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6 **Original Paper**
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11 **Concentration dependence of translational diffusion**
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13 **coefficients for globular proteins**
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[ABSTRACT]

This investigation examines published results of traditional diffusion experiments on ovalbumin and bovine serum albumin to determine the extent to which assumed concentration independence of the translational diffusion coefficient is a reasonable approximation in the analysis of boundary spreading in sedimentation velocity experiments on proteins. Although significant positive concentration dependence of the diffusion coefficient (D) for both proteins is predicted by current theories, none has been detected in these experimental diffusion studies performed under the constraints of constant temperature and solvent chemical potential (those also pertinent to sedimentation velocity). Instead, the results are better described by the relatively minor concentration dependence predicted by considering solution viscosity to be an additional source of D - c dependence. Inasmuch as the predicted variation in D for solutions with concentrations below 10 mg/mL is within the uncertainty of experimental estimates, these findings support use of the approximate solution of the Lamm equation developed by Fujita for the quantitative analysis of boundary spreading in sedimentation velocity experiments on proteins.

Introduction

The combination of a new-generation analytical ultracentrifuge and the massive advances in computer technology has rekindled interest in the quantitative description of the concentration dependence of translational diffusion coefficients (D) for proteins – a topic that has attracted only spasmodic attention in the past five decades. At that stage Fujita^{1,2} had reported an approximate analytical solution of the continuity equation describing solute migration in a centrifugal field³ that took into account the boundary sharpening that arises from negative, linear concentration dependence of the sedimentation coefficient for a system with D independent of solute concentration (c), which is a realistic situation inasmuch as the neglect of D - c dependence seems to be a reasonable experimental approximation. However, the situation is less satisfactory from a theoretical viewpoint in that the predicted positive concentration dependence of D^{4-6} contradicts observations of slight negative D - c dependence⁷⁻¹⁰ when experiments are conducted under the constraints of constant temperature and solvent chemical potential – the conditions relevant to sedimentation velocity experiments. This investigation attempts to resolve that disparity between theory and experiment.

Theoretical considerations

Although concentration dependence of the translational diffusion coefficient for macromolecular solutes has received considerable theoretical scrutiny, much of that attention has been directed towards the description of diffusion in quasi-elastic lights scattering spectroscopy (dynamic light scattering) studies,^{6,11-16} where the solute chemical potential is being monitored under the constraint of either constant pressure¹⁷⁻¹⁹ or constant volume:²⁰ either option applies to the incompressible

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3 solutions being considered here. As noted by Phillies,¹⁵ the disparate theoretical description of D - c
4 dependence reported by Batchelor⁴ refers to the situation involving flux of solute molecules under
5 the influence of an applied force, the gradient in solute chemical potential under the constraint of
6 constant solvent chemical potential – a constraint that allows all buffer and small electrolyte
7 components to be regarded as part of the solvent.^{21–23} This simplification does not apply to light
8 scattering experiments, where the constraint of constant pressure (or volume) dictates the
9 consideration of these small components as additional cosolutes.^{17–19}

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11 The most detailed consideration of the effects of hydrodynamic interaction on Brownian
12 diffusion involving net flux of solute appears in the above-mentioned article by Batchelor,⁴ which
13 provides an alternative derivation of the standard expression for independent translational diffusion
14 of molecules,

$$D = \frac{RT}{N_A 6\pi\eta a} \quad (1)$$

15
16 where the diffusion coefficient is described in terms of the hydrodynamic (Stokes) radius a and the
17 dynamic viscosity of the medium η . N_A is Avogadro's number. This expression for ideal diffusion
18 was developed initially by Einstein²⁴ on the basis of the osmotic pressure of the system, and hence
19 on thermodynamic considerations of solute chemical potential under the constraints of constant
20 temperature and solvent chemical potential (μ_1).^{25,26} These are certainly the constraints that apply in
21 traditional diffusion experiments involving an initially sharp boundary between protein solution and
22 the diffusate against which it has been extensively dialyzed. Furthermore, a similar situation applies
23 to boundary spreading in sedimentation velocity studies, where the constraints of constant
24 temperature and solvent chemical potential also seem to apply.^{27–29}

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An expression for D - c dependence of based on thermodynamic considerations

Attempts to incorporate the effects of solute concentration into eqn (1) followed the Einstein line of reasoning¹¹ by regarding the nonideality as a thermodynamic problem. On the grounds that the driving force for diffusion is the gradient in solute chemical potential the diffusion coefficient, D has been expressed⁴⁻⁶ as

$$D = \frac{M}{N_A f} \left(\frac{\partial \Pi}{\partial c} \right)_{T, \mu_1} = \frac{RT(1 + 2BMc + \dots)}{N_A f} \quad (2)$$

where the second representation of D follows from the standard virial expansion of osmotic pressure under the same constraints,

$$1 \quad \left(\frac{\Pi}{RT} \right)_{T, \mu_1} = c/M + BMc^2 + \dots \quad (3)$$

in which $B = B_{22}/M^2$ is the osmotic second virial coefficient for solute self-interaction expressed in the usual experimental units of mL mol g⁻² rather than its molar counterpart (B_{22}). Upon introducing the relationship

$$f = f^0(1 + k_s c) \quad (4)$$

for the concentration dependence of the translational friction coefficient,⁵ eqn (2) becomes

$$D = \frac{RT(1 + 2BMc + \dots)}{N_A f^0(1 + k_s c)} = \frac{RT(1 + (2BM - k_s)c + \dots)}{N_A f^0} \quad (5)$$

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4 By defining D^0 as $RT/N_A f^0$ and expressing the concentration dependence of the diffusion
5 coefficient as
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$$D = D^0(1 + k_D c) \quad (6)$$

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12 it follows^{5,30} from eqn (5) that
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$$k_D = 2BM - k_s \quad (7)$$

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21 Harding and Johnson⁵ provided two expressions for k_D : eqn (7) above, which corresponds to
22 sedimentation coefficients corrected for *solvent* density, and another expression for sedimentation
23 coefficients corrected for *solution* density in which an extra term ($-\bar{v}$) appears. The appropriate
24 correction (for solvent density) was first recognized by Fujita³ who amended the basic
25 sedimentation equilibrium equation³¹ to take into account the concentration dependence of solution
26 density – a parameter originally regarded as a constant in the integration of the buoyancy term. A
27 consequence of this amendment was the erroneous consideration of the experimental second virial
28 coefficient as $(2BM - \bar{v})$ until the existence of a second (compensating) error in the original basic
29 sedimentation equilibrium equation was detected.^{26,32,33}
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44 In statistical-mechanical terms the osmotic second virial coefficient for protein self-
45 interaction may be calculated by assuming spherical geometry for the hydrated solute (radius a)
46 with net charge Z spread uniformly over its surface. Specifically, the expression for B_{22} comprises
47 two terms: a relatively simple term for the covolume of two identical impenetrable spheres, and a
48 term to cover the potential-of-mean-force, $u_{ij}(x)$ between charged pairs of molecules (i and j) that
49 are separated by distance x .³⁴ Specifically,
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$$B_{22} = \left(\frac{16\pi N_A a^3}{3} \right) - 2\pi N_A \int_0^\infty f_{ij}(x) x^2 dx \quad (8a)$$

$$f_{ij}(x) = \exp[-u_{ij}(x)/(kT)] - 1 \quad (8b)$$

in which k is the Boltzmann constant: Avogadro's number is introduced to convert B_{22} from a molecular to a molar basis. The potential energy of the two molecules, $u_{ij}(x)$, can be calculated from the expression^{35,36}

$$\frac{u_{ij}(x)}{kT} = \frac{1000Z^2\kappa^2(-\kappa(x-2a))}{8\pi N_A I(1+\kappa a)^2 x} \quad x \geq 2a \quad (9)$$

where κ , the Debye–Hückel inverse screening length (cm^{-1}), is related to the molar ionic strength of the solvent (I) by the expression $\kappa = 3.27 \times 10^7 \sqrt{I}$. The factor of 1000 in eqn. (9) takes into account the units of ionic strength (mol/L) in an expression where the unit of volume is cm^3 .

An expression for D - c dependence based on hydrodynamic considerations

From hydrodynamic considerations of the concentration dependence of the translational diffusion coefficient for a rigid spherical particle in sedimentation velocity experiments (which also involve a gradient in solute concentration), Batchelor⁴ has derived the relationship [see eqns (6.10) and (6.12) therein]

$$D = \frac{(1 + (8\phi - 6.55\phi))RT}{N_A 6\pi\eta a} \quad (10)$$

which, after replacement of the volume fraction of solute (ϕ) by the product $v_s c$, becomes

$$D = \frac{(1 + (8v_s - 6.55v_s)c)RT}{N_A 6\pi\eta a} = \frac{(1 + (8v_s - k_s)c)RT}{N_A 6\pi\eta a} \quad (11)$$

where the alternative formulation takes advantage of the Batchelor expression for the coefficient (k_s) describing linear concentration dependence of the sedimentation coefficient, $s = s^0(1 - k_s c)$. In that regard we note that Batchelor's value of 6.55ϕ for k_s has since been decreased to 5ϕ by Brady and Durlofsky³⁷ – an amendment that is incorporated into subsequent considerations.

Upon noting that $2BM = 8v_s$ for an uncharged solute, the numerators of eqns (5) and (11) are equivalent for an isoelectric protein – as are the denominators in that Batchelor⁴ has merely made the Stokes–Einstein substitution for f^0 . Whereas f^0 was regarded as a constant in order to obtain eqn (7) as the quantitative expression for k_D , it is actually the frictional coefficient at zero protein concentration in a medium with viscosity η . Inasmuch as η is a function of solute concentration, f^0 becomes a concentration-dependent variable of which account needs to be taken in the quantitative expression for D – c dependence. As noted by Rowe,³⁷ the Batchelor treatments of migration^{4,38} take no account of the solute contribution to viscosity of the medium.

An effect of viscosity on the magnitude of the translational diffusion coefficient is thus manifested in eqn (11), which we now write (with k_s taken as $5v_s c$) in the form

$$D = \frac{(1 + 3v_s c)RT}{N_A 6\pi(\eta/\eta_b)\eta_b a} \quad (12)$$

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3 where inclusion of the term for the ratio of solution to buffer viscosities. η/η_b , allows separation of
4 this effect from that of buffer viscosity (η_b), which is routinely eliminated by expressing D as the
5 value applying to diffusion in a medium with the viscosity of water at the temperature of interest.
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11 Although a is a hydrodynamic parameter, its magnitude is frequently taken as that of its
12 thermodynamic counterpart [a in eqn (8a)] in sedimentation equilibrium studies of interacting
13 systems,^{32,33,40,41} a practice for which there is supporting experimental evidence in the few instances
14 where thermodynamic and hydrodynamic estimates of protein radius have been compared.⁴² Such
15 assumed identity of the Stokes (hydrodynamic) radius and the effective thermodynamic (excluded
16 volume) radius is also supported by recent calculations based on coarse-grained models (4 beads per
17 amino acid residue) for a range of proteins with widely differing sizes, shapes and non-uniform
18 charge distribution.⁴³ Their assumed identity is also seemingly reasonable in that both parameters
19 define the effective size of the protein molecule, especially when account is taken of the fact that
20 sedimentation equilibrium reflects a balance between the two hydrodynamic processes of
21 sedimentation and diffusion. On the grounds that the thermodynamic nonideality for a non-
22 associating protein finds rational explanation in terms of physical interaction between solute
23 particles rather than a change in radius,^{33,34} we shall regard a as a constant in eqn (12).
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44 To take into account the concentration dependence of viscosity we introduce the
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$$\eta/\eta_b = 1 + \eta_{sp}c = 1 + [\eta]c + k_{sp}c^2 \quad (13)$$

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54 where the quadratic term in c reflects concentration dependence of specific viscosity η_{sp} , the
55 intrinsic viscosity ($[\eta]$) being the limiting value of η_{sp} as $c \rightarrow 0$. For a spherical solute the intrinsic
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viscosity is readily obtained as the product $2.5\nu_s$, whereupon the expression for the concentration dependence of the diffusion coefficient becomes

$$D = \frac{(1 + 3\nu_s c)RT}{N_A 6\pi\eta_b a(1 + 2.5\nu_s c + \dots)} \approx D^0(1 + 0.5\nu_s c) \quad (14)$$

for the concentration dependence of diffusion coefficient correct to first order in protein concentration. It should be noted that D^0 is indeed the diffusion coefficient in the limit of zero protein concentration in that f^0 now refers to the frictional coefficient at zero protein concentration in a medium with the buffer viscosity η_b . A much smaller concentration dependence of D is now predicted.

Consideration of experimental diffusion studies

In view of the ambiguity surrounding the quantitative description of the concentration dependence of the diffusion coefficient, this aspect of experimental studies clearly needs to be examined. For that purpose we need experimental data obtained under the constraints of constant temperature and solvent chemical potential in order to comply with expression of the solute chemical potential gradient in terms of osmotic pressure [eqns (2) and (3)]. Unfortunately, that requirement excludes consideration of diffusion coefficients obtained by dynamic light scattering (photon correlation spectroscopy), for which the pertinent solute chemical potential is either $\mu_{T,P}$ ¹⁷⁻¹⁹ or $\mu_{T,V}$.²⁰ We must therefore go back half a century to the classical free diffusion experiments involving the spreading of an initially sharp boundary between protein solution and the diffusate against which it had been dialyzed extensively.^{7-10,45} Furthermore, this was an era when the accuracy of diffusion coefficient measurement reached its peak in that several hours were spent generating the initial sharp boundary,

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3 after which diffusion was allowed to proceed for several days to generate concentration
4 distributions with up to 5 cm between the solvent and solution plateaux.⁹ Indeed, the definition of
5 the concentration distributions is sufficiently precise to allow delineation of the concentration
6 dependence of D from the extent of boundary asymmetry.⁹
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12 13 14 **Experimental studies of isoelectric ovalbumin**

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17 Results¹⁰ obtained from Rayleigh interference records of boundary spreading for ovalbumin in a
18 Tiselius electrophoresis cell⁴⁶ are presented in Fig. 1. Most of the diffusion coefficients (\blacklozenge) refer to
19 ovalbumin under isoelectric conditions (pH 4.59, I 0.16), but similar results (\diamond) have also been
20 obtained⁴⁵ under conditions (pH 7.5 and 8.5, I 0.10) where the protein bears a net charge of -14 .⁴⁷
21 Because the diffusion coefficients refer to the mean concentration across the boundary,⁴⁸ the
22 abscissa of Fig. 1 is expressed in terms of the mean protein concentration \bar{c} rather than that ($2\bar{c}$) in
23 the solution plateau. Substitution of a value of 2.9 nm for a ^{47,49,50} into eqns (12) and (13) [with $f_{ij}(x)$
24 = 0 for $Z = 0$] gives an estimate of 246 L mol⁻¹ for B_{22} , or 1.27×10^{-4} mL mol g⁻² for the usually
25 reported second virial coefficient (B) of this glycoprotein with a molecular mass of 44 kDa.⁵¹
26 Support for the use of 5ϕ rather than 6.55ϕ for k_s comes from a comparison of the respective
27 estimates of 7.0 and 9.2 mL/g with the experimentally determined value of 7.6 mL/g.⁵²
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46 We are now in a position to compare the experimental results with the concentration
47 dependence predicted by the three theoretical expressions [eqns (5), (12) and (14)]: because of the
48 identity of $2BM$ and $8v_s$ for an uncharged solute, eqns (5) and (12) necessarily lead to the same
49 predicted concentration dependence of D . For non-spherical particles the covolume term, $u_{red}v_s$
50 where the coefficient u_{red} (known as the reduced covolume) is 8 for spheres and greater than 8 for
51 non-spherical particles, can be evaluated in terms of the axial dimensions of a particle based on
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3 theory for co-excluding triaxial ellipsoids under dominant Brownian motion⁵³ – theory implemented
4 in the algorithm COVOL.⁵⁴ The experimental results have therefore been subjected to linear
5 regression analysis according to eqn (6) with two assigned values of k_D to obtain D^0 and hence their
6 best-fit description in terms of that model: $k_D = +4.20$ mL/g for eqns (5) and (12); and $k_D = +0.70$
7 mL/g for eqn (14), which incorporates the viscosity correction. For the first model the concentration
8 dependence (---), corresponding to the relationship $10^7 D = 3.90(1 + 4.20 \bar{c})$, provides a poorer
9 description of the experimental data than that (—) emanating from the best-fit to eqn (14), $10^7 D$
10 $= 3.93(1 + 0.70 \bar{c})$. Linear least-squares analysis of the results in Fig. 1 to eqn (4) with D^0 and k_D as
11 curve-fitting parameters yields a negative value (-2.0 mL/g) for the concentration coefficient; but
12 the uncertainty ($\pm 2SD$) of 2.6 mL/g exceeds its absolute magnitude. Inasmuch as the variation in
13 diffusion coefficient predicted by eqn (14) is considerably smaller than the experimental uncertainty
14 inherent in its measurement, the consideration of D to be a concentration-independent parameter for
15 this system is justified.
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36 As noted above, the results for an isoelectric protein do not distinguish between $8v_s$ and $2BM$
37 in the expressions for D - c dependence. However, the fact that similar estimates of D were obtained
38 at neutral pH (where $Z = -14$ and hence $2BM$ is much larger) certainly implicates the covolume
39 ($8v_s$) as the more appropriate parameter. This aspect is now examined further by considering the
40 results from traditional diffusion studies of bovine serum albumin under conditions where the
41 protein is decidedly anionic.
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Experimental studies of bovine serum albumin bearing net charge

An English-designed diffusimeter incorporating Gouy optics to record concentration gradient distributions⁵⁵ was employed⁸ to obtain diffusion data for bovine serum albumin in phosphate buffer (pH 6.8, I 0.10), conditions under which the protein bears a net charge in the vicinity of -20 .⁵⁶ From Fig. 2 it is evident that the measured diffusion coefficients, taken from Table 4 of Creeth,⁸ exhibit no discernible concentration dependence; and the resultant average value of $6.14 (\pm 0.04) \times 10^{-7} \text{ cm}^2 \text{ s}^{-1}$ for $D_{20,w}$ indicates a Stokes radius of 3.5 nm. Incorporation of this value for a and -20 for Z into eqns (12) and (13) yields a molar second virial coefficient (B_{22}) of $765,000 \text{ mL mol}^{-1}$, whereupon $B = 1.76 \times 10^{-4} \text{ mL mol g}^{-2}$ for the bovine serum albumin, which has an analytical molecular mass of 66 kDa.⁵⁷ In keeping with the observations for ovalbumin, the lower estimate of k_s (8.3 cf 10.9 mL/g) is closer to the generally accepted range of 7–8 mL/g that is considered to describe this parameter for globular proteins.

Analysis of the experimental results in accordance with eqn (6) using the calculated value of k_D (+15.0 mL/g) from eqn (5) to obtain D^0 and hence the best-fit theoretical description according to eqn (5) yields the expression $10^7 D = 5.93(1 + 15.0 \bar{c})$, which provides a relatively poor fit (•••••) of the data. The disparity between experiment and prediction is certainly decreased by employing eqn (12), a change which leads to the best-fit description $10^7 D = 6.07(1 + 4.92 \bar{c})$ and the broken line (---) in Fig. 2; and decreased still further (—) by incorporating the viscosity correction to obtain the best-fit relationship $10^7 D = 6.13(1 + 0.82 \bar{c})$. Linear least-squares analysis of the results in Fig. 2 to eqn (6) with D^0 and k_D as curve-fitting parameters yields a slightly negative value (-1.80 mL/g) for the concentration coefficient; but the uncertainty ($\pm 2.6 \text{ mL/g}$) again exceeds its absolute

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3 magnitude. As with ovalbumin (Fig. 1), the variation in diffusion coefficient predicted by eqn (14)
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5 is within the experimental uncertainty of an experimental measurement, whereupon the
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7 consideration of D to be concentration-independent is an acceptable approximation for this system
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9 as well.
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14 An interesting point to emerge from this assessment of diffusion data for a charged protein
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16 system is the much better theoretical description that is obtained by regarding the covolume term,
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18 $u_{red} \cdot v_s$, rather than the excluded volume ($2BM$) as the parameter contributing to hydrodynamic
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20 nonideality. In other words, the nonideality of Brownian motion seems to be governed by the actual
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22 size of the spherical solute, whereas the covolume has to be supplemented by the contribution
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24 arising from considerations of the potential-of-mean-force between charged molecules [see eqns
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26 (12) and (13)] in order to describe thermodynamic nonideality. The consequent lack of an effect of
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28 charge on D , which was noted for ovalbumin (Fig. 1), is also observed with bovine serum albumin
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30 in that the diffusion coefficients ($D_{20,w}$) of 6.02×10^{-7} and $6.10 \times 10^{-7} \text{ cm}^2 \text{ s}^{-1}$ inferred from
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32 traditional studies of essentially isoelectric protein^{58,59} are encompassed by the envelope of scatter
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34 in Fig. 2.
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41 These findings of minimal dependence of diffusion coefficients on either the charge or
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43 concentration for both ovalbumin and bovine serum albumin are clearly at variance with
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45 conclusions drawn from studies of the extent of boundary spreading in sedimentation velocity
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47 experiments,³⁰ for which a better quantitative description was obtained by assigning a magnitude to
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49 k_D in the above eqn (6) as the expression for D - c dependence – a reasonable course of action in
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51 view of the latest theoretical treatment of D - c dependence⁵ at the time. However, such observation
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53 of a decrease in the sum-of-squares-of-residuals¹⁷ merely establishes that better agreement between
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3 experiment and theory can be achieved by introducing an additional curve-fitting parameter to
4 decrease slightly the predicted extent of boundary sharpening; and the present conclusions based on
5 considerations of boundary spreading arising solely from a gradient in solute chemical potential
6 render less likely the validity of identifying that additional curve-fitting parameter as k_D . Although
7 the only other recent sedimentation velocity investigation to take account of boundary sharpening
8 resulting from s - c dependence⁶⁰ also incorporated eqn (6) to allow for concentration dependence of
9 the diffusion coefficient for pegasys, such action may be vindicated by the attachment of a polymer
10 chain (polyethylene glycol) to the pegasys polypeptide chain, a feature which disqualifies its
11 consideration as a globular protein – the only system for which the present claims about D - c
12 independence have been substantiated.
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28 Discussion

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32 A major purpose of this investigation has been to show limitations of a theoretical treatment that has
33 been developed for the concentration dependence of translational diffusion coefficients for globular
34 proteins.^{5,6} Although seeming agreement between experiment and prediction was observed,^{61,62}
35 those comparisons (on spherical plant viruses) entailed diffusion coefficients obtained by dynamic
36 light scattering – a technique involving the measurement of D under the constraint of constant
37 pressure rather than constant solvent chemical potential. Because the latter constraint is specified in
38 the theoretical treatment by virtue of expressing the gradient in solute chemical potential in terms of
39 the corresponding gradient in osmotic pressure [eqn (3)], diffusion studies of proteins under
40 conditions that comply with the specified constraint (constant solvent chemical potential) have been
41 sought in the current investigation. Whereas significant positive concentration dependence of the
42 diffusion coefficient for both proteins is the theoretical prediction of eqn (5), none has been detected
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3 experimentally in traditional diffusion studies of either bovine serum albumin⁸ or ovalbumin.^{10,45}
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5 Instead, the results tend to favor description in terms of the relatively minor concentration
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7 dependence predicted by eqn (14), which substitutes the covolume ($8v_s$) for the thermodynamic
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9 second virial coefficient term ($2BM$) in eqn (5) and also includes a viscosity correction term. In that
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11 regard we note that such interpretation is also supported by the report⁹ of only slight negative
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13 concentration dependence ($k_D = -1.0 \text{ cm}^3 \text{ g}^{-1}$) based on interpretation of measurements of boundary
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15 skewness measurements for serum albumin. Indeed, essentially the same concentration coefficient
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17 ($k_D = -1.1 \text{ cm}^3 \text{ g}^{-1}$) also applies to the diffusion of β -lactoglobulin.^{7,9} On the grounds that these
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19 predicted variations in D for plateau concentrations in the range $1\text{--}10 \text{ mg ml}^{-1}$ ($0.5 \leq \bar{c} \leq 5$) are
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21 also within the uncertainty of experimental estimates, they provide additional justification for
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23 neglecting D - c dependence in analyses of boundary spreading by globular proteins in
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25 sedimentation velocity studies, where the constraints of constant temperature and solvent chemical
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27 potential also seem to apply.²⁷⁻²⁹ On the other hand, reports of significant positive concentration
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29 dependence of D ^{61,62} have emanated from dynamic light scattering measurements, where the
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31 constraint of constant pressure rather than constant solvent chemical potential applies to the solute
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33 chemical potential being monitored. Because the latter constraint is specified in the theoretical
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35 treatment by virtue of expressing the gradient in solute chemical potential in terms of the
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37 corresponding gradient in osmotic pressure [eqn (3)], diffusion studies of proteins under conditions
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39 that comply with the specified constraint (constant solvent chemical potential) have been sought in
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41 the current investigation. Whereas significant positive concentration dependence of the diffusion
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43 coefficient for both proteins is the theoretical prediction of the current expression for D - c
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45 dependence [eqn (5)], none has been detected experimentally in traditional diffusion studies of
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47 either bovine serum albumin⁸ or ovalbumin.^{10,45} As noted above, the results tend to favor
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3 description in terms of the relatively minor concentration dependence predicted by eqn (14), which
4 substitutes the covolume ($8v_s$) for the thermodynamic second virial coefficient term ($2BM$) in eqn
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8 (5) and also includes a correction term for solute viscosity. In that regard we note such action is
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10 counter to that adopted in dynamic light scattering studies, where buffer viscosity is regarded as the
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12 appropriate parameter.¹¹⁻¹⁶ However, because of the constraint of constant pressure that applies in
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14 those experiments, the buffer constituents must be regarded as additional solutes¹⁷⁻¹⁹ whose
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16 contribution would dominate the solution viscosity relative to that of water (the solvent). In
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18 diffusion studies under the constraint of constant solvent chemical potential the buffer constituents
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20 become part of the solvent, whereupon the only contributor to the relative viscosity (η_2/η_1) is the
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22 protein solute.
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28 Another factor that could also decrease the D - c dependence from that predicted by the
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30 Batchelor expression⁴ [eqn (10) as modified by Brady and Durlofsky³⁷] is the operation of
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32 solute-solvent interaction arising from the coupling of ion flows.^{63,64} Indeed, for a protein bearing
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34 net charge the condition of constant solvent chemical potential generates a reverse concentration
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36 gradient in buffer/electrolyte ions to accommodate the Donnan effect.⁴⁵ On the grounds that the
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38 magnitude of this reverse concentration gradient in buffer and electrolyte ions becomes greater with
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40 increasing protein concentration, the mediation of overall protein diffusion by such coupling of ion
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42 flows could also be partly responsible for the concentration independence of D that is observed
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46 experimentally.
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50 Inasmuch as the current interest in quantifying the concentration dependence of D resides in
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52 analysis of boundary spreading in sedimentation velocity studies of proteins,^{30,60} we again draw
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54 attention to the fact that an approximate analytical solution of the Lamm equation³ already exists^{1,2}
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56 for the situation encountered above in which D is essentially concentration independent and s
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3 exhibits linear concentration dependence: this study provides a sound theoretical basis for
4 reintroducing such an analysis. Alternatively, it provides the basis for simplifying current simulative
5 procedures^{30,60} by the elimination of k_D , the coefficient describing the D - c dependence for globular
6 proteins [eqn (6)], as an additional curve-fitting parameter to be evaluated from the analysis.
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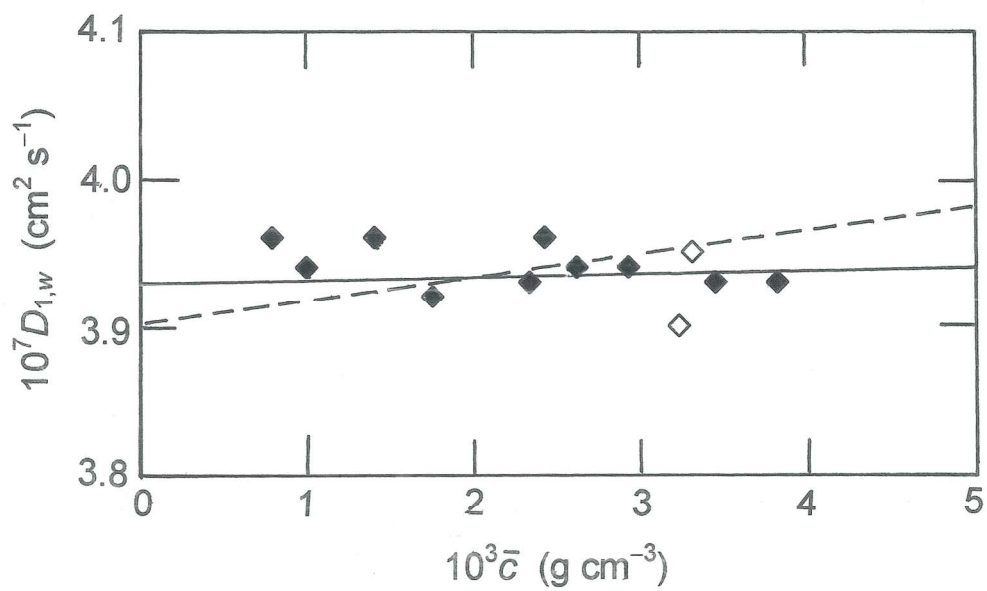
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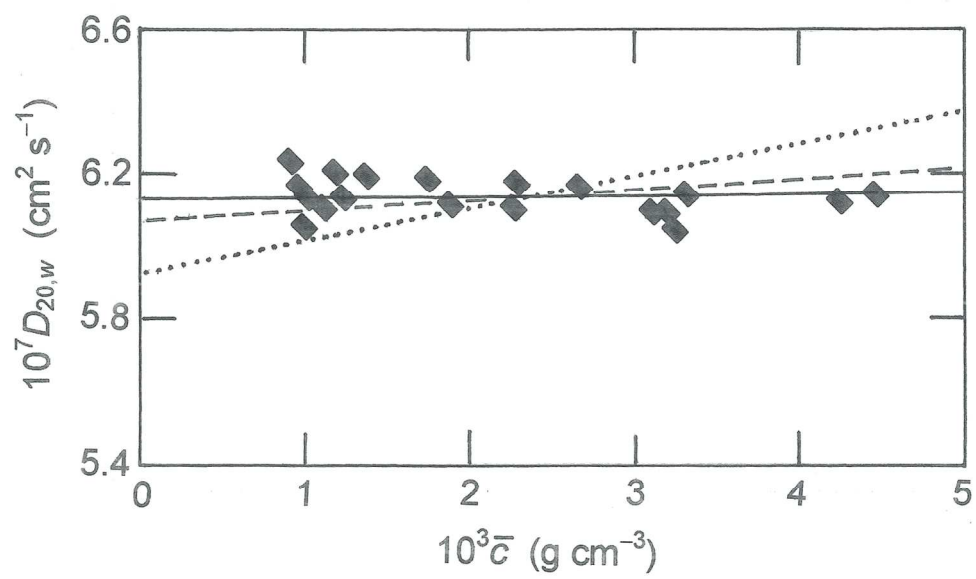
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8 Fig. 1 Concentration dependence of the translational diffusion coefficient for ovalbumin under isoelectric
9 conditions (◆) and at neutral pH (◇), together with theoretical relationships predicted by eqn (5) or eqn (12)
10 (---), and eqn (14) (—). [Data taken from Table 1 of Creeth et al.¹⁰ and Table 1 of Nichol et al.⁴⁵
11 respectively.]
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17 Fig. 2 Concentration dependence of the translational diffusion and sedimentation coefficients for bovine
18 serum albumin. (A) Experimental diffusion coefficients⁸ obtained at neutral pH (pH 6.8, I 0.10), together with
19 the theoretical relationships predicted by eqn (5) (•••••), eqn (12) (---), and eqn (14) (—).
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21 [Experimental data taken from Table 4 of Creeth.⁸]
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