over-all ΔH should be less negative than in the case of a dipeptide. It is unfruitful to attempt to argue these points in any more detail at the present time.

The chief significance of the result reported here is that it constitutes further indication that the heat of hydrolysis of a typical peptide bond in a protein is a smaller negative quantity than has usually been supposed, probably lying between -1000 and -2000 cal. per mole.

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[Contribution from the Virus Laboratory, University of California, Berkeley]

The Effect of Pressure on Sedimentation, and Compressibility Measurements in the Ultracentrifuge[1]

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For most ultracentrifuge work the large pressures, which may amount to several hundred atmospheres, produced at the bottom of the centrifuge cells cause so little change in the physical properties of the solute or the solvent that the effect of pressure on ultracentrifugal analysis is almost negligible. If, however, the solvent has a density almost equal to that of the sedimenting particles, then the change in density of the solvent throughout the cell can cause marked variations in the effective centrifugal force on the particles in different regions of the cell. In studies on polystyrene latex particles in a solvent of almost equal density, the change in density of the solvent throughout the cell was sufficiently large to cause the particles to float in the high pressure part of the cell while the particles at the top of the cell were sedimenting. Thus sedimenting and floating boundaries could be obtained in a single run and the rate of movement of each boundary could be measured. The flotation rate of the particles in a D_2O–H_2O mixture was, however, much less than that expected from considerations based on the size and density of the particles and the compressibility of the solvent. From the difference between the observed and expected flotation rates, the coefficient of compressibility of the particles was calculated and found to be in good agreement with other compressibility data on solid polystyrene. Other areas of ultracentrifugal analysis in which there is a significant pressure effect are discussed. The application of the ultracentrifuge to the measurement of the compressibility of solids is described.

Introduction

As Svedberg and Pedersen[2] pointed out, the forces developed in modern ultracentrifuges cause sufficient compression of the liquid in ultracentrifuge cells to produce variations in both the density and viscosity of the liquid throughout the cell. These changes in viscosity and density must be considered in the quantitative evaluation of sedimentation data since particles in different regions of the cell are subject to different forces as a result of the pressure gradient. For most systems, and especially those employing aqueous solvents, the density and viscosity variations are almost negligibly small, but increasing use of ultracentrifugal techniques in recent years has encompassed substances for which these compression effects can be very important.

Mosimann and Signer[3] considered the effect of pressure on the viscosity of the organic solvent, acetone, which they employed in a study of the sedimentation of nitrocellulose. They cited data to show that the viscosity of acetone increased by 28% from the meniscus to the bottom of the cell and they made appropriate corrections to the sedimentation velocity observed at different parts of the ultracentrifuge cell. From data on the compressibility of acetone they showed, further, that the buoyancy term in the Svedberg equation[2] varied only 2.4% throughout the cell due to the increase in density of the acetone under the same experimental conditions.

In a recent study on polystyrene latex particles (PSL)[4] in solvents of different densities we have observed that the effect of pressure on the buoyancy term is not negligibly small. In fact, the effect was sufficiently large to justify further study of this problem, and this communication presents the results of this study. As we will show, the sedimentation rate of the PSL is markedly affected by the change in density of the solution due to compression. Furthermore, the magnitude of the differences in sedimentation rate in different parts of the cell can be used, under certain circumstances, to determine the compressibility of the sedimenting solute particles. Though the technique is, at present, limited to certain rather large particles, it is fundamentally a differential method, based on differences in compressibility between solute and solvent, and therefore capable of high accuracy.

Materials and Methods

The suspensions of PSL were the same as those used previously[4] in studies on the validity of Stokes' law of sedimentation and the Einstein viscosity equation. It appears that the PSL are rigid, impermeable spheres of diameter 2640 Å., and density 1.0520 g./cc. Sedimentation coefficients were determined from plots of the logarithm of boundary position, as distance x in cm. from the axis of rotation, *versus* time t in seconds. Boundary positions were determined, as before,[4] by the change in blackening on the photographic plate. This change in blackening on the plate was generally very abrupt and was due to the variation in turbidity in the region between solvent and solution. Because of the large size of the PSL the scattering of light by the

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[1] (a) This work was supported by grants from the Corn Industries Research Foundation, Lederle Laboratories and the Rockefeller Foundation. (b) Presented before the Division of Polymer Chemistry at the 126th meeting of the American Chemical Society, September, 1954.

[2] T. Svedberg and K. O. Pedersen, "The Ultracentrifuge," Oxford University Press, 1940.

[3] H. Mosimann and R. Signer, *Helv. Chim. Acta*, **27**, 1123 (1944).

[4] P. Y. Cheng and H. K. Schachman, *J. Polymer Sci.*, in press.

solution was so great that the sections of the plate corresponding to regions in the ultracentrifuge cell containing the PSL were underexposed and hence white on the original plates. The figures in this communication are photographic positives and, therefore, those regions in the cell which appear black show the presence of the scattering particles.

In order to decrease the sedimentation rate of the PSL, D_2O was added to make the density of the solvent very slightly less than 1.0520 g./cc. at atmospheric pressure. The solutions in some experiments also contained sodium chloride to a concentration of 0.1 molar. To study the sedimentation of the PSL in this medium the ultracentrifuge was operated at speeds between 12,590 and 23,150 r.p.m. Pictures taken at 2-minute intervals showed almost linear plots of log x *vs.* t for total boundary displacements of about 0.15 cm. Sedimentation coefficients corresponding to zero time, or the meniscus in the cell, were then calculated from these data. These results correspond to pressures of one atmosphere. After about 20 minutes the ultracentrifuge rotor was rapidly accelerated to speeds of about 44,770 to 52,640 r.p.m. and another boundary moving from the bottom of the cell toward the axis of rotation was then followed for a period of about 10 minutes. Again plots of log x *vs.* t were constructed, and the slopes of these curves, which were linear for only a small range of distances, were obtained at a position corresponding to the bottom of the cell. In this way, the negative sedimentation rate corresponding to pressures in the neighborhood of 200 atmospheres were obtained in the same ultracentrifuge studies.

The entire ultracentrifuge run consumed a period of less than one hour, and the heating of the rotor was only about 0.5°. However, the rotor cools due to stretching at high speeds, as shown by Waugh and Yphantis,[5] and appropriate corrections were made for this effect.

Theory.—If a given mass m of material occupies a volume v_0 at atmospheric pressure P_0 and the volume becomes v at a pressure P, we can write

$$\rho = \frac{\rho_0}{1 - \beta(P - P_0)} \qquad (1)$$

where ρ and ρ_0 are the densities of the material at the pressures, P and P_0, respectively, and β is the coefficient of compressibility defined in the usual manner. Now considering a solution of two components with solute denoted by the superscript, p, and solvent with the superscript, s, we can write for the relative compressibility

$$\frac{\beta^p}{\beta^s} = \frac{\rho^p - \rho_0{}^p}{\rho^s - \rho_0{}^s} \times \frac{\rho^s}{\rho^p} \qquad (2)$$

Under the experimental conditions chosen for this work, the solvent has a density very close to that of the solute ($\rho^s - \rho^p < 0.005$), and equation 2 reduces to

$$\frac{\beta^p}{\beta_s} = \frac{\rho^p - \rho_0{}^p}{\rho^s - \rho_0{}^s} \qquad (3)$$

For spherical particles of radius r and density, ρ^p, Stokes' law can be written

$$\frac{4}{3}\pi r^3(\rho^p - \rho^s) = 6\pi\eta r s \qquad (4)$$

where η is the viscosity of the solvent and s is the sedimentation coefficient of the particles. Equation 4 can be rearranged to yield

$$\rho^p - \rho^s = k\eta s(\rho^p)^{2/3} \qquad (4a)$$

where k is equal to $(162)^{1/3}\pi^{2/3}/M^{2/3}$ and M is the mass of a particle. We can also write an equation similar to 4a for conditions at atmospheric pressure and subtract the latter from 4a to give

$$\rho^p - \rho_0{}^p = \rho^s - \rho_0{}^s + k[\eta s(\rho^p)^{2/3} - \eta_0 s_0(\rho_0{}^p)^{2/3}] \qquad (5)$$

Since the compressibilities of solids are quite small, ρ^p will be almost equal to $\rho_0{}^p$ and there will be only a small error in writing equation 5 as

$$\rho^p - \rho_0{}^p = \rho^s - \rho_0{}^s + k(\rho_0{}^p)^{2/3}[\eta s - \eta_0 s_0] \qquad (5a)$$

Combinations of equations 3 and 5a leads directly to the final equation

$$\frac{\beta^p}{\beta^s} = 1 + k(\rho_0{}^p)^{2/3}(\eta s - \eta_0 s_0)/(\rho^s - \rho_0{}^s) \qquad (6)$$

Thus, the determination of the compressibility of a substance involves measurements of the sedimentation rate of the particles at two different pressures in a solvent of approximately equal density, and a knowledge of the compressibility, density and viscosity of the solvent at the two pressures, as well as the density of the particles at one of the pressures.

Results

Figure 1 shows representative ultracentrifuge patterns from a study demonstrating the effect of compressibility of the liquid on the sedimentation of PSL. The first two pictures at 16,200 r.p.m. show the movement of the boundary toward the right, the centrifugal direction, due to the sedimentation of the particles which are slightly *more* dense than the medium. Upon acceleration of the ultracentrifuge to 44,770 r.p.m. this boundary first moved more rapidly and then at a continuously decreasing velocity until the sedimentation velocity approached zero. This follows, of course, since the particles are entering regions of steadily increasing pressure, and hence they reach a point at which the density of the liquid equals the density of the PSL. At the same time, a second boundary appeared at the bottom of the cell, as shown in Fig. 1, at a speed of 44,770 r.p.m. and the boundary moved toward the left, in a centripetal direction, due to flotation of the PSL in a region of the cell in which the particles are *less* dense than the medium. Fig. 1 shows, therefore, that it is possible to obtain both a sedimenting and a floating boundary in a single ultracentrifuge experiment. In order to demonstrate that the flotation of the PSL in the bottom of the centrifuge cell was due to the pressure gradient and not to variations in density of the solvent caused by a preferential concentration of D_2O near

SEDIMENTATION AND FLOTATION OF PSL IN D_2O–H_2O MIXTURE

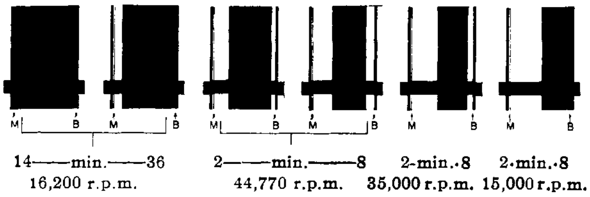

Fig. 1.—Ultracentrifuge patterns of the sedimentation and flotation of polystyrene latex particles in a D_2O–H_2O solution. Sedimentation is to the right and flotation to the left. The arrows marked M and B refer, respectively, to the liquid meniscus and the bottom of the ultracentrifuge cell. Pictures are photographic positives and the dark regions in cell result from scattering of light by the particles. From left to right the pictures correspond to 14 and 36 min. at 16,200 r.p.m., 2 and 8 min. at 44,770, 35,000 and 15,000 r.p.m., respectively.

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TABLE I

ω_{low}, r.p.m. 10^{-3}	x_{men}, cm.	s_{men}, S	ω_{high}, r.p.m. $\times 10^{-3}$	x_{bot}, cm.	s_{bot}, S	$P - P_0$, atm.	$\rho_{\text{sol}} - \rho_{0\text{sol}}$,[a] g./cc.	η, poise	β_{sol}[b] $\times 10^6$ atm.$^{-1}$	β_{part} $\times 10^6$ atm.$^{-1}$
12.6	5.88	61.7	47.7	7.06	−24.0	197	0.0081	0.0105	39.2	28
23.2	5.92	57.8	52.6	7.08	−41.3	242	.0099	.0099	39.2	28
16.2	5.84	64.7	44.7	7.22	−48.9	205	.0085	.0097	39.2	26

[a] In the calculations of the compressibility according to equation 6 we have assumed that the coefficient of compressibility of the D_2O–H_2O–NaCl solution is the same as that of the H_2O–NaCl solution. Justification for this assumption stems from results of comparative measurements of the compressibilities of the solvents directly in the ultracentrifuge cells. From pictures at low and high speeds we are able to measure the movement of the liquid meniscus upon raising the speed of the rotor. The movement of the meniscus can be attributed to three causes. First of all, the rotor stretches at the higher speed but this can be accounted for by the movement of reference marks on each picture, which result from two holes in the balance cell at a fixed distance from the axis of rotation. Secondly, the liquid is compressed and the expected fractional decrease in volume is about 1.3%. Thirdly, the cells distort due to bulging of the windows so that the cross-sectional area does not remain constant. This leads, of course, to a movement of the meniscus which makes the apparent compressibility of the liquid greater than the true compressibility. In measurements with water in the ultracentrifuge cell, the apparent decrease in volume upon acceleration of the rotor was 1.5 per cent. whereas calculations from the coefficient of compressibility indicate that the decrease in volume should be 1.3%. It was found, moreover, that the observed result was reproducible; *i.e.*, the distortion of the cell leading to the discrepancy between the observed and calculated decrease in volume was relatively constant in different runs. When D_2O–H_2O mixtures were studied in this way the fractional decrease in volume was the same as that observed for H_2O. Though this is not a precise method for measuring compressibilities of liquids, reasonably reliable results can be obtained if the same ultracentrifuge cell is used for both the unknown and the comparison standard and if the two liquids for comparison are not too different in density. [b] This value for the compressibility of water was obtained from Bridgman's value[6] after modification for the effect of sodium chloride. Bridgman's value, 39.6×10^{-6}, for β was obtained over the pressure range 0 to 970 atm. and at a temperature of 22°. From extrapolation of the data of Pohl[7] on the compressibility of salt solutions relative to water, we estimate that 0.1 *M* sodium chloride has a compressibility equal to 0.99 that of water.

the bottom of the cell, pictures were taken while the rotor was decelerated to low speed. Since the particles which had been floating in the high pressure region of the cell began to sediment, Fig. 1, when the pressure gradient was reduced, we can conclude that it was the increase in density of the solvent due to compression rather than a change in composition of the solvent which caused the original flotation. Furthermore, the optical system at high sensitivity indicated that there was no redistribution of the D_2O in the centrifuge cell. Table I shows the results of the calculation of the compressibility of PSL according to equation 6. It should be noted that the variation of viscosity of aqueous solutions with pressure is small enough to be considered negligible.

The value 28×10^{-6} atm.$^{-1}$ for the compressibility compares favorably with the values, 22×10^{-6} atm.$^{-1}$, obtained by Spencer and Gilmore[8] for a commercial polystyrene sample, and 17×10^{-6} atm.$^{-1}$ obtained by Bridgman[9] for another sample of commercial polystyrene. As a method to determine the difference in compressibility between a solute and solvent this ultracentrifugal technique is very reliable. It should be noted that the presence of the sodium chloride in some of these experiments may lead to a slight error in the measurement of the compressibility of the solute. At the high centrifugal fields used to measure the flotation rate of the PSL there is some redistribution of the sodium chloride in the ultracentrifuge cell. As a consequence, the concentration of the sodium chloride at the bottom of the cell will be slightly greater than 0.1 molar and the density of the solvent will be affected accordingly. Neglect of this redistribution of the sodium chloride causes the value of the compressibility calculated from equation 6 to be smaller than the true value. This error is small since there is only a slight enhancement of the concentration of sodium chloride at the bottom of the cell in the experiments described above. The redistribution of low molecular weight substances like sodium chloride or sucrose can, in fact, be studied by using the PSL as an indicator of the density in the ultracentrifuge cell.[10] For accurate measurement of the compressibility of PSL the solvent should be composed of D_2O–H_2O mixtures without any additional low molecular weight materials.

Discussion

Such pressure effects on sedimentation as that described in this communication are, of course, unusual; but it is important to examine other areas of ultracentrifugal analysis for the possible complicating effects of density changes throughout the cell.

For organic solvents which generally have coefficients of compressibility greater than that of water, the increase in density through the cell can cause a marked variation in sedimentation rate as the boundary moves through the cell. Although Mosimann and Signer[3] showed that this amounted to only a 2.4% correction in the sedimentation of nitrocellulose in acetone, this small effect is, in some respects, not typical of the sedimentation of synthetic high polymers in organic solvents. The reciprocal partial specific volume of nitrocellulose is much larger than the density of acetone; and, therefore, the buoyancy term, $1 - \bar{V}\rho$, where $\bar{V}$ is the partial specific volume of the solute and ρ is the density of the solvent, will be very different from zero. Variations in ρ throughout the cell due to compression of the liquid will thus have little influence on the buoyancy term which is one of the factors governing the rate of sedimentation of the

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macromolecules. Other polymer systems[11] might very well have values of $1/\bar{V}$ close to that of ρ and, as a consequence, the density change due to compression could cause an appreciable change in sedimentation rate during the run.

In the study of lipoproteins in salt solutions of density sufficiently high to cause flotation, the change in density of the solvent may have a marked effect on the sedimentation rate during a single run. This could complicate analysis of complex mixtures of lipoproteins considerably because of concomitant changes in the Johnston–Ogston effect.[12,13] With some of the lipoproteins the buoyancy term at atmospheric pressure is about -0.02 and variations in sedimentation coefficient during a run may amount to 30% due to compression of the solvent.

Sedimentation studies of other macromolecules in solutions of high density may also involve complications resulting from the increase in density of the liquid due to compression. This is most clearly indicated in the studies of Lauffer and co-workers[14] on the sedimentation of southern bean mosaic virus in solutions containing high concentrations of sucrose. At sucrose concentrations below 70% by weight the virus sediments, and sedimentation rates at the meniscus can be obtained. If the sucrose concentration is increased still further, the density of the solution becomes so high that the virus floats and negative sedimentation rates can be obtained from the movement of the boundary near the bottom of the cell where the pressure is several hundred atmospheres. Since the flotation data in their figure are plotted with densities corresponding to atmospheric pressure rather than the pressures operative under the experimental conditions, it seems as if the density value extrapolated from the flotation data to a value corresponding to zero flotation rate is higher than the corresponding extrapolated value from the sedimentation data. This would suggest that the virus particles were compressed under the high pressures at the bottom of the cell. Calculations of the compressibility of the particles cannot be made readily from these data since some of the runs were made using a boundary forming technique and the positions of the boundaries are not given. Furthermore, in these experiments there is a large redistribution of the sucrose molecules during the ultracentrifuge runs and the values of the density of the solution at different parts of the cell is uncertain.

A slight modification of this ultracentrifugal method might be useful for the determination of the compressibility of solids. If a small amount of finely ground solid is suspended in a solvent in which the solid is insoluble, the solid would tend to sediment in a centrifuge until it reached an equilibrium position at which the densities of the solid and solvent are equal. From the known density of the solvent at atmospheric pressure, the compressibility of the solvent, the speed of the centrifuge and the position in the cell, the density of the solvent at the equilibrium position could be calculated. Changing the speed of the centrifuge should cause the solid to move to another equilibrium position, and from measurements of the equilibrium positions at different speeds, or different pressures, the coefficient of compressibility of the solid could be calculated. This method is, to some extent, different from that described earlier in this communication, since it is based on the position of the particles at equilibrium rather than the velocity of the particles as indicated by the moving boundary. In many respects it is similar to the density gradient technique of Linderstrøm-Lang[15] which is used for the accurate determination of the densities of microdrops of liquids. In the ultracentrifugal technique the density gradient comes about as a result of the compression of the liquid. The main virtue of the technique stems from its differential nature, and it is probable that the accuracy in the determination of the compressibility of the solid would be limited by knowledge of the compressibility of the solvent in which it is suspended.

From the sharpness of the boundaries in the experiments illustrated by Fig. 1, we can conclude that the particles are homogeneous with respect to density. If there are differences in density among the particles, these differences would be exaggerated, in so far as the sedimentation rate is concerned, by reducing the average density difference between the particles and the solvent to a small value. When the average difference in density between particles and solvent is 0.052 g./cc., variations in particle density of 0.01% lead to differences of only 0.2% in sedimentation rate. If D_2O is added to the solvent so the average net density difference is 0.001 g./cc. then the same distribution in density of the particles leads to a 10% variation in sedimentation rate. Thus the ultracentrifuge, under certain circumstances, can be used to study the homogeneity of a solute with respect to density.

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