J. clin. Path. (1952), 5, 294.

A DIRECT SCANNING APPARATUS FOR READING ELECTROPHORETIC PAPER STRIPS

BY

L. L. GRIFFITHS

From the Department of Pathology, Farnborough Hospital, Kent

(RECEIVED FOR PUBLICATION APRIL 16, 1952)

Since the publication of papers on the electrophoresis of serum proteins in filter paper strips by Cremer and Tiselius (1950), Durrum (1950), Turba and Enenkel (1950), and Flynn and de Mayo (1951), several attempts have been made to simplify the technique by the elimination of the tedious elution and subsequent estimation of the protein fractions. Grassmann, Hannig, and Knedel (1951) rendered the strip translucent with anisol and it was then read in the special apparatus described by Knedel (1951). This is unobtainable in Britain and the method of construction results in a graph of small dimensions which is difficult to analyse. A much simpler apparatus was devised, which, while accurate, had the disadvantage that it was not permanent, but utilized apparatus already in the laboratory without interfering with its use for the original purpose for which it was designed. In a bigger laboratory where more estimations are carried out the need was felt for a more elaborate apparatus, and the one to be described was constructed with this end in view.

The apparatus consists of a wooden box 23 in. long and 19 in. wide and approximately 4 in. high. The top is made of plywood and is covered with a sheet of perspex. In these is cut a slot ½ in. wide and 12 in. long. One inch from the end of the slot is an oblong opening of such a shape as to take the mounting of an EEL 37 × 50 mm. barrier layer photoelectric cell. A second piece of perspex, half the size of the top, is now cut. This has only the long slot cut in it to correspond with the slot in the top of the box. A sheet of cardboard is cut to the same shape and in addition a slit 2 in. long and 1/10 in. wide is also cut out so as to be over the position of the photoelectric cell. Two paper rulers are pasted on the edges of the cardboard, and the exposed cardboard painted black. The smaller piece of perspex is placed over the card and the two bolted to the top of the box so that the three long slots coincide. Two narrow pieces of perspex are cemented to the top 3 in. apart so as to act as guide rails to the glass strip carrier. The lamp housing consists of a perspex box containing a 25 watt 250 volt lamp with a straight slot 2 in. long and ½ in. wide cut in the centre with its long axis at right angles to the guide rails. This opening is filled with a piece of ½-in. perspex rod cut lengthways so as to form a cylindrical lens. The opacity left by the saw can be easily removed by painting the cut surface with acetone or perspex cement. Between the lamp and the lens is mounted a 2 × 2 in. glass filter (Chance yellow OY).

The lamp house is mounted over the 1/10 in. slit so that the cylindrical lens projects a beam of light through it and on to the photoelectric cell. The house should be raised sufficiently so that the bottom of the lens just touches the glass carrier strips.

A single pole, single throw switch, a 20,000 ohm potentiometer and an EEL galvanometer with a logarithmic transmission scale, are mounted in convenient places on the top of the box. Under the box and parallel with the centre of the ½ in. slot runs a ¼ in. Whitworth screw 25 in. long, which has a pitch of 0.0200 in. This is held in position by suitable drilled strips of metal. The threaded rod projects for 2 in. through one of the sides and to this is fixed a small handle. A suitable brass nut is soldered to a metal strip which in turn is bolted to a perspex square, the top of which is cut to such a shape that ½ in. projects through the long slot in the top of the box. One of the outer contacts of the potentiometer is wired to the negative terminals of the photoelectric cell and the galvanometer. The other outer contact goes to the positive terminal of the meter. The centre contact of the potentiometer is connected to the positive terminal of the cell. The switch is wired across the two terminals of the metre and when shorted protects the movement. By making the connexions in this manner, a constant resistance is placed across the coil of the meter which therefore does not vary its sensitivity with each setting of the resistance.

The stained electrophoretic strip is rendered translucent and is placed between two glass strips which are 3 × 12 in. These are kept in apposition by "cellotape" and the glass is placed between the guide rails. A portion of the paper strip which has no protein bands is placed in front of the slit and the galvanometer adjusted to 100% transmission or zero by aid of the 20k potentiometer. Two revolutions of the handle move the paper strip 1/10 in. The deflections of the meter are recorded for each movement of 1/10 in. The graph is then plotted and measured by the usual technique. Similar scanners have been constructed with different screw-threads and consequently different slits. In all, slits of 1/32, 1/16, 1/20, 1/10, and 1/12 in. have been used. No advantage has been found from using a very narrow slit. 1/16 in. was found to give an excellent graph which was easy to analyse and to measure but suffered from the slight disadvantage that the size of the graph does not correspond with the separation of the bands when plotted on the usual 1/10 paper. With a 1/10 in. slit the length to be scanned as measured on the built-in rulers corresponds exactly with the graph, and this size of slit has been adopted as standard in this laboratory.

Figs. 1*a* and 1*b* show the construction. None of the dimensions is critical except that the size of the slit must be an exact multiple of the pitch of the screw.

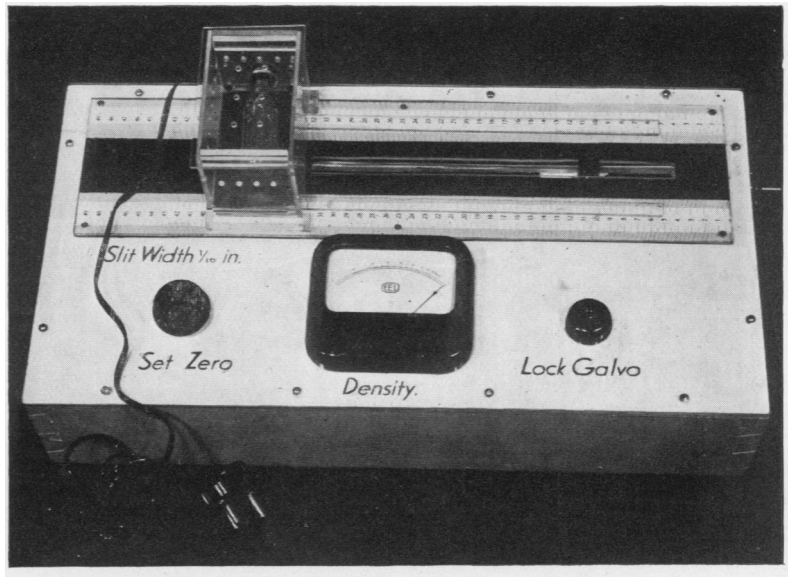

FIG. 1*a*

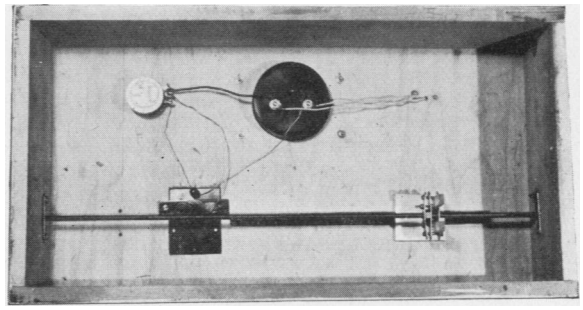

FIG. 1*b*

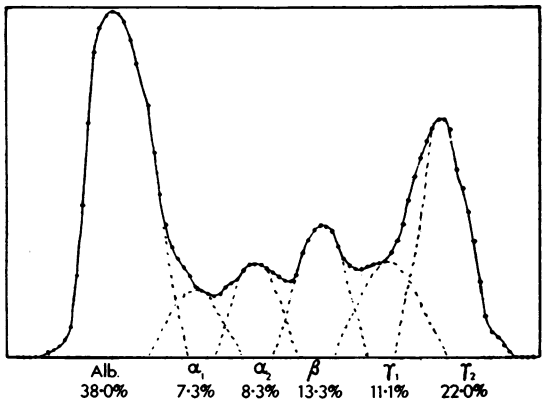

FIG. 2

Fig. 2 is an example of the scanning of a typical strip from a case with increased gamma globulins.

With this apparatus one person can record and plot 100 to 150 points in 10 minutes, and it can be operated by a relatively unskilled worker with ease and accuracy.

My thanks are due to Mr. E. G. Poynter for the three photographs.

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