XXII. "PLATE" FORMS OF ULTRAFILTRATION APPARATUS.

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The use of ultrafilters as accepted aids to investigation leads naturally to more practical forms of ultrafiltration apparatus than that in which observations of their properties were originally made. For particular experiments special designs will always be necessary: nevertheless, as an attempt at sound constructions for general laboratory use, which will doubtless be improved upon in the light of wider experience, I put forward those illustrated below. The larger pattern was briefly described in a previous communication in which details of a method of making suitable ultrafilters were also given [Walpole, 1915].

12" APPARATUS—PATTERN A. DIAMETER OF FILTER 10".

Construction of the Ultrafiltration Apparatus proper.

There are two circular metal plates 12" diameter and $\frac{3}{8}$ " thick called the *front plate* and the *back plate* (Fig. 1). These can be bolted together by nine $\frac{3}{8}$ " bolts and three $\frac{3}{8}$ " studs and wing-nuts arranged symmetrically, the former with their centres round an $11\frac{3}{8}$ " circle, and the latter round an $11\frac{5}{16}$ " circle. Between them is a $\frac{3}{16}$ " filter plate built up of two pieces—a ring 11" external and 10" internal diameter, and a 10" circular disc of exactly the same thickness over all and embossed with a pyramidal pattern on both sides. The ring just clears the bolts all round and has three small indentations cut in it enabling it to fit exactly between the three studs. These support the filter plate before the front plate is applied to it, and

ensure that when the films are in position the front plate may be slid on gently with all the bolt holes and the holes *b*, *d*, and *a* (Fig. 2) in perfect register.

In Fig. 2 the two filters themselves are shown in position. They are indicated by the white space between two thin black lines. The metal of the three plates is indicated by cross-hatching and the fluid to be filtered entering the nipple *b* under pressure is represented by dead black. Passing through channels *x* and *y* it spreads all over both filters. The raised "pyramidal pattern" of the filter plate acts as an excellent support for the filters under pressure, and when once the filtrate (not indicated) has passed through the filter it has an unobstructed flow to the edge of the filter plate. Here it can pass through the channel *c'* out of the apparatus. A similar channel *c*, not shown, is at the top of the filter plate and connects to the outside air. The pyramidal pattern filter plate has been used by permission of Messrs S. H. Johnson, the owners of the patent.

It will be noticed that *c*, *c'* are placed in the "median line," as it were, of the apparatus, while *a*, *d*, *b*, in order that the channel they form may not cut into *c*, are placed a little to one side. For convenience of illustration the top half of Fig. 2 is a vertical section through *d*, and the lower half a vertical section through *c'*, the outlet for the filtrate.

The two holes *a*, *b* and another at *a'* are tapped No. 2 B.A. thread. Fitting interchangeably into these three tapped holes are two screwed plugs and a screwed nipple all fitted with small fibre washers.

At *g*, *h*, *j* (Fig. 1) on the front plate, and at similar positions on the back plate, another set of holes is drilled and tapped. These are seldom used, but if, at the end of a filtration, the filter plate is found to adhere to either of the other plates, separation may be immediately and safely effected by screwing brass screws into these holes.

The apparatus may be made of iron varnished and baked, or of aluminium. In the latter case rolled sheet, and not cast metal, must be used, because of the porosity of the latter. Aluminium has many advantages including that of cheapness, for there is a good deal of turning to be done, and aluminium can be cut much more quickly than iron.

To the back plate is attached a bracket, by means of which it may be screwed or clamped to the bench. As a matter of convenience the apparatus is drawn in the position shown. It is used just as easily and just as often in the inverted position which is advantageous for certain operations described later.

Thick-walled rubber tubing of capillary bore is used to lead into the apparatus through the nipple the liquid to be filtered. No wiring is required on such a joint which is perfectly sound up to quite considerable pressures (50–80 lbs. per sq. inch) and may be readily made and unmade. Capillary cycle valve tubing is used to lead off the filtrate through the small nipple at c' , and to admit at c air blowing out of c' the last drops of filtrate.

Method of assembling Apparatus for use.

To facilitate description it will be supposed in the first instance that a filtration is to be performed using two filters, one on each side of the filter plate. The circle of plate glass on which the collodion was poured [see Walpole, 1915, pp. 291 et seq.] is 12" diameter so that the prepared films

Fig. 2.

lying under water are all this size. One is taken, laid on a clean piece of wood or glass and, using the filter plate as template, is cut round with a knife. The trimmed film 11" diameter is put back under water and from the cuttings a piece is quickly taken by means of scissors, taking care not to include any part from the extreme edge where oil containing collodion has been

employed to prevent the film curling off the glass during drying. This piece is dried between hard filter paper without using pressure and weighed, and then dried to constant weight at 100° C. and weighed again. The ratio of these two weights gives w the wetness of the film. The value m , milligrams of dry nitro-cotton per sq. cm., is known from the volume and concentration of the collodion taken and the area of the glass plate over which it was poured.

When the two circular films each 11" diameter are prepared, one is taken and applied carefully to the front face of the *back plate*. As it is wet it is readily manoeuvred into position and then held there by placing the *filter plate* in front of it. As has already been noticed the nipple of the *front plate* is not quite in the median line, so that in order that the continuous channel shown in the top half of Fig. 2 may be formed, care must be taken not to put the *filter plate* in back to front. The second film, thoroughly wet, is now applied to the back face of the *front plate*, the *front plate* slid on to the studs, and the whole apparatus tightened up by screwing up the wing-nuts and bolts seriatim, going round the apparatus several times. Wet films "give" considerably under pressure and the tightening up of the apparatus takes some time. The nipple on the *front plate* and the screw opposite it on the back plate are now removed and a $\frac{3}{32}$ " punch passed through so that two neat circular holes are made in the films. The screw and nipple are replaced, the rubber supply tube connected to the nipple, and filtration may be commenced forthwith. The liquid under pressure set up by the cycle pump and indicated by the gauge is forced through the passage shown in Fig. 2 over both films and the combined filtrates run away through c' . By connecting cycle valve tubing to c' the filtrate may be led to a measuring cylinder or other suitable receiver and it is certain that no small leak of the experimental liquid can pass into the filtrate.

Great trouble is experienced in summer time through flies attaching themselves to the drying collodion, so that a screen of muslin stretched on a wire frame should be provided.

A simple auxiliary device may be mentioned in passing. When say fifty cc. or less are to be handled the pressure bottle shown in Fig. 2 is obviously unsuitable. A piece of stout glass tube drawn out to a nipple at each end is clamped vertically close to the apparatus. The liquid to be forced into the apparatus may now be placed in this tube and the pressure bottle is simply used as a compressed air reservoir and is connected to the top of the tube.

Portion of the filtering area inoperative through presence of air bubbles.

The films are, of course, impervious to air and it may be thought that a certain quantity of filtering surface will not be utilised because the air in the passages will be forced on to the filter. Examination of the films, when the apparatus is taken to pieces after a filtration, shows that owing to the fineness of the passages the proportion of filtering area so rendered inoperative is very small, and may be entirely abolished if care is taken that all the channels are full of liquid before filtration commences. This being so it is a matter of indifference what the position of the apparatus is in use: in some experiments where it was desired to keep the temperature of filtration under control it was immersed horizontally in a large shallow basin: in others the bracket was screwed to the top of the table near the edge with the plates hanging down vertically.

Use of One Film only.

If one film only provides sufficient filtering area on any occasion it is convenient to put it between the *filter plate* and the *front plate*: the position of the other film between the *filter plate* and the *back plate* being occupied by a "dummy," an impervious film cut from rubber insertion. In order that the channel *da* may not be filled unnecessarily the filter plate should be put in back to front—the other channels *c* and *c'* which are symmetrically placed will of course be unaltered in position.

It is frequently desirable to recover the "ultraprecipitate," which is usually a thick gum, when the filtration is over. This may be done either by taking the apparatus to bits or by attaching a receiver to the nipple and aspirating. Washing out may be done directly by running water in and out. Except in so far as the films remain impregnated with the materials filtered off, they are recovered clean in this way, as the subsequent examination of the film plainly shows.

Although from the design of the apparatus it would appear at first sight as if the volume of fluid actually spread over the film at any one time was very small indeed, it is actually considerable, say 15 cc., and depends upon the pressure applied.

The film, of course, sags between the "pyramids" while filtration is in progress, though if the pressure is released, and still more so under suction, any remains of the liquid to be filtered are forced back through the nipple again.

Another and perhaps better way of going about the recovery of the "ultraprecipitate" is to screw in the nipple at a' and transfer the screw shown occupying a' to b . The liquid to be filtered is then pumped in at a' . The wash liquid is passed in and out at this point and b is used simply as an air vent.

Dialysis.

A simple procedure, which is frequently useful, for recovering a material, say a protein from a concentrated salt solution, may now be described by citing an actual example. The material, say a precipitate of crystalline egg albumin wet with ammonium sulphate solution, is to be handled with a view to obtaining the egg albumin in concentrated solution free from electrolyte. We will suppose again that only one filter is to be used. The precipitate is wetted with distilled water until fluid, and filled into a suitable glass tube drawn out at both ends (see page 258). Its lower end is attached to the nipple in b or a' and the liquid forced all over the film by air pressure from the bottle. Tap water and, later, distilled water are run across the filter plate, in at c and out at c' , care being taken to get the air out of the grooves between the pyramids first. Owing to the high osmotic pressure of the ammonium sulphate originally present some of the fluid being dialysed may regurgitate into the tube but as the dialysis and ultrafiltration proceed the ammonium sulphate is carried away. In fact, it is generally possible to regulate the pressure so that the space between the front plate and the filter is just full, *i.e.* the whole of the dialysing surface is operative. The design of the apparatus ensures extremely rapid dialysis for the volume of the liquid under treatment per sq. cm. of dialysing surface is as small as it very well can be made.

When examination of either the liquid being dialysed, or of the other liquid, or of both, shows that removal of the ammonium sulphate is sufficiently complete, then the liquid may be run off the filter plate and increased pressure applied. The egg albumin may, at the conclusion of the resultant ultrafiltration, be recovered as above.

4" APPARATUS—PATTERN B. DIAMETER OF FILTER 3".

For small scale operations, such as experiments on the properties of the individual members of a series of films, or to determine which film of a series is most suited for a particular operation, the simple apparatus (Fig. 3), which may be screwed up in any ordinary vice or clamp, has been found of service.

It takes one film between two pieces of aluminium, one recessed and fitted with a piece of pyramid pattern material to act as a filter plate, and the other left flat. The discs, supported and protected by being backed by pieces of iron, are suitably drilled and tapped to take the nipples, and the edges between which the film is held are ground face to face after being turned true. The perfection of this joint enables even the hardest nitro-cellulose films to be used without any leak at pressures from 50–80 lbs. per sq. inch. The films must, however, be uniform and flat.

Fig. 3.

Separation of chloride from sulphate by dialysis.

I am able to confirm with flat films of nitro-cotton (British Xylonite Co.) Brown's observation [Brown, 1915] made with films of test-tube shape that this separation may be readily carried out. For assistance in obtaining suitable nitro-cotton to work on and ready advice and help in its use I am indebted and grateful to Dr J. N. Goldsmith. The following is the procedure that I have found satisfactory.

157 cc. of collodion (3.5 per cent. dry nitro-cotton in equal parts by volume of alcohol and ether) are poured on to a 12" disc of levelled plate glass in the usual way. To prevent the drying film from curling off the glass the edge is painted round with collodion containing castor oil when half an hour has elapsed. Next day when the film is quite dry it is cut round with a penknife

using an $11\frac{1}{2}$ " circular template. It is found that when perfectly dry it detaches itself readily from the plate—a uniform film which may be preserved indefinitely. Following Brown's directions this must be softened and made of the right degree of permeability by soaking in alcohol of the required strength. If a whole film is so treated the result serves admirably in the 12" apparatus: but the small one does just as well on this occasion, so that from the original dry film four circles may be cut of ample size for this operation. Four trials may thus be made from one full-sized film. As a matter of fact twenty-four hours soaking in dilute alcohol, 50 % by volume, gives a film of the required properties. Such a film allows considerable quantities of chloride but no trace of sulphate to pass by dialysis against distilled water from a solution containing 1 % of sodium chloride and 1 % of sodium sulphate.

There have now been described two methods of preparing flat and uniform *standardised* ultrafilters of nitro-cotton and the question naturally arises as to which method is the more convenient and more reliable.

It is my opinion that where a "soft" filter is required—one, say, for separating an enzyme or a toxin from peptones, and other simpler substances—it is simpler to pour the film in the usual way and to plunge glass and all into water when drying has proceeded to the correct extent. On the other hand, where hard filters are required, and one must be prepared to differentiate, say, between those allowing dextrin to pass and those allowing the passage of sugar but not dextrin, Brown's method is certainly to be recommended.

REFERENCES.

- Brown (1915). *Biochem. J.*, **9**, 591.
Walpole, S. G. (1915). *Biochem. J.*, **9**, 284.