On the Preparation and Use of Collodion Osmometers.

BY

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IN an earlier paper¹ the writer described methods of preparing and grading collodion membranes for purposes of dialysis. It was there shown that by soaking the air-dried membranes in alcohol-water mixtures of different strengths and subsequently washing in water a series of membranes with a wide range of permeability could be obtained. At the one end of the series (using a grading mixture with a low concentration of alcohol) were membranes which were only slowly permeable to water and in a less degree to the simpler electrolytes, such as sodium chloride, potassium nitrate, &c.; at the other end (using a grading mixture with a high alcohol content) were those which allowed a slow diffusion of starch, aniline blue, and other substances which are highly colloidal in aqueous solution. Between these extremes any desired degree of permeability could be obtained. As was pointed out in the paper already referred to collodion membranes of certain grades are directly applicable to osmotic work, as it is possible with certainty to prepare them of such a permeability that they will allow a ready passage of water and at the same time hold back such solutes as cane sugar, copper sulphate, &c. They may thus be used to replace the well-known precipitation membranes (such as the copper ferrocyanide membrane of Traube and Pfeffer), which repeated attempts in this laboratory have shown to be difficult to prepare satisfactorily. The type of osmometer described in this paper has been used now for some years in the plant physiology practical classes in this laboratory, and has been found to be accurate and simple in manipulation. The following practical notes will serve to show how these osmometers are prepared and used. For convenience in description we shall deal in succession with:

1. Preparation of air-dried membranes or 'thimbles'.
2. Grading and method of attachment.
3. Method of using.

1. The type of membrane most convenient for the present purpose is that known as the thimble, which may be formed either inside or outside

¹ Biochem. Journ., 1915, ix, p. 591.

a test-tube. A full account of the method of preparation has been given in the paper already cited, and it is unnecessary to repeat the details here. Though the writer prefers to make the thimbles on the outside of the test-tube, as greater uniformity in successive membranes can be obtained by that method, it is probable that the alternative method will be found preferable for general purposes, as a certain amount of practice is required for success in stripping the membranes from the outside of the tube. A brief account was given in the original paper of a method of preparing membranes inside the test-tube, and it was stated then that these membranes tended to be too thin and fragile for practical purposes. Subsequent work has, however, shown that that difficulty can be overcome.

Schering's celloidin was formerly used in this work; latterly a preparation known as 'Necoloidine', and supplied by the New Explosives Company, has been found to be equally satisfactory. For preparing membranes by the 'inside' method, the arrangements given on p. 615 of the paper already mentioned will be found to give good results with the following alterations. Use a stronger solution of collodion (12 per cent.), and reduce the time of drainage to about two to three minutes. The membranes tend to be somewhat thin at the upper (closed) end, but this tendency can be checked by careful warming of the upper end of the tube during drainage. This has the effect of vaporizing some of the ether from the upper part of the membrane and preventing further running of the collodion sol in that part.

After drainage the membrane is dried off completely by blowing air into the tube. It is then separated from the tube by filling the latter with water when it is found that the collodion membrane readily separates from the glass. The membrane is now dried off completely, when it is ready for the next stage.

The important points to observe in drying the membrane inside the tube are: (1) not to touch the membrane until drying is well advanced, otherwise a hole will result; (2) to continue blowing in air until the membrane is well dried and may in fact begin to separate from the tube of its own accord. If the water is added before drying is well advanced, the subsequent drying in the open air will entail considerable shrinkage and distortion.

2. The membrane in its air-dried form is highly impermeable, and therefore, from the point of view of an osmometer, insensitive. To increase its permeability without at the same time making it permeable to the more slowly diffusible crystalloids, it is placed for twenty-four hours in (about) 60 per cent. alcohol—the maximum percentage varies somewhat with different makes of celloidin—then washed in several changes of water until the alcohol is removed. It must be emphasized that from the time the membrane is taken out of the grading mixture of alcohol and water it must not be allowed to undergo any drying. If dried out, it will of course revert to its previous highly impermeable condition.

In setting up this membrane as an osmometer use is made of the fact that whereas collodion will not adhere to glass in presence of water, it will under these circumstances adhere strongly to rubber. For the present purpose, rubber tubing is preferable to the ordinary rubber cork as the cylindrical shape of the former permits of a better joint. The method of procedure is given below.

A piece of pressure rubber tubing, about 1 inch long, is inserted into the open end of the thimble so that the edge of the membrane is about half-way up the 'cork'. The rubber tubing is chosen of such a diameter that it can just be inserted. A piece of ordinary glass tubing (about 2 to 3 inches long) is now passed through the cork until its inner end is flush with the inner surface of the latter. If the various parts have been suitably selected, the cork will be closely pressed against the membrane, all irregularities of which are thereby evened out so that a tight junction is effected. The membrane, filled with water to just below the lower end of the cork, is now set upright in water, so that the water-levels inside and outside are approximately the same. In this way the part of the membrane in contact with the rubber cork is allowed to dry out completely by exposure to the air, after which the junction is painted over with a collodion solution. This is in turn allowed to dry off and the junction is now watertight. A short length of capillary tubing is attached, and the osmometer is complete.

The simplest method of filling the osmometer is by pinching the membrane to drive out the air and running in the required solution from a funnel. The operation of filling must be carried out rapidly to avoid any risk of drying the membrane. For accurate work several fillings and emptyings are required each time a new solution is tested in the osmometer. As the membrane substance is tough it will stand repeated fillings and emptyings without damage, granted moderate care is exercised. Before using an osmometer for the first time it is advisable to keep it for a few days under an internal pressure of about two feet of water in order to even out any wrinkles that may be present, and to make sure that the membrane is properly sealed to the cork.

3. These osmometers are used in class work in the following manner. They are filled with the solution the osmotic pressure of which is to be determined (either unknown concentrations of cane sugar or copper sulphate, or a plant extract such as beet juice), the level of the meniscus being arranged somewhere in the middle of the capillary tube. The osmometer is now placed in a succession of dilutions of a standard cane-sugar solution, and by a process of trial and error one determines at what degree of dilution of the standard solution the meniscus remains steady. For class purposes the normality of the unknown solution is determined to within one unit in the second decimal place but a greater degree of accuracy than this is feasible. The method of procedure described below is more systematic, and

is recommended on account of the ease and rapidity with which the osmotic pressure of the unknown solution can be determined.

The determinations now to be described were carried out with a moderately thin osmometer of 7 c.c. capacity, attached to a capillary tube of 1 mm. internal diameter. For testing purposes a half-molar solution of copper sulphate was prepared, and the accuracy of the method determined by comparing known dilutions of this stock solution with each other.

In determining the osmotic strength of an unknown solution it is of great assistance to know certain constants appertaining to the particular osmometer. These should be determined when the osmometer is first used, for it will be found that a knowledge of them will greatly facilitate all future work with that particular osmometer.

The rate of passage of water through the membrane is proportional to the difference of osmotic pressure of the liquids on opposite sides. If, therefore, the rate of passage corresponding to a known difference has been determined, it is possible to tell the whereabouts of an unknown concentration by a single test of it against a known solution. The concentration suggested by this first reading can now be set up and compared with the unknown solution. By this means a closer approximation is obtained at the second trial, and thus one rapidly arrives at the concentration of the unknown solution.

With the particular osmometer under consideration, and with half-molar copper sulphate inside and water outside, the rise of the meniscus in 1 hour is 3.3 cm. at 17° C.

The rate of intake of water varies considerably with temperature, as is shown by the following figures :

<i>Temperature.</i>	<i>Rise of meniscus in 1 hour.</i>
14°	3.0
17°	3.3
24°	4.4

Thus a constant temperature bath, if available, is advantageous, but if laboratory temperature does not vary more than a few degrees, and if a rough correction is made for this temperature effect, the rapidity with which one obtains a close approximation to the unknown solution is not seriously affected.

In addition to the effect of temperature on the rate of endosmosis, account is to be taken of its more direct effect on the osmometer. In the present case the meniscus was found to rise 1.5 mm. for each 1° C.

In dealing with a solution of an electrolyte such as copper sulphate it is well to remember that the osmotic pressure is not directly proportional to the concentration of salt present. As it is a function of the number of molecules plus ions present, and as the degree of ionization depends on concentration (in accordance with the law of mass action), one can easily prove

that the osmotic pressure of an electrolyte is not a linear function of the concentration, but falls short of a linear relation as the concentration rises. Thus the osmotic pressure of half-molar copper sulphate is less than twice that of a quarter-molar solution. The following determinations with this particular osmometer illustrate this point :

Concentration inside.	Concentration outside.	Difference.	Endosmosis.	Endosmosis $\div$ concentration difference.
n/2	0	n/2	3.3	3.3
n/4	0	n/4	1.8	3.6
n/2	n/4	n/4	1.5	3.0

Thus a given increase of concentration produces a greater effect on the osmotic pressure when the concentration is low than when it is high. Attention to this fact enables one to approach more rapidly the concentration of an unknown solution.

A particular determination will now be described.

A solution, x , of concentration unknown to the experimenter, was prepared by dilution of the stock half-molar copper sulphate solution. The following readings were then obtained :¹

Inside.	Outside.	Rise in 1 hour at 17°.
m/2	water	3.3 cm.
m/2	x	0.65, 0.7

Assuming as a first approximation that osmotic pressure is directly proportional to concentration, we find that the value of x is in the neighbourhood of 0.40 molar ($\frac{m}{2} - \frac{0.7}{3.3} \times \frac{m}{2}$). In view of the effect of ionization above mentioned, this value is too high. A slightly weaker solution than 0.40 molar was therefore set up, i.e. a 0.38 molar solution. The solution x was now put inside the membrane and the 0.38 solution outside, with the following result :

Temperature at start = 17.0°.

After 18 hours, rise = 0.3 cm. Temp. = 14.0°.

The rise in 18 hours (corrected for temperature) was therefore approximately 0.75 cm. Calculating as above, we find the approximation to the unknown solution to be 0.385 molar. This concentration was now made up and tested against the x solution. A slight drop, amounting to 0.2 cm. in 24 hours, was recorded. Using this correction as before, the final value obtained was 0.384 molar. The correct value according to the original measurements was 0.383 molar. Thus with three trials the molecular strength of the unknown solution was found correct to one unit in the third decimal place. In a second experiment, a solution was estimated as 0.368 molar as against the correct figure of 0.366 molar.

¹ In any determination the whole of the membrane must be completely submerged in the outside solution.

It may be said generally that by means of three or four determinations performed as above the method will give the correct molecular value to within two units in the third decimal place. This degree of accuracy is thus somewhat greater than that claimed by Barger¹ for his method—viz. five units in the third decimal place. Each method has its own particular merits, but the present method has the great advantage for demonstration purposes that it measures osmotic pressure directly, i. e. by diffusion across a semipermeable membrane, and not indirectly through the effect of osmotic pressure of the solute on the vapour tension of the solvent. It thus illustrates more clearly the functioning of the living cell as an osmotic unit.

The carrying out of the above determinations requires about two days. In the course of the finer determinations which run over a period of about twenty-four hours the solutions must be protected against evaporation. It is advisable to have the unknown solution inside the osmometer during the longer periods, as it is thereby protected from evaporation, and in any case its concentration can be brought back to its original value. It is probable that an accuracy of 0.002 molar is about the best that can be obtained short of elaborate arrangements for preventing change in concentration through evaporation. It also approaches very close to the limits of accuracy which usually obtain in solutions prepared for quantitative work.

In taking the above readings, the level of the meniscus was kept at about 10 cm. above the outside level. This head of water represents a molecular concentration of about 0.0005, and thus if greater accuracy is attempted than that claimed above, this factor will require to be allowed for.

In order to bring back the meniscus in the capillary to the same point, and thus ensure that no alteration in concentration has taken place either by evaporation or by diffusion of water across the membrane, the osmometer is placed in water if the level has fallen, or, in the case where the level has risen, first washed on the outside with water, the excess of water drained off by means of filter-paper and the contents of the osmometer allowed to concentrate to the required degree by evaporation of water through the membrane. This latter process can be repeated time and again without affecting the permeability of the membrane; that is, no drying out of the membrane takes place at ordinary temperatures so long as one side of it is maintained wet.

Strictly speaking, these osmometers can only be used to determine the osmotic pressure of solutions the solutes of which do not diffuse across the membrane. As, however, the diffusibility of water is much greater than that of even the simplest electrolytes, considerable accuracy can be obtained even in the case of such substances.

It will be noticed that the method of determining osmotic pressure here described is one of comparison and not of absolute measurement. For

¹ Journ. Chem. Soc. (Trans.), 1904, lxxxv, 286-324.

most biological purposes the former is sufficient, as the osmotic pressure of a biological fluid may be determined in terms of a standard substance such as cane sugar, and thereby obtained either from tables or from theoretical calculation. For the absolute measurement of osmotic pressure (except where it is very small, as in the case of colloidal solutions) these osmometers cannot be used, as they stretch under moderate pressure. Semi-permeable collodion membranes in sheet form could, however, be so used, but some type of apparatus to take up the pressure developed would obviously be necessary.

These osmometers, as far as is known, last indefinitely. If kept in water they tend to become coated with fungoid growths so that they should be kept in a dilute solution of an antiseptic, e. g. dilute copper sulphate.

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