SIZE AND DENSITY OF POLYSTYRENE PARTICLES MEASURED BY ULTRACENTRIFUGATION*

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A material currently in use (1) for calibration of electron magnification is a polystyrene latex, characterized by a remarkably high degree of particle uniformity with respect to size and shape. The particles, spherical in shape and stable in water suspension, are in a range suitable not only for electron micrography but for independent determinations of size by sedimentation velocity, viscosity, and light-scattering methods. Consequently, the material offers opportunities for checking the theories underlying measurements of size by these various methods. In the work reported here,1 studies have been made on the size of the particles estimated from data obtained with the ultracentrifuge. In order to make the calculations of size, studies were made also on the density and state of dispersion of the particles. These accessory data are of interest for comparison with the results previously reported on analogous investigations of the size and density of the influenza (2, 3) and other viruses (4).

Materials and Methods

The polystyrene latex was of Batch 580G, prepared by The Dow Chemical Company, Midland, Michigan, and obtained in water suspension from Dr. Robley C. Williams. An electron micrograph of the material is shown in Fig. 1. The suspension contained about 4 per cent by weight of the particles. Sedimentation velocity studies were made on the particles in 0.9 per cent NaCl, in Ringer's solution, and in 0.06 M phosphate buffer of pH 7.2. There was no evidence of aggregation or precipitation of the particles in these media. Studies on the relation of sedimentation rate to concentration of the particles were made in 0.9 per cent NaCl.

Density of the particles was investigated to provide data for calculation of particle diameter. These studies were made by sedimenting the particles in suitable concentration, 6.66 mg. per ml., in D2O (3) and in bovine serum albumin (2-4) by the techniques previously described in analogous

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studies on viruses. Solvent density was varied by suitable dilutions of the D₂O or bovine serum albumin with water. In all instances, the content of NaCl was maintained at the constant level of 0.9 per cent. The maximum density obtained with D₂O was 1.092 and that with bovine serum albumin was 1.073.

Sedimentation velocity studies were made in the air-driven ultracentrifuge, carrying a rotor cell of 5 mm. thickness and 4° arc width, turning on a mean radius of 6.5 cm. Photographs of the sedimenting boundary were made on Ansco process film by use of a high pressure capillary mercury arc with the lens and filter arrangements of Svedberg and Pedersen (5).

![Electron micrograph of polystyrene latex spheres, shadow-cast with chromium.](http://www.jbc.org/)

**Results**

For studies on the influence of concentration on sedimentation rate, a series of dilutions of the particles was made with 0.9 per cent NaCl solution. The preparations contained 20, 10, 6.66, 2.5, 0.2, and 0.04 mg. of polystyrene per ml., respectively. In this series of studies, the particles were sedimented at 2890 X g. The results are illustrated in Fig. 2.

At all of the concentrations down to and including 2.5 mg. per ml., there was a single, exceedingly sharp boundary (Fig. 3), indicative of a high degree of particle uniformity in the suspensions. At concentrations of 0.2, Fig. 4, and 0.04 mg. per ml., there was still sufficient light absorption for photography, but the sedimentation rate was substantially less at 0.2 mg. per ml. than at 2.5 mg. per ml., and at 0.04 mg. per ml. there was a further decrease accompanied by erratic splitting of the boundary.
These last two runs have been excluded in calculations from these data (Fig. 2).

The results of the density studies are shown in Fig. 5. It is seen that when the density of the medium was 1.033 sedimentation still proceeded away from the axis of rotation, but at 1.06 and above the direction was reversed, the particles moving in toward the axis from the cell bottom. The line was drawn by the method of least squares through the data obtained with D$_2$O, because relatively small viscosity corrections were needed (6), and these data are, therefore, regarded as more reliable than those obtained with bovine serum albumin. The data obtained with bovine serum albumin, however, are in close agreement. The intersection of this line with the zero axis indicates a particle density of 1.053 for the sedimenting particles in the D$_2$O-H$_2$O mixtures containing 0.9 per cent NaCl.

The mean value of particle size can be calculated approximately from the data of Fig. 5 as follows

\[
\frac{4}{3} \pi r^3 (\rho - \rho_s) \omega^2 R = 6 \pi \eta r \frac{dr}{dt} \tag{1}
\]

\[
S = \frac{1}{\omega^2 R} \frac{dr}{dt} \tag{2}
\]

\[
2r^2 (\rho - \rho_s) = 9 \eta S \tag{3}
\]
**Fig. 3.** Boundary of the polystyrene spheres sedimenting in 0.9 per cent NaCl at a concentration of 20 mg. of the material per ml.

**Fig. 4.** Sedimenting boundary of the material of Fig. 3 diluted with 0.9 per cent NaCl to 0.2 mg. of polystyrene per ml.

**Fig. 5.** Relationship between corrected sedimentation rate ($\eta S$ in centimeter-gram-second units) of polystyrene spheres and the density of the suspending medium. The concentration of polystyrene was 6.66 mg. per ml. and that of NaCl was 0.9 per cent for all preparations. The density of the suspending medium was adjusted with D$_2$O in one series of sedimentations (△) and with bovine serum albumin in the other (□). The circle is the value of $\eta S$ for this polystyrene concentration in 0.9 per cent NaCl taken from the line of Fig. 2.

where $4/3\pi r^3$ is the volume of the spherical particles of radius $r$; $\omega^2 R$ is the centrifugal acceleration at distance $R_{cm}$ from the axis of the rotor.
running at \( \omega \) radians per second; \( 6\pi \eta r \) is the frictional resistance to sedimentation of a sphere of radius \( r \) through a medium of viscosity \( \eta \); \( S \) = the sedimentation rate per unit acceleration (5); \( \rho \) = the density of particle; and \( \rho_s \) = the density of the suspending medium.

Clearly, \( \eta S \) is linearly dependent on \( \rho_s \), and it vanishes when \( \rho_s \) is adjusted to \( \rho \). The data bear this out, giving intersection with the zero axis at \( \rho_s = \rho = 1.053 \). From the first derivative of \( \eta S \) with respect to \( \rho_s \), it is seen that the magnitude of the slope of this line is

\[
\frac{d(\eta S)}{d(\rho_s)} = \frac{2r^2}{9} = \text{slope}
\]

a simple measure of the radius squared. Particle diameter, \( D = 6 \sqrt{-(\text{slope})/2} \). A slight correction must be made in measurement of the slope, because the sedimentation rate is not independent of the concentration of suspended particles. The correction is made by increasing the measured slope by the factor 1820/1790, which is the ratio of \( \eta S \) extrapolated to infinite dilution (Fig. 5) to its value at 6.66 mg. per ml., the concentration used in these density experiments. When this is done, the calculated sedimentation constant is 1940S, and the mean particle diameter is 253 m\( \mu \) with an estimated variation of \( \pm 3 \) m\( \mu \). It is, therefore, highly probable that measurement of a very few images of these spheres in an electron micrograph is sufficient, for most purposes, to establish the magnification.

**DISCUSSION**

It is difficult to say with precision how closely the particle sizes are grouped about the mean value associated with the sedimenting boundary in the present experiments. It is estimated that the total spread of boundary in Fig. 4 (0.2 mg. per ml. concentration) is not over 2 per cent, some of which is attributable to mechanical and photographic imperfections in the centrifuge arrangement. This figure, 2 per cent, at face value, would reflect only a 1 per cent spread in the diameters of the sedimenting spheres. Furthermore, if some particles sediment substantially more slowly than the main body of the material, their concentration must be considerably less than one-hundredth part of the total, as seen from Fig. 3 which shows the sedimentation on a sample containing 20 mg. of the particles per ml. Light penetrated the region of the supernatant fluid and the air space above the solution with equal ease but, had 1 part in 100 of slower sedimenting particles been present, this quantity should have absorbed as much light here as was absorbed in Fig. 4 when the concentration of sedimenting material was 0.2 mg. per ml. No such critical test
for particles larger than the mean is supplied by these pictures, but any homogeneous component greater than a few per cent could have been seen below the principal boundary of Fig. 4.

The particle diameter calculated from the sedimentation data, $253 \pm 3 \mu$, was somewhat lower than the value, $2590 \pm 25 \AA$, which has been obtained (1) by direct measurements in the electron microscope.

In the present experiments, it was noted that the sedimenting boundary, at low concentrations of the particles (0.04 mg. per ml.), tends to break up in a manner suggestive of instability owing to lack of sufficient density differential at the boundary between the supernatant fluid and the solution below. The low sedimentation rate seen at 0.2 mg. per ml. may be evidence of the onset of this condition. Preparations in which the density of the medium was near 1.05 show multiple boundaries with the higher concentrations of polystyrene particles, in contrast with the single boundaries seen in media of density near 1.0 or 1.1, both of which values are far from the density of the particles. It would appear from this that the effect is indeed one of boundary instability in the ultracentrifuge at these low density increments, and this may explain similar behavior previously observed in low concentrations of both the T2 (7) and the T7 (8) bacteriophages of Escherichia coli.

The procedure for measuring the density of particles by sedimentation in the ultracentrifuge has been employed in earlier studies on the properties of viruses. In the study of virus density, it was observed (2, 9-11) that the sedimentation properties of the virus particles changed in solutions of densities varied by solutes of low molecular weight such as NaCl, sucrose, and others, so that the relation between sedimentation rate and density of the suspending medium was not linear. It was supposed (2, 10) that this finding was due to osmotic effects of the solute on the virus, resulting in changes in the water content of the agents, and it was for this reason that the relatively high molecular weight bovine serum albumin was employed (2, 4) for measurement of the wet density of the virus particles. With this material, linear relations were observed. The use of D$_2$O was based on the supposition (3, 12) that this material might freely penetrate the virus particles and, consequently, that measurements of sedimentation rate in solutions would give the dry density of the particles or, perhaps, more correctly, the reciprocal of the partial specific volume. Results have been reported that appear to give weight to these hypotheses.

It will be observed in Fig. 5 that the albumin and D$_2$O curves obtained with polystyrene latex are essentially identical, in contrast with the analogous relations found with influenza virus (2). This finding indicates that little, if any, water is associated with the polystyrene particles in
these solvent mixtures. The value, 1.053, obtained by means of D₂O was not greatly different from that, 1.052, determined by an entirely different method (13). These results with polystyrene particles support the validity of the previous interpretations relative to the wet and dry density of viruses and the theories concerned with the use of bovine serum albumin and D₂O in the determination of these respective properties of virus particles.

**SUMMARY**

Studies have been made on the size of polystyrene latex particles by means of sedimentation in the ultracentrifuge. Single, sharply sedimenting boundaries were observed in the region of particle concentration of 2.5 to 20 mg. per ml., indicating high uniformity of particle size, and in this region the sedimentation rate was linear with concentration. At lower concentrations the rate diminished and the boundary became unstable. The calculated sedimentation constant was 1940 s⁻¹.

Particle density was determined by sedimenting the material in D₂O solution which gave the value 1.053. Data in close agreement with those obtained with D₂O were observed with bovine serum albumin. The calculated diameter of the particles was 253 μm.

The findings with polystyrene latex particles are compared with the results of analogous studies with virus particles.

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