Time saving applications in horizontal paper chromatography of amino acids

The method published previously by the authors[1] separates the mixture of amino acids with the first solvent system into several groups, transfers these onto another paper by capillary elution and effects the separation of the groups into individual amino acids with other solvent systems.

This method has been improved. A solvent system[2] was found which eliminates the need for rechromatography and a simple apparatus which dries the spots during the process of elution permits one worker to control six such procedures simultaneously (Fig. 1).

The dimensions of the paper for the separation into groups are given in Fig. 2.

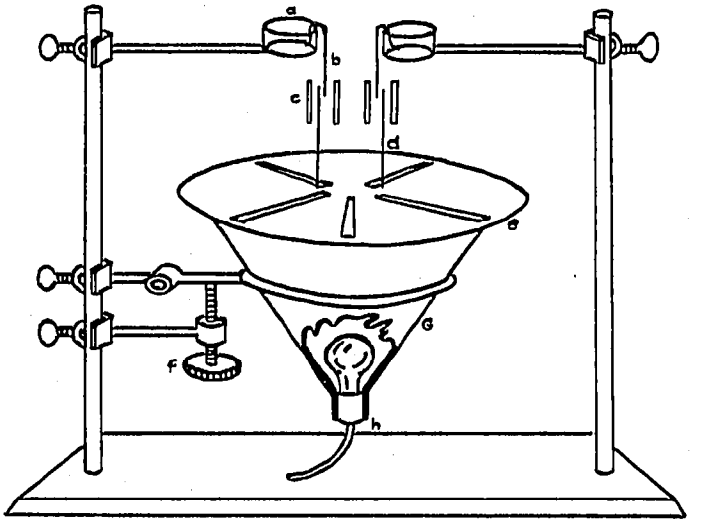

Fig. 1. Simultaneous elution and drying of "group" on K.C.T. paper. (a) Solvent vessel; (b) leader strip; (c) microscope slides; (d) cut out band from first chromatogram; (e) K.C.T. paper; (f) adjustment; (g) light metal cone, base and height 24 cm; (h) 40 W bulb.

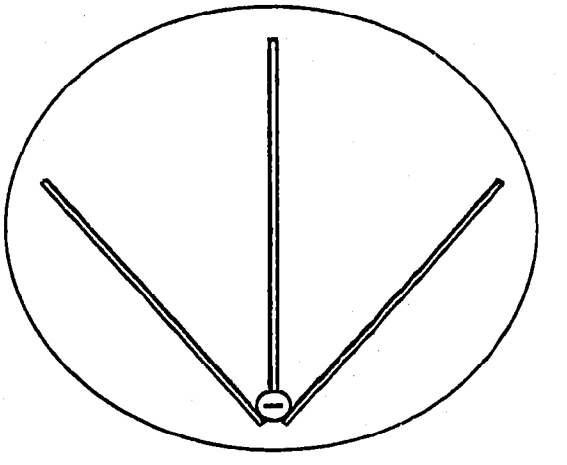

Fig. 2. Circular paper with two segments, one for ninhydrin development the other for cutting out and eluting groups of amino acids. Diameter 33.3 cm, of small circle 2.2 cm; slit in same 2 cm; paper tongue 2 × 5 cm; angles 37° each.

The chromatogram is run with the solvent system *n*-butanol–methyl ethyl ketone–diethylamine–water (20:20:2:10, v/v) which travels about 230 mm within 18 h at 19°. This is very convenient, permitting to start the run in the late afternoon and to take it off next morning.

It was found advisable to increase the size of the box slightly to 37.6 × 37.6 × 7.5 cm and to saturate the atmosphere therein by an additional 40 ml solvent, divided in five regularly spaced, flat dishes. The paper is supported by glass needles of 4 cm length.

Diethylamine should be removed quantitatively in order to avoid complications during the separation of the groups. This is achieved by drying the paper in a ventilated oven at 70° for 30 min and by washing it three times with chloroform.

The distribution of the amino acids and the separation of the various groups with lutidine–water (3:2, v/v) and *tert.*-butanol–formic acid–water (75:0.8:24.2, v/v) are shown in Table I.

TABLE I

GROUP-SEPARATION AND SUB-SEPARATION OF AMINO ACIDS BY HORIZONTAL PAPER CHROMATOGRAPHY

Group-separation			*Sub-separation*	
BuOH–Me Et ketone–H_2O–diethylamine (20:20:10:2, v/v)			*Lut–H_2O (3:2, v/v)*	*tert.-BuOH–HCOOH–H_2O (75:0.8:24.2, v/v)*
Group	*amino acids*	R_F	R_F	R_F
I	Cystine	10–16		17–19
	Arginine			23–34
	Asp. acid			40–44
	Glut. acid			50–54
II	Cysteic acid	18–21	44–50	
	Lysine*		13–20	
III	Glycine*	23–25	37–41	
	Methiosulfone		45–49	
IV	Alanine	30–37		46–50
	Histidine			18–21
	Serine			38–41
V	Tyrosine*	42–44		
VI	Valine	47–50	59–64	
	Methionine		48–53	
VII	Threonine	57–69		45–49
	Phenylalanine			67–70
	Leucine and isoleucine			75–80

* Determined immediately after the first run, provided no H_2O_2 has been used.

Lysine, glycine and tyrosine appear as single bands and can be determined immediately after the first run. If, however, the sample spot has been treated with a drop of H_2O_2, cysteic acid will appear with lysine (group II) and methiosulfone with glycine (group III). Each pair is easily separated by lutidine–water (3:2, v/v) as shown in Table I.

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1 J. CHARTIER, F. W. VAN KLAVEREN AND G. VAILLANCOURT, *J. Chromatog.*, 1 (1958) 317.
2 N. M. SISAKYAN, E. N. BEZINGER, P. G. GARKAVI AND G. Y. KIVMAN, *Doklady Akad. Nauk. S.S.S.R.*, 96 (1954) 343; *C. A.*, 48 (1954) 10821.

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