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A modified pyridine–formic acid gradient for automated column chromatography of amino acids and small peptides

The use of sodium citrate buffers for the separation of amino acids has the disadvantage that before unusual or radioactive amino acids in the eluate can be identified and measured, it is almost invariably necessary to remove the salts. This is time consuming and may result in losses (*e.g.* some sulphur-containing amino acids are oxidised on the ion-exchange resins used for desalting). A volatile buffer system offers obvious advantages[1]. However, the resolution obtained with these buffers is not as good as that obtained with citrate buffers. This paper describes a system that is simple to operate and gives good separation of most of the common amino acids.

Experimental

A single column Technicon amino acid analyser (Technicon Instruments Co. Ltd., Chertsey, Surrey) was used. The column (140 cm × 0.6 cm) was packed with Technicon "Chromobeads, Type A" and maintained at 60°. Three buffers (A, B and C) were prepared from pyridine (A.R., British Drug Houses Ltd., Poole, Dorset; purified by redistillation over ninhydrin) adjusted to pH with formic acid (A.R., B.D.H. Ltd., 90 %): (A) pH 2.88, 0.1 *N* pyridine; (B) pH 3.80, 0.1 *N* pyridine and (C) pH 5.00, 1.0 *N* pyridine. Thiodiglycol (0.5 %) was added to buffers A and B, but no detergent was added. The nine-chambered Varigrad was charged according to Table I.

In the trace illustrated in Fig. 1 is represented the analysis of a synthetic mixture containing 0.5 μmole of each amino acid with a buffer flow rate of 1 ml/min of which 0.1 ml/min was passed into the analytical system for reaction with ninhydrin and the remainder was diverted to a fraction collector. The column will, however, tolerate considerably larger amounts of amino acids (2.5–3 μmole) without loss of resolution which is advantageous when minor components of a mixture are being investigated. In the latter case, 0.05 ml/min of the effluent was reacted with nin-

TABLE I

VARIGRAD BUFFER COMPOSITION

Chamber	*MeOH* (*ml*)	*A* (*ml*)	*B* (*ml*)	*C* (*ml*)
1	10	140	—	—
2	6	144	—	—
3	—	150	—	—
4	—	150	—	—
5	—	—	140	10
6	—	12	18	120
7	—	—	—	150
8	—	—	—	150
9	—	—	—	150

hydrin. After each analysis, the column was washed with 0.2 *N* NaOH for 30 min followed by pH 2.88 buffer for 2 h.

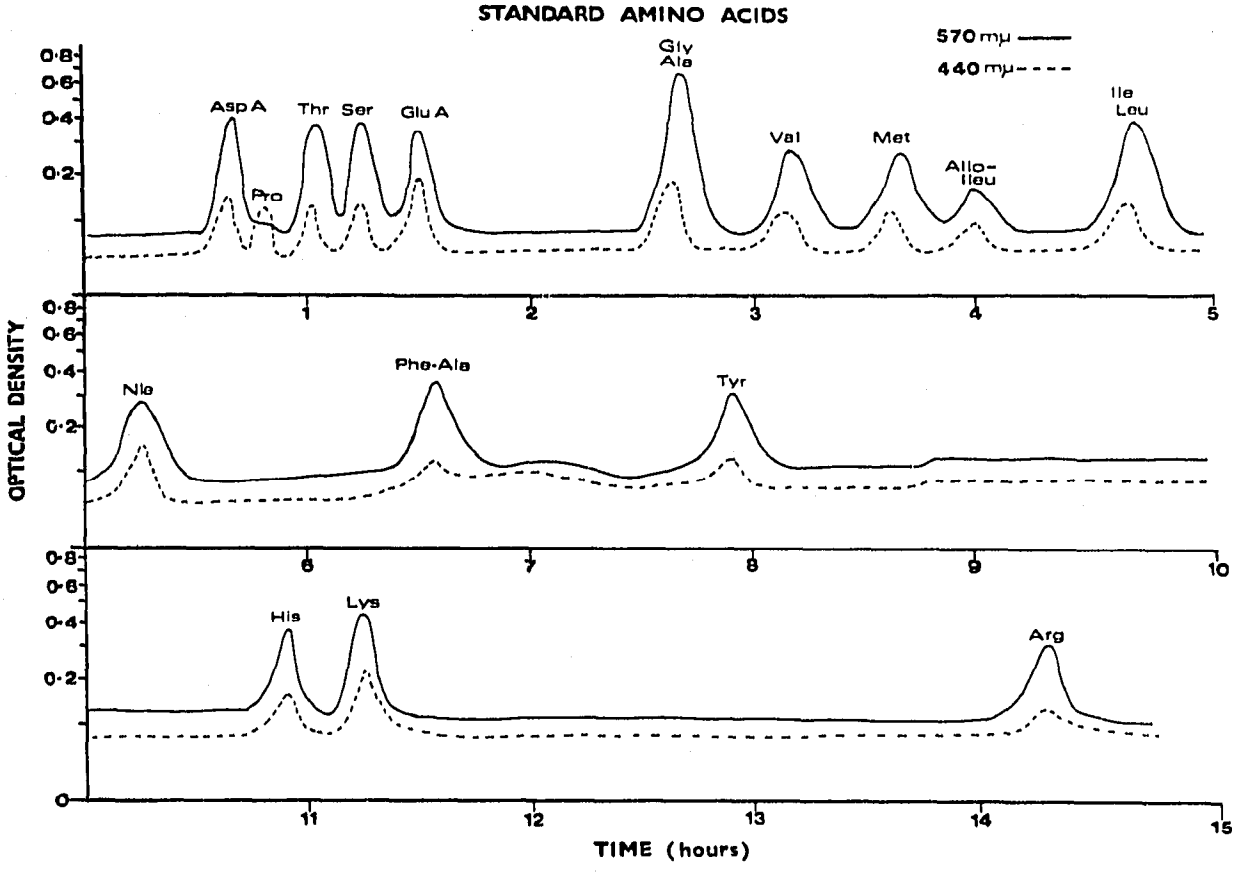

Fig. 1. Chromatogram of a standard mixture of amino acids using the procedure described in the text. The time scale commences 1 h after application of the sample to the column.

Results and discussion

The elution times of some amino acids not shown in Fig. 1 were: hydroxyproline 75 min; S-methylglutathione 65 min; S-methylcysteine 140 min; citrulline 180 min and cystine 400 min. It has not been possible to separate glycine from alanine or isoleucine from leucine by altering the gradient. Indeed, the system is remarkably insensitive to changes in pH in this region. Separation can be effected by lowering the temperature of the column after glutamic acid has emerged, but since the main use of the system is to isolate relatively large amounts of amino acids in a form which facilitates their identification, this is rarely necessary.

Radioactivity in the fractions can be located and measured by scintillation counting, as the buffers give clear solutions when mixed with methyl cellosolve (0.1 ml:2 ml) and scintillator (2,5-diphenyloxazole, 0.5 % in toluene, 10 ml). Although these buffers are supposed to be volatile, in practice it is often difficult to remove the last traces of solvent, especially in early fractions when thiodiglycol is present. Spots applied to paper chromatograms may appear "greasy" and thiodiglycol will

interfere with reagents used to aid identification of sulphur-containing amino acids. Washing the papers in acetone and drying before chromatography removes thiodiglycol and traces of buffer and good chromatograms can be obtained.

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