

contains neither active hydrogen nor acetyl group. The methoxyl test is positive and the substance does not react with diazomethane. It seems not impossible that the substance is related to 'kellin'<sup>2</sup>, which is used in the treatment of angina and bronchial asthma<sup>3</sup>.

Further work from the pharmacognostical and chemical sides is in progress.

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<sup>1</sup> Fahmy, I. R., and El-Keiy, M., *Rep. Pharmaceut. Soc. Egypt*, **3**, 72 (1931).

<sup>2</sup> Spaeth, E., and Gruber, W., *Ber.*, **71**, 106 (1938).

<sup>3</sup> Anrep, G. V., Barsoum, G. S., Kenawy, M. R., and Misrahy G., *Lancet*, **1**, 557 (1947).

## An Apparatus for Preparative Electrophoresis

A CONSIDERABLE number of suggestions have been made<sup>1</sup> of arrangements for the separation of ionized substances of different mobilities in an electric field, involving, for example, (1) diaphragm cells with three or more compartments<sup>2</sup>, (2) a series of beakers connected by inverted U-tubes<sup>3</sup>, (3) tubes filled with sintered glass<sup>4</sup>, (4) slabs of silica gel<sup>5</sup>, (5) modifications of the Tiselius apparatus which permit the withdrawal of liquid from any section of the advancing front<sup>6</sup>.

(1) and (2) suffer from the disadvantage that convective and electro-osmotic streaming occur in the compartments, so that the useful separative process is confined to the membranes or narrow tubes; (3) has an unduly high resistance; (4) has been successfully employed for comparatively small peptides. It is anticipated that the gel may hinder the free migration of larger molecules; and the problems of detection within the jelly and separation from it become formidable when only a few milligrams of material are available. The Tiselius apparatus is essentially one for the analysis of the moving fronts, and though samples can be withdrawn for analysis, it does not permit a complete separation of the components of a mixture.

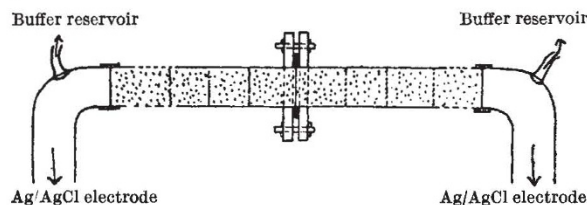


Fig. 1. PREPARATIVE ELECTROPHORESIS APPARATUS

After a number of trials of other types, we have developed a simple apparatus which gives satisfactory results so far as it has been tested (Fig. 1). Migration is made to occur down a tube of thin polystyrene plastic material, about an inch in diameter and a metre long, which is fairly loosely filled with asbestos fibre of the type usually used in Gooch crucibles. This does not greatly increase the resistance of the

column when filled with the buffer solution, and effectively prevents convective spreading. The asbestos is divided into sections by filter paper barriers at 1-inch intervals. These permit the wads of asbestos to be separated with ease when removed from the tubes. The tube is divided into two halves, fitted with plastic end-pieces, which enable them to be clamped together, making with a rubber washer a water-tight junction. The tube can be divided into as many sections as may be convenient, and sections can be removed or added during the electrolysis without disturbing the remainder. The tube is cooled by a water sprinkler placed above it.

The solution to be examined (about 5 c.c. in this apparatus) is mopped up with dry asbestos and introduced into the tube at the middle join. It is important that the solution and the buffer present in the rest of the column should be at the same pH and have approximately equal specific conductances; otherwise migration anomalies occur. The electrodes are of silver-silver chloride in concentrated sodium chloride similar to those used with the Tiselius apparatus. They are completely closed, so that no transport of the solvent occurs through the column.

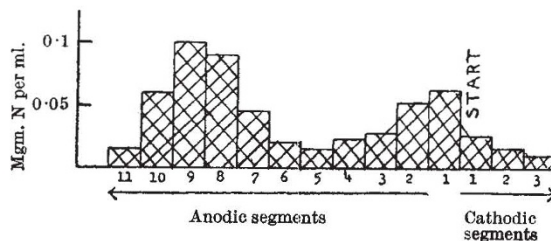


Fig. 2. ELECTROPHORETIC SEPARATION OF GLYCINE AND GLYCYLGLYCINE at pH 9.3

In tests with coloured substances such as dinitrophenylglycine and coloured indicators it was found that the asbestos effectively prevents the convective dispersion of the coloured ions, which move down the tube without undue spreading. The asbestos wads are blown out of the tube by air pressure or pulled out by tongs after the experiment. It is a simple matter to squeeze the liquid contents of each segment into a test tube for analysis. Fig. 2 shows the analysis of the segments after electrolysis of a mixture of glycine and glycylglycine at pH 9.3 for 24 hours with a potential difference of 250 volts between the electrodes (approximately 2 volts per cm. in tube). It is clear that a good separation has been obtained, the fraction which has migrated towards the anode at this pH being glycylglycine. A more detailed account will be given elsewhere.

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<sup>1</sup> For review, see Martin, A. J. P., and Synge, R. L. M., in "Advances in Protein Chemistry", Vol. II (1945).

<sup>2</sup> For example, Gordon, A. H., Martin, A. J. P., and Synge, R. L. M., *Biochem. J.*, **35**, 1369 (1941); **37**, 92 (1943).

<sup>3</sup> du Vigneaud, V., Irving, Dyer and Sealock, *J. Biol. Chem.*, **123**, 45, 485 (1938); *J. Amer. Chem. Soc.*, **63**, 503 (1941). See also Williams and Truesdail, *ibid.*, **53**, 4171 (1931); **55**, 2912 (1933).

<sup>4</sup> Coolidge, T. B., *J. Biol. Chem.*, **127**, 551 (1939).

<sup>5</sup> Conden, R., Gordon, A. H., and Martin, A. J. P., *Biochem. J.*, **40**, 33 (1946).

<sup>6</sup> Theorell, H., *Biochem. Z.*, **275**, 1 (1934). Svensson, H., *Arkiv. för Kemi, Min. o. Geol.*, **22A**, No. 10 (1946). Bunn, D., *Proc. Biochem. Soc.*, **41**, 24 (1947).