THE value of ultrafiltration as a research weapon is indicated by its increasing use in biological science for separating colloid-free filtrates from colloidal solutions. The modern tendency, following Bechhold [1907], is towards the use of metallic apparatus embodying flat membranes through which filtration is effected under high pressure. For many purposes such technique is invaluable provided due attention is paid to the fundamental principles underlying ultrafiltration [see Augsberger, 1925; Elford, 1933]. However, when ultrafiltrates are required for quantitative analysis, ultrafiltration through collodion tubes at rather low pressures (about 100 mm. Hg) is often to be preferred for the following reasons.

It is not to be expected that ultrafiltration of biological fluids under several atmospheres pressure will yield filtrates typical of living processes—as witness the significantly higher values for ultrafiltrable calcium obtained by Nicholas [1932] who ultrafiltered blood-serum under 10 atmospheres pressure, compared with those reported by numerous investigators using much lower pressures.

An unavoidable defect of high pressure ultrafilters is that the solution being filtered must come into contact with metallic surfaces and usually with rubber-fibre gaskets. Further, metallic ultrafilters are costly to construct.

The technique to be described was primarily devised for measuring ultrafiltrable blood-serum-calcium though it may be adapted to other similar purposes. For the reasons indicated above it involves filtration under 120 mm. Hg pressure through collodion tubes of standard permeability.

**Experimental.**

*Ultrafiltration apparatus.* One unit of the all-glass apparatus is illustrated in Fig. 1. The filtration head carrying a collodion tube of about 10-5 cc. capacity fits into the receiver by means of a standard ground joint. The collodion tubes are made of such a diameter that they will slide nicely over the widened end of the tube A. They can be made closely to adhere to the latter by carefully heating round the groove over a micro-flame. A binding of thin twine gives added security to the joint.

Filtration under excess positive pressure (120 mm. Hg) is preferred to the use of suction as recommended by Greenberg and Gunther [1930] as the risk of concentration of the filtrate by evaporation is thereby minimised. For this purpose nitrogen at a known constant pressure is supplied to tube A (Fig. 1) by means of the apparatus described by Folley and Peskett [1933]. As a further precaution against evaporation of the filtrate the side-tube B is connected to a wash-bottle containing a liquid approximately isotonic with the ultrafiltrate.
A battery of six identical units connected in parallel to the pressure supply and wash-bottle is in use at the present time.

Procedure. A collodion tube taken from store under ice-cold water is tied to a filtration-head and the last traces of water removed from its interior by means of a blunt-tipped pipette introduced through A. These operations must be done as rapidly as possible so that the membrane does not dry. The latter is thrice washed out with 3 cc. portions of the fluid to be filtered the last traces remaining from the third washing being removed with the special pipette as before. Violent shaking during washing is avoided so as to minimise frothing. Then 10 cc. of the solution to be filtered are measured into the membrane, the outside of the latter dried with filter-paper and gas pressure applied to A. After 10 minutes the liquid which appears on the outside of the membrane (mainly water expressed from its pores) is removed by filter-paper and collection of the filtrate in a dry receiver begun.

![Fig. 1. All-glass ultrafiltration apparatus.](image)

In determinations of ultrafiltrable calcium in blood-serum, filtration is allowed to proceed for $2\frac{1}{2}$ hours by which time, with the grade of membrane used, about 2-3 cc. of filtrate are obtained. For analysis 2 cc. are required, the remainder being used to test for absence of protein. Analyses of ultrafiltrates obtained by careful use of this technique show good agreement between duplicates, as the following results indicate. The numbers of cc. of $N/200$ KMnO$_4$ required to oxidise the oxalate, obtained by the Clark and Collip [1925] method from 2 cc. of each of four ultrafiltrates of a sample of bovine blood-serum, were 1-20, 1-21, 1-21 and 1-09 respectively. The titres for three ultrafiltrates of another bovine blood-serum were 1-26, 1-16 and 1-26 cc.

Preparation of collodion tubes. Factors which influence the permeability of collodion membranes include the nature and concentration of the collodion solution, its water content and the time for which evaporation of solvents from the hardening film is allowed to proceed. In quantitative work the importance of using membranes with standard properties needs no emphasis and since the usual technique of making collodion membranes allows little control of the aforementioned factors the following standard method of preparation was devised. In some respects it is similar to a method described by Pierce [1927].
A clean and dry test-tube ground flat at the top is filled with collodion and at once covered by a glass square. When the solution is free from bubbles the tube is inserted into the glass evaporation chamber shown in Fig. 2. It is held in the position shown in Fig. 2 by means of a suitable holder made from wire. The tube is uncovered and the evaporation chamber closed by inserting a rubber bung carrying a metal funnel closed at the bottom by a rubber sleeve and spring-clip. The membrane is cast by rapidly inverting the apparatus and rotating slowly in the hands at a suitable angle for precisely one minute, after which it is clamped in the inverted position, the collodion which has collected in the funnel run off and a metal tube pushed through the stem of the funnel into the position shown in Fig. 3. It is important that the funnel and tube be made of metal since glass components frequently break. The rubber connections shown in Fig. 3 are rapidly made, and at the end of 2 minutes from the start of the casting the taps are opened so that a stream of dry air is drawn through the apparatus and flows over the collodion film. The air is dried by passage through a train consisting of two sulphuric acid wash-bottles, two soda-lime tubes and two calcium chloride tubes. The efficiency of the drying train was tested by placing a weighing bottle containing sulphuric acid in the evaporation chamber, while the air stream was passed for two hours. The sulphuric acid did not appreciably increase in weight during this time.

Variations in the rate of flow are eliminated by constant manipulation of the fine-adjustment valve (Fig. 3), which consists of a large screw-clip with a long handle and fitted with springs to prevent backlash, in such a way that the flow-meter reading remains steady. After an accurately measured period of time the air-flow is cut off, the tube containing the membrane immersed in distilled water, the membrane extracted from the tube and washed in running distilled water for some 24 hours.

Collodion tubes of varying permeability may be made by utilising different evaporation periods or air-flow velocities. For the purpose mentioned above these values are so chosen that the membranes are quite protein-tight and strong enough to withstand the ultrafiltration pressure while giving a satisfactory filtration rate. The membranes referred to in an earlier section are prepared by evaporating at 22° for 15 minutes with an air flow of 13 litres per hour.

The ether-alcohol nitrocellulose solution "Necol" supplied in \( \frac{1}{2} \) lb. cans by Nobel Industries Ltd. has proved satisfactory for making both protein-tight and protein-passing membranes. The dilution used is given below, the whole contents
of each can being diluted immediately it is opened. In making membranes the excess collodion must not be used again.

<table>
<thead>
<tr>
<th>Colloidal</th>
<th>Ether</th>
<th>Alcohol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Necol</td>
<td>...</td>
<td>40 g.</td>
</tr>
<tr>
<td>Dry ether</td>
<td>...</td>
<td>12 cc.</td>
</tr>
<tr>
<td>Dry alcohol</td>
<td>...</td>
<td>8 cc.</td>
</tr>
</tbody>
</table>

Membrane permeability. Information as to the reproducible nature of the membranes can be obtained by comparison of the rates of flow of water through a given area under given conditions of temperature and pressure. These measurements are made by means of the apparatus shown in Fig. 4, the necessary flat membranes being obtained by opening up collodion tubes with scissors. The apparatus when assembled is connected to the constant pressure nitrogen supply and the flow of water along the 1 cc. pipette timed with a stop watch.

It is of interest to note here that if these measurements are made on membranes unsupported by metal gauze, the velocity of water flow shows a gradual decrease with time, finally becoming constant. This is partly due to a slow reversible stretching of the membrane since when the latter is mechanically supported the phenomenon is not so pronounced.

The following rates of flow were observed for membranes selected at random from two batches made on different days:

Temperature 21°. Pressure 50 mm. Hg.
Constant membrane area (approx. 6.2 cm.²).
Flow 0.46, 0.50, 0.41, 0.46, 0.43, 0.41 mm.³ per sec.

Keeping in mind the conditions under which these measurements were made, these results show that membranes of reproducible permeability can be made by the method described.

Summary.

A technique has been evolved whereby collodion tubes of standard and reproducible permeability can be made. Details are given of a method of low pressure ultrafiltration making use of these membranes. The method has been used to determine ultrafiltrable calcium in bovine blood-serum.

I wish to express my thanks to Mr G. L. Peskett for helpful suggestions.

REFERENCES.

Folley and Peskett (1933). *J. Physiol.* 78, 13 P.