AN APPARATUS FOR LARGE SCALE PREPARATIVE CHROMATOGRAPHY WITH ESPECIAL APPLICATION TO THE SEPARATION OF A LONG CHAIN FLUORO-FATTY ACID

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In recent work[1] a new apparatus has been used for the separation of a long chain unsaturated fluoro-fatty acid by reversed phase chromatography.

It was developed when it was found that cylindrical columns of a diameter greater than 1 cm were unsuitable for the isolation of the fluoro-fatty acid from the seed fat of *Dichapetalum toxicarium*. The apparatus is described here because it is likely that wider uses could be found for it than that of the particular research for which it was constructed. The description given of its use is for the separation of the fluoro-fatty acid.

The essential feature of the apparatus was the utilization of a large narrow block of supporting medium impregnated with the stationary phase, and held together by plates of glass and "Tufnol", a fabric-based synthetic resin.*

THE APPARATUS

This consisted of a sheet of $^3/_8$ in. or ½ in. thick plate glass (not Triplex), 21¾ in. long by 11 in. wide, with a sheet of ¼ in. thick "Tufnol-carpbrand" of similar size separated by four glass or "Tufnol" strips 1½ in. wide and of a thickness varying from a ¼ in. to 1½ in. according to requirements, and bolted together by O BA chromium-plated brass bolts with grooved heads and wing nuts. Two strips were placed in a vertical position along the length of the plates and two shorter base strips were set at 45° angles to meet each other, forming an arrow with a $^1/_8$ in. gap through the point. Thus a narrow wedge-shaped vessel was constructed. In the tip was a perforated block of "Tufnol" covered by a piece of filter paper. This block was of the same thickness as the strips separating the main plates. Before assembly the surfaces of the "Tufnol" or glass strips were smeared liberally with a paste of 7 % w/v paraffin wax in liquid paraffin.

The threads of the brass bolts were covered by thin translucent rubber sleeves and between the wing nuts and the glass plates were placed, first a hard rubber

* The apparatus can be obtained as described from the Shandon Scientific Co. Ltd., 6 Cromwell Place, London, S.W. 7.

washer 1 in. in diameter with a $^5/_{16}$ in. hole, then a brass or duralumin washer of similar size.

It was important to tighten the nuts and bolts evenly so that they were just "finger-tight", this being sufficient to effect a seal between the plates and strips. The excess sealing paste was easily removed from the inside of the apparatus by rubbing untreated cellulose powder along the edges of the strips and removing it with a thin length of wood. All the nuts, bolts and washers were lightly smeared with the sealing paste.

The apparatus prepared for a descending separation is shown in Fig. 1, and detailed diagrammatically in Fig. 2.

Packing and loading the column for ascending chromatography

The column was packed with treated ashless "Whatman" cellulose powder (Standard grade) in amounts of about 50 g at a time and compressed tightly with a flat rectangular stainless steel plate at the end of a suitable handle. If the column was required to be 1 in. thick, about a kg of paper was required. Packing was continued to within 2 in. of the open end. There is a full description elsewhere of the preparation of the cellulose powder by treatment with 85% v/v acetic acid–water followed by impregnation with liquid paraffin[1].

To load a 1 in. thick column, 10 g of an oil fraction soluble in acetone to 1% w/v at —20° (as free fatty acids) were mixed with 25 ml of 0.1% Oil Red 4B (George Gurr Ltd.) in ether as a marker and to this were added 80 g of the treated cellulose. Oil Red 4B was chosen for the marker because it contains an orange coloured component which travels chromatographically to the same position as the fluoro-fatty acid. The whole was mixed together thoroughly with a pestle and mortar, and put on to the column as a compressed band. The remaining ½ in. of the column was then filled with treated cellulose. A piece of thick filter paper was wrapped over the end to prevent the cellulose from falling out and kept in position with cellulose adhesive tape. The column was developed at room temperature for 18 h with a solvent consisting of acetic acid, formic acid and water in the proportions of 2:2:1 by volume.

It was convenient to stand the apparatus on its open end in a large glass chromatography tank containing about 1 in. depth of the solvent. After development, a broad orange band could be seen half way up the column. This band contained the fluoro-fatty acid. Oleic and linoleic acids remained near the place of application in a bright red band.

The components were isolated by cutting out ½ in. or 1 in. wide sections of the pad and extracting these with 200 ml of 85% v/v acetic acid. The cellulose was separated on a sintered glass funnel and the acetic acid removed by distillation under reduced pressure ensuring that the temperature did not rise above 30°. Fatty acids were extracted from the residue by shaking with 10–20 ml of methanol which left any liquid paraffin undissolved. Demonstration of the fluoro compound in the clear solutions was achieved by paper chromatography already described[1]. Those fractions which contained only fluoro-fatty acid were pooled and further purified by

precipitation as the calcium salt[1]. Fractions containing traces of other fatty acids were pooled until several grams were available and refractionated on a fresh column.

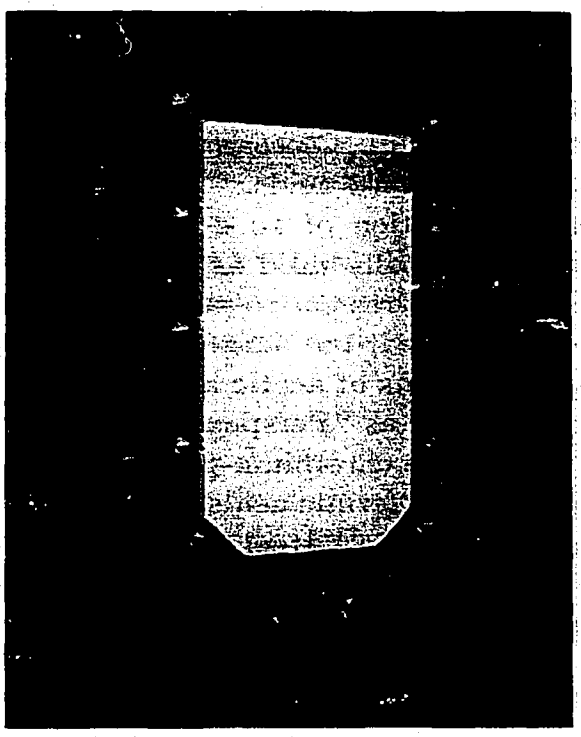

Fig. 1. The apparatus prepared for the separation of long chain fatty acids by descending technique.

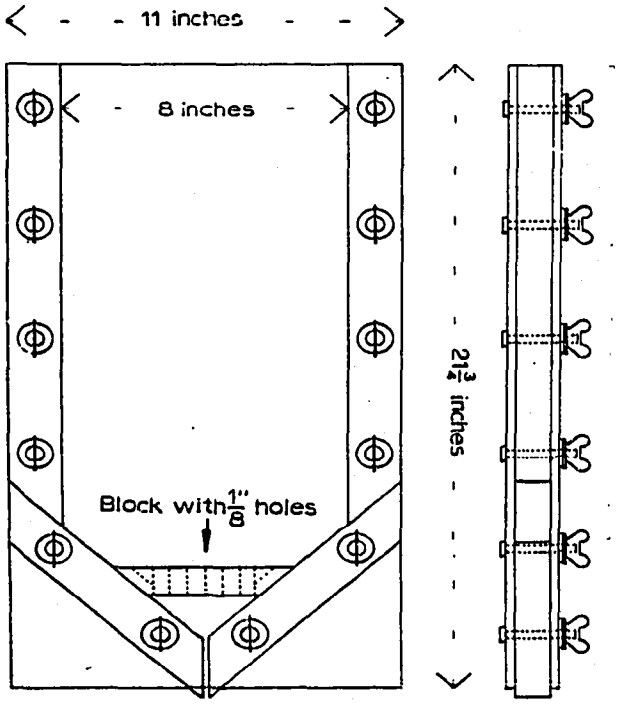

Fig. 2. Diagrammatic detail of the apparatus.

For descending chromatography

More discrete separations were obtained by descending technique which had the further advantage of being quicker. This mode of use, however, necessitated constant attention to the apparatus throughout the developing period. The apparatus was packed with the treated cellulose to within 4 in. of the end. The oil, mixed with dye and cellulose, was applied as for ascending development and then covered with 1 in. or so of packed treated cellulose. The apparatus was supported on blocks and held by clamps fitted to a retort stand on each side of it. The developing reagent was supplied from two 500 ml stoppered separating funnels having 8 mm delivery tube stems with 5 mm holes in the stop-cocks. The funnels, with their stoppers inserted, are fixed with their stems just below the surface of a 1 in. layer of solvent on top of the cellulose. When the level of the solvent fell below the tip of the stems it was automatically replaced from the reservoirs.

The first eluate usually came through after approximately 2 h at the rate of about 5 ml/min. Successive eluates varying from 20 ml to 200 ml were collected from which the solvent was removed by distillation under vacuum. Latterly, it was found adequate to extract the eluates with small volumes of light petroleum (boiling range 40–60°). The long chain fatty acids and some dye went into the petroleum leaving a large amount of dye in the acid solvent. The petroleum could be evaporated off with a

stream of compressed air in a fume cupboard, and the oil subsequently redissolved in methanol to remove traces of liquid paraffin.

DISCUSSION

Techniques of reversed phase column chromatography for long chain fatty acids have been described by HOWARD AND MARTIN[2] and CROMBIE, COMBER AND BOATMAN[3], separating the components of no more than 60 mg of mixed acids.

The present apparatus is described for the separation of 10 g of mixed fatty acids on a 1 in. thick column, and it has been successfully used as a $1^5/_8$ in. thick column when 15 g of oil were put on. There seems no reason to suppose that the overall dimensions of the apparatus could not be varied, but larger models might require thicker glass front plates. The high concentrations of acetic and formic acids had no effect upon the "Tufnol" but other plastics such as "Perspex" were softened after a short time. Experience has shown that whilst the chromium-plated wing nuts and bolts resisted corrosion by the vapours of acetic and formic acids for a long time, it would probably be better for them to be made of stainless steel. Small cylindrical glass columns still proved useful for the isolation of a small quantity of material, but, when large cylindrical columns were tried, components tended to separate initially and then run into each other during elution in the form of a series of concentric cones.

BÉKÉSY[4] observed the same effect during studies on the chromatographic separation of plant extracts with circular columns, and concluded that a larger surface area was required to overcome the problem. He constructed a very small apparatus consisting of lengths of thin sheet glass held only 5 mm apart by cork strips, the whole assembly being maintained by clamps. Although BÉKÉSY's apparatus was very different from that described in this paper, the principles and reasons involved in its design were similar. Such an apparatus, however, could not be constructed on a larger scale. In this laboratory two attempts to make the large apparatus described in this paper entirely of ¼ in. plate glass were unsuccessful; the glass plates rapidly developed lateral cracks. By using a "Tufnol" sheet in conjunction with plate glass this problem was overcome. It is not clear why a block of supporting medium should function so well and not a large round column of it. It is possible that more uniform packing is achieved with a narrow rectangular column.

Experiments in this laboratory with cellulose powder impregnated with silicone MS200/16CS, the reagent employed by SCHLENK *et al.*[5] with filter paper sheets, showed that this system could give a more satisfactory separation of the long chain fluoro-fatty acids from oleic, linoleic and linolenic acids than did silane-treated kieselguhr[2]. Partition was effected with 85 % v/v acetic acid. The same reagents were employed with Whatman No. 531 filter paper sheets for the identification of the fluoro-acid. Later work showed that better results could be obtained by employing liquid paraffin instead of silicone and for the preparative technique, substituting the acetic–formic acid mixture for 85 % acetic acid. If, however, 85 % acetic acid was employed as the

developing solvent, complete separation of oleic, linoleic, linolenic, ricinoleic and fluoro-oleic acids was achieved, and these were collected chromatographically pure. By this method about 2 g of chromatographically pure fluoro-oleic acid were prepared as well as large quantities of oleic acid.

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SUMMARY

An apparatus is described for the separation and purification by reversed phase chromatography on cellulose powder treated with liquid paraffin, of 10 g or more of mixed long chain fatty acids. Its application to the isolation of a long chain unsaturated fluoro-fatty acid is described and it is suggested that the apparatus may find wider uses in other preparative chromatographic separations.

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