Solvation and the Determination of Molecular Weights by Means of the Svedberg Ultracentrifuge

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In a recent paper,[1] Professor McBain considers the error due to solvation in molecular weights determined by means of the ultracentrifuge and concludes that the error may amount to "hundreds of per cent." Although Professor McBain is not clear on this point, by emphasizing the dependence of sedimentation on a difference in the partial specific volumes of solvated and unsolvated molecules, he implies that solvation affects in a different manner molecular weights determined by means of the ultracentrifuge and by conventional methods such as osmotic pressure or change in freezing point, boiling point, or vapor pressure.

In contrast to this point of view, we shall show that the effect of solvation is the same for the ultracentrifuge as for conventional methods; that as a matter of fact, the error owing to solvation in binary systems is independent of the partial specific volumes of the components; and that in no case can the error amount to "hundreds of per cent." in a binary system for the reason that the apparent molecular weight, ignoring solvation, is always less than the true molecular weight of the unsolvated molecules, regardless of the values for the partial specific volumes. Furthermore, we shall show that whenever the molecular weight determinations are made in low concentrations, as is generally admitted to be necessary for valid results by any method, enormous solvations lead to a relatively small error. We therefore believe that, in practice, solvation very rarely, if ever, leads to any significant error in ultracentrifuge determinations of molecular weight on binary solutions.

The effect of solvation in a multicomponent system may be considerably more complicated than for a binary solution. Since Professor McBain discusses such systems also, without however presenting any mathematical treatment, we add a brief quantitative discussion of ternary systems in which combination occurs between the sedimenting component and one of the other two.

Solvation in Binary Systems.—Considering a binary solution, in which s grams of component 2

(1) J. W. McBain, THIS JOURNAL, **58**, 315 (1936).

(solvent) combines with one gram of component 1 (solute), the following symbols and definitions may be set up

x_1, x_2 = number of grams of components 1 and 2 in solution.

y_{12} = $(s + 1)x_1$ = grams of compound (solvate) in solution.

y_2 = $x_2 - sx_1$ = grams of free solvent.

v = volume of solution; V = specific volume of solution = $v/(x_1 + x_2)$.

$\overline{V}_1$, $\overline{V}_2$ and $\overline{V}_{12}$ = partial specific volumes of solute, solvent and solvate respectively.

Remembering that $v = f(y_{12}, y_2)$, one obtains by partial differentiation

$$\frac{\delta v}{\delta x_1} = \overline{V}_1 = \frac{\delta v}{\delta y_{12}}(s + 1) - \frac{\delta v}{\delta y_2} s \qquad (1)$$

and

$$\frac{\delta v}{\delta x_2} = \overline{V}_2 = \frac{\delta v}{\delta y_2}$$

Combining these, and replacing the differentials by the corresponding specific volume symbols, one finds that

$$(s + 1)\overline{V}_{12} = s\overline{V}_2 + \overline{V}_1 \qquad (2)$$

That is to say, the partial specific volume of the solvate can be calculated only if the degree of solvation s is known.

Turning now to the question of molecular weights, it is apparent that

$$M_{12} = (s + 1)M_1 \qquad (3)$$

where M_1 is the molecular weight of the unsolvated solute and M_{12} is the molecular weight of the solvated solute. Since in calculating molecular weight by the usual equation for sedimentation equilibrium, the partial specific volume of the unsolvated solute is used, an "apparent molecular weight" M' is obtained, which is related to M_{12} and the partial specific volumes by the equation

$$M_{12}(1 - \overline{V}_{12}/V) = M'(1 - \overline{V}_1/V) \qquad (4)$$

Combining equation 4 with equations 2 and 3, and eliminating M_{12} and $\overline{V}_{12}$, we get

$$M_1 = M' \frac{(1 - \overline{V}_1/V)}{(1 - \overline{V}_1/V) + s(1 - \overline{V}_2/V)} \qquad (5)$$

Introduction of the definitions for $\overline{V}_1$ and $\overline{V}_2$ ex-

pressed in terms of weight fractions, c_1 and c_2, for solute and solvent,[2] namely

$$\bar{V}_1 = V + c_2 dV/dc_1 \tag{6}$$
$$\bar{V}_2 = V - c_1 dV/dc_1$$

finally gives

$$M_1 = M' \frac{1 - c_1}{1 - c_1 - sc_1} \tag{7}$$

It may readily be shown that the same equation describes the effect of solvation on molecular weights by osmotic pressure and other conventional methods. This of course is to be expected in view of the thermodynamic equivalence of osmotic pressure and sedimentation equilibrium.[3]

From eq. 7 it is evident that the error due to solvation is independent of the partial specific volumes of the components for the ultracentrifuge method, as well as for osmotic pressure and related methods. Furthermore, inasmuch as the apparent molecular weight M' is always smaller than the true molecular weight M_1 of the unsolvated solute, the error can never amount to "hundreds of per cent." In fact, since low concentrations should always be used for molecular weight determinations by means of the colligative properties of solutions, quite enormous degrees of solvation have an insignificant effect on molecular weight. For example, from eq. 7, if c_1 is about 0.001, as it commonly has been in our ultracentrifuge work, the solvation would have to be about 990% to make an error of 1% in the molecular weight. We therefore believe that solvation needs never lead to any serious error in ultracentrifuge determinations of molecular weight for binary solutions.

Three–Component Systems with Solvation or Compound Formation.—Three–component systems containing but one sedimenting component and with no combination between the components may be considered as a binary system without solvation. If the sedimenting component combines with the other two components in the same proportions as they exist in the solution, the solution is equivalent to a binary solution with solvation, as treated above. If, however, combination occurs between the sedimenting component and one of the other components, or if the sedimenting component combines with both of the other components in proportions different from those in which they exist in the solution, a new case arises.

(2) Lewis and Randall, "Thermodynamics," McGraw-Hill Book Co., New York City, 1923, p. 38.
(3) A. Tiselius, *Z. physik. Chem.*, **124**, 449 (1926).

Calling the sedimenting or colloidal solute component 1, and considering the case where r grams of component 2 combines with one gram of component 1, we may set up the following definitions

$$y_{12} = (r + 1)x_1$$
$$y_2 = x_2 - rx_1$$
$$y_3 = x_3$$

where y_{12}, y_2, and y_3 are the grams of compound, free component 2, and component 3 in the solution.

By operations entirely similar to those used in the discussion of binary solutions, we obtain an equation which is analogous to eq. 2; namely

$$(r + 1)\bar{V}_{12} = r\bar{V}_2 + \bar{V}_1 \tag{8}$$

and an equation which is analogous to eq. 5, namely

$$M_1 = M' \frac{(1 - \bar{V}_1/V)}{(1 - \bar{V}_1/V) + r(1 - \bar{V}_2/V)} \tag{9}$$

In other words, the true molecular weight of component 1 in its uncombined state can be determined only if the component with which the colloidal solute combines, the composition of the compound, and the partial specific volumes of both components of the compound, are known. It may be noted that for this case, as well as for the binary solution, the error in molecular weight is not dependent on the partial specific volume of the compound formed by the colloidal solute.

The partial specific volumes in question are defined by a set of equations analogous to those of eq. 6 and similarly derivable

$$\bar{V}_1 = V + (1 - c_1)\frac{\delta V}{\delta c_1} - c_2 \frac{\delta V}{\delta c_2}$$
$$\bar{V}_2 = V - c_1\frac{\delta V}{\delta c_1} + (1 - c_2)\frac{\delta V}{\delta c_2} \tag{10}$$
$$\bar{V}_3 = V - c_1 \frac{\delta V}{\delta c_1} - c_2 \frac{\delta V}{\delta c_2}$$

where the partial derivative with respect to c_1 is taken with c_2 constant, and *vice versa*. Since $c_1 + c_2 + c_3 = 1$, changes in c_3 may be ignored. By substituting the values for $\bar{V}_1$ and $\bar{V}_2$ as given by eq. 10 into eq. 9, one may calculate the error corresponding to various values of r.

Whereas for the binary case, as Professor McBain points out, the partial specific volumes of the colloidal component and its compound with the solvent must necessarily differ if sedimentation is to occur, the same is not in general true for multicomponent systems. Specifically, in a ternary system, it is possible for the colloidal solute and its compound with one of the components to have

the same partial specific volume without eliminating the difference between $\overline{V}_{12}$ and V, which is the condition for sedimentation to occur. In such a case, as shown by eq. 8, $\overline{V}_1 = \overline{V}_2 = \overline{V}_{12}$, and as shown by eq. 9, the molecular weight calculated from ultracentrifugal data is then the true molecular weight of the *compound*. The relationship, under these conditions, between $\overline{V}_{12}$ and V may be shown from eq. 10 to be

$$\overline{V}_{12} = V + c_3(\delta V/\delta c_1) \qquad (11)$$

The question regarding the formation of a compound between the colloidal solute and another component of a solution has concerned us a good deal in connection with our ultracentrifugal studies of cellulose in cuprammonium.[4] Although these solutions contain at least four components, we have assumed that they could be considered as ternary systems with combination occurring between the cellulose and the copper. We know of no entirely satisfactory way of determining directly the composition of the compound or its partial specific volume, so our conclusions concerning the molecular weight of cellulose in cuprammonium involve other considerations. As pointed out above, however, if the partial specific volumes of cellulose and of the compound with copper happen to be the same, a true molecular weight of the compound is obtained. We attempted to give general expression to this thought in our previously cited paper, but unfortunately we did not limit our remark to ternary or more complicated systems, and we are in complete agreement with Professor McBain's criticism of our statement, in so far as binary systems are concerned.

From Professor McBain's remarks concerning "bound water" and its determination in ternary systems, it might be thought that his method offers a direct way of measuring at least the partial specific volume of the cellulose–copper compound. We fear, however, that such is not the case. The partial specific volume of the cellulose is considerably less than the specific volume of the solution. As a result, very considerable

(4) E. O. Kraemer and W. D. Lansing, *J. Phys. Chem.*, **39**, 165 (1935).

amounts of an additional substance with a definitely lower partial specific volume than the cellulose would have to be added to the solution in order to reduce the specific volume of the solution to equality with that of the cellulose–copper compound and thus to prevent sedimentation. Whether the elimination of sedimentation could actually be accomplished in this way we do not know, but if it could be done, we feel that there would in general be very little chance that the partial specific volume of the colloidal solute would remain unchanged, especially if the colloidal component formed a compound with some other component of the system.

However, whenever the partial specific volume of the compound containing the colloidal solute can be determined by Professor McBain's method, one may replace $\overline{V}_{12}$ of eq. 8 with the value of V, substitute the values of $\overline{V}_1$ and $\overline{V}_2$ as given by eq. 10, and calculate r. This assumes, of course, that $\overline{V}_1$, $\overline{V}_2$ and $\overline{V}_{12}$ are not changed by the additional component required to bring V and $\overline{V}_{12}$ into equality, that one knows which component combines with the colloidal solute, and that $\overline{V}_3$ and c_3 refer to the mixture of all components other than the colloidal solute and the component with which it combines.

Summary

For binary solutions the change in partial specific volumes resulting from solvation or compound formation does not affect molecular weights determined by the sedimentation equilibrium method in any way different from that for conventional methods, in spite of the fact that sedimentation equilibrium is dependent on differences in partial specific volumes. Under normal conditions, with a binary solution, solvation is probably rarely if ever great enough to lead to an appreciable error in molecular weights. For ternary and higher order systems, also, the error in molecular weight by the sedimentation equilibrium method may be evaluated quantitatively without considering the partial specific volume of solvates or compounds formed by sedimenting component.