

## On Osmotic Pressure of Macromolecules

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Elementary considerations show that the osmotic pressure of a solution containing non permeating molecules in a solvent consisting of permeating molecules can be calculated *as if* the former constituted a gas at the same temperature and concentration, *provided* that the form and magnitude of and the forces between them be maintained. The abridged van der Waals equation  $p(V-B) = RT$  is derived by introducing in the equation  $V dp = n d\mu$  (J. W. Gibbs, 1897) local concentrations and local activity-coefficients.

It is shown anew that if  $2b$  be the unavailable volume for a pair of congruent hard molecules and  $N$  be the number of molecules in the volume  $V$ , the available volume is  $V - Nb$  as shown by van der Waals 1899. A short history of this statement is given. It is emphasized that two different definitions of the unavailable volume  $2b$  of the pair exist. In the case of hard, spherical molecules they yield the same result,  $2b = 8v_m$ , where  $v_m$  is the volume of one sphere. In all other cases they yield different results. The two definitions are:

I.  $2b$  is the volume inside which the centre of one molecule must be if it be required that the one has points in common with the other one, supposed fixed.

II.  $2b$  is the volume inside which the center of one molecule can not come without expenditure of work by the surroundings, the other molecule supposed fixed.

Def. I is used by B. H. Zimm and A. Isihara. Def. II is a consequence of the present author's derivation of the abridged van der Waals equation.

It is shown that for long rods Def. II agrees with experiments on long chain-molecules. The condition for this agreement is  $b\rho \geq 8$ , where  $b$  is in  $\text{cm}^3/\text{g}$  and  $\rho$  is the density in  $\text{g}/\text{cm}^3$  of the solid solute or is the reciprocal of its partial specific volume in the solution.

The discussion of experimental values yields suggestions regarding the constitution of some chainlike macromolecules. The suggestions seem to be confirmed by knowledge from other sources.

Two corrections mentioned in a paper by the author and the late Dr. C. E. Jensen are withdrawn. The first one relates to the difference in density between the inner and the outer liquid, the other one to the difference in surface-tension between the same two liquids.

Both corrections relate to measurements with the "inverted osmometer". A third correction, subtraction of one third of the diameter of the capillary from the height measured, is introduced.

## PART I. THEORY

In the following we shall understand by the osmotic pressure (O.P.) of a solution of macromolecules that measured by means of a membrane which is impermeable and only impermeable to macromolecules, say molecules of molecular weights from some ten thousand and upwards.

It seems to be desirable to survey the basic ideas underlying our present knowledge of O.P. because in the rapidly expanding literature more or less controversial interpretations of the same phenomena have turned up so that the reader sometimes finds himself caught in a maze. The following is an attempt to illustrate different aspects of O.P. Not all of the author's views agree with views expressed by recent authors and his sincere hope is that this presentation should induce readers to meditate themselves on the problems involved.

The thermodynamic definition of osmotic pressure  
and its realisation by experiments

Fortunately, there are no controversies regarding the thermodynamic definition of O.P. It may be expressed as follows: The osmotic pressure is that excess pressure which must be exerted on the inner liquid, the solution, in an osmometer to prevent flow of the outer liquid, containing only permeating solvent molecules, through the membrane. This is the same as to say, that at equilibrium the chemical potentials of the permeating components of the inner liquid must be equal on both sides of the membrane.

The aim of all direct methods of determination of the O.P. is to realize this definition by experiment. As one example the reader may be referred to the method described by Christiansen and Jensen<sup>1</sup> in which the buoyancy of an air column in a vertical capillary produces the excess pressure on the inner liquid. If that pressure does not equal the O.P. there will be a flow of liquid through the membrane. The rate of flow (positive outwards, negative inwards) is measured by means of a horizontal microscope provided with an ocular micrometer scale. Experiment shows, that it depends linearly on the height of the air column, the resistance of the membrane against flow of the permeating liquid thus being independent of the direction of movement.

In exceptional cases the resistance proved to be greater for outward motions than for inward ones. Such experiments were discarded. In most cases the effect could be eliminated by removal and rinsing of the membrane. It is therefore probable that this valve effect is due to a layer of macromolecules, partly blocking the outward, but not the inward flow.

By means of a graph it is easy to find by interpolation the length of the column which corresponds to zero flow and from this by calculation the O.P.

Even at the largest rates of flow the linear velocity of the permeating liquid through the membrane (a collodion membrane) is extremely small, so small that, if the outer liquid be a mixture, diffusion-equilibrium is extremely nearly maintained in the displaced elements of the permeating liquid.

This follows from the following rough estimate: An exceptionally high rate of movement of the meniscus in the capillary is 20 divisions in the ocular micrometer in 10 min, which corresponds to 0.4 mm<sup>3</sup>/10 min. The surface of the membrane is about 200 mm<sup>2</sup> and consequently the linear velocity across the membrane is about 0.002 mm/10 min. As now diffusion coefficients in gels like the water containing collodion membranes used are known not to be much smaller than those in water, we arrive at the result mentioned.

The permeation is therefore very nearly reversible. From this follows, that the liquid which permeates the membrane from the outside must have very nearly the same composition as that which permeates from the inside. In other words, the solvent, the permeating part, of the inner liquid is (very nearly) identical in composition with that of the outer liquid, or we may say that the outer liquid is the solvent of the inner liquid even if the former is a mixture.

#### Calculation of the osmotic pressure

The thermodynamic definition does not tell us whether or how the O.P. may be calculated from the concentration of the (non permeating) solute. Let us therefore consider the equilibrium situation more closely. It is a fact that at equilibrium there is a pressure-difference between the inner and the outer liquid. It is also a fact, that at equilibrium there is no flow of solvent through the membrane. Furthermore the (permeating) solvent can only contribute to the pressure difference by flowing through the membrane. Consequently its contribution to the pressure difference must be zero at equilibrium. Therefore we should get the same pressure difference if we removed the solvent from both sides of the membrane, *provided* that we maintain not only the temperature and the concentration of the solute molecules, but also their form and magnitude and the forces between them. In other words we may calculate the excess pressure at equilibrium, the osmotic pressure, as if it were the pressure of a gas with the prescribed properties.

From this follows immediately, that the limiting law of van't Hoff must tend to be valid at such dilutions where the effects of the finite volume of the solute molecules and of the forces between them have vanished. Whether we may say, that the pressure difference at equilibrium be "due to" the bombardment of the (non permeating) solute molecules against the semipermeable membrane remains a matter of taste, the essential thing being that it can be calculated as described.

To treat the problem in more detail we shall use a method proposed by Gibbs<sup>2</sup>:

At constant temperature we have generally for a solution containing  $k + 1$  components

$$Vdp = n d\mu + \sum n_i d\mu_i \quad (i = 1, 2, \dots, k) \quad (1)$$

where  $V$  is the volume, and  $n$  and  $\mu$  are, respectively, the number of moles and the chemical potential per mole of the non permeating solute and  $n_i$  and  $\mu_i$  the corresponding quantities for the  $k$  permeating molecules. If now this solution is contained in an osmometer surrounded by the semipermeable

membrane and is in equilibrium with an outer liquid containing only the solvent substances at constant temperature, pressure and composition all  $d\mu_i$ 's are zero and we get

$$Vdp = nd\mu \quad (2)$$

exactly the same equation which is valid for a one component system. It is eqn (2) which we owe to Gibbs. The responsibility for the following must remain with the present author.

Let us now assume as usual that

$$d\mu = RT \, d \ln (cf) \quad (3)$$

where  $c$  is the concentration of the solute and  $f$  its activity coefficient. Now, if  $c$  means the overall concentration it is evident that

$$dn/n = dc/c \quad (4)$$

But (4) is also true if  $c$  means a local concentration, which may be different in different volume elements. This follows from the consideration that a small relative increase in the number of molecules of solute in the volume  $V$  cannot alter their distribution on the different volume elements.

It should be added that if we let  $c$  mean the local concentration it must be understood as the number of centers of gravity of solute molecules in a certain volume-element divided by the volume of that element.

Let us now assume that the solution is so dilute that  $f$  equals the same constant everywhere in the solution. Without loss of generality we may put this constant equal to one. Combining (2), (3) and (4) we then get by integration

$$Vp = RTn \quad (5)$$

because  $V$  at the small pressures in question is certainly independent of  $p$ . We thus arrive at van't Hoff's limiting law, or the gas-law. But evidently this is no proof of that law, because (3) has been chosen so as to lead in the limit to (5). The real proof of (5) rests of course on the validity of Maxwell's law of equipartition.

Let us now proceed one step further. Using again (3) and (4) we get by integration of (2)

$$Vp = RTn + RT \int n \, d \ln f \quad (6)$$

$f$  is assumed to be defined by

$$f = \exp(g/RT) \quad (7)$$

where  $g$  is a molar potential energy depending on the space coordinates of the center of gravity of a solute molecule. In accordance with the Boltzmann principle we shall assume that the activity  $a$  of the solute is constant everywhere inside the osmometer, *i.e.*

$$a = cf \quad (a \text{ constant}) \quad (8)$$

We shall only treat the case in which the solute molecules at a distance do not exert forces on each other, but that to force them nearer to each other, when they are already in contact, requires expenditure by the surroundings of an amount of work (per mole) which is several times as large as  $RT$  even for very small displacements. In other words, we consider the molecules to be hard bodies having well defined finite volumes. In that case  $g$  is either zero (when the molecules are out of contact) or  $g/RT$  is so large that  $f$  to all intents and purposes can be considered as infinitely large, the interval between these two cases being so small that it can be neglected. Therefore we have everywhere inside the osmometer

$$c \ln f = a \ln f/f = 0 \quad (9)$$

because if one factor is different from zero the other one is zero. Therefore

$$c \, d \ln f + \ln f \, dc = d(c \ln f) = 0 \quad (10)$$

We have seen before that  $dc/c = dn/n$  so that (10) can also be written

$$n \, d \ln f + \ln f \, dn = 0 \quad (11)$$

As now  $n = \int c \, dV$ , (6) can be rewritten

$$\int (p/RT - c(1 - \ln f)) \, dV = 0 \quad (12)$$

If in (8)  $f$  equals one we have  $a = c'$ , where  $c'$  is the concentration in volume elements where the solute molecules are "free". Therefore

$$\int (p/RT - c'(1 - \ln f)/f) \, dV = 0 \quad (13)$$

Now for those parts of the volume, where  $\ln f$  is different from zero we get

$$c' \int ((1 - \ln f)/f) \, dV = 0 \quad (14)$$

For those parts of the volume, where the centers of the solute molecules are "free", *i.e.* where  $f$  equals one

$$\int (p/RT - c') \, dV = 0 \quad (15)$$

which means that  $p/RT = c'$ .

Now

$$n = \int c \, dV = c' \int dV/f \quad (16)$$

The contributions to the latter integral evidently comes only from those volume-elements in which  $f$  equals one. Denoting that part of the volume, where  $f$  is different from one (the unavailable volume) by  $B$ , we evidently get

$$c' = n/(V-B) \quad (17)$$

or

$$p(V-B) = nRT \quad (18)$$

That this equation agrees exactly in form with van der Waals's equation for the case where intermolecular forces are absent is no wonder as it has been derived from exactly the same assumptions as the latter.

### The unavailable volume

As emphasized by all authors on the subject there are two kinds of unavailable volumes. The first kind is due to the fact, that the centers of the solute molecules cannot approach the inner surfaces of the osmometer beyond a certain distance. The second kind arises from the circumstance that the centers of two solute molecules cannot approach each other beyond a certain distance. A rough estimate shows at once, that if the osmometer be not microscopically small we may completely neglect the first kind. We shall now try to survey the situation concerning the unavailable volume of the second kind.

*Statistics.* Let us consider at first one pair of molecules identical in magnitude and shape, and let it be assumed that we have somehow succeeded in determining the unavailable volume  $2b$  for the pair. Let us further assume that the volume  $V$  contains  $N$  single molecules of the solute. If we number these and take out, *e.g.*, number one it is evident that we may combine this with the  $N-1$  others to  $N-1$  pairs with the total unavailable volume  $2b(N-1)$ . However, as all the  $N-1$  pairs contain molecule number one, only half of this volume belongs to the  $N-1$  second partners, those with the numbers 2,3,... $N$ . Consequently we must ascribe to each of these partners the part  $b$  of the unavailable volume. If furthermore we ascribe the same part  $b$  to molecule number one, this paired with any one of the other molecules will form a pair with the unavailable volume  $2b$  as it should. It follows from this argument that the space available to the center of molecule number one or to any other of the  $N$  molecules is  $V-(N-1)b-b = V-Nb$ . Thus  $Nb$  equals  $B$ , the unavailable volume introduced before.  $B$  evidently means the same whether we let  $b$  mean the unavailable volume for one molecule and  $N$  the number of molecules or we let  $b$  mean the unavailable volume for one mole and replace  $N$  by  $n$ , meaning the number of moles. Using the latter convention (18) can be rewritten in the form

$$1-bc = RTc/p \quad (19)$$

where  $c = n/V$  is the overall molar concentration of solute molecules. We may also let  $c$  mean the concentration in g/litre in which case (19) becomes

$$M(1-bc)/RT = c/p \quad (20)$$

where  $M$  is the molecular weight of the solute. In that case  $b$  becomes the unavailable volume of the solute in litre/g.

The expression  $V-Nb$  for the available volume has a rather complicated history. J. D. van der Waals has it in the second edition of his book: *Die Continuität des Gasförmigen und Flüssigen Zustandes* (1899) I, p. 45. His argument, in which he corrects an error committed by R. Clausius, is different from that given above.

L. Boltzmann in his: *Vorlesungen über Gastheorie*, 1st Ed. 1898, reprinted 1912, part II, p. 7, has (our notation)  $V - 2(N-1)b$  for the same quantity. In his argument, which is similar to ours, he counts, however, the contribution to the unavailable volume from what we have denoted as molecule number one  $N-1$  times in stead of only once. Then in his last work (with J. Nabl) in *Encyclopädie der Mathematischen Wissenschaften V* (1907) p. 551, Boltzmann quotes, without remarks, from the above mentioned book of van der Waals the latter's value  $V - Nb$ . It seems evident, therefore, that Boltzmann accepted this value before he died.

Finally, in his *Dynamical Theory of Gases*, 2nd Ed. (1916) p. 138, J. H. Jeans quotes Boltzmann's value from 1898,  $V - 2(N-1)b$  without mentioning the error.

We shall therefore stick to the value for the unavailable volume  $B = Nb$  and not use  $B = 2(N-1)b$ . It must be added that the expression "an unavailable volume of the second kind" has only a meaning if  $N$  is at least two.

*Geometry.* The magnitude of the unavailable volume, depends on the geometrical form of the solute molecules. Van der Waals and his first successors calculated  $2b$ , the unavailable volume for a pair of molecules, only for the case of hard spherical bodies which is by far the easiest one to treat. It is easy to see that the volume around a sphere A inside which the centre of another sphere B cannot come without the expenditure of work is a sphere whose radius is  $d$ , where  $d$  is the diameter of one of the spheres. In other words, the unavailable volume  $2b$  for the pair is eight times  $v_m$  where  $v_m$  is the volume of one of the molecules forming the pair if they are both spherical. For the following it is of interest to note that in this case and only in this case two different definitions of the unavailable volume  $2b$  lead to the same result, *viz.*

Def. I:  $2b$  is the volume inside which the centre of molecule B must be if molecule A be fixed and it be required, that they shall have points in common.

Def. II:  $2b$  is the volume inside which the centre of one molecule cannot come if the other one be fixed, unless work is performed by the surroundings.

For non spherical molecules definitions I and II lead to different results, the more so the more the form of the molecules deviate from a sphere. Def. II evidently is the one required by our derivation of the abridged van der Waals equation.

As examples of approximately spherical molecules certain globular macromolecules, *e.g.* certain proteins might be mentioned, but we shall focus our attention on a quite different type, molecules of the pearl-string type. Most or at least many of them are not at all stiff. If they were they might reasonably be considered as cylindrical rods, a case which seems next to the sphere in simplicity. However they must often be expected to perform not only intermolecular Brownian motions but also intramolecular motions of a similar irregular kind, resulting in incessant changes in "curliness" and end to end length. To take this accurately into account in an attempt to calculate the unavailable volume for a pair of such molecules seems nearly hopeless. The only problem one may reasonably expect to be able to solve is the tremendously simplified problem of determining  $2b$  for a pair of stiff circular cylinders with given height and length. Isihara<sup>3</sup> has suggested that for the solution of the corresponding problem for flexible molecules one might use information concerning average dimensions of such molecules obtained by means of light-scattering experiments. To the present author this seems doubtful because it seems to him that the lengths measured by optical methods may be quite

different from those which are decisive for the magnitude of the unavailable volumes. Actually, just because of the flexibility of the pearl-string model one would expect the decisive length for this to be the length of the string, while the average lengths measured by optical methods may be much shorter. Anyhow, we may not commit gross errors by replacing the pearl string by the same in its stretched form and then replace this by a cylinder of the appropriate dimensions, diameter  $d$  and height  $h$ .

#### The unavailable volume for a pair of solid cylinders

B. H. Zimm<sup>4</sup> and A. Isihara<sup>5,6</sup> have solved, the former approximately, the latter exactly, the following problem: Given two congruent cylinders, A and B, A being fixed in space and B movable. Find the volume inside which B must move if it be required that the common volume of A and B does not vanish. Zimm and Isihara consider the volume so defined to be the unavailable volume  $2b$  for a pair of cylinders, in other words they use Def. I. Isihara's result<sup>3,6</sup> is

$$2b/v_m = \pi + 3 + \pi d/2h + 2h/d \quad (21)$$

Now  $b$  can be determined by measurements of the osmotic pressure  $p$  at several concentrations  $c$ . We then plot  $c/p$  against  $c$ . According to (20) the graph obtained should be a straight line and seems to be so when only reliable experiments are taken into account. For details of the experiments and evaluation of the sources of error in osmotic experiments the reader is referred to Part II of this paper. From the intercept of the line with the axis of ordinates we may calculate the molecular weight and from its slope we get  $b$  in  $\text{cm}^3/\text{g}$  if  $c$  is in  $\text{g}/\text{cm}^3$ . Furthermore  $v_m$  might be taken as the partial specific volume in  $\text{cm}^3/\text{g}$  in the solvent in question or failing that, as the reciprocal density ( $\rho$ ) of the solid substance. However, measured in this way  $v_m$  may very probably be low because evidently the solute molecule may be covered with a sheath of solvent or it may be wound up in a "helix" whose effective volume at collisions may be several times larger than that of the densely packed molecules. Values of  $b\rho$  therefore represent an upper limit to the value of  $b/v_m$ . The former values usually lie in the range from about ten to less than one hundred. On the other hand the number of "pearls" on the "string" can be estimated from our knowledge of the chemical composition of the substance in question and its molecular weight. This number cannot be very different from the ratio  $h/d$ . A low estimate of this ratio is 100. From (21) it is seen that for this value of  $h/d$ ,  $b/v_m$  becomes 100, and by actual calculations in the particular examples it is found to be much greater. This disagreement between values of  $b\rho$  determined experimentally and  $b/v_m$  predicted by the calculation of Isihara<sup>5,6</sup> is so large, that serious doubt arises whether the definition (I) of the unavailable volume be the right one. What can be said at the present stage is that it is certainly different from Def. II adopted by the present author. This definition may be repeated here as follows: Let cylinder A be fixed and B be movable. The volume unavailable to B is then that volume inside which the center of B cannot be brought unless work is performed on the system.



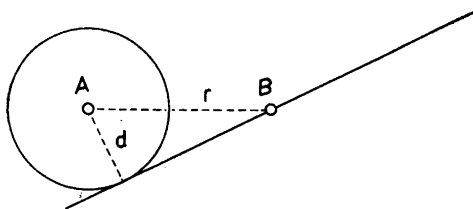


Fig. 1.

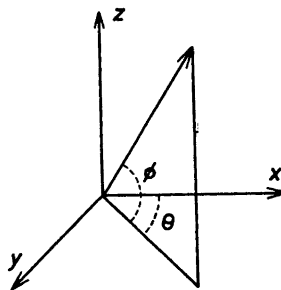


Fig. 2.

To illustrate the difference between the two definitions let us proceed in the following manner: We replace cylinder A by one of the same height but with diameter  $2d$  and replace simultaneously B by its axis of length  $h$ . Furthermore we consider the particular case that the axes of A and B are perpendicular to each other. The projection on a plane perpendicular to the axis of A is shown in Fig. 1. In this plane we may evidently speak of an unavailable area instead of an unavailable volume. Now according to Def. I the middle point of B certainly belongs to the unavailable area if the situation is as illustrated. According to Def. II, however, the same point does not belong to the unavailable area because we may shorten the radius vector  $r$  from the center of A to the middle point of B without performing work. Of course the line B will then slide or roll on the circle A but this does not require work as no forces resist a motion of that kind. But the radius vector cannot be decreased to a value less than  $d$ , the radius of the circle, unless work is performed on the system. Therefore, according to Def. II, the area unavailable to the centre of B is the area of circle A, which is certainly less than the unavailable area defined according to Def. I.

In this argument we have neglected the fact that both A and B are endowed with kinetic energy, translatory and rotatory. Strictly speaking we should therefore consider the probability of finding the two centers in between distances  $r$  and  $r + dr$  from each other. This probability depends partly on the kinetic and partly on the potential energy, both corresponding to the distance  $r$ . We know, however, from general theory that the average kinetic energy at a given temperature does not depend on  $r$ . Consequently our result must remain the same as that obtained under the assumption that the two cylinders are at rest. It seems therefore that both experimental results and theoretical considerations indicate that Def. II and not Def. I. must be the right one.

#### Calculation of the unavailable volume for a pair of cylindrical molecules.

The unavailable volume evidently depends on the orientation in space of the axes of the cylinders. Let the axis of A be parallel to the  $x$ -axis in a system of rectangular space coordinates as in Fig. 2 and let its centre be placed in the

origin. We want now to determine the volume which is unavailable to the centre of B. On account of the cylindrical shape of A and B the unavailable area is for all orientations of the axis of B equal to  $\pi d^2$ . The unavailable length however depends on the orientation of the B-axis. If this be parallel to the  $x$ -axis the length is evidently  $2h$ , beginning at  $x = -h$  and ending at  $x = +h$ . The vectors indicating the possible directions of the B-axis must always be positive and must therefore lie inside the corner limited by the three positive half-axes of coordinates. Denoting geographical longitude by  $\Theta$  and geographical latitude by  $\varphi$ , the surface element on a sphere with radius unity is  $dS = (\cos \varphi d\Theta) (d\varphi)$ . As we shall assume that all orientations (inside the octant) are equally probable the element of probability  $dw$  is  $A dS$ . As the vector in question ( $dw$ ) must have its endpoint somewhere inside the equilateral spherical triangle whose sides are  $\pi/2$  we have

$$\int dw = 1 = A \int dS = A 4\pi/8 \quad (22)$$

Consequently

$$dw = 2 \cos \varphi d\varphi d\Theta/\pi \quad (23)$$

The components of the unavailable length multiplied by the element of probability thus becomes for the axes indicated

$$\begin{aligned} (x): 4h \cos^2 \varphi d\varphi \cos \Theta d\Theta/\pi \\ (y): 2h \cos^2 \varphi d\varphi \sin \Theta d\Theta/\pi \\ (z): 2h \sin \varphi \cos \varphi d\varphi d\Theta/\pi \end{aligned} \quad (24)$$

where it be remembered that when the B-axis is parallel to either the  $y$  or to the  $z$ -axis the unavailable length is  $h$ , the end correction being already included in the contribution from the ( $x$ ) position. Performing now all the integrations from zero to  $\pi/2$  we get as contributions to the average length unavailable to the center of B

$$(x): h; (y): h/2; (z): h/2.$$

Thus the volume unavailable to the center of B, when A is fixed becomes  $2h \cdot \pi d^2$ . To get the volume unavailable to the center of A when B is fixed we must fix the axis of B perpendicularly to the  $x$ -axis. It is immaterial whether we choose the  $y$ - or the  $z$ -axis. Let us choose the latter and let furthermore the fixed B-axis extend from the origin along the two branches of the  $z$ -axis. The calculation need not be repeated as we evidently get the same result as before. We cannot, however, simply add the two results to get  $2b$ . Each of the two volumes  $2\pi h d^2$  represents the volume of a cylinder with height  $2h$  and radius  $d$ , but their axes intersect at right angles and divide each other in the ratio one to one. In the case of cylindrical bodies this ratio is of course immaterial so long as the two cylinders intersect but if the bodies were *e.g.*, prolate ellipsoids this would not be so. Now the total volume of two cylinders intersecting each other as described is equal to  $2b$  and we get

$$2b = 4\pi h d^2 (1 - 4d/3\pi h) \text{ if } d \leq h \quad (25)$$

For  $d > h$ , that is if the molecules are coin-shaped,  $2b$  is represented by two "coins" each of radius  $d$  and thickness  $2h$  intersecting each other symmetrically at right angles. The volume of such a body is

$$2b = 4\pi h d^2 - 16(d^3 - (d^2 - h^2)^{3/2})/3 \quad (26)$$

For small values of  $h/d$  this converges against

$$2b = 4\pi h d^2 - 8dh^2 = 4\pi h d^2(1 - 2h/\pi d) \quad (27)$$

Thus for very long "rods" and for very thin "coins" we get

$$2b = 16v_m \quad (28)$$

while for spherical molecules

$$2b = 8v_m \quad (29)$$

## PART II. COMPARISON WITH EXPERIMENTS

In the last two or three decades an immense number of determinations of O.P. particularly such of the filament or the rod type have been carried out. It would take too much time and space to review them all and the following examples are therefore chosen more or less at random. Peculiarly enough many of the results quoted were obtained by the time-consuming and not always quite reliable static method, although the principle of the dynamic method had been known for many years. Even when the present author at the Carlsberg Laboratory constructed an osmometer<sup>7</sup> according to the latter, the principle had been used more than twenty years before.<sup>8,9</sup>

The apparatus constructed by Fuoss and Mead<sup>10</sup> (1943) has since been used by several authors in U.S.A. It works according to the static principle, but with the important improvement, that the osmotic equilibrium is reached in a relatively short time and from both sides.

Some years ago the late Dr. Jensen and the present author<sup>1</sup> constructed, as mentioned before, a new type of a dynamic osmometer, an inverted osmometer, so called because the membrane is at the upper end of the observation capillary. This has since been used by different persons for different purposes and on a number of different solutes, but hitherto only with water or aqueous solutions as solvents. It is believed to have several advantages as compared to older models, particularly also to the Carlsberg model described by Sørensen<sup>7</sup> in 1917. First of all its volume is only about 400 mm<sup>3</sup>, its active surface being about 200 mm<sup>2</sup>. The Carlsberg model contained about 15 cm<sup>3</sup>, including about 4 cm<sup>3</sup> dead space, and had an active surface of about 30 cm<sup>2</sup>. The diameters of the cylindrical parts were, respectively, about 7 and about 14 mm. In the new model therefore in which there is practically no dead space the time necessary to reach diffusion equilibrium has been reduced by a factor of about four. Also the mechanical properties have been improved: On changing the hydrostatic pressure on the inner liquid the strain in the membrane is changed. This would be harmless if there were no elastic aftereffects in the collodion membranes used. Sørensen<sup>7</sup> showed that actually such effects seem to be present, but he showed also that the errors they produce were insignificant.

It can be estimated from experiments quoted in detail by Sørensen <sup>7</sup>, that his results are reliable to within one in about three hundred. In the new model the small membranes are about as thick as those in the Carlsberg model which are much larger and the resulting strains are therefore smaller. Accordingly elastic after-effects were not observed in experiments with the new model. To avoid these effects some recent osmometers <sup>10</sup> have a plane membrane supported by one or two perforated discs. This, however, has the drawback that the active surface becomes much reduced, or the volume strongly increased, because evidently, even if the whole surface of the disc were active it would be, for the same overall dimensions of the apparatus, less by a factor about two than the test tube shaped membrane.

Experience with the new apparatus has shown that osmotic pressure may be determined with an accuracy better than 0.1 mm water pressure for pressures below 5 mm, the range of the ocular micrometer. Above 5 mm the accuracy is limited to 0.1 mm, the heights being measured by means of a millimeter scale with Vernier on the vertical stem of the microscope. In any case the accuracy obtained can be read from the graph connecting the rate of movement of the air column with its height.

In most other, particularly older apparatuses, including those of both types, static and dynamic, the accuracy seems not to be much better than 1 mm, and sometimes to be worse. Furthermore, at some measurements performed according to the static method and with one-sided approach to equilibrium, complete equilibrium may not always be reached. For these reasons many measurements of O.P. lower than about  $10^{-3}$  atm, reported in the literature, may be off by 10 % or more.

Some earlier authors have used the abridged van der Waals equation to represent their results, *i.e.* they have plotted  $c/p$  against  $c$ , where  $c$  usually is the concentration in g/litre and  $p$  the pressure in some arbitrary unit. When points relating to the lowest pressures (less than  $10^{-3}$  atm) are neglected reasonably straight lines are usually obtained. More recent authors often calculate and plot  $p/c$  against  $c$ , thereby obtaining curves with an upward bend. Some such experiments have been recalculated by the present author who, again omitting low and therefore doubtful pressures, got nearly straight lines.

Fortunately earlier authors gave their numerical results in tables. More recent authors seem to have abandoned this useful habit and give their results in graphs, from which it is difficult to read their numerical results accurately enough for recalculations. In all the following examples the present author has performed a recalculation and redrawn the corresponding graphs, thereby obtaining the values indicated for the molecular weight,  $M$ , and the values of  $b$  in  $\text{cm}^3/\text{g}$ .

According to theory  $b = 8v_m$  if the molecules are (nearly) cylindrical. If  $v_m$  were the specific volume of the substance in question we should expect to get  $b\rho = 8$  if  $\rho$  be the density in  $\text{g}/\text{cm}^3$  of the substance in question. In stead of that one finds in most cases that  $b\rho$  is two to five or even ten times as large as that. The explanation is evident:  $v_m$  is the volume of a cylinder, which cannot penetrate a similar one. If now the principal chain in the molecule has many short branches, or if it be wound up in a helix, two such molecules can only penetrate each other to a distance which may be two or three times

that calculated from the densest possible packing of the constituents, as in the corresponding solid. This means that the factor in question may easily be four or even nine. It should of course be more correct to use the partial specific volume of the solute in the solvent in question instead of the reciprocal density of the solid substance, but even the volume measured that way may easily be less than  $v_m$  because the small solvent molecules can accommodate themselves in the structure of the solute macromolecules while other solute molecules can not. Therefore, the only thing we can state with some certainty is that the product  $b\rho$  must be equal to or greater than eight, provided that attractions between the rod-molecules can be neglected.

#### Example 1

Experiments by Kroepelin and Brumshagen<sup>11</sup>. Recalculated by J. A. Chr.  
Solute: Caoutchouk. Solvent: Benzene.

Temp.	$M$	$\rho$	$b$	$b\rho$
11°C	210 000	0.91 g/cm <sup>3</sup>	about 40 cm <sup>3</sup> /g	about 36
40°C	210 000	0.91 »	about 70 »	about 62

*Remarks:* As mentioned by the authors themselves, the  $b$  values are rather inaccurate, but it seems certain that  $b$  at 40°C is essentially larger than  $b$  at 11°C.

As an explanation it may be suggested that different rubber-molecules be curled into helixes of different diameters, those with the larger diameters having the greater energy-contents.

#### Example 2

Experiments by Schultz<sup>12</sup>. Recalculated by J. A. Chr.  
Solute: Cellulose-nitrate. Solvent: Acetone.  
Temp. 27°C. Density,  $\rho$  : 1.35 g/cm<sup>3</sup>

Specimen	$M$	$b$	$b\rho$
N66	about 54 700	about 25 cm <sup>3</sup> /g	about 34
N65	about 36 700	about 21 »	about 28

*Remarks:* Polysaccharide chains are probably straight, but the nitro-groups substituted for hydrogen in the hydroxy-groups may act as short side-chains.

#### Example 3

Experiments by Schultz<sup>12</sup>. Recalculated by J. A. Chr.  
Solute: Polystyrene. Solvent: Toluene.  
Temp. 27°C. Density,  $\rho$  : 1.06 g/cm<sup>3</sup>

Specimen	$M$	$b$	$b\rho$
P.S. 20°	about 141 000	about 30 cm <sup>3</sup> /g	about 32
P.S. 135°	» 160 000	» 29 »	» 31
P.S. 200°	» 57 500	» 23 »	» 24

*Remark:* The aromatic side-chains must increase the effective diameter of the principal chain but may prevent its curling up.

#### Example 4

Experiments by Schultz<sup>12</sup>. Recalculated by J. A. Chr.  
Solute: Polyethylene oxide. Solvent: Water.  
Temp. 27°C. Density,  $\rho$  : 1.12 g/cm<sup>3</sup>.

Specimen	$M$	$b$	$b\rho$
II	about 64 400	about 54 cm <sup>3</sup> /g	about 60
III	» 26 600	» 26 »	» 29
IV	» 17 700	» 26 »	» 29

*Remark:* In the book by H. Staudinger, *Die hochpolymeren organischen Verbindungen* (1932) p. 293, one author, H. Lohmann, gives arguments for zigzag formulae of the polyethylene oxides illustrated in a plane. This illustration may very naturally be taken as the projection formula of a helix.

#### Example 5

Experiments by Jensen and Marcker<sup>13</sup>. Recalculated by J. A. Chr.  
Solute: Dextrane, fractionated. Solvent: 0.2 M KCl in Water.  
Temp. 20°C. Density taken to be 1.588 g/cm<sup>3</sup> (sucrose), (starch about 1.5)

Specimen	$M$	$b$	$b\rho$
Dextrane	about 140 000	4.93 cm <sup>3</sup> /g	7.84

*Remarks:* Unfortunately no determinations of the density of the solid or of its specific volume in the solution were performed. Assuming that different polysaccharides have the same specific weights the value of  $\rho$  for sucrose has been taken. The value for starch, 1.5, is probably low, because it refers to granules of native starch which may include some air.

It is said in the paper<sup>13</sup> that the (commercial) specimen was very polydisperse and had to be freed from the low molecular fraction by dissolution and reprecipitation, but even so it became certainly not monodisperse. As, by experience, unavailable volumes are usually independent of the molecular weights for rod-molecules derived from the same structural unit, the degree of polymerisation is probably irrelevant for the value of  $b\rho$ .

The deviation of  $b\rho$  from 8 is certainly less than the probable error. The result therefore suggests that the polysaccharide chain be not coiled up. Because of the cyclic constitution of the units this seems intrinsically probable.

#### Addendum to Example 5, and amendments to a paper on the inverted osmometer

In Table 2 in the paper by Jensen and Marcker<sup>13</sup> there is one misprint (19.40 for 10.94) and one line is missing. The complete table shall therefore be reprinted below. Furthermore, the heights of the air-column had not been

corrected for curvature at its ends. This has now been done by subtracting one third of the diameter of the capillary (0.07 cm) from the heights ( $p$ ) in the table. The corrected values of  $c/p$  are under the heading  $(c/p)$ . Values of  $c/p$  have been calculated from the formula  $c/p = A - Abc$  with  $A = 5.68$ ,  $Ab = 0.0280$ . They are under the heading  $(c/p)_{\text{calc}}$ . Finally, values of the difference  $(c/p) - (c/p)_{\text{calc}}$  are in the last column.

Table 2 by Jensen and Marcker<sup>13</sup>, revised and enlarged by J. A. Chr.

$c$ mg/ml	$p$ cm	$c/p$	$(c/p)$	$(c/p)_{\text{calc}}$	
0				5.68	
5.19	1.05	4.94	5.05	5.54	-0.49
10.94	2.07	5.31	5.37	5.37	0.00
19.40	3.90	4.98	5.01	5.14	-0.13
31.51	6.45	4.89	4.91	4.83	+0.08
40.00	8.80	4.55	4.56	4.56	0.00

The deviations in the last column are such that if caused by errors in the determination of  $p$  three of them would correspond to errors of about 1 mm. Such errors are, however, nearly impossible and it is therefore much more probable that they have arisen from lack of homogeneity in the fraction investigated. It should be recalled in this connection, that it was certainly not monodisperse. If this be the cause of the deviations it is quite natural that it is the determination in which the smallest amount of substance (5 mg) was weighed in, which shows the largest deviation.

#### A m e n d m e n t s   t o   t h e   p a p e r   b y   C h r i s t i a n s e n   a n d J e n s e n<sup>1</sup>

On p. 1252 the authors mention three corrections which should be applied:

1. A correction for the finite density of air (saturated with water vapour at 20°C). This correction varies with the varying total pressure in the apparatus, but it is so small that its value at one atmosphere, 0.0011 g/cm<sup>3</sup>, can safely be used in all cases. Therefore the significant figures in the value of  $q$  on p. 1254, 246.8, should be replaced by 247.1.

2. A correction for the difference in density between the outer and the inner liquids. This correction is at any rate very small, and if complete equilibrium in the gravity-field has been established, not only across the membrane but also in the column of inner liquid extending from the upper end of the capillary to the upper end of the air-column, only the density of the outer liquid is relevant. This item be therefore withdrawn.

3. A correction for the difference in surface-tension between the solution at the upper end of the air-column and the solvent at the lower end. This be also withdrawn for the following reason: Water adheres strongly to glass. We must therefore assume that the cylindrical part of the air-column is surrounded by a liquid film, which, as known from the works of M. Vollmer, most probably is easily movable along the glass wall. The capillary forces at the ends of this cylindrical film must tend to drag it in opposite directions, and at osmotic equilibrium the forces must counterbalance each other exactly.

## Example 6

Experiments by Jensen and Marcker<sup>13</sup>. Recalculated by J. A. Chr.  
 Solute: Potassium hyaluronate. Solvent: 0.2 M KCl in water.  
 Temp. 20°C. Density, : 1.16 g/cm<sup>3</sup> (spec. vol. det. by Jensen)

Specimen	$M$	$b$	$b\rho$
Prep. I	122 000	35.8 cm <sup>3</sup> /g	41.6
Prep. II	144 000	35.8 »	41.6
Prep. III	144 000	35.8 »	41.6
Prep. IV	1 066 000	43.0 »	50.0

*Remarks:* At a former occasion Jensen<sup>14</sup> determined the specific volume of another specimen of potassium hyaluronate by pycnometric determinations in 0.2 M KCl in water. Result: 0.86 cm<sup>3</sup>/g. It is evident from the table that  $b$  depends only slightly on the molecular weight of the preparation. It is also evident that the product  $b\rho$  is 5—6 times larger for the hyaluronate than for dextran, although the former is a derivative of a polysaccharide and the latter is a polysaccharide. This may partly be due to the circumstance that the former contains carboxyl-groups as side-chains, but the effect of this may be masked by the property of its being a polyelectrolyte. In a forthcoming paper, Graae and Marcker point out that the large unavailable volume of the hyaluronate may be due to electrical forces from the ionic atmosphere surrounding the negatively charged "backbone" of the polyion. The effective diameter of the cylinder may be expected to be not very different from the Debyelength  $1/\kappa$  and  $b$  should therefore increase with decreasing ionic strength of the solvent. Graae and Marcker have found experimental evidence in the literature that this be true for another chain-like polyelectrolyte.

## Addendum to Example 6

The values for  $c/p$  in Table 3 of the paper in question have not been corrected for curvature at the ends of the air column. Table 3 has therefore been recalculated by the present author (see below) and the values for  $M$  and  $b$  in the table above are the results of this recalculation. It should be noted that in the columns marked  $p$  in the original table 3 the number of significant figures should have been increased by one in some cases. The figures in the column marked  $c/p$ , however, have in all cases been calculated from the original data and have sufficient numbers of significant figures. The recalculation, therefore, is based on the figures in that column. One misprint in the original  $p$ -column, 1.00 in stead of 1.06, has been found. As in the addendum to Example 5  $c/p$  is the original value,  $(c/p)$  the corrected one and  $(c/p)_{\text{calc}}$  that calculated according to the formula  $p/c = A - Abc$ . The differences (exp) — (calc) are in the last column.



Table 3 by Jensen and Marcker<sup>13</sup>, revised and enlarged by J. A. Chr.Prep. I.  $A = 4.92$ ;  $b = 0.0358$  litre/g.

$c$ mg/ml	$p$ cm	$c/p$	$(c/p)$	$(c/p)_{\text{calc}}$	
0				4.92	
16.00	7.89	2.03	2.03	2.10	-0.07
13.00	4.91	2.65	2.66	2.63	+0.03
11.00	3.70	2.97	2.99	2.98	+0.01
7.00	1.85	3.78	3.83	3.69	+0.14
5.00	1.292	3.87	3.94	4.04	-0.10

Prep. II.  $A = 5.85$ ;  $b = 0.0358$  litre/g.

0				5.85	
16.00	6.50	2.46	2.47	2.50	-0.03
13.00	4.19	3.10	3.12	3.13	-0.01
11.18	3.21	3.49	3.52	3.51	+0.01
5.00	1.059	4.72	4.83	4.80	+0.03

Prep. III.  $A = 5.85$ ;  $b = 0.0358$  litre/g.

0				5.85	
7.51	1.85	4.06	4.12	4.27	-0.15
5.10	1.10	4.64	4.74	4.78	-0.04
3.53	0.710	4.97	5.14	5.11	+0.03
2.56	0.490	5.22	5.49	5.31	+0.18

Prep. IV.  $A = 43.0$ ;  $b = 0.0432$  litre/g.

0				43.0	
19.99	3.49	5.72	5.76	5.80	-0.04
16.00	1.20	13.33	13.59	13.22	+0.37
13.78	0.85	16.21	16.68	17.38	-0.70
10.21	0.450	22.69	23.90	23.98	-0.08
7.48	0.280	26.71	29.15	29.10	+0.05

It should be noted how surprisingly accurately the van der Waals abridged formula holds, even in cases where the available volume is less than half of the total volume, in the case of Prep. IV even less than one seventh.

It should be added, that in recalculating the tables for Preps. I—III the present author has taken advantage of the fact found by Jensen and Marcker that the  $b$ -values are, inside the experimental errors, the same for these preparations.

It is seen that in all the examples quoted  $b\rho$  is equal to or greater than eight. On the other hand it is less than one hundred. Now according to the formula of Zimm<sup>4</sup> and Isihara<sup>3,6</sup> the lower limit for the product should be, for long molecules, the height of the cylinder divided by its diameter. The lowest value of this ratio occurring among the examples is probably the one in Example 4, specimen IV, and even here it can hardly be below 150. In the other examples rough estimates yields much larger values for the same ratio. This confirms the suggestion that Def. II and not Def. I be the right one to use.

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