Destaining apparatus for disc electrophoresis gels by current at right angles to gel axis*

In the original apparatus supplied by Canalco** for destaining of disc electrophoretic gel preparations, according to the method of ORNSTEIN[1] and DAVIS[2], the gels are arranged with their long axes vertical, in a glass tube through which current is passed lengthwise. This has been criticized on several grounds: destaining takes hours; it requires that very low current and refrigeration be used to avoid loss of enzyme activity due to heating; diffusion and bowing of the bands occur, however, slightly. To overcome these objections MATSON[3] and SCHRAUWEN[4] have described apparatus whereby the current passes at right angles to the long axis through the column. Their techniques do present very sharply defined bands, even the finest; the current density is not critical and permits a combination of low amperage and short destaining time. The present communication concerns a simple and inexpensive device which may be obtained commercially***, whereby as many as 12 columns of any length up to 4 in. may be accommodated, but which may be used, if desired, for single runs.

This device is made of a plexiglas cylinder, 5 in. high, 4 in. internal diameter, with walls 0.5 in. thick. Twelve holes are bored in this cylinder from top to bottom, their centers being 30° apart and 0.25 in. diameter. The top of this hole is smoothly widened to 7/16 in. to permit easy sliding of the column into place. Opposite each hole, slits 0.25 in. wide and 4.5 in. long are cut through into the plexiglas, from the internal to the external surface of the cylinder. Rims of tygon tubing are made of 0.25 in. external diameter tubing around the cylinder, one just below and the other just above the row of slits, and fixed in place by fitting the ends over a short glass rod. Strips of stainless steel, 0.25 in. wide and 6 in. long, are bent over the tygon rims. These are held firmly by a strip of stainless steel at the upper end and another rim of tygon tubing at the lower end. To the upper fixation strip a lead wire is attached and covered with GE Clearseal plastic. A cover of plexiglas is cut from a plate of convenient thickness and a hole 0.5 in. in diameter is cut in the center. This accommodates a carbon electrode removed from a dry cell battery. This electrode is soldered to a lead wire and the connection covered with GE Clearseal plastic.

To use this device a glass rod of 0.25 in. diameter and 0.5 in. long is placed in the lower end of each hole which is to accommodate a gel column; into all other holes a glass rod of 0.25 in. diameter and 5 in. long is inserted. The gel columns are then slid into the prepared holes, and a length of 0.25 in. glass rod of length suitable to complete the closure of the slit opening is inserted above the gel. In this way all current must pass through the gels. The cylinder may have previously been permanently attached at the bottom to a circular plate of plexiglas by GE Clearseal plastic, or may now merely be set on a solid rubber pad, and the whole placed in a suitable beaker, evaporating dish or the like, and the container and central well of the cylinder filled to within 0.25 in. of the top with 7 % acetic acid. The cover is placed over the cylinder, wires connected to power, and the current, of some 20 mA, allowed to run 20–30 min. If the destaining fluid is continuously run through charcoal the process of destaining may be readily observed and stopped when complete.

* Supported in part by NIMH grant MH 05096-04.
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Gels, such as 12 % polyacrylamide, swell with this method so that they are not readily removed from their positions, but others are very simply removed by inserting a fine probe beneath the bottom glass rod and lifting. The gels come out easily, without tear or deformation. Even the finest bands are distinct, sharp and flat.

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Received January 31st, 1966

J. Chromatog., 24 (1966) 204–205

Use of "lipophilic" Sephadex in peptide synthesis

In the synthesis of peptides the elimination of reaction by-products from the desired peptide derivative is often a cumbersome task. It occurred to us that in cases where the by-products are soluble in organic solvents filtration through methylated Sephadex[1] might be a useful procedure.

As an example we have chosen the widely used "*p*-nitrophenyl ester method" of peptide synthesis[2,3]. One of the advantages of this method is the ease with which it may be ascertained whether or not the main by-product, the yellow *p*-nitrophenol, has been removed. In the early steps of the elongation of a peptide chain, however, the removal of the nitrophenol may be quite time-consuming. It is usually accomplished by repeated extraction of the solution of the reaction mixture in ethyl acetate or ether with aqueous bicarbonate or ammonia. Often some half a dozen extractions are necessary, during which troublesome emulsions may form. Even then traces of nitrophenol are left and for the removal of these filtration through aluminium oxide has been recommended[4].

We have synthesized two peptides, L-leucyl-L-leucine and L-leucyl-L-valine, by the *p*-nitrophenyl ester method, with filtration of the reaction mixtures containing *p*-nitrophenol and benzyloxycarbonyl-L-leucyl-L-leucine methyl ester or benzyloxycarbonyl-L-leucyl-L-valine methyl ester, respectively, through a column of methylated Sephadex. In each case complete separation of the *p*-nitrophenol from the protected dipeptide was achieved. Fig. 1 shows this for the derivative of L-leucyl-L-leucine. The picture was practically identical for benzyloxycarbonyl-L-leucyl-L-valine methyl ester. The latter was isolated in crystalline form. As far as we know it has only been described as a syrup earlier[5].

It is obvious that filtration through lipophilic Sephadex may also find application in analytical work on peptides when derivatives soluble in organic solvents are formed.