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The Density Correction of Sedimentation Constants

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Introduction

It is well known that sedimentation constants of proteins and other materials depend upon the density and the viscosity of the medium. For this reason, it is customary to measure sedimentation rates under convenient experimental conditions, and then correct the rates to correspond to what they would have been if sedimentation had been carried out in a medium with the viscosity and density of water at 20°. Svedberg and Pedersen[1a] have presented an equation, exactly analogous to equation 4 of this communication, for carrying out this correction. It was pointed out by them that hydration might affect the validity of the equation, but the subject was not treated quantitatively. Since it is recognized that proteins are probably hydrated to an appreciable extent, it seemed worthwhile to consider the possible effect of hydration upon the corrected value of the sedimentation constant obtained through the use of equation 4. It was found that the error can be quite appreciable.

Theory and Discussion

Consider a particle of mass m which displaces a volume v in a medium of density ρ. Suppose that this particle becomes solvated in such a way that the increase in the volume displaced is h and the increase in mass is hd. In this notation, d amounts to the density of that portion of the medium attached to the particle. It could be the same as ρ, but it also could be different from ρ; for example, if the medium contained inorganic salts or sucrose and the solvating medium was solely water, then d would not equal ρ. In general, in a ternary system, d will differ from ρ unless h has the same composition as the two-component solvent and other sources of difference are negligible.

In a centrifuge, the force acting on the solvated particle is $\omega^2x[(m + hd) - (v + h)\rho]$, where ω is the angular velocity and x is the distance from the axis of rotation. The frictional force will be $f'\eta\ dx/dt$, where f'[1b] is the coefficient of friction of the solvated particle, η is the coefficient of viscosity of the solvent and dx/dt is the velocity of sedimentation. These two forces can be equated, and one can write

$$(m + hd) - (v + h)\rho = sf'\eta \qquad (1)$$

where s, the sedimentation constant, is defined as $(dx/dt)/\omega^2x$. Since v/m is the partial specific volume, V, of the solute at the concentration present in the solution, and since $Nm = M$, where N is Avogadro's number and M is molecular weight, the following equation results.

$$s = \frac{M[1 - V\rho + (h/m)(d - \rho)]}{Nf'\eta} \qquad (2)$$

If sedimentation experiments are carried out in two media with viscosities η_1 and η_2 and densities ρ_1 and ρ_2,[1c] respectively, in general two different sedimentation constants, s_1 and s_2, will be observed. If M and f' are the same in the two media, one can write

$$\frac{s_1}{s_2} = \frac{\eta_2[1 - V_1\rho_1 + (h_1/m)(d_1 - \rho_1)]}{\eta_1[1 - V_2\rho_2 + (h_2/m)(d_2 - \rho_2)]} \qquad (3)$$

This is the equation for correcting an observed sedimentation constant, s_2, to that, s_1, at some standard condition such as the viscosity and density of water at 20°. If h_1 and h_2 are 0, *i.e.*, there is no solvation, or if $d_1 = \rho_1$ and $d_2 = \rho_2$, *i. e.*, the density of the solvent material attached to the particle is the same as the density of the medium, regardless of the density of the medium, equation 3 reduces to equation 4, the one usually given for correcting sedimentation constants.

$$\frac{s_1}{s_2} = \frac{\eta_2(1 - V_1\rho_1)}{\eta_1(1 - V_2\rho_2)} \qquad (4)$$

V_1 and V_2 are usually assumed to be the same. Svedberg and Eriksson-Quensel,[2] in studies of the sedimentation of hemocyanin in heavy water, found that correction of their observed sedimentation constants according to equation 4 gave values for s^0_{20} equal to that obtained in ordinary water. It seems likely that the solvating medium in each mixture of heavy and ordinary water had a density equal to that of the medium. Thus equation 4 held.

Equation 5 can be derived from equation 1 in the same manner as equation 3 was derived.

$$\frac{s_1f'_1\eta_1}{s_2f'_2\eta_2} = \frac{(m + h_1d_1)\left[1 - \left(\frac{v + h_1}{m + h_1d_1}\right)\rho_1\right]}{(m + h_2d_2)\left[1 - \left(\frac{v + h_2}{m + h_2d_2}\right)\rho_2\right]} \qquad (5)$$

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(1a) Svedberg and Pedersen, "The Ultracentrifuge," Oxford Press, England, 1940.

(1b) The coefficient of friction, as defined by Svedberg, includes the viscosity coefficient and a term related to the size, shape, and hydration of the sedimenting particles. In this treatment the two terms are separated in order to relate the sedimentation constant to the viscosity of the solvent. Thus, our $f'\eta$ is the same as Svedberg's f.

(1c) Some discussion has been directed to the density term in equation 1. The work of Gofman, *et al.*, (*J. Biol. Chem.*, **179**, 973 (1949)) on the sedimentation of lipoproteins indicates that the presence of other protein molecules influences the direction as well as the rate of sedimentation of the lipoprotein molecules, thereby showing that solution density is important. In most studies the densities of the solvent and solution are very similar and either can be used without introducing a significant error. There is general agreement that the density of the solution is the controlling factor in sedimentation equilibrium.

(2) Svedberg and Eriksson-Quensel, *Nature*, **137**, 400 (1936).

If m, v, h, d and f' (which depends upon $v + h$) are all constant in different media, and if V_h is defined as $(v + h)/(m + hd)$, equation 5 reduces to equation 6.

$$\frac{s_1}{s_2} = \frac{\eta_2 (1 - V_h\rho_1)}{\eta_1 (1 - V_h\rho_2)} \qquad (6)$$

In such a case, ηs will be a linear function of ρ and V_h will equal the reciprocal of ρ when s is 0. Data obtained with tobacco mosaic virus[3] meet this requirement, and data obtained with Southern bean mosaic virus[4] deviate only slightly from this requirement. If m, v, h and f' are held constant but d is a linear function of ρ ($d = a + k(\rho - a)$, where a and k are constants), as in the experiments of McMeekin, *et al.*,[5] equation 5 reduces to equation 7, in which V_h is defined as $(v + h - hk)/(m + ha - hka)$.

$$\frac{s_1}{s_2} = \frac{\eta_2 (1 - V_h'\rho_1)}{\eta_1 (1 - V_h'\rho_2)} \qquad (7)$$

In this case, too, $s\eta$ will be a linear function of ρ and V_h' will equal the reciprocal of ρ when s is 0. It is possible that other assumptions can be made which yield equations of the form of 6 and 7 except that V_h or V_h' will be replaced by V_h'', V_h''', etc. This means that, while a constant equal to the reciprocal of ρ when sedimentation rate is zero, can be evaluated in experiments like those of Schachman and Lauffer, its meaning, in terms of the composition and amount of solvate, is ambiguous in the absence of additional information.

In spite of the ambiguity in the meaning of V_h, V_h', etc., equation 6 is an exact equivalent of equation 3 whenever ηs is a linear function of ρ. Table I shows the results obtained in the studies on the sedimentation of tobacco mosaic virus in sucrose solutions of different densities.[3] In the calculations, the value for V_h (or V_h', etc.) was obtained from the density of solution, obtained by extrapolation, in which the virus would have zero sedimentation rate. Comparison of column 4 with 5 shows the magnitude of the error if V is used instead of V_h. It is clear that reasonably constant values of s^0_{20} are obtained for all solutions if V_h is used, whereas an error of 26% results if V is used for studies in 40% sucrose. It is, of course, not surprising that correcting the s values in Table I by equation 6 leads to essentially constant values while correcting by equation 4 does not; the original sedimentation data[3] show that ηs is proportional to $(1 - V_h\rho)$ and not to $(1 - V\rho)$. The point to the calculations involving equation 4 is that they illustrate how large the error can be in an extreme case when the usual procedure (involving equation 4) is used to correct sedimentation constants. Similar calculations can be made from the data of Sharp, Beard and Beard[6] on the sedimentation of swine influenza virus in D_2O, sucrose and serum albumin solutions.

TABLE I

SEDIMENTATION OF TOBACCO MOSAIC VIRUS IN 0.01 *M* PHOSPHATE BUFFER SOLUTIONS CONTAINING VARYING AMOUNTS OF SUCROSE

Sucrose concn., %	$s_{meas.}/S$	ρ, g./cc.	s^0_{20}/S eq. 4, $V = 0.73$	s^0_{20}/S eq. 6, $V_h = 0.789$
0	182	0.999	169	169
0	188	0.999	170	170
5	148	1.018	163	166
12.4	98	1.045	159	168
20	71.4	1.079	152	170
30	41.3	1.113	139	168
40	22.5	1.151	128	174

Since it is probable that many proteins are hydrated to an appreciable extent, it is evident that many of the published sedimentation constants corrected to water at 20° by means of equation 4 may be in error. The larger the value of V, the greater will be the error if hydration is neglected. The only solutions to the problem are to carry out sedimentation experiments under conditions such that the density correction is not important or else to attempt to obtain a measure of V_h (or V_h', etc.). In general, sedimentation studies are carried out in dilute buffers of density about 1.01 g./cc. Under such circumstances the error in s^0_{20} will be small, amounting to only a few per cent.

The preceding discussion deals only with the question of obtaining the right value for s^0_{20} from s_t when the sedimentating particles are solvated. The next question is whether the correct value for the unsolvated molecular weight can be calculated from the correct value of s^0_{20} and the diffusion constant, D^0_{20}. The relationship between the diffusion constant and the coefficient of friction is given by the Einstein–Sutherland equation, equation 8.

$$D = kT/f'\eta \qquad (8)$$

In this equation, T is the absolute temperature, k is the Boltzman constant, and f' has the same meaning as in equation 2. By combining equations 2 and 8, one obtains equation 9, where R is the gas constant.

$$M = RTs/D[1 - V\rho + (h/m)(d - \rho)] \qquad (9)$$

When $d \rightarrow \rho$ or when $h/m \rightarrow 0$ or both, equation 9 reduces to the familiar Svedberg equation, equation 10. Under these conditions the molecular weight of the unsolvated particles

$$M = RTs/(1 - V\rho)D \qquad (10)$$

can be determined from the correct values of s^0_{20} and D^0_{20} of the solvated particles and the partial specific volume, V, of the unsolvated particle. Since $V\rho$ usually has a value of about 0.7 for

(3) Schachman and Lauffer, THIS JOURNAL, **71**, 536 (1949).

(4) Taylor and Lauffer, paper read before Division of Biological Chemistry, American Chemical Society, in Atlantic City, September, 1949.

(5) McMeekin, Groves and Hipp, THIS JOURNAL, **72**, 3662 (1950).

(6) Sharp, Beard and Beard, *J. Biol. Chem.*, **182**, 279 (1950).

experiments carried out on proteins dissolved in aqueous media, when h/m is less than 1 and $d - \rho$ is less than 0.01, a value for the unsolvated molecular weight, M, within about 3% of the correct value should be obtained. Whenever $(h/m)(d - \rho)$ exceeds 0.01, unsolvated molecular weights of proteins determined by means of equation 10 will be too low by 3% or more.

When allowance is made for the differences in symbols, it is evident that equation 9 divided by equation 10 yields the equation derived previously by Lansing and Kraemer[7] for sedimentation equilibrium. Their equation 9 applies to the case of sedimentation equilibrium in a ternary system where the solute combines with only one of the other two components. In the same paper, of course, Lansing and Kraemer showed that the molecular weight of solute determined by sedimentation equilibrium of a dilute binary solution was virtually independent of solvation. Just as Lansing and Kraemer's equation 9 in no way contradicts their own equation 7, our treatment is in no way inconsistent with theirs.

(7) Lansing and Kraemer, THIS JOURNAL, **58**, 1471 (1936).

Summary

The effect of hydration on the density correction of sedimentation constants is considered. Equations are presented to show the influence of hydration and a comparison is made between these new equations and the equation of Svedberg and Pedersen which is shown to represent a special case. Calculations involving previously published data show errors due to neglecting hydration ranging from 5 to 26% in an extreme case.

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NOTES

Synthesis of C^{14}-Sodium Cyanide from Carbon Dioxide[1]

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The two methods in current use for the conversion of radiocarbonate to radiocyanide, namely, the interaction[3] of $C^{14}O_2$, NH_3 and K in a sealed tube at 620°, and the fusion of $BaC^{14}O_3$ with sodium azide,[4] have in our experience proved to be tricky, inconvenient and distinctly limited in size [see also[5]]. Just recently an improved synthesis has been reported by Abrams[5]; $C^{14}O_2$ is reduced with magnesium to elementary carbon, which is then converted in 60–70% over-all yield to cyanide by treatment with ammonia at 1000°. The desirability of producing radiocyanide by simple organic reactions not requiring high temperature or pressure techniques prompted the adaptation of the following sequence of reactions.

$$(C_6H_5)_3CNa \xrightarrow[90\%]{C^{14}O_2} (C_6H_5)_3CC^{14}OOH \xrightarrow[95\%]{SOCl_2 \text{ then } NH_4OH}$$

I II

$$(C_6H_5)_3CC^{14}ONH_2 \xrightarrow[97\%]{P_2O_5} (C_6H_5)_3CC^{14}N \xrightarrow[90\%]{Na,\ EtOH}$$

III IV

$$(C_6H_5)_3CH + NaC^{14}N$$

V VI

Triphenylmethylsodium (I)[6] carbonates[7] readily to the crystalline triphenylacetic acid II. The corresponding amide III,[8] prepared in the usual way through the acid chloride,[8] dehydrates smoothly to the nitrile IV, which, because of the pronounced electrophilic properties of the component groups facilely undergoes[9] hydrogenolysis to triphenylmethane (V) and cyanide (VI) rather than hydrogenation to triphenylethylamine. A consistent yield of 90% or better has been achieved in each of the steps, with the over-all realization of 68 to 72% of $NaC^{14}N$ from $BaC^{14}O_3$. The purification of intermediates is not necessary.

The method offers several worthwhile advantages: (a) in one convenient reaction, a gaseous low molecular weight starting material ($C^{14}O_2$; m. w., 46) leads to a solid high molecular weight product (II, m. w., 290, m. p., 267°); (b) it is equally adaptable to a micro- or a macro-scale; and, (c) other useful one carbon compounds such as formate, methylamine, etc., may be obtainable directly (in the course of exploration).

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(3) Cramer and Kistiakowsky, *J. Biol. Chem.*, **137**, 547 (1941); Loftfield, *Nucleonics*, **1**, No. 3, 54 (1947).

(4) Adamson, THIS JOURNAL, **69**, 2564 (1947).

(5) Abrams, *ibid.*, **71**, 3835 (1949).

(6) Renfrow and Hauser, *Org. Syntheses*, Col. Vol. II, 607 (1943).

(7) Schlenk and Marcus, *Ber.*, **47**, 1668 (1914).

(8) Schmidlin and Hodgson, *ibid.*, **41**, 445 (1908).

(9) Biltz, *Ann.*, **296**, 253 (1897).