Sedimentation of Spheres in a Suspension of Finite Concentration

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The sedimentation velocity in a centrifugal field supplies valuable information for the size and shape determination of colloidal particles and large molecules. Sedimentation velocity, however, is dependent on the concentration of particles in the suspension. Therefore, experimental values obtained by measurements performed on solutions or suspensions of finite concentration should be extrapolated to a value for infinite dilution in order to obtain information concerning the particle size and shape using the Stokes' law.

Stokes' law was calculated by using a model in which a sphere moved through a viscous fluid medium which extended infinitely in all directions, and the Reynolds' number was assumed to be very small. In cases of sedimentation experiments on molecular or colloidal suspensions the Reynolds' number is usually very small, and the latter condition is always satisfied. However, the cells used in sedimentation experiments are not infinite in size. Since the size of particles we are concerned with is so small, the volume of the sedimentation cell can be regarded as infinite when the concentration is very low. When the concentration is high, however, the distance between particles is comparable to the size of the particles, and the condition of flow around each particle is different from that in a case in which a particle is moving through an infinite medium.

When sedimentation coefficients vs. concentration curves for various substances are examined, they seem to coincide if the concentration axis is multiplied by a suitable factor. Thus if we exactly know the situation of sedimenting spheres, we can imagine a similar situation for sedimenting molecules and colloidal particles.

A number of studies on the concentration dependene of sedimentation coefficients[1-5] have appeared in the past and

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[1] R. E. Powell and H. Eyring, *Advances in Colloid Science*, **1**, 183 (1942).

[2] J. M. Burgers, *Proc. Acad. Sci. Amsterdam*, **44**, 1045, 1177 (1941); **45**, 9, 126 (1942).

[3] J. H. Fessler and A. G. Ogston, *Trans. Faraday Soc.*, **47**, 667 (1951).

[4] P. G. W. Hawksley, " Some Aspects of Fluid Flow ", Paper 7, Edward Arnold, London (1951).

[5] S. Uchida, *Reports of the Institute of Science and Technology, University of Tokyo*, **3**, 97 (1949); An outline by J. Happel, *Ind. Eng. Chem.*, **46**, 1194 (1954).

various equations have been proposed. However, it has been difficult to verify any such equations, because of concentration ambiguity due to particle solvation or lack of particle size uniformity in the experimental cases.

Therefore an experiment was performed on polystyrene latex of uniform spherical shape. The polystyrene latex, Dow Latex 580 G, has been studied with various methods and the size and shape of particles are well established⁶⁾. Particles are perfectly spherical and the diameter of particles is uniform within a small range. It was also shown that polystyrene particles are not hydrated to any observable amount⁷⁾, and the volume of the latex particle can be used as its hydrodynamic volume.

Experimental

Materials and methods: By adding concentrated solution of sodium chloride, Dow Latex 580 G suspension was made 0.14 M in regard to sodium chloride, and then it was diluted to various concentrations by adding suitable amounts of 0.14 M sodium chloride solution. The solution was centrifuged in an ordinary laboratory centrifuge in a field of about 1000×g. for five minutes in order to remove aggregates.

For the determination of the concentration of latex particles, a few drops of the solution were weighed with a micro-balance, gently dried over phosphorus pentoxide and weighed again. Since the concentration of sodium chloride was known, the volume fraction concentration of latex particles was calculated using the value⁷,⁸⁾ for the density of polystyrene latex.

Sedimentation experiments were performed with an air-driven centrifuge of Pickels type⁹⁾ at a speed of 9000 r.p.m. The centrifugal field was about 6000 times that of gravity. The speed was controlled manually by changing the driving air pressure while observing a Lissajou's figure on a cathode ray oscillograph. The Lissajou's figure was formed by the potential caused by a magnet fixed on the air turbine, and the frequency of power supply was used as the reference. By this method the speed of rotation was readily kept within an error of 0.5%. The frequency of power supply was checked with a frequency meter.

The sedimenting boundary was observed and photographed through a schlieren optical apparatus. However, since polystyrene latex suspension was very opaque at all concentrations used in the experiment, we actually measured the absorption edge of the sedimenting boundary. The light source was a high pressure mercury discharge lamp, and was very intense. The exposure time was three seconds.

Results: sedimentation coefficients calculated and reduced for water at 20°C are shown in Fig. 1. Density and viscosity of solvent was used for the calculation. When the curve through measured points in Fig. 1 was extrapolated to zero concentration, a sedimentation coefficient of 2180 S was obtained.

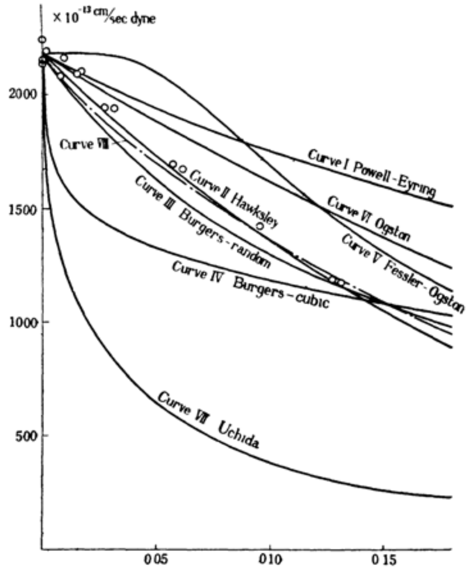

Fig. 1. Sedimentation coefficient for polystyrene latex particles.

Discussion

The value of the sedimentation coefficient for Dow Latex 580 G at infinite dilution obtained above agrees well with the value obtained by Hogeboom and Kuff¹⁰⁾. The work of Cheng and Schachman¹¹⁾ was published after our completion of the experiment, and their value agrees well with the value obtained here when it is corrected for the temperature difference.

By using Stokes' law, the diameter of the polystyrene latex was calculated as 268 mμ from the sedimentation coefficient value extrapolated to infinite dilution. This is in good agreement with values obtained by other methods⁶,¹²,¹³⁾.

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6) C. H. Gerould, *J. Appl. Phys.*, **21**, 183 (1950).

7) D. G. Sharp and J. W. Beard, *J. Biol. Chem.*, **185**, 247 (1950).

8) R. C. Williams and R. C. Backus, *J. Am. Chem. Soc.*, **71**, 4052 (1949).

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10) G. H. Hogeboom and E. L. Kuff. *J. Biol. Chem.*, **210**, 733 (1954).

11) P. Y. Cheng and H. K. Schachman, *J. Polymer Sci.*, **16**, 19 (1955).

12) W. B. Dandliker, *J. Am. Chem. Soc.*, **72**, 5110 (1950).

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In Fig. 1, together with the experimental values, curves are shown which were calculated from formulae which have been proposed by various authors.

Curve I was calculated using the formula of Powell and Eyring1).

$$s=s_0/(1+\nu\phi) \quad (1)$$

ϕ: Volume fraction concentration.
s: Sedimentation coefficient at ϕ.
s_0: Sedimentation coefficient at infinite dilution.
ν: Volume fraction limiting viscosity number for solute particles.

This formula was calculated by using a model in which only one particle was assumed to be sedimenting while all the other particles were assumed not to be in motion. However, such a situation is never realized in actual cases, as all the particles sediment at the same time. The fact that curve I does not agree with experimental points shows that the model used by Powell and Eyring was not suitable. It has also been pointed out14) that in cases in which ν was very large, the term $(1+\nu\phi)$ of formula (1) was too large to explain experimental values.

Curve II in Fig. 1 was obtained from the formula of Hawksley4),

$$s/s_0=(1-\phi)^2\exp.(-k\phi/(1-Q\phi)) \quad (2)$$

k: Einstein's shape factor for viscosity. 2.5 for spheres.
Q: Vand's interaction constant. 39/54 for spheres.

and is quite well fitted to the experimental values. This formula was derived by considering three effects: apparent increase of the viscosity of the medium by the presence of particles, apparent increase of buoyancy due to apparent increase of the density of the medium, and the back flow of the medium. However, when we consider the fact that all the particles sediment at the same time and there is no relative velocity between particles, the first and the second effect are not likely to exist. Hawksley calculated the first effect according to Vand's modification15) of Einstein's formula for viscosity.

$$\eta_{\text{rel.}}=\exp.(k\phi/(1-Q\phi)) \quad (3)$$

$\eta_{\text{rel.}}$: Viscosity ratio.

However, it was reported by Cheng and Schachman11) that formula (3) does not agree with experimental values for Dow Latex 580 G. If actual values11) of the viscosity of the suspension of Dow Latex 580 G were substituted into formula (2), the sedimentation velocity at a concentration of 8% will be about 5% lower. Thus, the excellent coincidence of the values calculated by the formula (2) with experimental values seems to be fortuitous.

Burgers derived a relation for the dependence of sedimentation velocity on concentration assuming a hypothetical function of flow2). Curve III and IV show results obtained by Burgers. Curve III was calculated assuming a random arrangement of sedimenting particles and curve IV was calculated by assuming a cubic arrangement of particles. Curve III follows the experimental points fairly well.

The formula of Fessler and Ogston3) is shown in Fig. 1 as curve V. This formula was derived by considering that when the concentration is very high the resistance of sedimenting particles approaches that of a porous plug, but is unsatisfactory in two points. The equation by Sullivan ane Hertel16) for fluid flow through a porous plug is used in the formula, and it includes the effect of back flow of the medium by the nature of the equation. The use of the Enoksson's factor17) in the derivation of the formula was theoretically unnecessary. It is also not justifiable to add the term $1/s_0$ to the term derived from the equation by Sullivan and Hertel. Recently Ogston18) has used a modified form, and it is shown as curve VI in Fig. 1.

Curve VII shows the result numerically calculated by Uchida5) on a model of spheres sedimenting in a cubic arrangement. The resistance calculated by using such a model is far larger than that for actual sedimenting latex particles, and this fact suggests that the actual arrangement shows less resistance than a cubic arrangement although particles are likely to be in some kind of arrangement while sedimenting. When particles are distributed at random in space and time, the average resistance against sedimenting particles should be, as was pointed out by Hawksley4), inversely proportional to the volume fraction of the suspending medium. The increase of resistance due to this effect is too small to explain the

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14) I. Jullander, *J. Polymer Sci.*, **2**, 329 (1947).
15) V. Vand, *J. Phys. Coll. Chem.*, **52**, 277 (1948).
16) R. R. Sullivan and K. L. Hertel, *Advances in Colloid Sci.*, **1**, 37 (1942).
17) B. Enoksson, *Nature*, **161**, 934 (1948).
18) A.G. Ogston, *Trans. Faraday Soc.*, **49**, 1481 (1953).

TABLE I

APPARENT VOLUME FOR MOLECULES WITH SOLVATION CALCULATED FROM THE CONCENTRATION DEPENDENCE OF SEDIMENTATION COEFFICIENT

Substance	v/v_0	$\sqrt[3]{v/v_0}$	f/f_0	$(f/f_0)/\sqrt[3]{v/v_0}$
Collagen[19]	8.5×10	4.4	9.7*	2.2
Herring sperm deoxyribonucleic acid. (Preparation 1b)[20]	6.4×10	4.0	5.0	1.25
Cellulose acetate[21] (in acetone)	4.3×10	3.5	3.5	1.0
Cellulose nitrate (Fraction I/6)[22] (in acetone)	3.0×10	3.1	4.6	1.5
Sodium cellulose xanthate[23] (Preparation No. 8)	2.0×10	2.7	3.4	1.3
Polystyrene (Fraction PSAF)[24] (in decaline)	1.4×10	2.4	3.3	1.3
Bovine serum albumin[25]	2.2	1.3	1.3	1.0
Calf thymus histone[26] (Histone I)	3.4	1.5	1.9	1.3
Bovine fibrinogen[27]				
Native	4.9	1.7	2.2	1.3
Denatured, at pH 5.5	1.2×10	2.3	3.0	1.3
Denatured, at pH 3.5	2.2×10	2.8	3.0	1.1

concentration dependence of the sedimentation coefficient, and suggests that the particles are in some kind of spacial arrangement with each other.

Curve VIII was drawn according to the formula*:

$$s_0=s\{1+\phi^{2/3}/(1-\phi^{1/3})^2(1+2\phi^{1/3})\} \qquad (4)$$

Within the range of concentration in which experiments were possible with polystyrene latex, formula (4) agreed well with the experimental points. Therefore formula (4) can be used as an experimental formula for the concentration dependence of the sedimentation coefficient.

Since the relation of sedimentation coefficient to concentration was experimentally established, and can be represented by formula (4), we came to be able to find the apparent volume of particles by measuring the concentration dependence of the sedimentation coefficient. The apparent volume obtained in such a way may be different from that for the substance in dry condition. The ratio of such apparent volume to the dry volume, v/v_0, was calculated for various substances from available values of sedimentation experiments and the results are shown in Table I. In all cases the curves for the dependence of sedimentation coefficient were very similar to that for the polystyrene latex, when s/s_0 values were plotted against volume fraction concentration calculated from the apparent volume in solution. This fact seems to suggest that the particles with solvation are nearly spherical in sphape in these cases. In the fourth column of Table I, values of the frictional ratio of particles calculated from values of the sedimentation coefficient and diffusion constant are shown. The cube root of v/v_0 and the frictional ratio have a very close relation with each other. This suggests that values of the frictional ratio larger than unity can be explained for the most part by the increase of

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* When the concentration is high and each sphere is sedimenting surrounded by many other spheres of the same size at close distances, we may assume that at the middle point of a line which connects the center of the sphere to the center of one of the surrounding spheres, the line of flow is at a right angle to the line. Such a condition may be represented by a model in which a sphere moves at the center of a spherical shell on the surface of which the liquid slips perfectly. A simple calculation was performed on such a model using the Stokes' function of flow, and the resistance against the movement of the sphere was calculated. Then the sedimentation velocity of the sphere at the center of such a spherical shell was calculated as follows:

$$s=s_0(1-a/b)^2(1+2a/b)$$

where a is the radius of the sphere and b is the radius of the spherical shell. Thus a/b is approximately $\phi^{1/3}$. This model was too crude, and the calculated result did not agree with experimental results.

* This value is different from the value reported in the article cited. By later experiments the diffusion constant for collagen was found to be 0.3×10^{-7}cm^2./sec.

19) H. Noda, *Biochim. Biophys. Acta*, **17**, 92 (1955).

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21) S. J. Singer, *J. Chem. Phys.*. **15**, 341 (1947).

22) H. Mosiman, *Helv. chim. Acta*, **26**, 61 (1943).

23) N. Gralen, Inaugural Dissertation, Uppsala 1944.

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25) V. L. Konig and J. D. Perrings, *Arch. Biochem. Biophys.*, **41**, 367 (1952).

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27) H. A. Scheraga, W. R. Carroll, L F. Nims, E. Sutton, J. K. Buckus and J. M. Saunders, *J. Polymer Sci.*, **14**. 427 (1954).

apparent volume of molecules or particles by solvation. The great amount of solvent which accompanies molecules in the cases of collagen and a preparation of deoxyribonucleic acid is surprising, but if such molecules are considered to be composed of long chains and to be in the shape of a random coil, it is possible for them to be accompanied by such an amount of solvent.

When the above method of estimating the amount of solvation was applied to the sedimentation data of fibrinogen by Scheraga et. al.(27), as is shown in Table I, the amount of solvation obtained for denatured fibrinogen was larger than that for native fibrinogen, and the increase in the frictional ratio due to denaturation was explained by the increase in the apparent volume of molecules. Scheraga et. al. reached a similar conclusion using their data on various hydrodynamic properties on the basis of the work of Scheraga and Mandelkern(28).

It should be noted that formula (4) was verified only for the case of sedimenting spheres. Therefore the above discussion is not decisive, especially in cases in which the ratio $(f/f_0)/\sqrt[3]{v/v_0}$ is far from unity. In order to determine exact contributions by the molecular shape and solvation to the increase of frictional ratio, we should know the relation between the molecular shape and the concentration dependence of the sedimentation coefficient.

Summary

Experiments were performed in order to find out the relation between the volume fraction concentration and the sedimentation coefficient in a suspension of spheres. The polystyrene latex suspension, Dow Latex 580 G, was used as the suspension of spheres of known hydrodynamic properties. No theoretical equation proposed in the past corresponds well with the experimental result obtained here. Therefore an experimental equation,

$$s_0=s\{1+\phi^{2/3}/(1-\phi^{1/3})^2(1+2\phi^{1/3})\}$$

is proposed. Using this equation the amount of solvation in solution of some proteins and other molecules was calculated. The amount of solvation found is nearly enough to explain the frictional ratio for such molecules..

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28) H. A. Scheraga and L. Mandelkern, *J. Am. Chem. Soc.*, **75**, 179 (1953).