## 149. *The Osmotic Pressure of Solutions of Polysaccharide Derivatives. Part I. A New Form of Osmometer.*

By SYDNEY R. CARTER and BASIL R. RECORD.

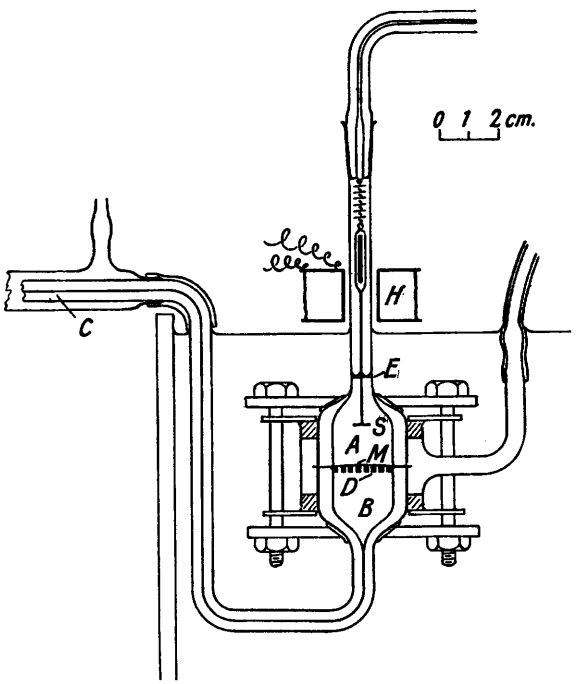
An osmometer has been designed for the investigation of polysaccharide derivatives of molecular weights between 3000 and 1,000,000 in solutions of organic solvents. Semipermeable membranes are described whose permeability may be controlled over a wide range down to complete impermeability.

RECENT studies on the structure of the polysaccharides have emphasised the need for physical methods suitable for the direct determination of their molecular or particle weights. Polysaccharides in the form of their methylated and acetylated derivatives are generally insoluble in water but can in most cases be dissolved or dispersed in certain organic solvents. The direct determination of the osmotic pressure of these solutions or dispersions provides a method well adapted for the investigation of particle weights of the order encountered in the field of polysaccharides, namely, between 3000 and 1,000,000.

Osmometers using organic solvents have already been described, but they did not meet our requirements (Herzog and Spurlin, *Z. physikal. Chem.*, Bodenstein Festband, 1931, 239; Van Campen, *Rec. Trav. chim.*, 1931, **50**, 915; Büchner and Samwel, *Trans. Faraday Soc.*, 1933, **29**, 32; Dobry, *J. Chim. physique*, 1935, **32**, 46).

The present osmometer works on the counter-pressure principle of Berkeley and Hartley (*Phil. Trans.*, 1906, *A*, **206**, 486), in which the osmotic pressure is applied externally. It is more rapid than the "self-registering" type of osmometer, in which the equilibrium pressure is built up by the osmosis itself, a process which becomes increasingly retarded as the equilibrium is approached, particularly with membranes of low permeability. The method furnishes valuable information on membrane permeability for a comparison of membranes as well as the detection of temporary diffusion pressures. It has the further advantage that measurements can easily be made over a wide range of pressures and concentrations, enabling the deviation from the ideal-solution laws to be studied. The osmometer has been designed for use with the flat membranes later described. Ground-glass joints, taps, packing washers, etc., have been avoided in the solvent–membrane–solution system, since they are a potential source of error in measurements involving organic solvents.

FIG. 1.




## EXPERIMENTAL.

*The Osmometer.*—The osmometer (Fig. 1) consists of two stout glass bells *A* and *B*, each of 5 c.c. capacity, with accurately ground ends between which is compressed the flat "Viscacelle" membrane *M*. The lower chamber *B* containing the solvent is fused to a stout horizontal capillary tube *C* of 0·5 mm. bore and 50 cm. long. The solvent meniscus in *C* is observed by means of a microscope fitted with an eye-piece carrying an etched scale of 100 divisions, enabling meniscus movement to be estimated to 0·01 mm. Both the microscope and the capillary tube are rigidly clamped to the same heavy iron stand. The upper chamber *A* contains the solution under test and it is connected to an air reservoir and manometer of the Sörensen type (*Z. physiol. Chem.*, 1919, **106**, 1). Pressures up to 100 cm. of water are obtained by using water in the reservoirs and manometer. A similar apparatus containing mercury provides pressures up to 1 atm.

In an osmometer working on the counter-pressure principle, a perfectly rigid membrane is essential to prevent membrane movement being superimposed on the flow of liquid through the membrane. The perforated brass membrane support *D*, having a convex face of 7·5 cm. radius on the upper surface, effectively eliminates all membrane movement under changing pressures. Similarly, volume changes in the solvent due to temperature variations must be reduced to

negligible proportions compared with the observed transport of solvent through the membrane. The mean temperature of the glass water-tank thermostat (30 l.) is maintained within ± 0·001° by means of a control (D.R.-P. 448,786) of the mercury thermometer type operating a 60-watt heater through a valve relay. The constancy of the mean bath temperature during measurements is checked by a Beckmann thermometer. Accuracy of control was improved by mounting the instrument in the vertical position by light springs to damp out vibrations.

The exposed osmometer capillary tube is also maintained at uniform temperature by circulating bath water through a narrow glass jacket surrounding the tube, by means of a Luther centrifugal pump immersed in the thermostat.

A satisfactory membrane seal could not be realised by compressing the membrane between the two ground faces of the osmometer unless the membrane edges were surrounded with a glass cylinder containing mercury under pressure. By means of a mercury bulb attached by rubber tubing to a side arm, a pressure of 1 atm. was applied. The two rubber rings closing the cell are backed by brass plates firmly clamped together. This method provides a positive membrane seal at all points and eliminates any doubtful packing devices.

To reduce the risk of air-bubble formation in the solvent chamber, the solvent in which is immersed the membrane support is placed under reduced pressure for ½ hour before use. The osmometer is assembled by placing the glass cylinder and lower bung in position round the cell *B* to provide an outer receptacle for the solvent. The prepared membrane is rapidly transferred to its position, the upper bell *A* filled, and the mercury seal is clamped into place. Some mercury is then poured into the outer chamber and a reduced pressure applied for a few seconds to remove air bubbles from the membrane joint. The mercury bulb is then attached, and the osmometer placed on horizontal rails in the thermostat. The capillary tube is levelled and, by imposing a suitable pressure on the solvent in the solution chamber, the meniscus is brought to a position in the horizontal capillary not far from the bend, to minimise evaporation from the open end. The solution in *A* is adjusted to a mark *E* etched a short distance above the shoulder of the cell. Connexion is made with the air-pressure reservoir and manometer by means of a length of capillary tube of 1 mm. bore and an unlubricated ground-glass joint, over which is drawn a short piece of rubber tube. The manometer reading must be corrected to give the effective pressure at the membrane interface by the addition of the capillarity of the solvent in the horizontal capillary. The latter is of uniform bore, so that the correction remains substantially constant over the length of tube used. To be deducted are (*a*) the difference in level between the horizontal plane of the capillary tube bore and the mark *E*, and (*b*) the small surface-tension effect at *E*. The effect of the difference in density between solution and solvent has been ignored, since it becomes appreciable only at high concentrations and therefore high pressures. The correction thus worked out may be checked by determining the manometer reading for zero meniscus movement, an open-pore membrane being used, and care being taken that temporary diffusion pressures due to slight differences between the solvent on the two sides of the membrane have been equalised. Osmotic pressures may be determined to the nearest 1 mm. of water, depending on the permeability of the membrane.

*The Solution.*—Chloroform is perhaps the best general solvent for the methylated and acetylated polysaccharides and has the additional advantages of (*a*) low surface tension, (*b*) low viscosity, (*c*) a rapid passage through the membranes used. A good-quality chloroform B.P. has been used in this work, as the presence of the small quantity of alcohol ensures its stability and there is no objection to mixed solvents provided that the solvent has the same composition on both sides of the membrane. Carbon tetrachloride is less volatile, but it has not a good solvent power in all cases and also has a lower rate of flow through the membrane.

Most methylated and acetylated polysaccharides are hygroscopic and are also prone to retain traces of the organic solvents used in their purification. Before use, therefore, the specimen is brought to a constant weight in a Pregl drier at a suitable temperature.

The concentration of the solution to be tested may be determined either during its preparation before being placed in the osmometer or after the test by withdrawing for analysis a sample (say, 1 c.c.) and weighing the evaporated residue on the microbalance. If the former method is adopted it is necessary to rinse out the cell with the solution before filling it, since the solution chamber must not be dried of solvent for fear of reducing the membrane permeability; this method is therefore more wasteful of material. The latter method is often unsuitable with solutions difficult to evaporate either through the specimen being deposited as a glassy film, or in the case of high-boiling solvents. An analysis is often useful as a check on the predetermined concentration, however, and this method alone must be used when the specimen is only available in small amount. Micro-analytical techniques with special precautions

against evaporation losses have been developed for both methods. Emphasis is here laid on the importance of securing solutions of homogeneous composition by thorough mixing at all stages. Concentrations are obtained as g. per 100 g. of solvent, and are converted into g. per 100 c.c. of solvent by a density determination for each specimen of solvent used. The absence of dissolved matter in the solvent is also confirmed by a concentration analysis.

*Determination of the Osmotic Pressure.*—The osmometer is left overnight after assembly, and then, before introduction of the solution, the permeability of the membrane to the solvent is measured, the proportionality of rate of flow to applied pressure being tested. The solution is run into the cell to the level of the mark *E*, and the pressure apparatus again connected. The pressure is frequently adjusted to keep the capillary meniscus as far as possible stationary. When no further increase is observed, the equilibrium pressure is determined from the rates of meniscus movement under applied pressures in the immediate vicinity of the equilibrium pressure, the uniformity of these small movements over a period of time being taken as the criterion that the final osmotic pressure has been developed.

Measurements on methylated inulin and methylated lichenin showed that the apparent osmotic pressure of the solution increased slowly with time, reaching its ultimate value only after several days. Agitation of the solution during the test considerably reduced the time required to reach equilibrium, one hour being sufficient in most cases. The electromagnetic stirrer consists of a soft-iron armature sealed in a thin-glass tube drawn out to a fine rod, at the end of which is attached a platinum disc *S*, sufficiently small to permit its insertion into and withdrawal from the osmometer cell. When the circuit of a solenoid *H* surrounding the osmometer tube is closed, the stirrer is drawn against a light, phosphor-bronze spring from which it is normally suspended. Thus a sharp movement of the stirrer occurs at the make and break of the solenoid circuit. Adjustments were made so that the solenoid current passed for only about one-tenth of the total time in order to minimise the heating effects from the solenoid. This device proved invaluable in giving a sharply defined end pressure, and the improved results obtained were manifest in the closer conformity of the points to a smooth pressure–concentration curve. Although emphasis is given to the advantages of stirring, yet too violent an agitation, especially in the case of the higher osmotic pressures, occasionally leads to the formation of an air bubble in the solvent chamber due to solvent containing air dissolved under pressure in *A*, diffusing to the solvent side *B* of the membrane. This behaviour is not likely to lead to erroneous results, since the air bubble when once formed develops rapidly and it is readily detected. It does, however, entail a complete reassembly of the osmometer.

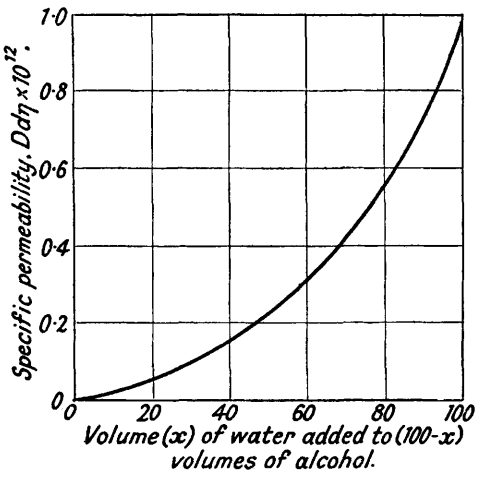
In the case of a homogeneous substance and a membrane perfectly semipermeable to its solution, the equilibrium pressure remains unchanged after switching off the agitator even after a lapse of a week. An equilibrium pressure greater for an agitated solution than for one at rest is a certain indication of the diffusion of some of the solute through the membrane, pointing either to the need for a membrane of lower permeability or to marked heterogeneity in the specimen examined. This provides a useful criterion of the validity of the results obtained. Other valuable criteria are here enumerated : (1) The constancy of the equilibrium pressure of a given solution when kept at constant temperature in the osmometer over a period of days or even weeks. (2) The absence of dissolved matter in the solvent chamber at the end of a series of measurements. (3) Reproducibility of results when measurements are repeated in independent experiments and with membranes of lower permeability. (4) Reproducibility of results when passing from a low concentration to a higher one and *vice versa*.

*The Membrane.*—Although denitrated collodion membranes have sometimes been used with organic solvents (Duclaux and Wolleman, *Compt. rend.*, 1911, **152**, 1580; Büchner and Samwel, *Trans. Faraday Soc.*, 1933, **29**, 32), we found that the control of permeability exercised in the production of the collodion membrane was destroyed during denitration, and that, although there appeared to be no difficulty in obtaining cellulose membranes semipermeable towards cellulose derivatives, smaller particles such as methylated inulin would pass through the membrane, even though a collodion membrane of zero permeability formed the starting point.

Cellulose film prepared by the viscose process is obtainable commercially in sheets of uniform characteristics, and McBain and his co-workers (*e.g.*, *Trans. Faraday Soc.*, 1930, **26**, 157) have controlled its porosity in ultrafiltration experiments. Dry " Viscacelle " or " Cellophane " (the non-moisture proof variety) swells up in water to a definite maximum, being now quite permeable to water and ordinary solutions. On the other hand, alcohol and organic liquids exert little or no swelling effect. Hence, any degree of swelling between these two extremes may be attained by treating the dry membrane with the appropriate alcohol–water mixture as the swelling medium. After transference to absolute alcohol, the membrane may be used

with any miscible organic liquid, the swelling remaining practically unaltered. A membrane prepared by immersion in a mixture of $x$ c.c. of water and $(100 - x)$ c.c. of absolute alcohol is described as a "$(100 - x)/x$ membrane." We may consider the sequence of operations in preparing a 50/50 membrane for use in chloroform. Discs of suitable size are cut from the "Viscacelle" sheet with a sharp cork borer, the sheet not being handled with the fingers, and placed in:

FIG. 2.




*Permeability of "Viscacelle 600" to chloroform.*

Bath I. A 60/40 alcohol–water mixture for 1 hour to remove the small amount of glycerol incorporated in the commercial sheet.

Bath II. A similar mixture containing in addition 1% of ammonia to remove traces of fatty acids. After a few hours' immersion the discs are transferred to

Bath III. The final swelling mixture of 50/50 alcohol–water in which the discs are left overnight.

Bath IV. This consists of absolute alcohol to which the discs are transferred through increasing concentrations of alcohol and allowed to remain for 4 hours or more, to ensure complete removal of water from the pores.

Bath V. The organic solvent—chloroform. Care is necessary during the transference to this bath to avoid evaporation of alcohol, as this would reduce the permeability. This is also very liable to occur with a volatile liquid, and in solvents denser than "Viscacelle" the discs must be kept immersed by a gauze cage.

Care has similarly to be exercised when fitting the membrane into the apparatus, the original permeability being rapidly reduced by evaporation of solvent from its pores. The effect of the different alcohol–water mixtures on the resulting permeability to chloroform of No. 600 "Viscacelle" is shown in Fig. 2. The No. 600 quality, by virtue of its greater strength and freedom from ripples, is preferable to the No. 300 for general use.

The permeability of a membrane is expressed in terms of specific permeability $= Dd\eta$, where $D$ is the permeability in c.c. per second for 1 cm.$^2$ of membrane surface at an applied pressure of 1 cm. of water, $d$ is the membrane thickness in cm., and $\eta$ the viscosity of the liquid (Bjerrum and Manegold, *Kolloid-Z.*, 1927, **42**, 97). A specimen of "Viscacelle" of thickness No. 600 was found to swell up to slightly more than double its dry thickness on immersion in water, and to have a specific permeability to water of $2{\cdot}0 \times 10^{-12}$. A sample of No. 300 (half the thickness) had a specific permeability of only half this value.

The authors thank the Department of Scientific and Industrial Research for a grant to one of them (B. R. R.)

UNIVERSITY OF BIRMINGHAM, EDGBASTON. [*Received, March 8th*, 1939.]