions via SOLPRO, *only* an arbitrarily sized or scaled bead model is required, having just the desired shape. In this case arbitrary units for the bead coordinates and radii suffice.

HYDRO requests other solution data such as temperature and solvent viscosity, as well as the partial specific volume of the macromolecule and its molecular weight. Again, if only evaluation of the Universal shape functions is required via SOLPRO, these additional data are not required, and dummy values should be given. Of course, since SOLPRO also provides the option of evaluation of all the rotational decays times, if the user wants these in addition, this information is necessary along with absolute bead coordinates and radii (in cm).

A main program, written by the user, will either calculate the coordinates and radii, or read them from a file, along with the other solution data. All these values will be placed in the FORTRAN data variables listed in the COMMON/DATA of subroutine HYDRO (García de la Torre et al. 1994 a). The statement CALL HYDRO will carry out all the HYDRO calculations, with the result for the hydrodynamic coefficient placed in COMMON/PROPERTIES. Nothing is new up to this point; this is instead the standard use of HYDRO, which has not been modified for the present work. For more details, consult the HYDRO Users Manual (García de la Torre 1994 b).

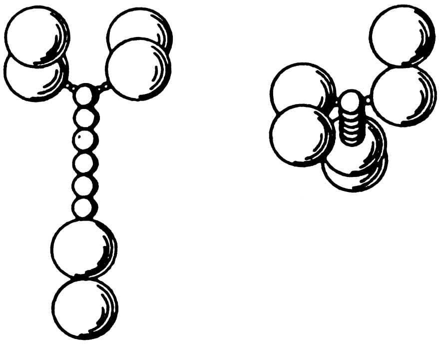


Fig. 1 Two views of the bead model for human immunoglobulin IgG3

```
IMMUNOGLOBULIN IGG3                 !Title
298.,       !Temperature, K
0.01,      !Solvent viscosity, poise (water, 20C)
1.E-07,     !Unit of length for coordinates and radii, cm (1 A)
1.58E5,        !Molecular weigth (-1=unknown)
0.239,         !Bouyancy factor for sedimentation (-1=unknown)
15,         !Number of beads
-12.9000  0.0000  0.0000  2.05
 -8.8     0.      0.      2.05
 -6.0     0.      0.      0.75
 -4.5     0.      0.      0.75
 -3.0     0.      0.      0.75
 -1.5     0.      0.      0.75
  0.      0.      0.      0.75
  0.5     0.      0.8660  0.25
  0.75    0.      1.2990  0.25
  0.5     0.     -0.8660  0.25
  0.75    0.     -1.2990  0.25
  1.9     0.     -3.2909  2.05
  3.95   -3.5507 -3.2909  2.05
  1.9     0.      3.2909  2.05
  3.95    3.5507  3.2909  2.05
1,                 IPRIN
1,                 IREF
1,                 IFLU
0.7071,0.7071,0.,     PHI
0.7071,0.,0.7071,   OME
-1,               Number of values of time
1,                IBIR
3.,0.,0.,        ALF
0.,2.,0.,
0.,0.,1.,
2.5,0.,0.,        CHI
0.,2.5,0.,
0.,0.,0.5,
-1,                Number of values of time
1,                 ISCA
-1,                Number of values of H
```

Fig. 2 Input file with the data for the SOLPRO calculation. The upper part is the input for standard HYDRO, and the lower part is specific for SOLPRO

3.2 Calling SOLPRO: time-dependent properties, form factor and shape-dependent functions

In the second step, the statement CALL SOLPRO in the user's main program will execute the calculation of time-dependent properties, solution scattering and shape-dependent functions. The transfer of data and results are made by means of the following COMMON blocks, which should be declared in the main program:

COMMON/BIREF/ALF(3,3), CHI(3,3), CB(5), SCB, AB(5), TINIB, TMEANB, NTB, TB(500), BIR(500), BIRN(500)

COMMON/FLUOR/PHI(3), OME(3), CF(5), SCF, AF(5), TINIF, TMEANF, NTF, TF(500), ANI(500), ANIN(500)

COMMON/SCATTER/NH, H(500), P(500)

COMMON/SHAPEFUN1/T0, TH, PERRIN, SIMHA, TIT0(5), THT0, TINBT0, TMEBT0, TINFT0, TMEFT0

COMMON/SHAPEFUN2/G, PFLORY, PHIFLORY, BETASM, RR, TR(5), TRH, TRINB, TRMEB, TRINF, TRMEF

COMMON/SHAPEFUN3/PSII(5), PSIH, PSIINB, PSIMEB, PSIINF, PSIMEF, RLAI(5), RLAH, RLAINB, RLAMEB, RLAINF, RLAMEF

In COMMON/BIREF, the data for the birefringence calculations are:

ALF, the optical anisotropy tensor, α in Eq. (27)
CHI, the electric alignment tensor, χ in Eq. (27)
NTB, is the number of values of time
TB, contains the values of time

and the results are:

CB(K), absolute amplitudes, c_k in Eq. (26)
SCB, sum of absolute amplitudes
AB(K), normalized amplitudes, a_k in Eq. (10)
BIREF, is the absolute birefringence for each time
BIREFN, is the birefringence for each time normalized to the initial value, $\Delta n(t)/\Delta n(0)$
TINIB, initial relaxation time, τ_{ini}
TMEANB, mean relaxation time, τ_{mean}

In COMMON/FLUOR, the data for anisotropy calculations are:

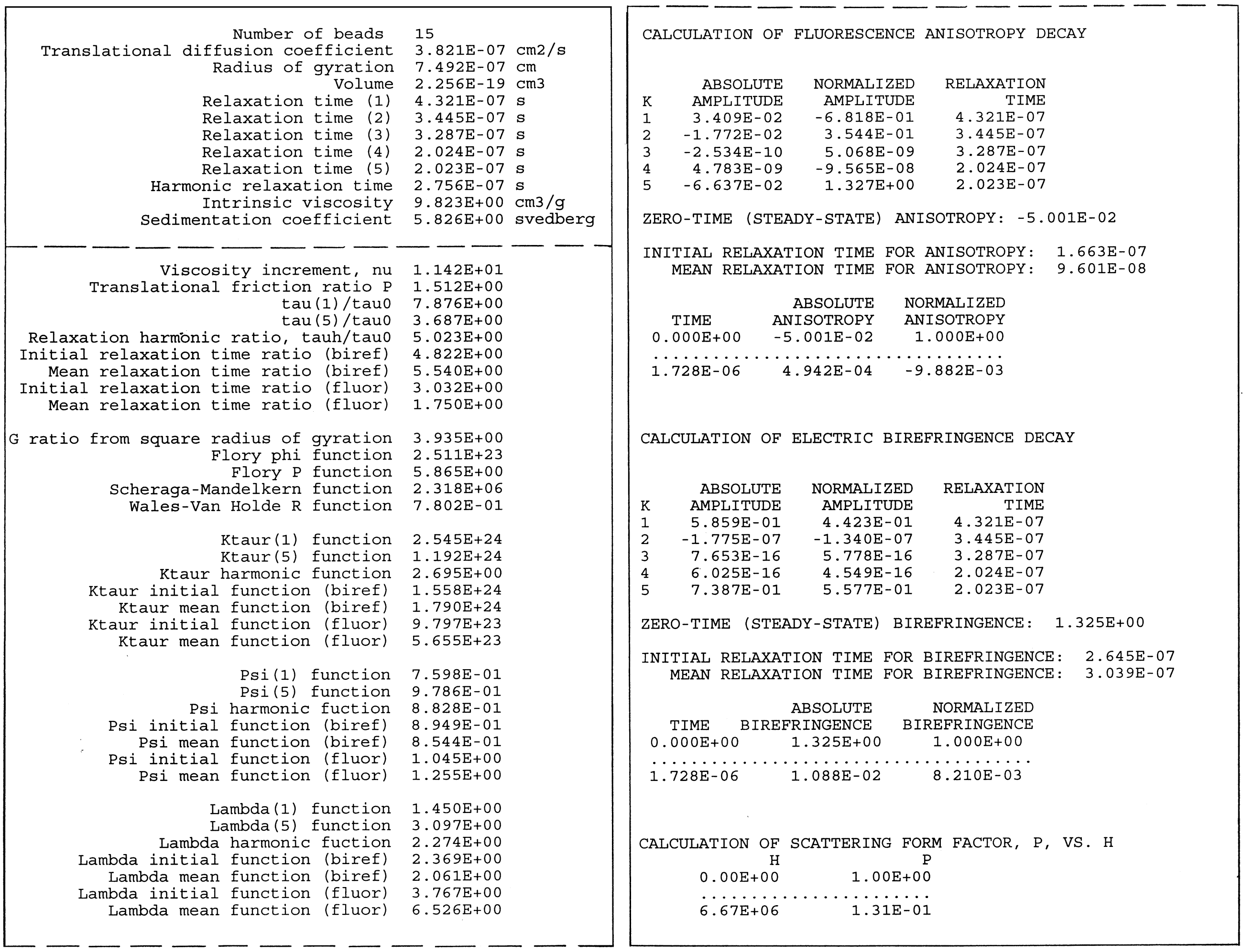
PHI, the absorbing dipole moment, ϕ in Eq. (42)
OME, the emitting dipole moment, ω in Eq. (42)

and NTF and TF has the same meaning for fluorescence as NTB and TB for birefringence, although they do not have necessarily the same values.

The results are:

CF(K), absolute amplitudes, c_k in Eq. (40)
SCF, sum of absolute amplitudes
AF(K), normalized amplitudes, a_k in Eq. (10)
ANI, anisotropy decay, $r(t)$
ANIN, normalized anisotropy decay, $r(t)/r(0)$

TINIF and TMEANF are τ_{ini} and τ_{mean}, the initial and mean relaxation time for fluorescence, which in general will be



```
               Number of beads  15
Translational diffusion coefficient  3.821E-07 cm2/s
            Radius of gyration  7.492E-07 cm
                        Volume  2.256E-19 cm3
            Relaxation time (1)  4.321E-07 s
            Relaxation time (2)  3.445E-07 s
            Relaxation time (3)  3.287E-07 s
            Relaxation time (4)  2.024E-07 s
            Relaxation time (5)  2.023E-07 s
       Harmonic relaxation time  2.756E-07 s
            Intrinsic viscosity  9.823E+00 cm3/g
       Sedimentation coefficient  5.826E+00 svedberg
```

```
            Viscosity increment, nu  1.142E+01
        Translational friction ratio P  1.512E+00
                        tau(1)/tau0  7.876E+00
                        tau(5)/tau0  3.687E+00
  Relaxation harmonic ratio, tauh/tau0  5.023E+00
 Initial relaxation time ratio (biref)  4.822E+00
    Mean relaxation time ratio (biref)  5.540E+00
 Initial relaxation time ratio (fluor)  3.032E+00
    Mean relaxation time ratio (fluor)  1.750E+00

G ratio from square radius of gyration  3.935E+00
                  Flory phi function  2.511E+23
                    Flory P function  5.865E+00
          Scheraga-Mandelkern function  2.318E+06
          Wales-Van Holde R function  7.802E-01

                  Ktaur(1) function  2.545E+24
                  Ktaur(5) function  1.192E+24
              Ktaur harmonic function  2.695E+00
        Ktaur initial function (biref)  1.558E+24
           Ktaur mean function (biref)  1.790E+24
        Ktaur initial function (fluor)  9.797E+23
           Ktaur mean function (fluor)  5.655E+23

                    Psi(1) function  7.598E-01
                    Psi(5) function  9.786E-01
                 Psi harmonic fuction  8.828E-01
          Psi initial function (biref)  8.949E-01
             Psi mean function (biref)  8.544E-01
          Psi initial function (fluor)  1.045E+00
             Psi mean function (fluor)  1.255E+00

                 Lambda(1) function  1.450E+00
                 Lambda(5) function  3.097E+00
              Lambda harmonic fuction  2.274E+00
        Lambda initial function (biref)  2.369E+00
           Lambda mean function (biref)  2.061E+00
        Lambda initial function (fluor)  3.767E+00
           Lambda mean function (fluor)  6.526E+00
```

```
CALCULATION OF FLUORESCENCE ANISOTROPY DECAY

      ABSOLUTE   NORMALIZED   RELAXATION
K    AMPLITUDE    AMPLITUDE         TIME
1    3.409E-02   -6.818E-01    4.321E-07
2   -1.772E-02    3.544E-01    3.445E-07
3   -2.534E-10    5.068E-09    3.287E-07
4    4.783E-09   -9.565E-08    2.024E-07
5   -6.637E-02    1.327E+00    2.023E-07

ZERO-TIME (STEADY-STATE) ANISOTROPY: -5.001E-02

INITIAL RELAXATION TIME FOR ANISOTROPY:  1.663E-07
   MEAN RELAXATION TIME FOR ANISOTROPY:  9.601E-08

                ABSOLUTE   NORMALIZED
   TIME       ANISOTROPY   ANISOTROPY
 0.000E+00    -5.001E-02    1.000E+00
 .................................
 1.728E-06     4.942E-04   -9.882E-03


CALCULATION OF ELECTRIC BIREFRINGENCE DECAY

      ABSOLUTE   NORMALIZED   RELAXATION
K    AMPLITUDE    AMPLITUDE         TIME
1    5.859E-01    4.423E-01    4.321E-07
2   -1.775E-07   -1.340E-07    3.445E-07
3    7.653E-16    5.778E-16    3.287E-07
4    6.025E-16    4.549E-16    2.024E-07
5    7.387E-01    5.577E-01    2.023E-07

ZERO-TIME (STEADY-STATE) BIREFRINGENCE:  1.325E+00

INITIAL RELAXATION TIME FOR BIREFRINGENCE:  2.645E-07
   MEAN RELAXATION TIME FOR BIREFRINGENCE:  3.039E-07

                 ABSOLUTE      NORMALIZED
   TIME     BIREFRINGENCE   BIREFRINGENCE
 0.000E+00      1.325E+00       1.000E+00
 ....................................
 1.728E-06      1.088E-02       8.210E-03


CALCULATION OF SCATTERING FORM FACTOR, P, VS. H
            H               P
      0.00E+00        1.00E+00
      ......................
      6.67E+06        1.31E-01
```

Fig. 3 Edited output from SOLPRO. The three parts are the HYDRO output, the shape-dependent quantities and the decay and scattering functions

essentially different from those measured in electric birefringence or dichroism.

COMMON/SCATTER contains the data and results from scattering calculations:

NH, number of values of h
H, array with the h values
P, array with the resulting P values

SOLPRO provides an ‘automatic’ mode for filling the values of time in the decays and the values of h in the scattering. If the user gives NTF=−1, the program will calculate the fluorescence anisotropy decay for 101 values of time from $t=0$ through a maximum value $t_{max}=4\tau_1$ where τ_1 is the longest relaxation time. This ensures that the normalized decay covers nearly three decades. For birefringence decay, setting NTB=−1 has the same effect. For scattering calculation, if the author gives NH=−1 or −2, the program will take 101 values of h between 0 y h_{max}. If NH=−1, $h_{max}=5/R_g$, useful for light scattering or low-angle x-ray scattering, while if NH=−2, $h_{max}=2/\sigma_{min}$, where σ_{min} is the radius of the smallest bead, that covers the wide-angle scattering region.

In COMMON/SHAPEFUN1, COMMON/SHAPEFUN2 and COMMON/SHAPEFUN3 we have the results for shape-dependent functions and other properties:

T0 is the relaxation time of the equivalent sphere, τ_0 in Eq. (15)
TH is the harmonic mean relaxation time, τ_h in Eq. (21)
SIMHA is the ν function in Eq. (67)
PERRIN is the P function in Eq. (68)
TIT0(K) is the τ_k/τ_0 ratio in Eq. (69)
THT0 is the similar τ_h/τ_0 ratio
TINBT0, TMEBT0, TINFT0 and TMEFT0 are the similar τ_{ini}/τ_0 and τ_{mean}/τ_0 ratios for birefringence and fluorescence
G is the G function in Eq. (70)
PFLORY is the P_0 function in Eq. (75)
PHIFLORY is the Φ function in Eq. (76)
BETASM is the Scheraga-Mandelkern parameter β in Eq. (71)
RR is the Wales-Van Holde R function in Eq. (72)

TR(K), TRH, TRINB, TRMEB, TRINF, TRMEF are the $K_{\tau r}$ functions in Eq. (77), based, respectively, on τ_k, τ_h, τ_{ini}, τ_{mean}, the latter two for birefringence and fluorescence

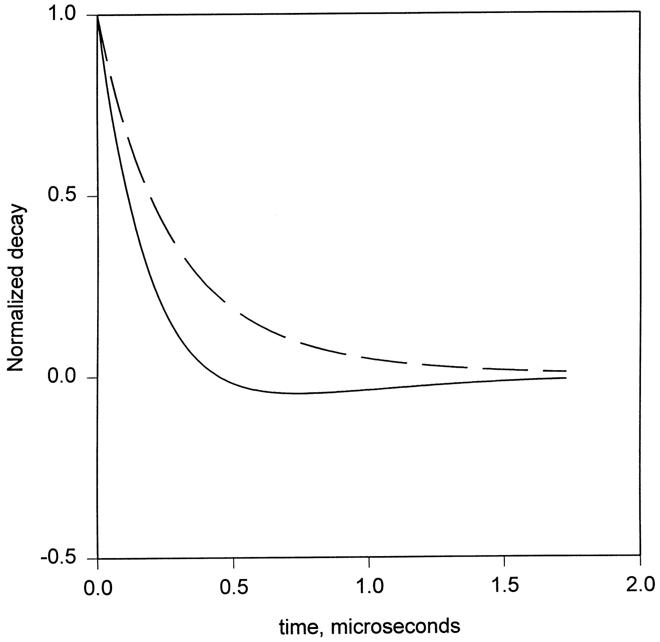


Fig. 4 Normalized decays of fluorescence, $r(t)/r(0)$ (continuous line) and birefringence, $\Delta n(t)/\Delta n(0)$ (dashed line)

PSI(K), PSIH, PSIINB, PSIMEB, PSIINF, PSIMEF are the Ψ functions in Eq. (73), based, respectively, on τ_k, τ_h, τ_{ini}, τ_{mean}, the latter two for birefringence and fluorescence.

RLA(K), RLAH, RLAINB, RLAMEB, RLAINF, RLAMEF are the Λ functions in Eq. (74), based, respectively, on τ_k, τ_h, τ_{ini}, τ_{mean}, the latter two for birefringence and fluorescence.

4 Results

We present here an example of the use of SOLPRO, where we calculate the properties of a bead model for immunoglobulin IgG3 (Gregory et al. 1987). This model has been used as an example of bead modelling in previous reviews (García de la Torre et al. 1989, 1992). The model is displayed in Fig. 1. The input data for the calculations of this model are presented in Fig. 2. In addition to the data required for HYDRO, we supply the additional values for birefringence, fluorescence and scattering as required by SOLPRO, (we recall that all these data can be omitted if one just wants the SOLPRO calculation of universal functions). As this is just a computational example, we have given arbitrary values to the spectroscopic and electrooptic quantities, ϕ, ω, α and χ. Nonetheless, the model dimension and other solution quantities are real properties of IgG3. In this example we have used the ‘automatic’ mode for t and h. The user supplied main program or the sample supplied with SOLPRO will read the input file before calling HYDRO and SOLPRO.

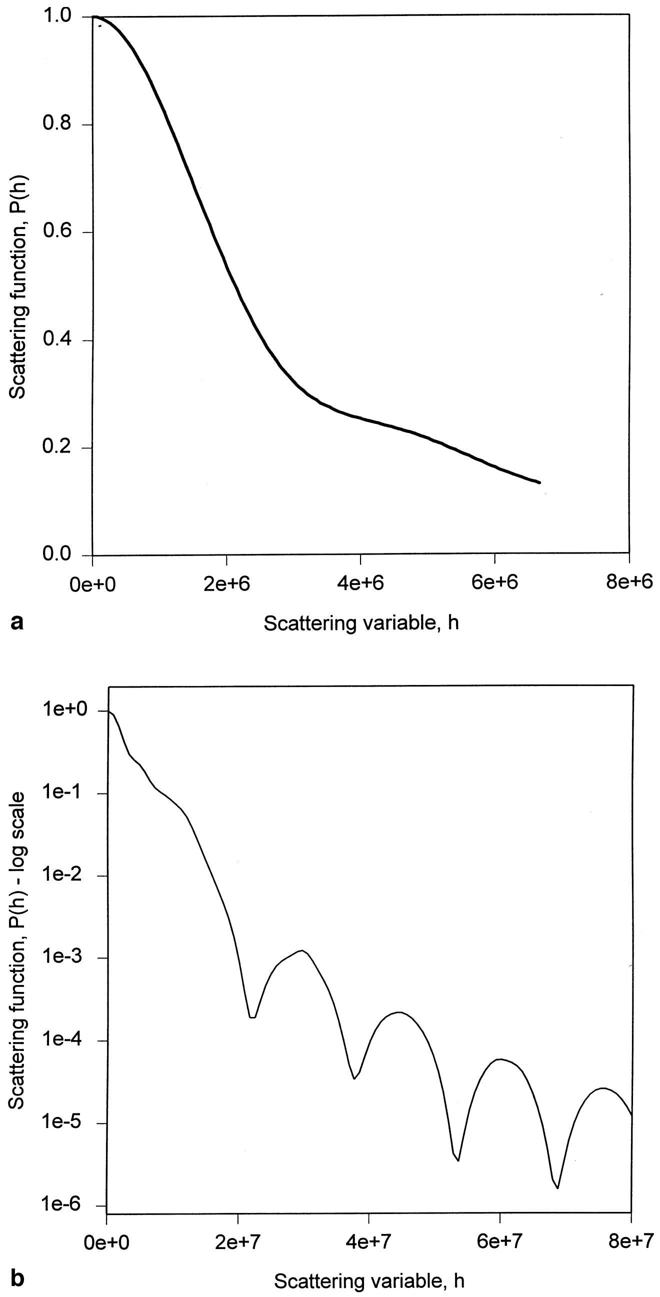


Fig. 5 a, b Scattering function calculated for the bead model of IgG3. **a** Low angle scattering. **b** Wide angle-scattering

The output file obtained from SOLPRO is shown in Fig. 3. We note first the standard output for HYDRO, followed by the values for the shape-dependent functions, and finally we see the results for the fluorescence and birefringence decays and for the scattering functions. Actually, Fig. 3 is an edited form of the long SOLPRO output: for the sake of brevity we have omitted in Fig. 3 the results derived from the intermediate relaxation times, (τ_2, τ_3 and τ_4) and we have cut the tables of $\Delta n(t)/\Delta n(0)$, $r(t)/r(0)$ and $P(h)$.

The decays for birefringence and fluorescence of the IgG3 model are shown in Fig. 4. The decay functions are clearly multiexponential. The fluorescence anisotropy decay shows the possibility of a change of sign in $r(t)$ during the decay to zero. The scattering diagrams are presented in Fig. 5. The light-scattering or low-angle x-ray scattering diagram is in Fig. 5a, while Fig. 5b displays the wide-angle x-ray scattering diagram, showing the typical minima and maxima in intensities observed at wider angles.

5 Discussion

From the case example presented in the preceeding section, it is clear that the combination of the new subroutine, SOLPRO, with the existing HYDRO program, enables the calculation of a great variety of solution properties. Using just the geometrical data of the bead model (coordinates and radii) it is possible now to calculate with SOLPRO not only the simple properties supplied by HYDRO, but also the various combination of properties and shape-dependent quantities, and the scattering diagram. If the spectroscopic and electro-optic quantities that determine electric birefringence or dichroism, and fluorescence anisotropy are also given, SOLPRO can be used to predict the time-decay of these properties.

More details on the use of SOLPRO will be found in the comments intercalated in the FORTRAN code. An ASCII computer file containing the SOLPRO subroutine plus a typical main program, along with sample input and output data files will be freely available. The latest versions of both SOLPRO and HYDRO can be freely downloaded from the INTERNET Web page http://leonardo.fcu.um.es/macromol.

Acknowledgements This work has been supported by grant PB93-1132 from Direccion General de Investigacion Cientifica y Tecnica, Ministerio de Educacion y Ciencia to J.G.T. B. Carrasco is the recipient of a predoctoral award from Plan de Formacion de Personal Investigador. Dr. Pilar Lillo (CSIC, Madrid) supplied us with valuable information. The support of the BBSRC is also acknowledged, and some helpful discussions with Dr. O. Byron.

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