Studies on Phosphatidylcholine Vesicles

DETERMINATION OF PARTIAL SPECIFIC VOLUMES BY SEDIMENTATION VELOCITY METHOD*

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SUMMARY

The partial specific volume of phospholipid vesicles was determined by the sedimentation velocity method in various concentrations of hydrogen and deuterium oxide mixture. Since the density of D₂O is very much greater than the reciprocal of the specific volume of phospholipids, the density of D₂O-H₂O medium corresponding to zero redistribution of phospholipids during sedimentation can be obtained with high precision by interpolation of the data. This method is therefore direct and accurate for phospholipids. Similar measurements on phospholipid vesicles in various concentrations of potassium chloride and sodium chloride solutions have also been made. The apparent isodensity point in the KCl solution was found to be approximately equal to that obtained in NaCl solution but smaller than that obtained in D₂O-H₂O medium. This difference can be attributed to the influence of preferential interaction of the vesicle with water. The partial specific volume and effective specific volumes of phosphatidylcholine vesicles are $\tilde{\nu} = 0.9814 \pm 0.0004$ ml per g, $\phi'_{\text{KCl}} = 0.9883 \pm 0.0002$, and $\phi'_{\text{NaCl}} = 0.9886 \pm 0.0006$ ml per g.

The accuracy of the calculation of molecular weight and other related hydrodynamic properties obtained from sedimentation measurements depends heavily upon a knowledge of the partial specific volume, $\tilde{\nu}$, of the macromolecule under study. This is the case because all the basic sedimentation equations contain the term $(1 - \tilde{\nu} \rho)$, where $\rho$ is the density of the solution. For proteins, $\tilde{\nu}$ is typically near 0.74 ml per g, and thus the usual error in $\tilde{\nu}$ of 0.3% results in an error of 1% in the molecular weight. For phosphatidylcholine vesicles, a closed shell-like sphere with a continuous phosphatidylcholine bilayer surrounding a volume of solvent, the situation is much less satisfactory. The partial specific volume of these objects is very close to unity, and hence the accuracy of the term $(1 - \tilde{\nu} \rho)$ becomes extremely sensitive to the accuracy of $\tilde{\nu}$. An error of 0.3% in $\tilde{\nu}$ magnifies to an error of 15% in the molecular weight.

The partial specific volume is calculated from density determinations on solutions of varying concentrations, according to the definition, $\tilde{\nu} = (\partial \rho/\partial \rho_i)_{T,P,W_i}$, where $\rho_i$ is the weight of the $i$th component added at constant temperature, pressure, and at constant weight of the other components of the system. Densities are, in general, determined by means of a pycnometer. It was recently reported that the determination of the partial specific volume of phosphatidylcholine vesicles by this method is unsatisfactory because the density of phosphatidylcholine vesicles is very close to that of the aqueous solvent (1). The magnetic densitometer (2, 3), perhaps the most sensitive method for determining $\tilde{\nu}$ accurately, may not be entirely satisfactory because the possibility of phospholipid adsorption to the surface of the glass buoy could introduce an appreciable error in the measurements.

This communication describes the determination of the partial specific volume of phosphatidylcholine vesicles by sedimentation in D₂O-H₂O mixtures of differing density. At the zero value of the viscosity corrected sedimentation coefficient, where the phosphatidylcholine vesicles neither sediment nor float, the corresponding density of the suspending D₂O-H₂O medium is taken as the density of the vesicle. The partial specific volume of the vesicle is the reciprocal of this density.

The sedimentation velocity method for determining partial specific volumes has been applied to viruses (4), proteins (5), and serum lipoproteins (6). Similar determinations in D₂O-H₂O media on lipids per se, however, have not been reported. This method should be ideal for phospholipid vesicles, since the density of the D₂O is considerably greater than that of phospholipids and thus the vesicles can be caused to float in a wide concentration range of D₂O in H₂O. Consequently, the density of the D₂O-H₂O medium corresponding to zero redistribution of the phospholipid vesicles can be obtained with high precision by interpolation, not extrapolation, of the data. Furthermore, since there are no exchangeable hydrogen atoms present in phosphatidylcholine in the pH range of 5 to 8, no corrections for deuterium exchange are necessary. The fact that phosphatidylcholine bears no net charge in this pH range eliminates the effects of charge on sedimentation.

In addition to the measurements with D₂O-H₂O media, sedimentation rates of phosphatidylcholine vesicles suspended in potassium chloride and sodium chloride solutions of varying densities were also investigated. Using the effective specific volume of the vesicle obtained from this data together with the information obtained in the H₂O-D₂O medium, the preferential hydration of the phosphatidylcholine vesicles has been determined.

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EXPERIMENTAL SECTION

Materials—The phosphatidylcholine was isolated from hen egg yolk by the column chromatographic method (1). The purity of the preparation was checked by thin layer chromatography (7). Deuterium oxide (~90 mole per cent D_{2}O, lot 4566) was purchased from Bio-Rad, Richmond, California. D_{2}O·H_{2}O media of different densities were made by mixing volumetrically the deuterium oxide with doubly glass-distilled water. Other chemicals were of reagent grade.

Preparation of Phosphatidylcholine Vesicles—Lyophilized phosphatidylcholine (300 to 400 mg) was suspended in 8 ml of buffered 0.1 M KCl solution (0.1 M KCl in 0.01 M acetate buffer) at pH 5.0. The suspension was ultrasonically irradiated (20 kHz) under nitrogen at 20°C for 2.5 hours and then centrifuged at 105,000 × g for 60 min. The resulting supernatant was subjected to gel filtration, at 4°C, on a column (2.5 × 50 cm) of Sepharose 4B which had been previously equilibrated with the same buffered KCl solution. The elution diagram of the phosphatidylcholine dispersion consists of two distinct fractions (I and II). Those portions of Fraction II which show a linear relation between absorbance at 300 nm and lipid phosphorous content and that from which the linear regression line passes through the origin were collected and dialyzed overnight against redistilled water at 4°C. The detailed experimental procedures for sonic disruption, gel filtration, and collection of the homogeneous vesicles solution in those portions of Fraction II have been published elsewhere (8). For those experiments with various concentrations of sodium and potassium chloride as suspending media, the phosphatidylcholine vesicles were prepared in buffered 0.1 M NaCl or KCl solution at pH 8.0. The vesicle solution, collected from the effluent of those proper portions of Fraction II (8), was mixed volumetrically with the appropriate concentration of buffered KCl or NaCl solution. The mixed solutions were stirred gently at room temperature for ½ hour before the beginning of the centrifuge run to allow for the complete equilibration of KCl or NaCl between the inner volume of the vesicles and the outer suspending medium. The buffer used throughout this work was 0.01 M Tris·HCl.

Viscosity and Density Measurements—A Cannon-Ubbelohde semimicrowave viscosimeter with a shear rate of about 1500 sec^{-1} and a water flow time of about 257 sec was used in all the viscosity measurements on the different suspending media. No kinetic energy correction was necessary. The viscosity of the medium relative to that of water, τ_{w}, was calculated using the relation \(\tau_{w} = \left(\frac{t}{L} \cdot \rho_{w}\right)\), where \(t\) is the flow time and \(\rho\) the density of the medium. The subscript, \(w\), represents the measurements with redistilled water. Flow times determined with an electric timer were the result of a minimum of five experiments. The density measurements of each suspending medium was determined in triplicate with a 5-ml calibrated pycnometer. Weightings were made with a Mettler H30T analytical balance to an accuracy of ±0.01 mg. Both the viscosity and density measurements were carried out at 20°C in a thermostated bath with a temperature control of ±0.005°C.

Sedimentation Velocity Measurements—Sedimentation velocity studies were carried out at 20°C ± 0.05°C in a Beckman-Spinco model E analytical ultracentrifuge, equipped with a schlieren optical system fitted with a phase plate and a RTIC temperature-control unit. The An-D rotor, operated at 42,040 or 50,740 rpm, was employed; a double sector, capillary-type synthetic boundary cell with a 12-mm optical path was used in most of the experiments. The phosphatidylcholine vesicle solutions employed were freshly prepared. The relative amounts of vesicle solution and its corresponding suspending medium introduced separately into the two sectors of the centerpiece were so chosen as to allow the formation of the boundary near the center of the cell. When the density of the suspending medium was higher than that of the phospholipids, the bar angle was set to be above 90°, thus converting schlieren pattern from a trough to a peak (10).

Since the vesicles were homogeneous with respect to size (1),

![Fig. 1. Ultracentrifuge schlieren patterns of a sedimentation velocity experiment on the phosphatidylcholine vesicles suspended in 10% D_{2}O medium. Time in minutes after rotor attained a speed of 50,740 rpm. The phase plate angle was 70° throughout. Initial concentration 10.8 μmoles per ml (P_{2}).](http://www.jbc.org/)

and the schlieren patterns were highly symmetrical (Fig. 1), sedimentation coefficient, $s_{\text{medium}}$, was calculated from least squares of $t$ versus $\log r_H$, where $r_H$ is the radial distance from the center of rotation of the centrifuge rotor to the point of the maximum ordinate on the schlieren peak at time, $t$. The maximum ordinate positions on schlieren patterns were measured with a Nikon shadowgraph (model 6).

RESULTS

To determine the partial specific volume of phosphatidylcholine vesicles, the sedimentation coefficients of the vesicles are first measured over a large range of phospholipid concentrations in various mixtures of $D_2O-H_2O$. The $D_2O-H_2O$ suspended medium can reasonably be considered to be a one-component solvent in which preferential binding of the different forms of water would not occur (11). The sedimentation coefficient, $s_0^0$, obtained by extrapolation to zero phospholipid concentration in each $D_2O-H_2O$ mixtures, is multiplied by the relative viscosity of the corresponding $D_2O-H_2O$ medium. The values of $s_0^0$ are then plotted against the density of the suspending $D_2O-H_2O$ media. At the zero value of $s_0^0$ the density of the suspending $D_2O-H_2O$ medium is taken as the reciprocal of the partial specific volume of the vesicles.

The schlieren patterns presented in Fig. 1 are typical examples of a sedimentation velocity experiment in which the initial lipid phosphorous was 10.86 pmoles per ml (0.84% by weight of phosphatidylcholine) and the suspending medium was 10% $D_2O$ in water. As shown in Fig. 2, when the density of the suspending medium was less than that of the phosphatidylcholine vesicle, the positive sedimentation coefficient decreased with increasing vesicle concentration ($P_i$); on the other hand, when the density of the suspending medium was greater than that of the vesicles, the negative sedimentation coefficient increased linearly with increasing vesicle concentration. A single preparation of phosphatidylcholine vesicles was used to determine the sedimentation coefficients in 5%, 10%, and 15% $D_2O$. Similar slopes of the $s$

### TABLE I

Summary of results of sedimentation studies on phosphatidylcholine vesicles suspended in various $D_2O-H_2O$ mixtures

<table>
<thead>
<tr>
<th>Physical parameter</th>
<th>0% (D$_2$O % in H$_2$O)</th>
<th>5%</th>
<th>10%</th>
<th>15%</th>
<th>30%</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\eta_r$</td>
<td>1.0000 ± 0.0007</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$s_0^0$</td>
<td>2.94 ± 0.08</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$s_{\text{medium}}$</td>
<td>2.94 ± 0.08</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\rho$ (grams per ml)</td>
<td>0.99820 ± 0.00001</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\delta_{2\rho}$</td>
<td>2.94 ± 0.11</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Deuterium oxide with purity of ~90 mole per cent $D_2O$ was obtained from Bio-Rad (lot #4366).
versus \( P_1 \) curves determined in 5\% and 10\% \( \text{D}_2\text{O} \), shown in Fig. 2 as \( -- \), were observed. Several different vesicle preparations were used to determine the sedimentation coefficients in pure \( \text{H}_2\text{O} \), and in 15\% \( \text{D}_2\text{O} \) (Fig. 2). Although the slopes of the two regression lines obtained under these conditions are parallel to each other, they are not parallel to those obtained in 5\% and 10\% \( \text{D}_2\text{O} \). In addition, it is apparent that the data scatter is less when a single phosphatidylcholine vesicle preparation was used. Sedimentation coefficients for vesicles suspended in 30\% \( \text{D}_2\text{O} \) were determined with a single vesicle preparation.

As is evident from Fig. 2, the slopes of the \( s \) versus \( P_1 \) plot vary somewhat with different vesicle preparations, although there is a linear relationship between \( s \) and \( P_1 \) for all preparations. Since it is the \( s \) values that are used in the calculation of \( \bar{s} \), the slope of the \( s \) versus \( P_1 \) plot is not important provided each preparation gives the same value of \( s \) under identical conditions. In order to establish that phosphatidylcholine vesicles obtained from a different preparation show the same \( s \) value, sedimentation velocity experiments with a second vesicle preparation were performed in the 5\% \( \text{D}_2\text{O} \) medium. These experiments are shown in Fig. 2 as \( \cdots \cdots \). The results indicate that although the slope of the \( s \) versus \( P_1 \) line differs from that of the previous experiment, the value of \( s \) for the two preparations are identical within the limit of experimental error.

Fig. 3 shows the viscosity-corrected sedimentation coefficient, \( \eta_s \), of phosphatidylcholine vesicles versus density of the suspending medium, the \( \text{D}_2\text{O}-\text{H}_2\text{O} \) mixture. The least squares method was used to calculate the linear regression line for the plot. A correlation coefficient of 0.9992 was found, which is indicative of a nearly perfect conformity of the experimental data to a straight line. The value of \( \bar{s} \), the density of \( \text{D}_2\text{O}-\text{H}_2\text{O} \) medium corresponding to zero redistribution of phosphatidylcholine vesicles, can readily be obtained from the point where the least squares line intersects with the density axis. The availability of experimental data both above and below the point of intersection yields the value of \( \bar{s} \), accurate to \( \pm 0.0004 \text{ g per ml} \). The partial specific volume of phosphatidylcholine, which is equal to the reciprocal of the value of \( \bar{s} \), was calculated to be \( 0.9814 \pm 0.0004 \text{ ml per g} \).

The results of the sedimentation studies on phosphatidylcholine vesicles suspended in various proportions of \( \text{D}_2\text{O}-\text{H}_2\text{O} \) mixture and the density and viscosity data of the suspending medium are summarized in Table I. Values of \( \bar{s} \text{,m} \), were calculated from the experimental data by the relation \( \bar{s} \text{,m} = \bar{s} \eta_s (1 - \beta \text{PH}_2\text{O})/(1 - \beta \text{PD}_2\text{O-H}_2\text{O}) \), where \( \beta \) is taken as \( 1/\rho \) obtained from Fig. 3.

Fig. 4 shows the relationship between the corrected sedimentation rate of phosphatidylcholine vesicles and the density of potassium chloride solution employed as the suspending medium. The linear regression line, shown in Fig. 4, with a correlation coefficient of 0.9998 was determined by the method of least squares. In this case the density corresponding to zero sedimentation rate is 1.0118 \( \pm 0.0002 \text{ g per ml} \), which is smaller

![Graph](http://www.jbc.org/)

**Fig. 4.** Plot of \( \bar{s} \text{,m} \) versus \( \rho \) for phosphatidylcholine vesicles suspended in potassium chloride and sodium chloride solutions of various densities. The straight line was calculated by the method of least squares to fit all the experimental data obtained in KCl solutions (\( \bullet \)) and the value of \( \rho \text{,c} \) was obtained from the point of intersection of the least square line with the density axis. Results of the sedimentation studies in NaCl solutions (\( \Delta \)) are closely similar to those obtained in KCl solutions.

**Table II**

Summary of sedimentation results obtained for phosphatidylcholine vesicles suspended in \( \text{H}_2\text{O} \) solutions containing various concentrations of either KCl or NaCl

<table>
<thead>
<tr>
<th>Physical parameter</th>
<th>Molarity of KCl*</th>
<th></th>
<th>Molarity of NaCl*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.000 M</td>
<td>0.010 M</td>
<td>0.050 M</td>
</tr>
<tr>
<td>( \eta_s )</td>
<td>1.0000 ± 0.0001</td>
<td>1.0000 ± 0.0001</td>
<td>0.983 ± 0.0006</td>
</tr>
<tr>
<td>( \rho ) (g/ml)</td>
<td>2.94 ± 0.06</td>
<td>1.84 ± 0.04</td>
<td>2.24 ± 0.04</td>
</tr>
<tr>
<td>( \bar{s} \text{,m} )</td>
<td>0.08 ± 0.06</td>
<td>0.000000</td>
<td>0.000000</td>
</tr>
<tr>
<td>( \beta )</td>
<td>2.94 ± 0.06</td>
<td>2.99 ± 2.39</td>
<td>2.77 ± 2.82</td>
</tr>
</tbody>
</table>

* The salt solutions were adjusted to pH 8.0 by addition of 0.01 M Tris-HCl.
Comparison of values of specific volumes of phosphatidylcholine vesicles obtained by different methods at 20°C.

<table>
<thead>
<tr>
<th>Solvent</th>
<th>(\bar{V})</th>
<th>(V)</th>
<th>Method</th>
</tr>
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<tbody>
<tr>
<td>D2O-H2O</td>
<td>0.9814 ± 0.0004</td>
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<td></td>
</tr>
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<td>NaCl-H2O</td>
<td>0.986 ± 0.0003</td>
<td>Pyenometer (1)</td>
<td></td>
</tr>
<tr>
<td>NaCl-H2O</td>
<td>0.9833</td>
<td>Method not specified (12)</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) The solvent contained 0.01 \(M\) of Tris-HCl buffer (pH 8.0).
\(^b\) The solvent contained 0.01 \(M\) of Tris-HCl buffer (pH 8.5).
\(^c\) Aqueous solvent of unspecified composition.

The results of the sedimentation studies on phosphatidylcholine vesicles suspended in sodium chloride solutions of various densities are also presented in Fig. 4 and Table II. In this case, the linear regression line of the \(s_v^3\) versus \(p\) plot shows a slope of \(0.0072\) g per ml. The sedimentation results and their related density and viscosity data are summarized in Table II. Values of \(s_v^3\) and \(p\) presented in Table II were calculated according to the equation

\[
s_v^3 = s_v^3(1 - f'\rho_{DzO})/(1 - f'\rho_{NaCl-H2O}),
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where \(f'\) is the reciprocal of \(\rho\). (12)

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A comparison of the specific volumes of phosphatidylcholine vesicles obtained by the sedimentation method with those obtained by other methods is made in Table III. A value of \(\eta\) reported by Saunders, Perrin, and Gammack (13) is also included for comparison. The \(\eta\) values obtained by sedimentation exceed that obtained in D2O-H2O media by 0.0072 g per ml. The reciprocal of the density gives an effective specific volume of 1.0115 ± 0.0006 g per ml as the density, \(p\), at which zero sedimentation occurs. The effective specific volume, \(\phi'\), can thus be calculated from the reciprocal of \(\rho\) as 0.9886 ± 0.0006 ml per g, which is essentially identical, within the limit of experimental error, to the value obtained in potassium chloride solution.

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**DISCUSSION**

For a two-component system, the behavior of a hydrated, sedimenting vesicle component at infinite dilution in a medium (Component 1) of density, \(\rho\), and viscosity, \(\eta\), at constant temperature is described by

\[
(\rho + \rho_3) - (\rho + \rho_4)_s = f^2\eta\rho
\]

where \(m\) is the anhydrous mass of the vesicle, \(h\) the volume of the hydrated and enclosed medium with density, \(\rho_3\), and \(f'\) is a function of the size and shape of the solvated vesicle (12).

When the vesicles are sedimented in D2O-H2O medium, it seems likely that both the solvating medium and the medium enclosed within the vesicle have a density equal to that of the suspending medium, i.e. \(\rho_3 = \rho\), and since \(N_m = M\), where \(N\) is Avogadro's number and \(M\) is the molecular weight, Equation 1 reduces to Equation 2

\[
M(1 - \eta/\rho)N_f = \eta\rho\phi
\]

Dividing both sides of Equation 2 by the viscosity of water, \(\eta_w\), yields Equation 3

\[
M(1 - \eta/\rho)/N_f = \eta_w\rho\phi
\]

where \(\eta_w\) is the viscosity of D2O-H2O medium relative to that of water.

Equation 3 describes the sedimentation behavior of vesicles at infinite dilution in media of different densities, and provides the theoretical justification that the point of zero sedimentation corresponds to a D2O-H2O density (\(\rho\)) equal to the reciprocal of the partial specific volume.

Application of Equation 3 to the phosphatidylcholine vesicle depends on the plausible assumption that \(\rho_3 = \rho\). The linearity in the \(\eta \rho \phi\) versus \(\rho\) plot (Fig. 4) is a necessary, although not a sufficient, condition for this assumption to be valid. Moreover, the fact that the water permeability coefficients of the planar phosphatidylcholine bilayer membrane determined in H2O and D2O, respectively, are identical (14) indicates that the structure of the phosphatidylcholine bilayer is not perturbed by the presence of deuterium oxide. In addition, the self-diffusion coefficient of water molecules across the planar bilayer membrane is extremely fast (about 4 \(\mu\) per sec) (14); hence, the complete equilibration of different forms of water molecules between the inner volume of the vesicles and the suspending medium must have been reached within seconds. This further suggests that the D2O-H2O medium enclosed within the interior of the phosphatidylcholine vesicle can be regarded as a one-component medium with a density equal to the bulk suspending medium.

In a three-component system comprised of water (Component 1), phosphatidylcholine vesicles (Component 2), and monovalent salt (Component 3), the sedimentation velocity of vesicles at infinite dilution depends on the partial specific volume of the vesicle and on the preferential interaction with solvent components. For such a system we may write (15)

\[
\eta \rho \phi = (1 - \phi')M/N_f s
\]

where \(\rho\) is the density of the solvent mixture (Components 1 and 3) in the absence of Component 2, and \(\eta\) is the viscosity of the solvent mixture relative to that of water. Equation 4 is similar to Equation 3, except that the term \(\phi'\) used in two-component system is replaced by \(\phi\), an effective specific volume, which is related to the true partial specific volume of Component 2 and the influence of preferential interaction with solvent Components 1 and 3 (expressed in terms of \(\xi_1\) and \(\xi_2\), respectively) by the following Equation (16, 17)

\[
\phi' = \phi + \xi(\phi' - \phi) = \phi_1 + \xi_1(\phi_1 - \phi)
\]

where the interaction coefficient, \(\xi\), is the weight of a solvent component in grams to be removed (Component 3) or added (Component 1) per g of Component 2, and \(\phi_1, \phi_2, \phi_3, \xi_1, \xi_2, \xi_3, \xi_4\) are the partial specific volume of components 1, 2, and 3, respectively.

At the condition of \(\eta \rho \phi = 0\) and assuming that the partial specific volume of the vesicles is unaffected by the presence of

**Table III**

Comparison of values of specific volumes of phosphatidylcholine vesicles obtained by different methods at 20°C.

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\(^a\) The solvent contained 0.01 \(M\) of Tris-HCl buffer (pH 8.0).
\(^b\) The solvent contained 0.01 \(M\) of Tris-HCl buffer (pH 8.5).
\(^c\) Aqueous solvent of unspecified composition.
Based on purely geometrical reasoning, a sedimenting vesicle by the concept of steric exclusion of the more bulky component above calculated result of 1.6 layers of water molecules around the polar head region of the same structure. Therefore, the structure and the polar head region (8). In addition, this near the interface between the nonpolar hydrocarbon portion of the polar head region of the enclosed bilayer structure is flexible enough to permit the rapid anisotropic motions of the label (8).

4 A, the outer and inner radius of the vesicle are 124 A and 84 A, respectively (1), and the molecular weight of the sedimenting vesicle is 2.13 x 10^6. The molecular weight is calculated from the Svedberg equation [M = (RT\omega,)/((1 - Q)D^0)] using values of B = 20.15 x 10^8 and D^0 = 1.87 x 10^-7 cm^2 sec^-1 determined in 0.1 M NaCl solution (Table II) and D^0 = 1.87 x 10^-7 cm^2 sec^-1 determined in 0.1 M NaCl solution (1). This value of vesicle weight (2.13 x 10^6) based on new values for \( f \) and \( \delta_{20,w} \) is slightly larger (only 3%) than that reported previously (1).

Electron paramagnetic resonance studies of the spin-labeled vesicles show that the polar head part of the enclosed phosphatidylcholine bilayer structure is not an impermeable region to small uncharged molecules because the maleimide spin labels can penetrate into this region to react with -SH groups located near the interface between the nonpolar hydrocarbon portion of the structure and the polar head region (8). In addition, this polar head region of the enclosed bilayer structure is flexible enough to permit the rapid anisotropic motions of the label (8). Hence, the water molecules may easily penetrate into this flexible polar head region of the same structure. Therefore, the above calculated result of 1.6 layers of water molecules around the surfaces of the vesicle to be considered as an upper limit.

The presence of preferential hydration for vesicles suspended in dilute KCI medium can, in part, be qualitatively interpreted by the concept of steric exclusion of the more bulky component of the solvent from the neighborhood of the macromolecule (19). Based on purely geometrical reasoning, a sedimenting vesicle would be expected to behave as if it bound the water molecules in preference to hydrated salt ions because the larger molecules, i.e., the hydrated salt ions, cannot come as close to the surface of the sedimenting vesicle as the smaller water molecules. The deficiency of hydrated salt ions in the immediate neighborhood of the sedimenting vesicle results in a decrease in the effective density of the vesicle in KCI suspending medium.

The determination of partial specific volume of macromolecules by sedimentation equilibrium method has been described by Edelstein and Schachman (11). The sedimentation velocity technique in D_2O-H_2O media reported in this communication is extremely useful for phospholipids, because it is accurate, convenient, reproducible, and requires only small amounts of materials. In principle, the sedimentation rate of proteins and enzymes can also be carried out in D_2O-H_2O medium, but, in terms of accuracy the sedimentation velocity technique cannot operationally rival the standard methods. This is due to the fact that the density of D_2O, 1.1054 g/ml at 20°, is not sufficiently dense enough to reduce the sedimentation rate of proteins, with an averaged density of about 1.35 g/ml to zero. Consequently, the value of \( \phi^s \) can be obtained only by a long extrapolation of the experimental data in a \( \eta_g \) versus \( p \) plot. Recently it was reported that deuterium oxide can cause alterations of the association-dissociation equilibrium of subunit proteins (20). Such alterations would further severely limit the application of the sedimentation technique in the determination of the partial specific volume of these proteins.

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