Continuous Electrophoresis on Paper.

By G. DICASTRO and M. SAN MARCO.

[Reprint Order No. 5421.]

A new apparatus for continuous paper electrophoresis is described. With this apparatus it is possible to obtain a considerable separation of the components of mixtures.

PAPER electrophoresis has made rapid progress on account of its simplicity. Recently Tiselius and Flodin (*Adv. Protein Chem.*, 1953, **8**, 461) summed up its principles and applications. On the other hand continuous paper electrophoresis did not make the same progress, although it permits not only the separation of a mixture into its various components according to their electrochemical properties, but also their separate collection. We believe that this failure to make progress was caused by the fact that with no proposed apparatus is it possible to reproduce strictly the experimental conditions. The simplest apparatus is Durrum's (*J. Amer. Chem. Soc.*, 1951, **73**, 4875) but in this the applied potential difference does not produce a uniform electric field because of the position of the electrodes in relation to the paper, the field being stronger in the lower part of the sheet. In this case the density of electric charge is not proportional to the applied potential difference.

Grassmann and Hannig (*Z. physiol. Chem.*, 1953, **292**, 32) applied the two electrodes along the two lateral margins of the sheet. In this way the electric field is uniform, but the products from the electrolysis of the buffer solution diffuse directly on the paper causing pH variations which alter the experimental conditions. It is useless to wash the margins of the sheet where the electrodes are applied with a larger amount of buffer solution to remove these products. Svensson and Brattsten (*Arkiv Kemi*, 1949, **1**, 401) washed the respective electrode zones with a basic and an acid solution to neutralise the electrolysis products but it is impossible to regulate the washing of these zones with exactly the calculated amounts of the solutions. However, with this aplication of the two electrodes it is possible to obtain a better separation of the various components because of the

uniformity of the electric field which causes the substances to take a straight path from the start. It is possible to determine, as a characteristic of every substance, the angle of its deviation, the tangent of which is the ratio between the velocities due to the electric field and to the gravitational falling of the solution.

For continuous paper electrophoresis we used a new apparatus, with which it is possible to obtain a high uniformity of the electric field over most of the paper, and to retain the constancy of the pH and of the ionic concentration all over the sheet, so producing the greatest deviation of the substances.

Experimental

We used a sheet of paper with three strips at each side (Fig. 1). Each strip rests in the buffer contained in a Perspex cell through which there is a continuous flow of the buffer solution.

Each cell (50 ml.) is divided into three compartments by two Perspex plates. The buffer solution is compelled to flow under the first plate (*A*, Fig. 2) to reach the electrode placed on the

Fig. 1. *Complete apparatus.*

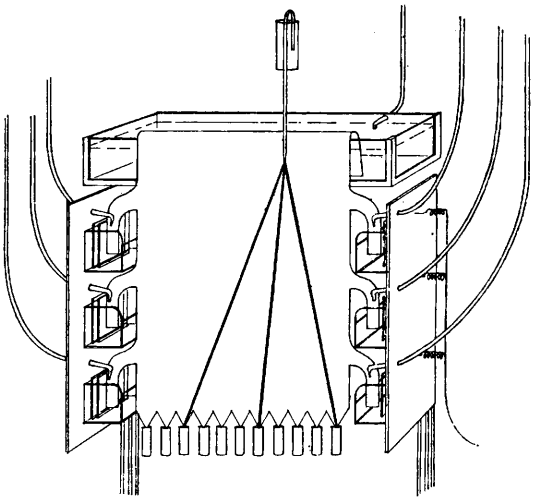
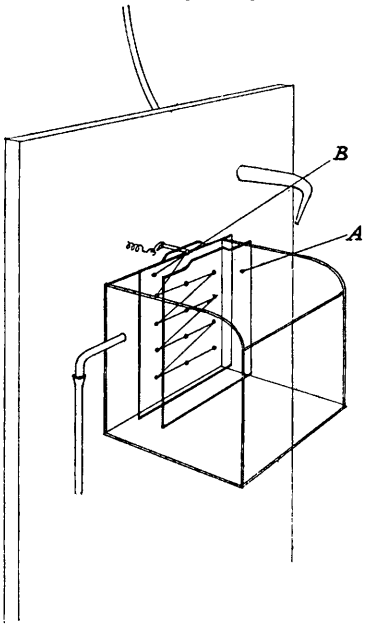


Fig. 2. *Detail of one of the cells.*



second plate (*B*). The three electrodes of the three cells on each side are joined to one of the poles of the electric supply. Fresh buffer solution drops from a capillary tube into the first compartment in which rests one of the three strips which provide the electric contact between the paper and the electrodes. An outlet tube in the third compartment removes the excess of the buffer solution and products from the electrolysis.

The three cells on each side are mounted on a large Perspex plate. The distance between these plates can be varied to obtain different electric fields with the same applied voltage. All the apparatus is contained in a damp chamber. The buffer solution for the cells and the upper vessel is supplied through regulating stopcocks from a reservoir, the quantity flowing being measured by collecting the effluents in graduated cylinders. A rate of 40—50 ml. of buffer solution per hr. per cell is sufficient to remove all the electrolysis products even when a high current strength is used.

With this apparatus, we tested a solution of bromophenol-blue in a phosphate buffer (pH 5·3; μ 0·4) and found that the deviation of bromophenol-blue (Plate 1) was almost twice that obtained with Durrum's apparatus (Plate 2) under the same electrical conditions (450 v) and with a flow rate of 0·2 ml. per hr. Before performing continuous paper electrophoresis it is best to subject the mixture to flat paper electrophoresis. This makes it possible to establish the best conditions for the analysis of mixtures of amino-acids (Durrum, *J. Colloid Sci.*, 1951,

PLATE 1. *Continuous electrophoresis of a bromophenol-blue solution with the new apparatus.*

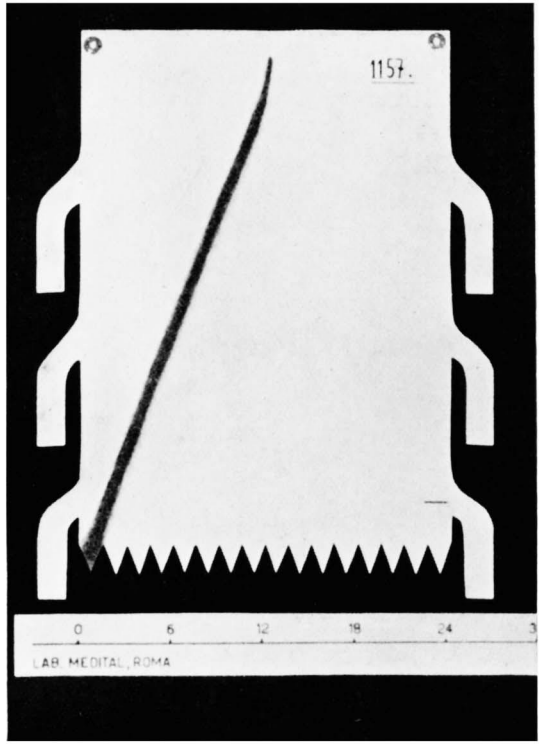
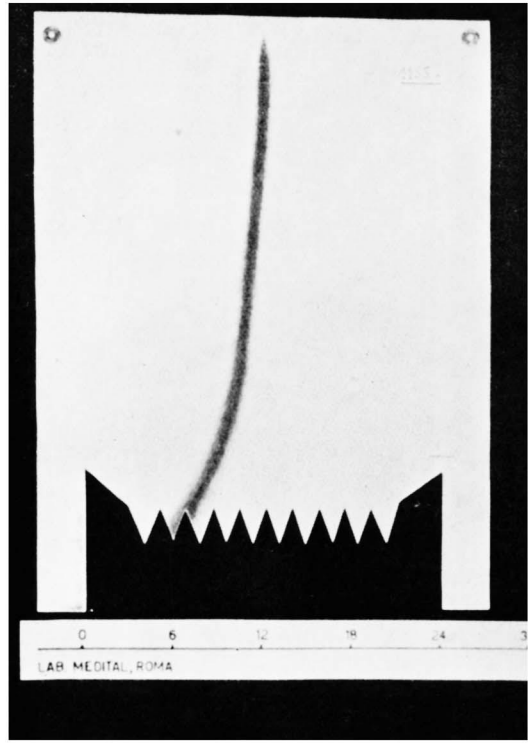


PLATE 2. *Continuous electrophoresis of a bromophenol-blue solution using Durrum's apparatus.*



[*To face p.* 4158.

6, 275), proteins (Turba and Enenkel, *Naturwiss.*, 1950, **37**, 93; Kunkel and Tiselius, *J. Gen. Physiol.*, 1951, **35**, 89), and mixtures containing substances sensitive to the electric field or which become so under certain conditions, *e.g.*, sugars in borate buffers (Consden and Stanier, *Nature*, 1952, **169**, 783).

The quantities which can be separated depend on many factors. We have separated mixtures of amino-acids in concentration 0·04M, and the proteins of dialysed human serum at 300 mg. in 12 hr.

MEDITAL LABORATORIES, RESEARCH DIVISION, ROME. [*Received, May 27th*, 1954.]