A simple system of multiple organ-baths

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Summary. A compact and easily assembled system of multiple organ-baths is described, in which use is made of readily available materials.

The principle requirement for an organ-bath is that of the maintenance of a tissue in a solution at a constant temperature. Many 'physiological' solutions require to be equilibrated with a gas mixture, usually accomplished by bubbling the solution. The organ-bath must be of size sufficient to accommodate the tissue, along with any auxiliary apparatus required, e.g., for the support or the electrical stimulation of the tissue. Further desirable features include facilities for filling and emptying the bath with ease. A system of multiple organ-baths is described in this article, in which an attempt is made to provide a convenient apparatus without some of the problems associated with glass organ-baths. The advantages and disadvantages of the apparatus are discussed.

The apparatus. The apparatus is based on the use, as organ-baths, of the barrels from disposable plastic syringes. The syringe barrels are affixed to 3-way taps which are, in turn, mounted on a perspex tube. The tube, construction details of which are given in figure 1, also serves to supply gas to a group of baths. The gas flow to each bath is regulated by the 3-way tap and limited by a cut-off syringe needle inserted through a rubber bung at the base of the tap. The apparatus is illustrated in figure 2.

The apparatus, containing, perhaps, 10 baths, can be placed in a thermostatically controlled water-bath.

Discussion. The advantages of the apparatus probably arise from 2 principle features. First, the advantages attributable to the use of plastic syringe barrels and, second, those due to the compact design of the apparatus.

Plastic syringes are readily obtainable, their cost being minimal as compared to glass organ-baths. Moreover, the relative cheapness and availability of syringe barrels facilitates the replacement of baths, necessary, e.g., after toxic contamination or after a temporary modification of bath design. The barrels are obtainable in a large range of sizes and are, in general, calibrated for volume. The standard taper (Luer) fittings of the syringes simplify the assembly and dismantling of the baths. Furthermore, the standardization also allows for a greater versatility in the use of different baths, taps and other Luer-type attachments. The plastic can usually be adapted by the use of heat or a suitable adhesive, in addition to the mechanical working of the material; the lugs on the syringe barrels can provide useful sites for the support of auxiliary apparatus. Thus, the baths may conveniently be adapted for special requirements, unaltered substitutes normally being readily available.

By utilizing the supporting rod as a means of supplying gas to a series of baths, the inconvenience of separate gas-lines is overcome. This feature, together with the use of a single water-bath in the place of separate water-jackets, creates a very compact apparatus; indeed, the present authors have fitted 30 organ-baths into a water-bath measuring 20×40 cm. Furthermore, the use of a single water-bath removes, to a large extent, the temperature gradient normally observed when glass baths are connected in series to a source of heating water. The dispensation with the large number of connecting pipes, otherwise necessary with water-jacketed organ-baths, facilitates the assembly and dismantling of the apparatus. A thorough cleaning of the apparatus can be accomplished in a few min; indeed, if contamination with a toxic drug is suspected, the syringe barrels may be disposed of with relative impunity. The syringe needles at the base of the taps serve both to equate the flow of gas to each organ-bath in a group, and to eliminate or minimize reflux of fluid into the supporting rod when the gas is turned off; they also help to prevent an excessive flow of gas to the baths. The flow of gas to any bath may be stopped by setting the 3-way tap in an appropriate position, and the rate of supply of gas to a group of baths is conveniently controlled by a valve in the gas supply-line.

The disadvantages of the apparatus are, probably, 2fold. First, a contamination of the 'physiological' solution with substances from the plastic of the organ-baths must be considered. Second, the baths may compare unfavourably with glass organ-baths with respect to the process of filling and emptying.

While in contact with the organ-baths, there is the possibility of a contamination of the 'physiological' solution with substances eluting from the plastic. This possible source of contamination is highlighted in the work of Rosseel and Bogaert[2]. However, this problem may be overcome by using washed plastic syringe barrels. 'Brunswick' syringes, as used by the authors, have been shown to be compatible with a large number of solutions and drugs (Sherwood Medical Industries Inc., Research Center, St. Louis [Mis-

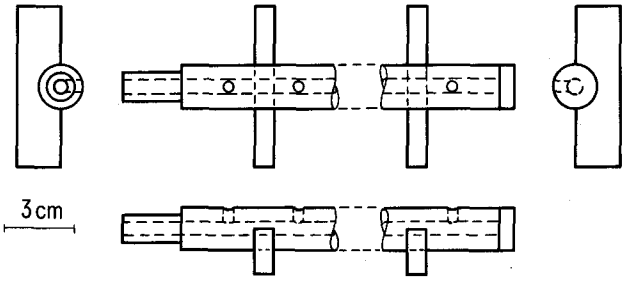

Fig. 1. Construction details of perspex supporting tube (1st angle projection).

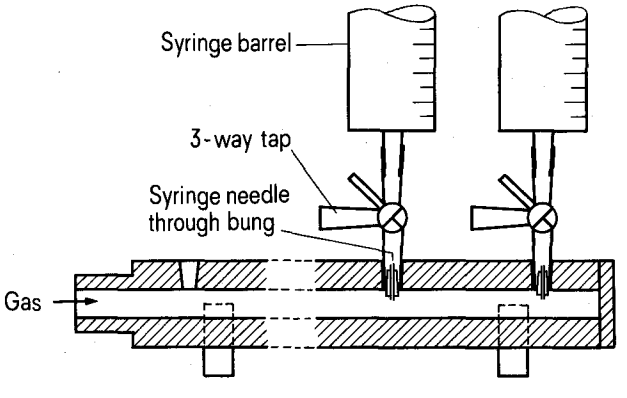

Fig. 2. Detail of the mounting for the organ-baths.

souri, USA]; Personal communication); in addition, of 10 toxic metal ions, only the elution of zinc was detectable when the plastic was autoclaved with water at 121 °C for 1 h, even this being at a very low concentration.

Filling of the organ-baths may be accomplished by passing the 'physiological' solution through a warming coil, using gravity feed; this arrangement is, perhaps, not as convenient as that of the glass organ-bath used in conjunction with a warming column. Similarly, the baths may be emptied by the use of a suction-line.

This paper is not intended to exhaust all the possible examples of form or usage of the apparatus, but rather to outline the principles behind the construction and use of a simple system of multiple organ-baths. Readers are urged to exploit the versatility of the apparatus, adapting it to their own requirements.

1 Acknowledgment. G.E.R. was in receipt of the Ruggles Gates Research Award.

2 M.T. Rosseel and M.G. Bogaert: J. Pharm. Pharmac. *28*, 942 (1976).

Use of arrow-root powder in starch gel electrophoresis

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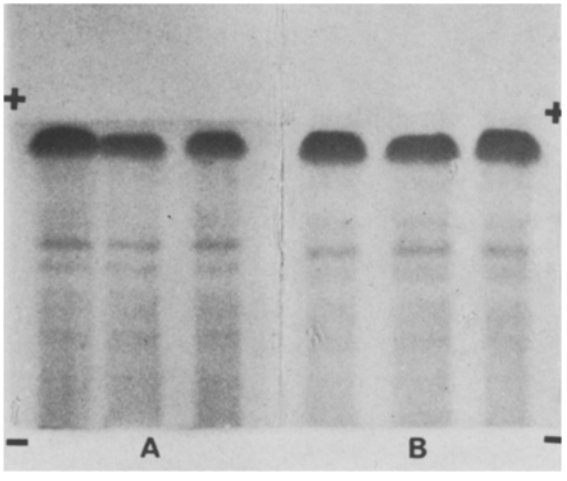
Summary. The arrow-root powder is used in electrophoresis as an alternative to potato starch. Its use is quite economical and this starch can be easily hydrolysed in any laboratory.

Since the discovery of Smithies[2] method for the hydrolysis of starch, potato starch is used frequently in various laboratories for electrophoretic separation of different substances. The introduction of any new gel medium for electrophoresis, must have some justification in view of the general use and satisfactory resolutions of the materials to be separated by this method. The present study presents considerable reasons for the use of arrowroot powder in place of potato starch.

In the present study, as the arrow-root powder is used for the first time, the properties of the gel made of this starch are tested by comparing it several times with the hydrolyzed potato starch supplied by BDH (England) by running human and frog serum[3] samples simultaneously in the 2 gels using same strength of buffers and current.

Materials and method. The commercially available arrow-root powder (Weikfields Ltd) was obtained from the local market and hydrolyzed in the laboratory by a modification of Smithies[2] method as follows.



Electrophoretic pattern of human serum in 2 starch media. *A* Arrow-root starch, *B* potato starch, after starch gel electrophoresis, in Tris (0.076 M) citric acid (0.005 M) buffer pH 8.7 for gel and boric acid (0.3 M) pH 8.6 for electrode vessel using the method of Gordon[4].

The arrow-root powder (200 g) was treated with 400 ml acetone - HCl (100:2) mixture and kept in an incubator at 37 °C for 2 h. The reaction was stopped by taking out the above mixture and adding 100 ml M sodium acetate. The starch was then filtered thoroughly with glass distilled water and finally dehydrated, in a cylinder, with acetone. 3 washings with acetone were found sufficient for complete dehydration. Lastly it was dried in an oven at 55 °C; this took about 48 h. The chemicals used were of analytical grade obtained from BDH.

Results. This starch was found suitable for the preparations of gels of the concentrations ranging from 10 to 20 g/100 ml of any desirable length. A concentration of 11% was found most suitable. It could be easily handled, sliced and stained with suitable dye. As shown in the figure to compare the properties of 2 gels, the human sera was separated into different fractions by running 3 samples in each gel kept parallel to each other using the same quantity of serum and keeping exactly similar strength of buffers and gels in the same tank. The results obtained were fairly comparable to each other. No difference could be observed in the pattern of electrophoretic separation. The experiments were repeated several times with different concentrations of gel; the results obtained were always identical.

Discussion. The new starch used for the separation of various protein fractions gives reasonably accurate results. These results are in good agreement with those obtained by Smithies hydrolyzed potato starch. In some cases the resolutions are better in arrow-root starch than in potato starch. Furthermore this arrow-root starch can be easily handled, and in some respects it is superior in certain properties to potato starch. This can be easily hydrolyzed in any laboratory.

The arrow-root starch can easily be adapted as an alternative to potato starch, and its use would cut down the cost several times.

1 The author is indebted to Dr M.M. Goil, Prof. & Head of the Department and Dr R.K. Sharma, Professor of Zoology, for their invaluable suggestions and help in various ways.

2 O. Smithies, Biochem. J. *61*, 629 (1955).
3 K. Singh, Zool. Anz. *197*, 39 (1976).
4 A.H. Gordon, Electrophoresis of proteins in polyacrylamide and starch gels. North-Holland Publ. Co., Amsterdam 1969.