A NEW ELECTROPHORESIS APPARATUS

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(Received September 11th, 1961)

INTRODUCTION

Of the various types of apparatus that have been devised for filter paper electrophoresis the enclosed strip method is probably the most versatile. The method was originally described by KUNKEL AND TISELIUS[1]. Their apparatus consisted of a filter paper strip enclosed between glass plates, the ends of the paper strip projecting beyond the ends of the glass plate and dipping into the electrode chambers. The glass plates were clamped together at several points. FOSTER[2] modified this apparatus by clamping the plates together and on to a metal cooling plate through which water flowed, thus effectively preventing evaporation and permitting the use of relatively high potential gradients.

In FOSTER's apparatus the glass sheets are clamped together with laboratory clamps, but experience with this method has shown that pressure gradients are produced across and along the paper. The pressure applied to the glass plates is unknown and no doubt varies from experiment to experiment. Irregular distribution of pressure results in local variations of buffer content in the paper and consequently compounds migrate in an irregular pattern so introducing unknown and sometimes considerable errors into determinations of mobilities. KUNKEL[3] has discussed the need to have an even distribution of pressure on the paper in order to reproduce exactly the same pattern from one experiment to another. He has described an apparatus in which the pressure is applied at 4 points to the top glass plate, the glass plates being 2 1/2 cm in thickness.

To achieve reproducible electrophoretic analyses it is necessary to have reproducible and uniform pressure conditions. The apparatus described below provides these and the design is simplified.

DESIGN PRINCIPLES

The requirement of a uniform pressure over the paper is met most logically by employing pneumatic or hydraulic means. An inflatable elastic bladder large enough to cover the paper is therefore used. A property of such a bladder is that it will communicate an equal pressure to all surfaces used to restrain it. By placing the paper directly between the bladder and the restraining surface use is made of this property and it eliminates the need for the surface machining of any components.

The use of an air or liquid medium to apply the pressure suggested that simplification would result if the same fluid were used for cooling the paper. Accordingly air or water at the appropriate pressure is allowed to flow through the bladder, the only barrier to heat removal being the thin wall of the bladder. The method of supplying air or water to the bladder allows the pressure to be readily reproduced or varied. The paper remains visible at all times because it is above the bladder and glass is used as the restraining surface.

CONSTRUCTIONAL DETAILS

Fig. 1 shows the general layout of the apparatus. The rigid brass body is 17 in. × 8 3/4 in. × 1 7/8 in. overall and has its top hinged on a 5/16 in. steel pin at the rear. After the paper and glass are placed in position the assembly is held together by a

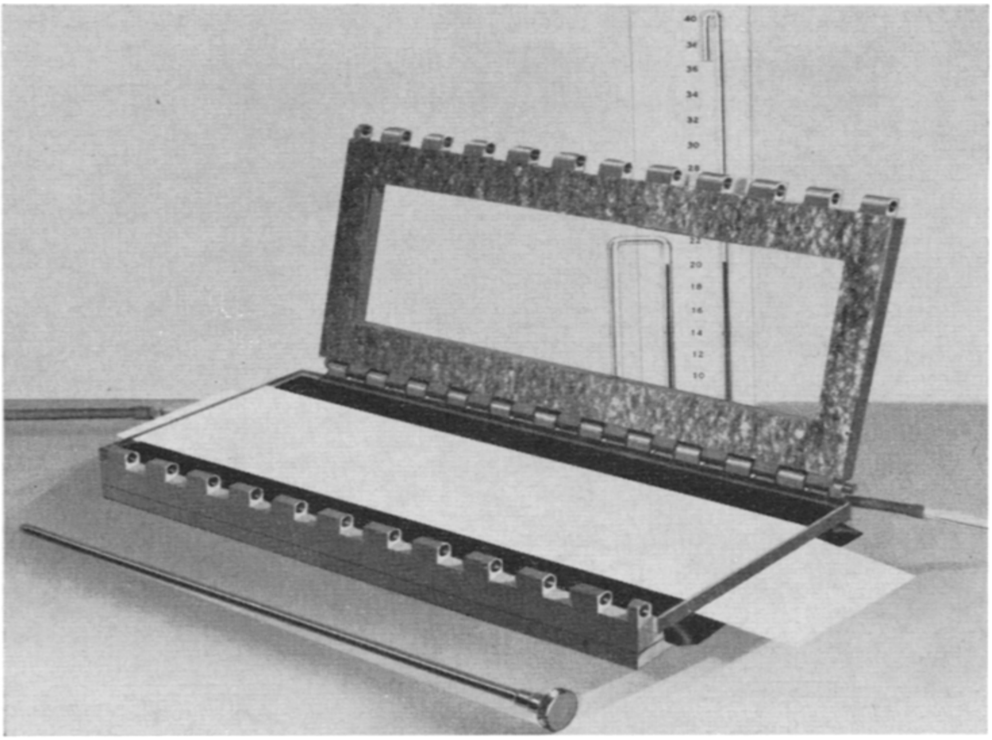

Fig. 1. General view of apparatus for paper electrophoresis.

5/16 in. steel locking pin in front. The apparatus is designed for 5 in. wide paper and the aperture in the top plate is 15 in. × 4 3/4 in. The 1/2 in. thick glass plate and the rubber bladder which are 18 in. long project 1/2 in. beyond the end of the frame and provide this amount of insulation between the paper and the frame. For safety the brass frame is earthed.

The bladder is made from two sheets of rubber 18 in. × 7 1/2 in. and 1/32 in. thick. They are vulcanised together for 1/2 in. at the edges to give a free inflatable area of 17 in. × 6 1/2 in., the surplus width preventing any possibility of pressure falling off at the edges of the paper. Three lengths of 3/16 in. internal diameter rubber tubing are vulcanised into the rear edge of the bladder and pass out through slots in the back of the brass frame. A tube is situated at each end of the bladder and these serve as inlet and outlet for the cooling and pressurising fluid. The third tube is in the centre and is connected to a manometer. The inlet and outlet tubes together constitute the main restriction to flow in the system as wider bore tubes are used elsewhere; for this reason these two tubes are kept as short as possible. The manometer is connected directly to the bladder so that the true pressure inside the bladder is

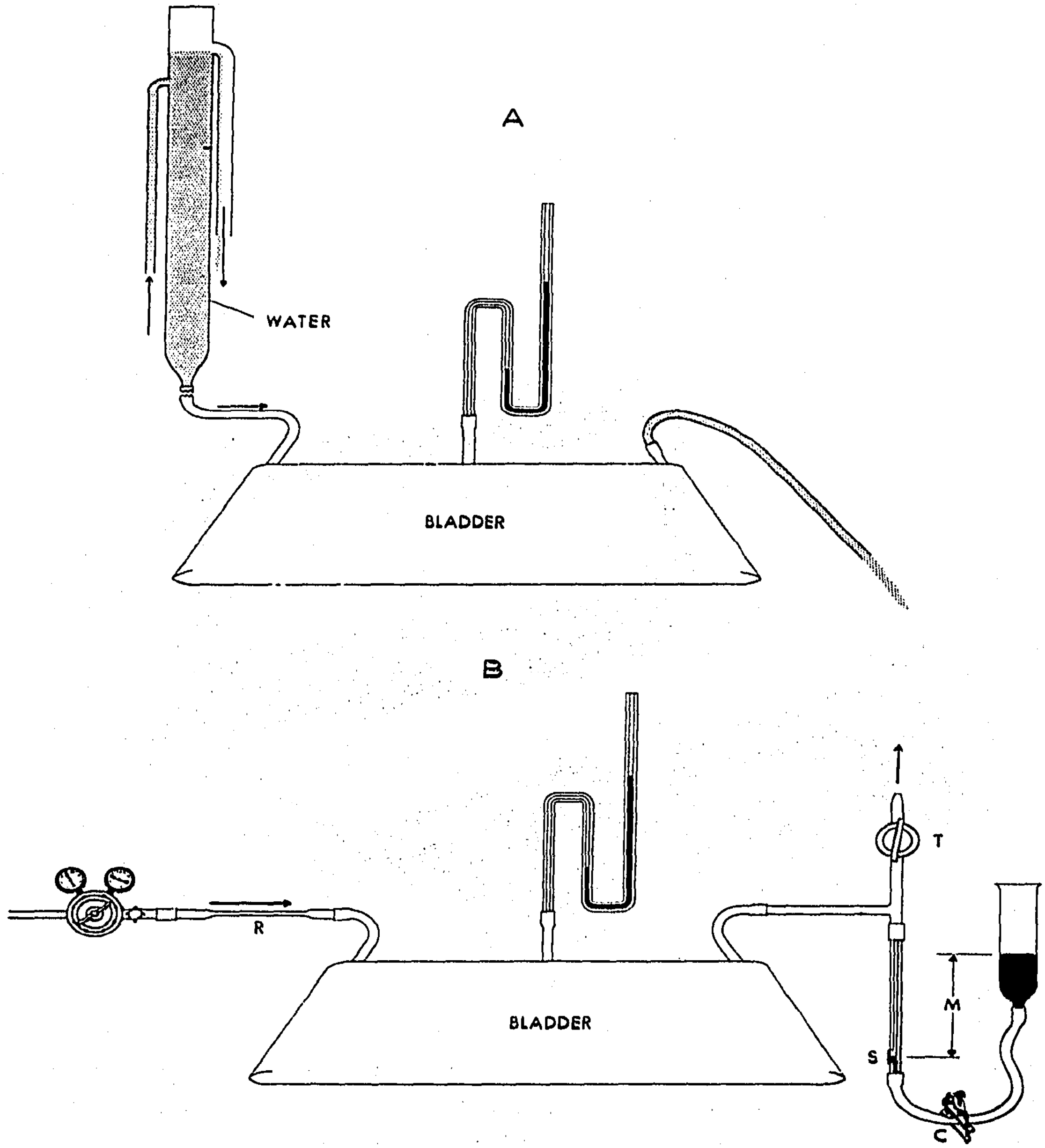

Fig. 2. Arrangements for cooling and for maintaining pressure in the new apparatus. A, with water. B, with air.

measured and errors due to flow restriction are avoided. The bladder occupies a 7 1/2 in. wide and 3/8 in. deep channel at the bottom of the frame. Above this the width increases to 7 13/16 in. and the two ledges so formed carry the 7 3/4 in. glass plate when the bladder is deflated. The underside of the hinged top plates has cemented to it a 1/32 in. thick sheet of asbestos which reduces the possibility of temperature gradients from the centre to the edge of the glass.

The methods of maintaining the desired pressure and providing a flow of fluid for cooling purposes depend on whether a liquid or a gas is used. With water a simple constant head device (see Fig. 2A) is satisfactory. This system lacks compactness but we have been unable to find a suitable pressure regulating valve to replace it. We have generally used water as this provides very high rates of cooling. For some purposes air or other gases may be preferred, and the device (see Fig. 2B), based on the principle described by JAMES[4] is used. Compressed air (or cylinder gas) is supplied to the bladder B through a pressure reducing valve and a capillary smoothing choke R. The outlet from the bladder is connected to the device. So long as the flow rate of air into the bladder is greater than that escaping to the atmosphere through tap T the column of mercury will be depressed and air will blow off through the sintered glass block S. Providing the lower end of the column of mercury partly uncovers the sintered block a constant pressure will be maintained in the system. The pressure and flow rate are determined by the height of the column of mercury M, the adjustment of tap T and the setting of the pressure reducing valve. In practice the mercury oscillates and the oscillations may be reduced by constricting the tube with a screw clip C as shown in the figure.

DISCUSSION

This apparatus appears to be unique in that the rubber bladder is used not only to apply the pressure to the filter paper but also as the means by which cooling is effected. If the pressure is maintained by a stream of gas the rate of cooling is sufficient for use with a potential difference of 1000 V (*i.e.* 20 V/cm) and a current of 2–3 mA. For higher currents it is necessary to use a stream of water to provide the pressure and to cool the paper. The rate of cooling, when water is used, is greater than that obtained by clamping a water-cooled metal plate to a 1/4 in. glass plate as in the apparatus described by FOSTER[2]. Since the rubber bladder is separated from the glass plate only by the filter paper, both it and the filter paper take on the shape of the glass surface. A uniform pressure is therefore applied over the entire surface of contact. Rubber, being more flexible, was found to be superior to plastic for the construction of the bladder as it gave better contact with the surface of the paper and the glass.

The uniformity of the pressure over the entire paper strip can be shown by the use of dyes. Electrophoretic runs were carried out using an aqueous solution of eosin and as buffer 0.1 *N* aqueous ammonia. A number of spots of the dye were placed across the paper strip and the dye in each spot travelled in a straight line parallel to the edges of the paper strip. That the rate of cooling was adequate was shown by the constancy of the current. The pressure applied can be readily determined from the manometer and can be accurately reproduced. The dielectric strength of the sheet rubber used in preparing the bladder provides a large margin of safety. The rubber bladder has not been affected by any of the buffer systems normally used in paper electrophoresis but should such a risk arise the rubber may be protected by a very thin sheet of suitable plastic.

A glass plate has been retained as the upper restraining surface of the paper because the procedure described by FOSTER[2] has in general been followed. This procedure has the advantage that the zones of the substances to be studied are concentrated on the origin line. In addition the movement of coloured compounds may be directly observed. Should visibility not be required then the apparatus could be modified by omission of the glass plate, reduction of the overall height by 1/2 in. and the use of a hinged restraining surface made of a solid plate of metal of reasonable flatness. If this solid plate be water cooled much higher potentials can be used. A sheet of insulating material, for example, polythene would have to be interposed between the metal surface and the filter paper.

In high voltage electrophoretic apparatus the filter paper strip, which is covered on both sides by polythene sheets, is held between two rigid metal cooling plates. Pressure is applied to one plate by an inflatable rubber or plastic bladder (GROSS[5] and commercially available instruments). This arrangement neglects to take advantage of the absolute uniformity of pressure inherent in pneumatic and hydraulic systems. Attempts to retrieve the situation are made by machining the surface of the metal plates and by arranging for one of the plates to float. Such attempts can only be partly successful. The design principles described in this paper overcome all these problems and, in addition, accommodate any lack of uniformity in the thickness of the filter paper and of any plastic sheets that may be included. A further advantage of these principles is that the pressure can be pre-set. This overcomes the risk of squeezing liquid from the paper by temporarily exceeding the working pressure during the inflation of the bladder.

ACKNOWLEDGEMENTS

The authors wish to acknowledge the assistance given by Mr. J. B. MCCABE during the development of this apparatus.

SUMMARY

An apparatus for paper electrophoretic analysis is described in which a uniform pressure is applied to the paper in a very simple manner. The pressure is applied by hydraulic or pneumatic means and can be readily and accurately reproduced.

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