CHROM. 4151

Ion-exchange thin-layer chromatography of amino acids

The thin-layer chromatographic separation of amino acids has been reported using the different stationary phases barium sulphate[1], aluminium oxide[2], starch[3], silica gel[4,5], cellulose[6–8], DEAE-cellulose[9], and Sephadex[10]. Cellulose ion-exchangers are frequently used for the column separation of peptides, proteins, and enzymes, but these materials are generally unsuitable for thin-layer chromatography due to their particle size and tendency to swell. Cellulose ion-exchange papers have been used successfully for the separation of amino acids[11].

In the present study a new DEAE-cellulose, which was especially prepared for thin-layer chromatography* and which yielded excellent separations was used. This material has a nominal small ion capacity of 0.25 mequiv./g, and a water regain value of 0.6 g/g. Plates of this material, prepared by conventional methods, give layers which are free of cracks and separations which are reproducible. A slurry of 10 g of DEAE-cellulose in 26 g of demineralised water is stirred for 2 min and spread over clean glass plates (20 × 20 cm) with a Desaga spreader set for 0.4 mm thick layers. The plates are then air-dried for 10 min followed by drying at 60° for 2–4 h. Standard solutions of 0.02 g amino acids are prepared in 100 ml 0.1 *N* hydrochloric acid and 10% isopropanol and stored at 4°.

A sample of 5 μl is applied to the layer with a micropipette and dried with a stream of air. Development for one-dimensional chromatography is by multi-development with *n*-butanol–acetic acid–water (4:1:5)[12] run three times. Development for two-dimensional chromatography is by twofold development for 60 min each with *n*-butanol–acetic acid–water (4:1:5) in the first direction and a single development for 60 min with pyridine–water (4:1)[13] in the second direction. Between each development the plates are dried at 60° for 10 min. The amino acids are located

TABLE I

R_F VALUES AND COLOURS OBTAINED WITH THE NINHYDRIN–COLLIDINE REAGENT

No.	*Amino acid*	$R_F \pm$ S.E.	*Colour*
1	Aspartic acid	0.05 ± 0.003	bright blue
2	Glutamic acid	0.12 ± 0.004	violet blue
3	Serine	0.13 ± 0.003	green blue
4	Glycine	0.17 ± 0.003	brown
5	Hystidine	0.28 ± 0.004	brown
6	Hydroxyproline	0.18 ± 0.004	yellow
7	Threonine	0.20 ± 0.003	grey blue
8	Lysine	0.26 ± 0.005	violet blue
9	Alanine	0.26 ± 0.00	violet blue
10	Proline	0.29 ± 0.003	yellow
11	Tyrosine	0.29 ± 0.004	grey
12	Methionine	0.37 ± 0.004	red violet
13	Valine	0.42 ± 0.00	violet blue
14	Phenylalanine	0.44 ± 0.004	grey brown
15	Leucine	0.52 ± 0.006	violet blue

* Prepared by the Whatman Research Laboratories, W. and R. Balston (Modified Cellulose) Ltd., Springfield Mill, Maidstone, Kent, Great Britain.

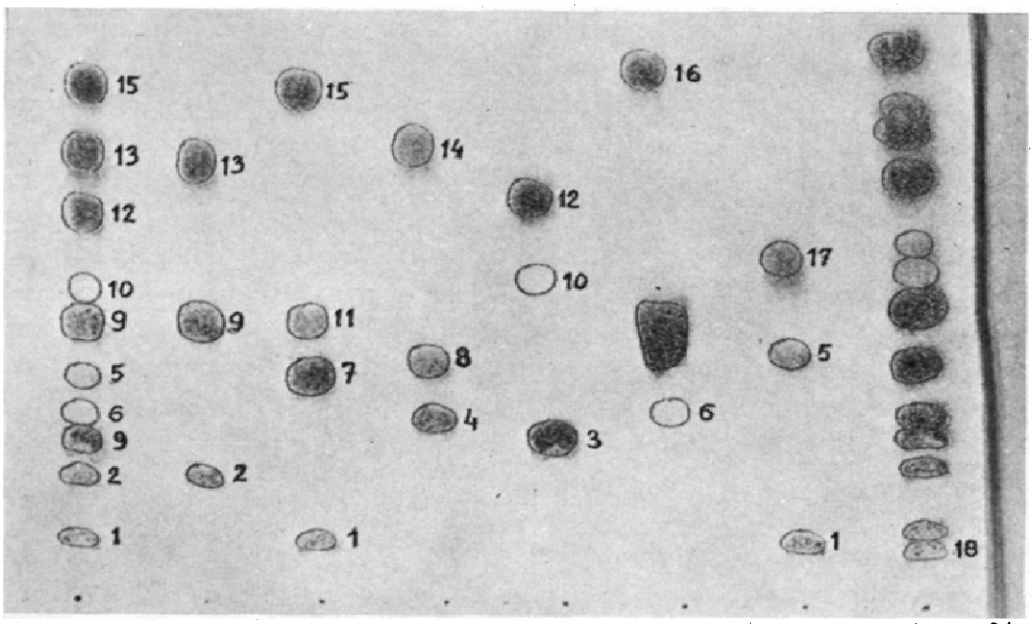

Fig. 1. One-dimensional separation of amino acids with *n*-butanol–100% acetic acid–water (4:1:5). 1 = aspartic acid, 2 = glutamic acid, 3 = serine, 4 = glycine, 5 = hystidine, 6 = hydroxyproline, 7 = threonine, 8 = lysine, 9 = alanine, 10 = proline, 11 = tyrosine, 12 = methionine, 13 = valine, 14 = phenylalanine, 15 = leucine, 16 = isoleucine, 17 = tryptophan, 18 = cystine.

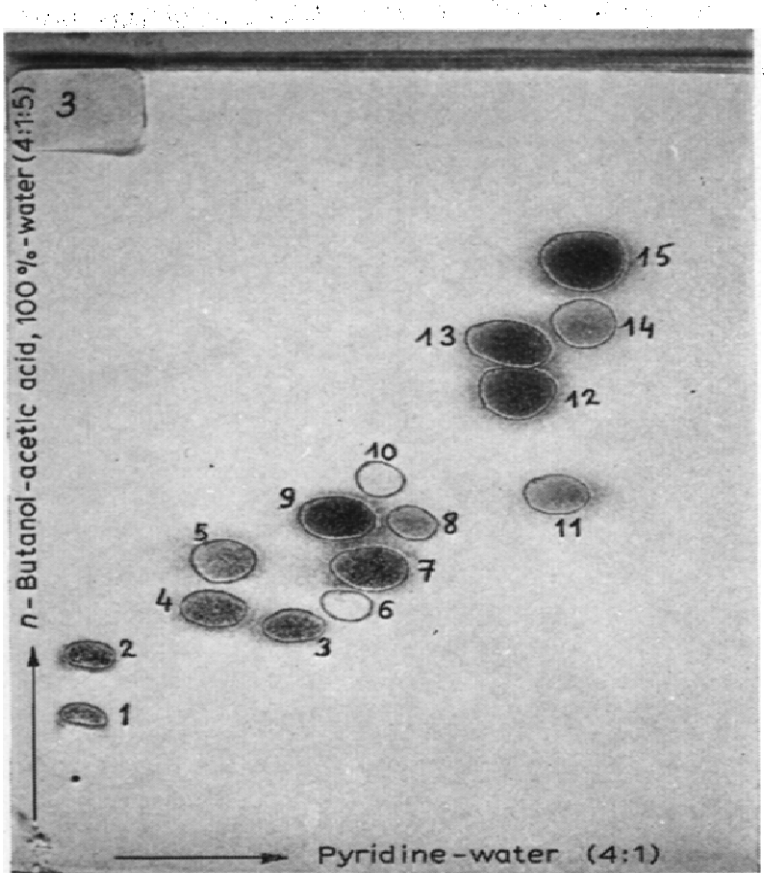

Fig. 2. Two-dimensional separation of amino acids. 1 = aspartic acid, 2 = glutamic acid, 3 = serine, 4 = glycine, 5 = hystidine, 6 = hydroxyproline, 7 = threonine, 8 = lysine, 9 = alanine, 10 = proline, 11 = tyrosine, 12 = methionine, 13 = valine, 14 = phenylalanine, 15 = leucine.

by spraying with ninhydrin–collidine[14], and the colour is developed by heating at 100–110° for 10 min.

Table I gives the R_F values in the one-dimensional separation and the colour of the amino acid spots obtained with the ninhydrin–collidine reagent. Fig. 1 shows the amino acid separation obtained with one-dimensional development, and Fig. 2 that obtained with two-dimensional development. Fig. 1 shows a good separation of valine and methionine, amino acids which are usually difficult to separate. The amino acid pairs aspartic acid and serine, with similar solubilities, and glutamic acid and glycine are well separated by ion-exchange (on non-modified cellulose these are not separated). When the ion-exchange cellulose is recycled, similar results are obtained.

This new DEAE-cellulose, especially prepared for thin-layer chromatography, is useful in screening the amino acids in urine and blood.

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Received April 20th, 1969