MODELLING BIOLOGICAL MACROMOLECULES IN SOLUTION: THE GENERAL TRI-AXIAL ELLIPSOID

Stephen Ernest Harding, M.A. (Oxon), M.Sc.

PhD Dissertation

University of Leicester

1980

C H A P T E R 5

Concluding Remarks

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

Despite the availability of the R function for determining the 'equivalent hydrodynamic ellipsoid of revolution' for a structure in solution to a reasonable precision (and also the Π function for prolate ellipsoids - Appendix III), it was clear from a perusal of the crystallographic dimensions given in Table 3 and a comparison of model dependent with model independent estimates for $\bar{v}_s/\bar{v}$ in Table 2, that for many macromolecules the assumption of two equal axes on the ellipsoid model is a poor

approximation to the real structure in solution. This stimulated my attempts to develop the necessary theoretical and data analysis techniques so that the restriction of two equal axes could be dispensed with and the subsequent research has shown that the more general tri-axial ellipsoid can now, in principle at least, be successfully employed for modelling biological macromolecules in solution.

The first step was to derive an explicit expression for the viscosity increment ν for a dilute suspension of general tri-axial ellipsoids in overwhelming Brownian motion, based on a model first given by Simha (1940) and improved by Saito (1951) for ellipsoids of revolution. Although the assumption of the particles rotating on average with the same local angular velocity of the fluid has only been rigorously proved so far for ellipsoids of revolution (Brenner, 1972a), it was assumed that this would be a very close approximation for tri-axial ellipsoids, particularly for low axial ratios (<3.0, i.e. the globular particle range). After the derivation of equation (88) a numerical procedure (involving complicated numerical matrix inversions), but based on a full statistical analysis of the angular motion was made available by Rallison (1978). It was explained in section 2.8. how the difference in the results predicted by equation (88) and Rallisons approach was negligible ($<.01\%$) for the globular particle range mentioned above, and for some particles of higher asymmetry discrepancies of not more than 1% arose. Rallison has also given a numerical procedure for calculating the normal stress coefficients in terms of axial ratio; normal stress effects are however second order in the shear rate, thus in order to measure these coefficients it is necessary to use high shear rates. However, the assumption of overwhelming Brownian motion with respect to the shear rate ceases to be valid, and hence, unfortunately, the normal stress coefficients cannot be applied.

It was described how the problem of the line solution (i.e. how a given value for ν does not uniquely fix a value for the axial ratios (a/b, b/c)) could be dealt with by combining it graphically with translational frictional or rotational relaxation line solutions. I was able to give the R function for tri-axial ellipsoids and also many other tri-axial functions whose experimental determination did not require a knowledge of the swollen molecular volume in solution. After a careful consideration of all these line solutions with regard to giving suitable intersections, experimental measurability, insensitivity to experimental error and sensitivity to axial ratio, it was decided that the best approach for determining a unique solution would be to combine the R line solution graphically with the δ_+ and δ_- line solutions, the latter to be determined from the two electric birefringence decay constants and the intrinsic viscosity.

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

of subsidiary minima located in the iteration procedure, even for data of two orders of magnitude more precise than that currently available from the best instrumentation.

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

Investigation of the theoretical reasons for the concentration dependence of the decay constants provides however both an interesting and important field for further work. It has been described (section 1.7.1. &

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

In section 1.6. the techniques of light and low-angle x-ray scattering were discussed as an alternative to the hydrodynamic techniques, and stated how Martin (1964) had given formulae relating the radius of gyration to

axial ratio for ellipsoids of revolution. Mendelson and Hartt (1980) have applied results from low angle x-ray scattering in terms of a general triaxial ellipsoid model to the regulatory light chains of scallop myosin, and determined axial dimensions of 16nm x 4.16nm x 1.26nm. We also mentioned however that the major disadvantage of the scattering approach was that it is necessary to assume the macromolecule to be of uniform electron density; this can lead to errors of the order of 3%, notwithstanding other errors in measurement as the simple calculation given in Appendix VII for a hypothetical spherical macromolecule with a cavity (based on the electron microscopy and x-ray diffraction results for apoferritin - Harrison, 1959) shows.

It is hoped however that the results of the research described here have now made it possible to determine the gross conformation of biological macromolecules in solution in terms of a general ellipsoid - independent of any assumptions concerning the internal homogeneity of the macromolecules - by combining the results of viscosity, sedimentation and electric (or acoustic) birefringence. There are some macromolecules however that apparently will never be modelled by an ellipsoid, even tri-axial. Bovine serum albumin (BSA) is a typical example; McCammon et al (1975) have attempted to account for a value for β below the theoretical minimum of 2.112×10^6 (and above the theoretical maximum for R of 1.6 - see Table 2) by assuming its structure to be porous with respect to the solvent, but found the discrepancy was still far too large. With the availabilty of the tri-axial ellipsoid model and a comparison with model independent estimates for the swollen molecular volume, a classification of proteins into those which do and those which do not behave as hydrodynamic tri-axial ellipsoids in solution can now be made.

BIBLIOGRAPHY.

Alexander, A.E. and Johnson, P. (1949)

'Colloid Science', Volume 2, Oxford University Press

Alpert, S.S. and Banks, G. (1976)

Biophysical Chem. 4, 287

Baghurst, P.A., Nichol, L.W., Ogston, A.G., Winzor, D.J. (1975)

Biochem. J. 147, 575

Ballinger, K.W.A. and Jennings, B.R. (1979)

Nature 282, 699

Batchelor, G.K. (1967)

'An Introduction to Fluid Mechanics', Cambridge University Press

Batchelor, G.K. (1970)

J. Fluid Mech. 41, 545

Batchelor, G.K. and Green, J.T. (1972)

J. Fluid Mech. 56, 375

Beeman, W.W., Koesberg, P., Anderegg, J.W. and Webb, M.B. (1957)

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

Benoit, H. (1951)

Ann. Physik. 6, 561

Berne, P.J. and Pecora, R. (1974)

Ann. Rev. Phys. Chem. 25, 233

Blake, C.C.F. (1975)

Essays in Biochem. II, 37

Blake, C.C.F., Geisow, M.J. and Oatley, S.J. (1978)

J. Mol. Biol. 121, 339

Blake, C.C.F., Koenig, D.F., Mair, G.A., North, A.C.T., Phillips, D.C. and Sarma, V.R. (1965)

Nature 206, 757

Bloomfield, V.A., Dalton, W.O. and Van Holde, K.E. (1967)

Biopolymers 5, 135

Bresler and Talmud (1944)

CR Acad. Sci. URSS, 43, 310

Brinkman, H.C., Hermans, J.J., Oosterhoff, L.J., Overbeek, J. Th. G., Polder, D., Staverman, A.J. and Wiebenga, E.H. (1949)

Proc. Int. Rheol. Congress (Schveningen) II, 77

Brenner, H. (1970)

J. Coll. Int. Sci. 32, 141

Brenner, H. (1972a)

Chem. Eng. Sci. 27, 1069

Brenner, H. (1972b)

Progr. Heat and Mass Transfer 5, 93

Cantor, C.R. and Tao, T. (1971)

Proc. Nucl. Acid Res. 2, 31

Cerf, R. and Scheraga, H.A. (1952)

Chem. Revs. 51, 185

Chapman, P.F. (1913)

Phil. Mag. 25, 475

Cheng, P.Y. and Schachman, H.K. (1955)

J. Polymer Sci. 16, 19

Chu, B. (1974)

'Laser Light Scattering', Academic, New York

Chwang, A.T. (1975)

J. Fluid Mech. 72, 17

Clenshaw, C.W. and Curtis, A.R. (1960)

Num. Math. 2, 197

Creeth, J.M. and Knight, C.G. (1965)
Biochim. Biophys. Acta 102, 549

Cummings, H.Z. and Pike, E.R. (1973)
(eds.) 'Photon Correlation and Light Beating Spectroscopy', Plenum, New York

Dickerson, R.E. and Geiss, I. (1969)
'The Structure and Action of Proteins', Benjamin, California

Dougherty, J. and Kreiger, I.M. (1972)
in Kreiger, Adv. Colloid Sci. 3, 111

Edsall, J.T. (1953)
in 'The Proteins' (Neurath, H. and Bailey, K. eds) 1B, Chapter 7, Academic, New York

Edwardes, D. (1892)
Quart. J. Math. 26, 70

Einstein, A. (1905)
Ann. Physik 17, 549

Einstein, A. (1906)
Ann. Physik 34, 591

Einstein, A. (1911)
Ann Physik 34, 591

Emes, C.H. (1977)

Ph. D. thesis, University of Leicester

Emes, C.H. and Rowe, A.J. (1978a)

Biochim. Biophys. Acta 537, 110

Emes, C.H. and Rowe, A.J. (1978b)

Biochim. Biophys. Acta 537, 125

Farrant, J.L. (1954)

Biochim. Biophys. Acta 13, 569

Feldman, R.J. (1976)

'Atlas of Macromolecular Structure on Microfiche', Traco Jitco Int., Rockville, Md. USA

Gans, R. (1928)

Ann. Physik 86, 628

Garcia Bernal, J.M. and Garcia de la Torre, J. (1980)

Biopolymers 19, 628

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

Biopolymers 16, 1747

Garcia de la Torre, J. and Bloomfield, V.A. (1977b)

Biopolymers 16, 1765

Garcia de la Torre, J. and Bloomfield, V.A. (1977c)

Biopolymers 16, 1779

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

Biopolymers 17, 1605

Gardner, D.G., Gardner, J.C., Laush, G. and Meinke, W.W. (1959)

J. Chem. Phys. 31, 978

Giesekus, H. (1962)

Rheol. Acta 2, 50

Gill, P.E. and Murray, W. (1976)

Nat. Phys. Lab. report NAC 72

Gold, O. (1937)

Ph. D. thesis, University of Vienna

Goodwin, J.W. (1975)

Colloid Science 2 (Chemical Society), 246

Guoy, L.G. (1910)

J. Phys. Chem. 9, 457

Hall, C.E. and Slayter, H.S. (1959)

J. Biophys. Cytol. 5, 11

Harding, S.E. (1980a)

Biochem. J. 189 (in press)

Harding, S.E. (1980b)

mss. submitted to J. Phys. Chem.

Harrison, P.M. (1959)

J. Mol. Biol. 1, 69

Herzog, R.O., Illig, R. and Kudar, H. (1934)

Z. Phys. Chem. A167, 329

Holtzer, A. and Lowey, S. (1956)

J. Am. Chem. Soc. 78, 5954

Jablonski, A. (1961)

Naturforsch 169, 1

Jeffrey, G.B. (1922)

Proc. Roy. Soc. (London) A102, 476

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

Biochim. Biophys. Acta 102, 476

Jost, J.W. and O'Konski, C.T. (1978)

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

Kartha, G., Bello, J. and Harker, D. (1967)

Nature 213, 862

Kendrew, J.C., Bodo, G., Dintzis, H.M., Parrish, R.G., Wycoff, H. and Phillips, D.C. (1958)

Nature 181, 665

Kim, S.H. (1974)

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

Kirkwood, J.G. (1967)

'Macromolecules' (Aueur, P.L. ed.), Gordon and Breach

Koenig, S.H. (1975)

Biopolymers 14, 2421

Kratky, O., Leopold, H. and Stabinger, H. (1969)

Z. Angew. Phys. 27, 273

Kratky, O., Leopold, H. and Stabinger, H. (1969)

in 'Methods in Enzymology' (Hirs, C.H.W. and Timasheff, S.N. eds.), 27, 98, Academic, London

Krause, S. and O'Konski, C.T. (1959)

J. Am. Chem. Soc. 81, 5082

Kuff, E.J. and Dalton, A.L. (1957)

J. Ultrastructure Res. 1, 62

Kuhn, W. and Kuhn, H. (1945)

Helvetica Chimica Acta 38, 97

Kynch, G.J. (1956)

Proc. Roy. Soc. (London) A237, 90

Labaw, L.W. and Wycoff, R.W.G. (1957)

Biochim. Biophys. Acta 25, 263

Lauffer, M.A. (1942)

Chem. Rev. 31, 561

Laurent, T.C. and Killander, J. (1964)

J. Chromatog. 14, 317

Lipscomb, W.N. (1971)

Proc. Robert A. Welch Found. Conf. Chem. Res. 15, 134

Lloyd, P.H. (1974)

'Optical Methods in Ultracentrifugation, Electrophoresis and Diffusion',

Clarendon Press, Oxford

Lucas, C.W. and Terrill, C.W. (1970)

Collected Algorithms from CACM, No. 404

Manley, R. and Mason, S. G. (1954)

Canad. J. Chem. 32, 763

Martin, R.B. (1964)

'Introduction to Biophysical Chemistry' Chap. 11, McGraw-Hill, New York

McCammon, J.A., Deutch, J.M. and Bloomfield, V.A. (1975)

Biopolymers 14, 2479

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

Science 92, 132

Mendelson, R. and Hartt, J. (1980)

EMBO Workshop on Muscle Contraction, Alpbach Conf. Austria

Mihalyi, E. and Godfrey, J. (1963)

Biochim. Biophys. Acta 67, 90

Memming, R. (1961)

Z. Physik. Chem. (Frankfurt) 28, 169

Mooney, M. (1951)

J. Coll. Sci. 6, 162

Mooney, M. (1957)

J. Coll. Sci. 12, 575

Munro, I., Pecht, I. and Stryer, L. (1979)

Proc. Nat. Acad. Sci. (USA) 76, 56

Nichol, L.W., Jeffrey, P.D., Turner, D.R. and Winzor, D.J. (1977)

J. Phys. Chem. 81, 776

Nisihara, T. and Doty, P. (1958)

Proc. Nat. Acad. Sci. (USA) 44, 411

Oberbeck, A. (1876)

J. reine. angew. Math. 81, 62

O'Connor, D.V., Ware, W.R. and Andre, J.C. (1979)

J. Phys. Chem. 83, 1333

Offer, G., Moos, C. and Starr, R. (1973)

J. Mol. Biol. 74, 653

O'Hara and Smith (1968)

Comp. Journal 11, 213

O'Konski, C.T. and Haltner, A.J. (1956)

J. Am. Chem. Soc. 78, 3604

Oliver, J. (1972)

Comp. Journal 15, 141

Oncley, J.L. (1940)

J. Phys. Chem. 44, 1103

Oncley, J.L. (1941)

Ann. New York Acad. Sci. 41, 121

Paradine, C.G. and Rivett, B.H.P. (1960)

'Statistical Methods for Technologists', English Universities Press, London

Pearce, T.C., Rowe, A.J. and Turnock, G. (1975)

J. Mol. Biol. 97, 193

Perrin, F. (1934)

J. Phys. Radium 5, 497

Perrin, F. (1936)

J. Phys. Radium 7, 1

Perutz, M.F., Rossmann, M.G., Cullis, A.F., Muirhead, H., Will, G. and North, A.C.T. (1960)

Nature 185, 416

Powell, D.R. and MacDonald, J.R. (1972)

Comp. Journal 15, 148

Pytkowickz, R.M. and O'Konski, C.T. (1959)

J. Am. Chem. Soc. 81, 5082

Rallison, J.M. (1978)

J. Fluid Mech. 84, 237

Riddiford, C.L. and Jennings, B.R. (1967)

Biopolymers 5, 757

Ridgeway, D. (1966)

J. Am. Chem. Soc. 88, 1104

Ridgeway, D. (1968)

J. Am. Chem. Soc. 90, 18

Rowe, A.J. (1977)

Biopolymers 16, 2595

Rowe, A.J. (1978)

Techniques in Protein and Enzyme Biochem. B105a, 1

Saito, N. (1951)

J. Phys. Soc. (Japan) 6, 297

Scheraga, H.A. (1955)

J. Chem. Phys. 23, 1526

Scheraga, H.A. (1961)

'Protein Structure', Academic, New York

Scheraga, H.A. and Mandelkern, L. (1953)

J. Am. Chem. Soc. 79, 179

Simha, R. (1940)

J. Phys. Chem. 44, 25

Simha, R. (1952)

J. Appl. Phys. 23, 1020

Shaw, D.J. (1970)

'Introduction to Colloid and Surface Chemistry' (2nd edn.), Butterworths

Small, E.W. and Isenberg, I. (1977)

Biopolymers 16, 1907

Sorenson, N.A. (1930)

CR Lab. Carlsberg 18, No. 5

Squire, P.G. (1970)

Biochim. Biophys. Acta 221, 425

Squire, P.G. (1978)

in 'Molecular Electro Optics' (O'Konski, C.T. ed.) Volume 2, 565

Squire, P.G., Moser, L. and O'Konski, C.T. (1968)

Biochemistry 7, 4261

Stacey, K.A. (1956)

'Light Scattering in Physical Chemistry', Butterworths, London

Stokes, Sir G. (1851)

Trans. Cambridge Phil. Soc. 9, 8

Stokes, Sir G. (1880)

'Mathematical and Physical Papers', Cambridge University Press

Svedberg, T. and Pedersen, K.O. (1940)

'The Ultracentrifuge', Oxford University Press

Tanford, C. (1955)

J. Phys. Chem. 59, 798

Tanford, C. (1961)

'Physical Chemistry of Macromolecules', Wiley, New York

Theorell, H. (1934)

Biochem. Z. 268, 46

Vand, V. (1948)

J. Phys. Coll. Chem. 52, 277

Van de Hulst, H.C. (1957)

'Light Scattering by Small Particles', Wiley, New York

Van Holde, K.E. (1971)

'Physical Biochemistry', Prentice Hall, New Jersey

Wahl, P. (1966)

Compt. Rend. Acad. Sci. (Paris) 263D 1525

Wales, M. and Van Holde, K.E. (1954)

J. Polymer Sci. 14, 81

Wilde, D.J. (1964)

'Optimum Seeking Methods', Prentice Hall, New Jersey

Williams, R.C., Ham, W.T. and Wright, A.K. (1976)

Anal. Biochem. 73, 52

Wilson, R.W. and Bloomfield, V.A. (1979a)

Biopolymers 18, 1205

Wilson, R.W. and Bloomfield, V.A. (1979b)

Biopolymers 18, 1543

Yang, J.T. (1961)

Adv. Protein Chem. 16, 323

Zimm, B.H. (1948)

J. Chem. Phys. 16, 1093

Zimm, B.H. (1956)

J. Chem. Phys. 24, 269