Chromatography of amino acids on plaster of Paris

The use of thin and thick strips of set plaster of Paris in the chromatographic separation of alkaloids has been described in an earlier paper[1]. It was decided to investigate the use of such strips in the chromatographic separation of amino acids and this paper deals with the method evolved and the results obtained.

Thin layer chromatography of amino acids has already been studied[2] and the method proved to be faster than the classical separation on paper. The thin layer plates require delicate handling throughout the chromatography operations while strips of set plaster of Paris are mechanically stronger and offer better conditions especially when developing coloured spots. The chromatographic separation of amino acids in set plaster of Paris is very fast and the medium can be used for ascending, descending, circular and two-dimensional chromatography. In the present paper, ascending and circular chromatography methods are described and it has been found that there was sharp and clear separation in a matter of minutes of amino acids from protein hydrolysate, mixtures of known amino acids and amino acids in urine. Since the size of the sample which can be applied to set plaster of Paris strips is much larger than that possible on paper, colour development with ninhydrin is fast and the tone and depth of the colours obtained is better. The results obtained with thick and thin plates of set plaster of Paris are essentially the same, but in this paper 1 mm thick plates have been used because the size of the sample was not of importance as no preparative purpose was envisaged.

Preparation of thin plates of set plaster of Paris

30 g of plaster of Paris were mixed with 27 cc. of distilled water. Plates of 1 mm thickness were prepared by the technique described previously[1]. The plate, when fully set, was allowed to dry overnight at room temperature, and was then cut as described previously[1] in strips 2 cm broad, 10 cm long, and also in squares of 5 cm. The strips and squares were dried at 100° for 3 h.

Application of sample

The sample of mixture of amino acids, protein hydrolysate and 24 h collection of urine, were applied to the strips as spots with a graduated capillary pipette, the end of which was wrapped in cotton wool to form a swab. For the squares, the sample was applied to one corner by touching the latter with the pipette. In all cases the size of sample was 5 μl. In order to obtain quickly uniform and concentrated spots, the strips and squares were heated for 3 min on either side by the radiant heat of a 700 watt open-coil heater at a distance of 5 cm above the heater. The sample was applied to the hot strip or corner of the squares and further drying was carried out over the heater for 2 min. The strips and squares were allowed to stand at room temperature for 10 min before use.

Apparatus for chromatography

For ascending chromatography of the strips, a conventional chamber was used. For circular chromatography of the squares, a chamber consisting of Petri dishes arranged as shown in Fig. 1 was used. The corner of the square of set plaster of Paris G where the sample has been applied touches the filter paper wick E which is fixed

to a polyethylene hollow bottle stopper D having a hole in the centre. The polyethylene stopper has holes on its sides to allow the solvent H in Petri dish cover C free access to the wick. Petri dish A with cover B has a small amount of solvent

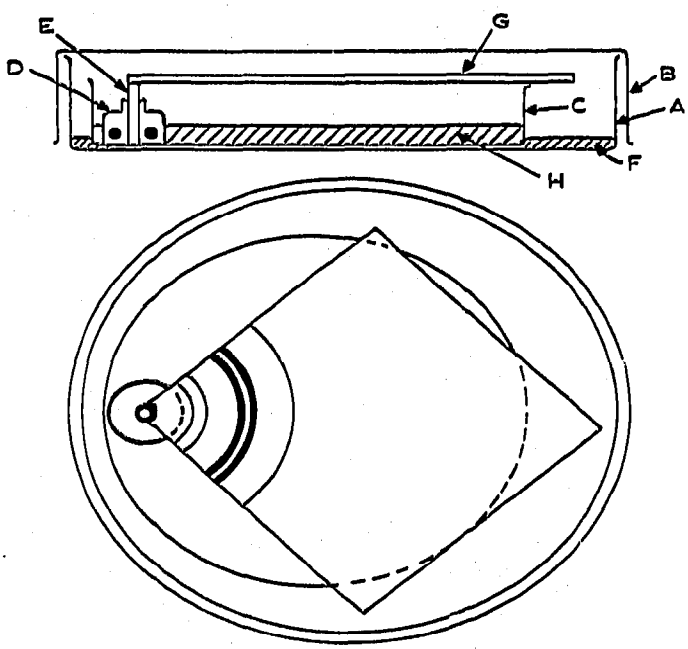

Fig. 1. Chamber for circular chromatography.

F which saturates the atmosphere easily. The square of set plaster of Paris rests on its three corners on Petri dish cover C and the fourth corner touches the wick.

Solvent

It is possible to work out different compositions of solvents suitable for separating different amino acids such as has been done on paper[3], but for the purpose of the material investigated here, the following solvent is suitable: acetone–chloroform–99–100 % acetic acid–acetate buffer[4], pH 4 (4 cc.:1 cc.:0.5 cc.:1.5 cc).

Instead of subsequently spraying or painting of the strips and squares with an 0.5 % acetone solution of ninhydrin, it has been found more convenient to add 35 mg of ninhydrin to the above solvent. The presence of ninhydrin does not affect the R_F values of the amino acids.

Chromatographic separations

Chromatography was carried out for 45 min in all cases. Longer times increase the distance between separated bands but decrease the colour intensity of the spots making photographic recording difficult.

Development of colour of spots and bands

As pointed out earlier, since ninhydrin is already included in the solvent, there is no need to spray or paint the dried strips and squares. If spraying or painting is needed for any purpose, it can also be carried out without smearing. The strips and squares are dried in an oven at 100° for 20 min for full colour development.

Results

Fig. 2 shows photographs of the following separations:

(A) Ascending chromatographic separation of a protein hydrolysate injection USP made by Bengal Immunity Co. Ltd. Calcutta.

(B) Circular chromatography of a mixture of pure amino acids showing (1) aspartic acid; (2) histidine; (3) tryptophan; and (4) tyrosine. The mixture applied contained 0.25 % aspartic acid; 0.5 % histidine; 0.5 % tryptophan; and 0.35 % tyrosine.

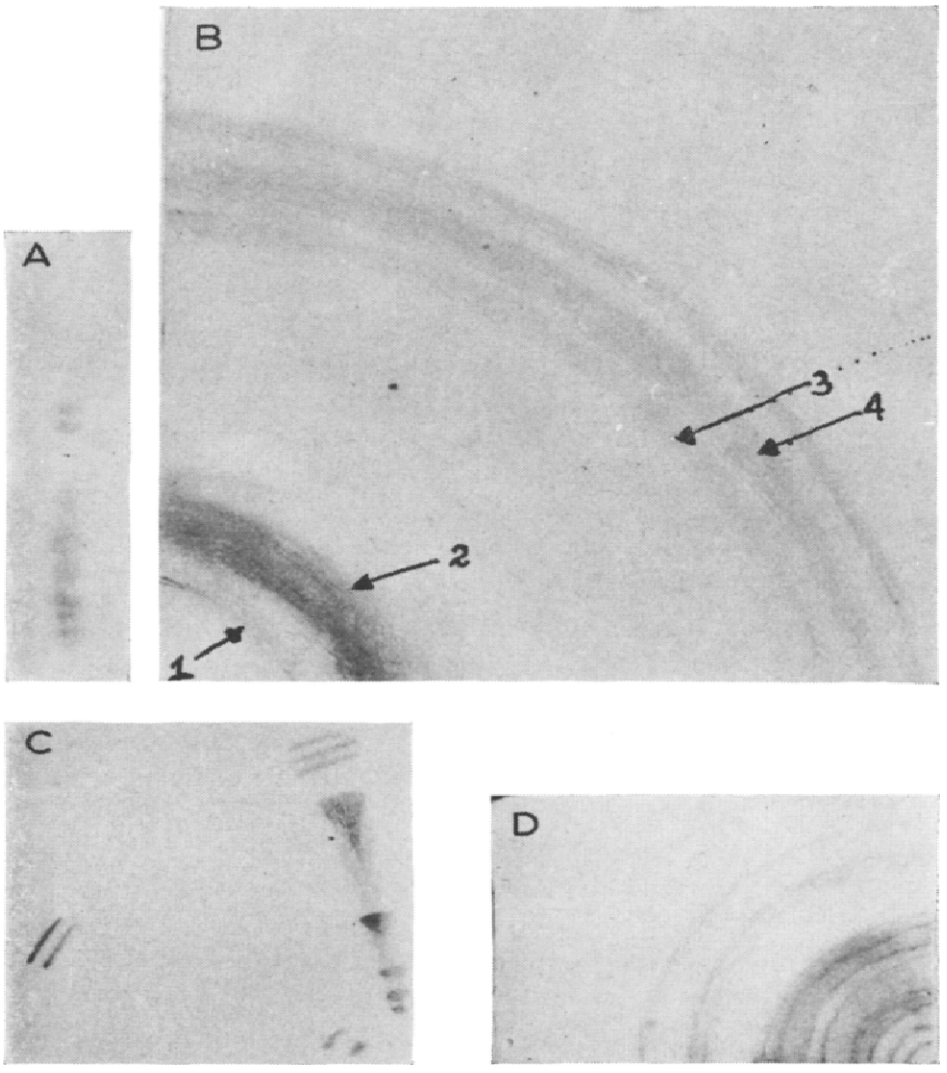

Fig. 2. (A) Chromatographic separation of amino acids in protein hydrolysate. (B) Circular chromatogram of a mixture of pure amino acids. (C) Simultaneous circular chromatogram of protein hydrolysate and a mixture of pure amino acids. (D) Circular chromatogram of protein hydrolysate.

(C) Circular chromatogram of simultaneous spots of (A) and (B). The four amino acids of (B) also exist in the protein hydrolysate (A). Only in this case the size of each sample was reduced from 5 μl to 3 μl.

(D) Circular chromatogram of protein hydrolysate as in A. Twelve amino acids could be distinguished clearly in the original square.

Fig. 3 shows the separation of amino acids present in a 24 h collection of urine from a normal healthy male on a non-vegetarian diet.

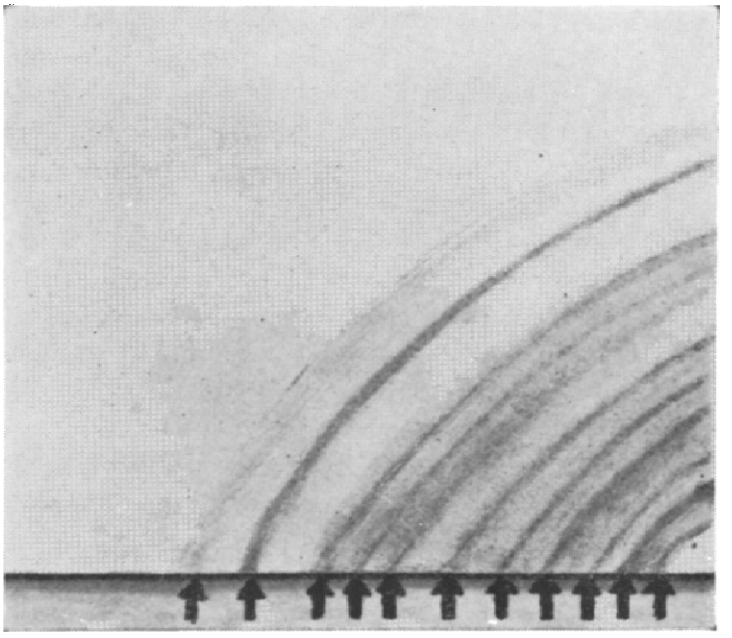

Fig. 3. Chromatogram of a sample of urine.

Discussion

Results show that the use of set plaster of Paris offers a convenient method of separating groups of amino acids very easily. It has been possible to separate clearly with the same medium individual amino acids by the uni-dimensional method, or with a longer time for chromatography by the bi-dimensional method; but the aim in the present work was to develop a quick method for screening most of the amino acids in urine or body fluids in an attempt to study the clinical aspects of amino acid excretion. Results of this investigation will be communicated later. Studies are also being made of the use of set plaster of Paris discs in centrifugal chromatography of amino acids for preparative purposes in much the same way as has been communicated in an earlier paper[5] for plant alkaloids.

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