

The concentration-dependence of macromolecular parameters

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Theories concerning the concentration-dependence of sedimentation and diffusion coefficients for macromolecules in dilute solution are compared and discussed, together with their experimental basis. An attempt has been made to clarify an important uncertainty still present in the literature as to whether sedimentation coefficients should be corrected for solvent or solution density. It is pointed out that the two processes yield the same extrapolation limit but different concentration-dependencies, which have, however, been related. A general expression is derived thermodynamically for the concentration-dependence of diffusion that includes the coefficient of the concentration term involved in sedimentation (on the basis of sedimentation coefficients corrected from solution density). For rigid spherical particles the expression is shown to be exactly equivalent to one given by Batchelor [(1976) *J. Fluid Mech.* **74**, 1–29], which was derived on the basis of sedimentation coefficients corrected from solvent density. Finally, we discuss the concentration-dependence of apparent weight-average relative molecular masses ('molecular weights') (from, e.g., sedimentation equilibrium) and note an important omission in some earlier representations.

INTRODUCTION

Over the last decade, considerable interest has developed over the details of sedimentation and other transport processes, as occurring in dilute solutions of suspended particles and macromolecules. In particular, the magnitudes of concentration effects on sedimentation, diffusion and viscosity have been calculated, as well as certain thermodynamic coefficients. Some comparisons with experiments have been undertaken [e.g. Cheng & Schachman (1955), Maude & Whitmore (1958) and Buscall *et al.* (1982)]. In the present paper we review the theoretical advances and discuss their experimental support, and in the following paper (Harding & Johnson, 1985) introduce new experimentally obtained data against which to test the theoretical values.

THEORY

Concentration-dependence of sedimentation velocity

It is usually assumed (e.g. Schachman, 1959) that the concentration-dependence of the sedimentation coefficient, s_c , correct to first-order in concentration, c (g/ml), is given by:

$$s_c = s(1 - k_s c) \quad (1a)$$

where s is the infinite dilution value, with s_c referring to a finite concentration c , k_s being an empirical constant (ml/g). An equivalent expression may, however, be written in terms of the volume fraction of suspended particles, ϕ :

$$s_c = s(1 - K_s \phi) \quad (1b)$$

where $\phi = cv_s$, v_s being the 'swollen' specific volume of the particles, in which allowance is made for bound or entrained solvent, and K_s is given by:

$$K_s = k_s/v_s \quad (1c)$$

Schachman (1959) reviewed the relevant theoretical work that had been performed to that time, including

particularly that of Kermack *et al.* (1929) and that of Burgers (1941*a,b*, 1942*a,b*). Both sets of workers had considered the backflow of solvent arising from the sedimentation of the assumed spherical, neutral, particles themselves and of the surrounding liquid dragged along by normal viscous forces. In all, this amounted to 4 times the volume of the particles according to Schachman (1959), but substitution in Burgers's (1941*a,b*, 1942*a,b*) equations of his evaluated constants gives an even larger value (up to 6.88 times the volume). Progress was also made by Pyun & Fixman (1964), who attempted to evaluate K_s for the sedimentation of soft as well as hard spheres.

A further theoretical advance was made by Batchelor (1972), who also considered dilute homogeneous (mono-disperse) suspensions of rigid, neutral, impermeable spheres and reviewed the difficulties associated with previous work. In Batchelor's (1972) work the occurrence of divergent integrals was circumvented and evaluations were performed without the uncertainties mentioned by Burgers (1941*a,b*, 1942*a,b*). Thus, with the above notation, Batchelor (1972) obtained:

$$s_c = s(1 - 6.55\phi) \quad (2a)$$

where the coefficient -6.55 contains contributions from several types of effect. Thus motion of the particles alone is responsible for $-\phi$, and the viscous dragging of the fluid surrounding each particle contributes -4.5ϕ , giving a total -5.5ϕ for these backflow terms. Two further smaller terms were considered. One, representing the mutual effect of the particles upon one another, yields a term -1.55ϕ , and a further term $+0.5\phi$ arises from Faxen's relation owing to the non-zero mean value of the second derivative of the fluid velocity in the environment of any sphere due to all the others.

A large measure of agreement occurs between Batchelor's (1972) work and that of Burgers (1941*a,b*, 1942*a,b*), as well as that of Pyun & Fixman (1964).

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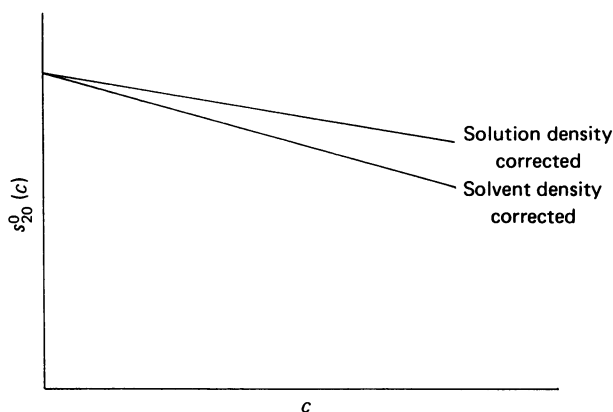


Fig. 1. Illustration of the effect of choice of density of the medium through which sedimentation occurs in correcting experimental sedimentation coefficients [$s_{20}^b(c)$] to water at 20 °C [$s_{20}^0(c)$]

Burgers (1941*a,b*, 1942*a,b*) estimated the numerical coefficient to be -6.88 , whereas Pyun & Fixman (1964) obtained -7.16 ; in both cases Batchelor (1972) offers an explanation for the discrepancies. In further theoretical work, Batchelor (1976, 1982) and Batchelor & Wen (1982) have now extended this treatment to Brownian diffusion and to the properties of dilute polydisperse systems of interacting spheres.

Sedimentation coefficients are normally obtained in buffer solution, indicated by superscript *b*, at a temperature *T*, indicated by the subscript. In order to consider concentration effects only, it is necessary to correct such coefficients to a common temperature, usually 20 °C, and to a solvent environment, usually water. In making this correction, an expression of the following type is utilized (see, e.g., Tanford, 1961):

$$s_{20}^0(c) = s_{20}^b(c) \frac{\eta}{\eta_{20}^0} \cdot \frac{(1 - \bar{v}\rho_{20}^0)}{(1 - \bar{v}\rho)} \quad (2b)$$

Here $s_{20}^b(c)$ is the actual measured sedimentation coefficient and $s_{20}^0(c)$ is the value corrected to water at 20 °C. The partial specific volume \bar{v} is usually taken as a constant. η refers to the viscosity of the solvent, but it is necessary to make an assumption regarding the density, ρ , of the medium through which sedimentation occurs during the measurement of $s_{20}^b(c)$. Is it merely the buffer solution at temperature *T*, whose density is usually well known, or should it be the whole solution (containing macromolecules), for which the density would require measurement for every *s* value? In each case it should be mentioned that the corrected value is hypothetical, for it does not correspond to a real situation and therefore cannot be experimentally checked. Further, the two corrected values will differ significantly at any finite concentration, though the difference will decrease with decreasing concentration and disappears at infinite dilution. Thus the general dependence of $s_{20}^0(c)$ on *c* in the two cases will be of the type shown in Fig. 1. For the remainder of the present paper we are concerned with $s_{20}^0(c)$ values, but for simplicity refer to them as *s*(*c*), and the values extrapolated to infinite dilution as '*s*'.

It should be made clear that, in his treatment, Batchelor (1972) considered the motion of the spheres through a medium with the density of the pure solvent

(rather than the whole solution), though he takes account of the distribution of spheres about a given sphere. Thus, in correcting experimentally measured *s* values to the solvent at a particular temperature (for comparison with Batchelor theory), pure solvent densities must be utilized. In such a case it is valid to compare the value of K_s derived with Batchelor's (1972) calculated value of 6.55. On the other hand, it has been argued by several authors [e.g. Schachman (1959), Fujita (1962, 1975), van Holde (1971) and Rowe (1977)] that the effective density of the fluid through which sedimentation occurs is the solution density. This view is strongly supported by the irreversible thermodynamic approach to the flow in the ultracentrifuge cell together with the thermodynamic nature of the partial specific volume (\bar{v}) normally utilized. However, correction to the density of pure water at a standard temperature (i.e. 20 °C or 25 °C), from solution density, yields (i) a corrected s_c value that is significantly higher than that s_c obtained from solvent properties and (ii) a k_s value that is numerically lower. Referring to the solvent-derived constant as k'_s Rowe (1977) obtained the following approximate inter-relation (which is exact for the limiting value at infinite dilution):

$$k_s = k'_s - \bar{v} \quad (3)$$

A proof of this formula is given in the Appendix. Thus K_s and K'_s differ significantly, and dividing eqn. (3) by v_s we obtain:

$$K_s = K'_s - \frac{\bar{v}}{v_s} \quad (4)$$

where K'_s refers to the solvent-derived parameter. Thus the two forms of correction give s_c (or s'_c)-versus-*c* plots of differing slope (as in Fig. 1), and either may be utilized. However, it is essential to be consistent in treating measured coefficients.

For solid, impermeable, inert and unsolvated spheres, $\bar{v} \approx v_s$, so that $K_s = (K'_s - 1)$. Thus, for Batchelor's (1972) model spheres, a K_s value of 5.55 is to be expected. Where solvation is appreciable, $(K'_s - K_s) < 1$. It should be noted also that either procedure of correction is valid in determining limiting s_c values at zero concentration.

In the paper already mentioned Rowe (1977) also dealt with the problem of the concentration-dependence of sedimentation and other processes. However, he assumed initially 'that for particles sedimenting at very low concentrations, the velocity relative to solvent is unchanged by varying the particle concentration'. This model therefore does not consider interaction terms that other workers have calculated and found significant. Rowe (1977) considered the backflow of solvent due to the sedimentation of the particles (cf. Enohsson, 1948), but, by imposing a boundary condition of 'infinite slip' (where the frictional ratio tends to 0) and by consideration of different frames of reference, he magnified this backflow term to give a K_s value for unhydrated spheres of 4. This is at variance, however, with Happel & Brenner (1965), who showed that 'infinite slip' corresponds in fact to a frictional ratio of $\frac{2}{3}$, not 0, so that the theoretical basis of the boundary condition is doubtful. It will be recalled that Batchelor (1972) calculated a contribution of 5.5 (≈ 4.5 if solution-density corrections are used) for the total backflow (including that due to solvent viscously dragged along), but that he also found significant interaction terms that bring the total term to 6.55 (i.e. $K_s \approx 5.5$ for $v \approx \bar{v}_s$).

In comparing experimental and theoretical values of K_s

or K'_s , one of the most serious difficulties is associated with the nature of \bar{v} , defined as the partial molar volume divided by the relative molecular mass ('molecular weight'). Although it is generally accepted that the term $(1 - \bar{v}\rho)$, ρ being the solution density, is valid in the Svedberg equation [$M = RTs/D(1 - \bar{v}\rho)$, D being the translational diffusion coefficient] for the calculation of relative molecular mass, M (see, e.g., Alexander & Johnson, 1949), or valid in correcting s_c values to standard conditions, it is generally agreed that the solute volume fraction, ϕ , cannot normally be equated with $c\bar{v}$ for the reason that a macromolecular solute is expected to bind and entrain solvent molecules, as suggested in connection with eqn. (1b), where v_s is used to denote the swollen specific volume of the solvated particles. The extent to which v_s differs from \bar{v} is expected to be very variable. Thus, for polystyrene-latex spheres in aqueous suspension, little or no hydration is through to occur, and Cheng & Schachman (1955), Maude & Whitmore (1958) and Buscall *et al.* (1982) were able to take $1/\bar{v}$ as the bulk density. For such systems, experimental support for the various theoretical values for K_s (or K'_s) has been mixed. Buscall *et al.* (1982) have obtained a value of 5.4 ± 0.1 for K'_s (4.5 for K_s) for latex spheres sedimenting under gravity. They argued that the discrepancy with Batchelor's (1972) theoretical result could be accounted for in terms of the formation of temporary doublets of spheres, although this is perhaps open to question. Maude & Whitmore (1958) had earlier obtained a value of 5 for K'_s (4 for K_s) for latex particles again sedimenting under gravity. Cheng & Schachman (1955), in a thorough study using the analytical ultracentrifuge in which all sedimentation coefficients had been corrected in terms of solution density, had also obtained a value for K_s of (4.06 ± 0.10) .

On the other hand, as stated above, most biological macromolecules are significantly solvated. For example, a human macroglobulin (Johnson & Miller, 1970) was found to have an effective specific volume in aqueous solution almost 5 times \bar{v} . In the more extreme case of a mucus glycoprotein (Harding *et al.*, 1983) the ratio v_s/\bar{v} was found to be greater than 100. With such a range of values for v_s/\bar{v} , it is clear that reliable values of ϕ cannot be obtained from accurate weight concentrations (c).

However, for a particle of given shape, v_s may be determined from the intrinsic viscosity $[\eta]$; in general:

$$[\eta] = \nu v_s \quad (5a)$$

where the coefficient ν for spherical particles is 2.5. With elimination of v_s from eqns. (5a) and (1c), a ratio R can be defined (Rowe, 1977):

$$R \equiv k_s/[\eta] = K_s/\nu \quad (5b)$$

The predicted theoretical values of this ratio (corrected to solution density) for spheres are $5.88/2.5 = 2.35$ (Burgers, 1941a,b, 1942a,b), $6.16/2.5 = 2.46$ (Pyun & Fixman, 1964), $5.55/2.5 = 2.22$ (Batchelor, 1972) and $4.00/2.5 = 1.60$ (Rowe, 1977). A comprehensive review of experimentally obtained data by Creeth & Knight (1965) has shown that, in general, globular macromolecules have a value for R of ≈ 1.4 – 1.7 , with lower values for more-asymmetric particles (see also Harding & Rowe, 1982). It may also be noted that for randomly coiled macromolecules Wales & van Holde (1954) have obtained a value for R of 1.66.

Concentration-dependence of diffusion

The variation in the sedimentation coefficient with concentration may be considered to arise from the increase in frictional coefficient with concentration, where:

$$f_c = f(1 + k_s c) \quad (6)$$

correct to first-order in concentration, where f_c is the frictional coefficient at finite concentration c and f is the corresponding value at infinite dilution; k_s is the same term as occurs in eqn. (1a). A similar consideration applies in the case of the diffusion coefficient, but, in addition, activity-coefficient terms are also involved, since the driving force for diffusion is essentially the gradient in chemical potential (μ). Using the subscript '1' to denote solvent and '2' to denote macromolecular solute, we have:

$$\mu_2 = \mu_2^0 + RT \cdot \ln \gamma \cdot c \quad (7)$$

γ being the (solute) activity coefficient at a particular concentration. Differentiating with respect to c we obtain:

$$\frac{c}{RT} \cdot \frac{\partial \mu_2}{\partial c} = \frac{d(\ln \gamma)}{d(\ln c)} + 1 \quad (8)$$

Now, from the Gibbs–Duhem Relation:

$$\frac{\partial \mu_2}{\partial c} = \frac{-M(1 - c\bar{v})}{\bar{V}_1 c} \cdot \frac{\partial \mu_1}{\partial c} \quad (9)$$

where \bar{V}_1 is the partial molar volume of solvent and M is the relative molecular mass of the macromolecule. This was first pointed out by Ross & Minton (1977), although in earlier work [e.g. Tanford (1961) and Tombs & Peacocke (1974)] the term $c\bar{v}$ is missing, presumably on the grounds that $c\bar{v} \ll 1$. However, as shown below, it should be retained in general. It could be argued that cv_s rather than $c\bar{v}$ should be used in this expression, but, because the source of the term in eqn. (9) is thermodynamic rather than hydrodynamic (μ_1 refers to the solvent irrespective of whether it is free or bound), $c\bar{v}$ appears to be correct.

Recalling that μ_1 is related to osmotic pressure (Π) by:

$$\mu_1 = \mu_1^0 - \Pi \bar{V}_1 \quad (10)$$

where μ_1^0 is the standard state chemical potential, we find:

$$\frac{\partial \mu_1}{\partial c} = -\bar{V}_1 \cdot \frac{\partial \Pi}{\partial c} \quad (11)$$

assuming \bar{V}_1 to be constant. Substituting eqns. (9) and (11) into eqn. (8), we obtain:

$$\frac{d(\ln \gamma)}{d(\ln c)} = \frac{M(1 - \bar{v}c)}{RT} \cdot \frac{\partial \Pi}{\partial c} - 1 \quad (12)$$

Since the diffusion coefficients at finite concentration, D_c , may be written:

$$D_c = \frac{RT}{Nf_c} \cdot \left(1 + \frac{d(\ln \gamma)}{d(\ln c)}\right) \quad (13)$$

with N as Avogadro's number, we have, on introducing eqn. (12):

$$D_c = \frac{RT}{Nf_c} \cdot \left(\frac{M(1 - \bar{v}c)}{RT} \cdot \frac{\partial \Pi}{\partial c}\right) \quad (14)$$

The concentration-dependence of osmotic pressure is usually written as (Tanford, 1961):

$$\frac{\Pi}{c} = \frac{RT}{M} \cdot [1 + BM \cdot c + O(c^2)] \quad (15)$$

where B is the second virial coefficient, and hence:

$$\frac{\partial \Pi}{\partial c} = \frac{RT}{M} \cdot [1 + 2BM \cdot c + 0(c^2)] \quad (16)$$

Introducing eqn. (16) into eqn. (14) and substituting for f_c from eqn. (6), we obtain:

$$D_c = \frac{RT(1 - \bar{v}c)[1 + 2BM \cdot c + \dots]}{Nf(1 + k_s c)}$$

which to a good approximation may be written:

$$D_c = D(1 + k_D c) \quad (17a)$$

where D (the value of D_c at $c = 0$) is RT/Nf , and where k_D is given by:

$$k_D = 2BM - \bar{v} - k_s \quad (18)$$

Writing eqn. (17a) in volume fraction terms:

$$D_c = D(1 + K_D \phi) \quad (17b)$$

where:

$$K_D \left(\equiv \frac{k_D}{v_s} \right) = \frac{2BM}{v_s} - \frac{\bar{v}}{v_s} - K_s \quad (18b)$$

From eqns. (18) it is apparent that omission of the \bar{v} term in the coefficient of c may be justifiable where asymmetry or solvation of the particles cause the excluded volume term BM to be very large, but in general it should be included. Its contribution for spherical particles of moderate solvation is significant. Since the arguments leading to eqns. (17) and (18) have been almost entirely thermodynamic, it is clear that k_s or K_s (rather than k'_s or K'_s) should appear in these equations. On the other hand, Batchelor (1976), on statistical-mechanical as well as thermodynamic grounds, considered the diffusion of hard neutral spheres [the same model as he used in sedimentation (Batchelor, 1972)] and obtained:

$$D_c = \frac{RT}{Nf} \cdot [1 + (8 - 6.55)\phi] \quad (19)$$

which can also be written for the particular model:

$$D_c = \frac{RT}{Nf} \cdot [1 + (8v_s - k'_s)c] \quad (20)$$

But $2BM$ for the same model has the value $8v_s$ (see, e.g., Tanford, 1961), and, from eqn. (3), $k'_s = k_s + \bar{v}$. Thus Batchelor's (1976) formulation (eqn. 19) and our formulation (eqns. 17 and 18) are identical.

Rewriting eqn. (18b) in terms of K'_s , we find in general:

$$K_D = \frac{2BM}{v_s} - K'_s$$

Now, since the first term is simply 8 for spheres, if we take the Batchelor value of K'_s as 6.55, then $K_D = 1.45$, as reported by Batchelor (1976). An earlier attempt to support this empirically had been made by Newman *et al.* (1974), with circular DNA, and was quoted by Batchelor (1976). The fact that circular DNA is far from a rigid impermeable unsolvated sphere in solution appears to have been overlooked by these authors. Since circular DNA possesses asymmetry relative to a sphere, the apparent agreement would tend to suggest that K'_s for a sphere should be lower than 6.55.

A more recent analysis has been given by Kops-Werkhoven & Fijnaut (1981) on silica spheres (coated

with long-chain hydrocarbons) in cyclohexane. These workers have obtained a value for v_s from measured values of D (by using photon correlation spectroscopy) and the corresponding Stokes radius, and apparently employed this to obtain a value for K'_s of 6 ± 1 and a value for K_D of 1.3 ± 0.2 . Their use of the parameter q_e , however, makes it clear that it is equivalent to our v_s , and is thus a solvated specific volume rather than an excluded volume, as these authors claim. Nonetheless, their experimental values for K'_s and K_D appear to support the calculated Batchelor (1976) coefficients (6.55 and 1.45 respectively).

Concentration-dependence of the apparent relative molecular mass from sedimentation equilibrium

The apparent relative molecular mass of a macromolecule in a two-component system is given by:

$$M_{\text{app.}} = \frac{M}{\left(1 + \frac{d(\ln \gamma)}{d(\ln c)}\right)} \quad (22)$$

Substituting for $d(\ln \gamma)/d(\ln c)$ using eqn. (12) and again using the virial expansion of eqn. (16), we obtain:

$$M_{\text{app.}} = \frac{M}{1 + (2BM - \bar{v})c + 0(c^2)} \quad (23)$$

Thus:

$$\frac{1}{M_{\text{app.}}} = \frac{1}{M} \cdot [1 + (2BM - \bar{v})c + \dots] \quad (24)$$

The \bar{v} term is missing from the coefficient of c in eqn. (16.20) of Tanford (1961), but is present in an expression given by Ross & Minton (1977) and in an equivalent expression for a self-associating system given by Wills *et al.* (1980). As previously, although the contribution of this term may be small for highly asymmetric, solvated or charged macromolecules, where $2BM$ may be $\gg \bar{v}$, in general this is not so. For example, for unsolvated spheres $2BM$ is only $8\bar{v}$. For solutions of more than one component of macromolecule the $M_{\text{app.}}$ and M values become weight averages [see, e.g., eqn. (34) in Teller (1973)].

DISCUSSION

In considering the sedimentation process, the thermodynamic nature of \bar{v} and the viewpoint from the thermodynamics of irreversible processes tend strongly to suggest that the solvated macromolecule or particle should be viewed as moving through a medium with the density of the solution rather than the pure solvent. As has been shown above, this leads to a coefficient, k_s , that differs significantly (see eqns. 3 and 4) from that, k'_s , obtained if solvent density had been utilized. Further, these coefficients are also involved in the concentration-dependence of diffusion coefficients (eqn. 18), even though there is no ambiguity in correcting diffusion coefficients to standard conditions. It is of interest that the coefficient in the diffusion case takes on a simpler form ($k_D = 2BM - k'_s$) where \bar{v} does not occur explicitly if solvent-corrected, k'_s , values are utilized. However, in sedimentation equilibrium, it is clear that the coefficient of the concentration term must contain \bar{v} . In the following paper (Harding & Johnson, 1985) an application of the various equations to monodisperse preparations of

spherical virus particles (turnip-yellow-mosaic virus) is attempted.

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APPENDIX

Proof of $k_s = k'_s - \bar{v}$

If solution density is used for the correction of sedimentation coefficients, we have:

$$s_c = s(1 - k_s c) \quad (1a)$$

If solvent density is used, we may write:

$$s'_c = s(1 - k'_s c) \quad (A1)$$

Therefore, using eqn. (2b), we obtain:

$$\frac{s'_c}{s_c} = \frac{1 - k'_s c}{1 - k_s c} = \frac{(1 - \bar{v}\rho')}{(1 - \bar{v}\rho)}$$

Re-arranging:

$$k_s = k'_s - \frac{\bar{v}(\rho - \rho')}{(1 - \bar{v}\rho')} \cdot \frac{(1 - k_s c)}{c} \quad (A2)$$

Take M' g of solvent with volume V' . The solvent density ρ' will be M'/V' . If a small quantity g g of solute of partial specific volume \bar{v} is added, there will be an increase in volume of solution of $g\bar{v}$. The solution density ρ is therefore $\rho = (M' + g)/(V' + g\bar{v})$, and:

$$(\rho - \rho') = \frac{M' + g}{V' + g\bar{v}} - \frac{M'}{V'} = c(1 - \rho'\bar{v})$$

where the concentration c (g/ml) = $g/(V' + g\bar{v})$. Substituting for $(\rho - \rho')$ in eqn. (A2) we obtain:

$$k_s = k'_s - \bar{v}(1 - k_s c) \quad (A3)$$

As 'infinite dilution' is approached (namely $c \rightarrow 0$) eqn. (A3) becomes:

$$k_s = k'_s - \bar{v}$$