A modified elution schedule for accelerated amino acid analysis

The resolution obtained with spherical resins when using the factory-recommended accelerated amino acid analysis elution schedules is inadequate for many plant samples. This difficulty is caused, in part, by the relatively large content of aspartic acid, amides, and ammonia. Moreover, in short column basic amino acid analyses, tyrosine, 3,4-dihydroxyphenylalanine and phenylalanine appear as a single peak, and tryptophan is resolved only as a broadly spreading peak. This paper describes changes in buffer pH and water jacket temperatures, as well as alterations in buffer and temperature change times which have eliminated these problems.

*Experimental**

Apparatus. A Beckman 120B amino acid analyzer was used at buffer flow rates of 50 ml/h and a ninhydrin reagent flow rate of 25 ml/h. Back pressures were 345 p.s.i. at 30° and 120 p.s.i. at 60° for the long and short columns, respectively. High pressure lines between the pumps and columns were 0.062 in. I.D. × 0.030 in. wall Teflon TFE (Penn. Fluorocarbon Co., Clifton Heights, Pa.). Adjustment of pH was done using a Beckman Expandomatic pH meter.

Reagents. Sodium citrate buffers were prepared according to the recommended procedures[1]. Modified pH's were obtained by adding either 50% NaOH or conc. HCl to the appropriate buffer. Caprylic acid, rather than pentachlorophenol, was used as a preservative. All other reagents were prepared according to Beckman specifications.

Procedure. Basic amino acids were separated on a 20 × 0.9 cm PA-35 resin column. Elution was started with a pH 4.62 (0.38 *N*) buffer which was changed to a pH 5.28 (0.35 *N*) buffer after 30 min. The water jacket temperature during the entire analysis was 60°. In this procedure, if the ninhydrin pump was started at the same time as column elution there was not sufficient time to set the recorder baseline before the appearance, of the first peak. Therefore, this adjustment was made just prior to the short column analysis while pumping buffer through the long column.

A 57 × 0.9 cm PA-28 resin column was used for analysis of the acidic and neutral amino acids. After regeneration at 60°, the column was equilibrated with pH 3.47 (0.20 *N*) buffer; a reproducible amount of this same buffer was used to wash and fill the column after the sample had been driven into the resin column. Elution was started with a pH 3.12 (0.20 *N*) buffer at 30°. A temperature change to 55° was started after 105 min and a buffer change to pH 4.25 (0.20 *N*) after 215 min.

Results and discussion

Figs. 1 and 2 illustrate the resolution obtained with a synthetic mixture of amino acids and amides. Peak elution times for compounds not shown in the figures are: (57 cm column) taurine 44 min; urea 49 min; 3,4-dihydroxyphenylalanine 306 min; (20 cm column) anthranilic acid 50 min; kynurenine 55 min; 5-hydroxytryptophan 58 min; hydroxylysine 72 min; 5-hydroxytryptamine 91 min; carnosine and creatinine 109 min; 1-methylhistidine 115 min; L-3-methylhistidine 119 min; tryptamine 122 min.

* Reference to a company or product name does not imply approval or recommendation of the products by the U.S. Department of Agriculture to the exclusion of others that may be suitable.

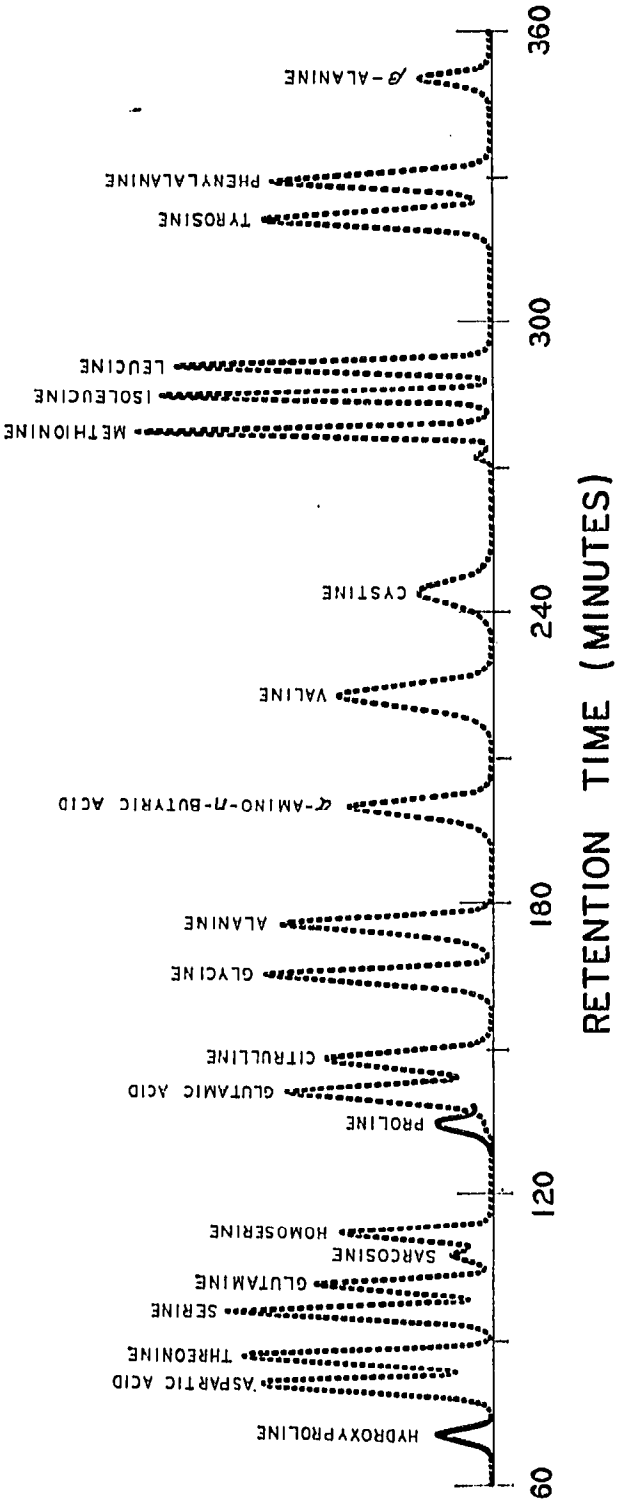

Fig. 1. Chromatogram of a synthetic mixture of acidic and neutral amino acids. 0.25 μmole of each compound was used except for hydroxyproline (0.10 μmole) and glutamine, sarcosine, homoserine, citrulline, α-amino-n-butyric acid and β-alanine (0.20 μmole).

The use of the pH 3.47 buffer for equilibration, as well as for filling, gives better reproducibility than if a buffer with pH higher than 3.28 is used for filling only. Automation for the addition of this buffer can be done by using a system similar to that described by HUBBARD[2] for accelerated regeneration and equilibrium.

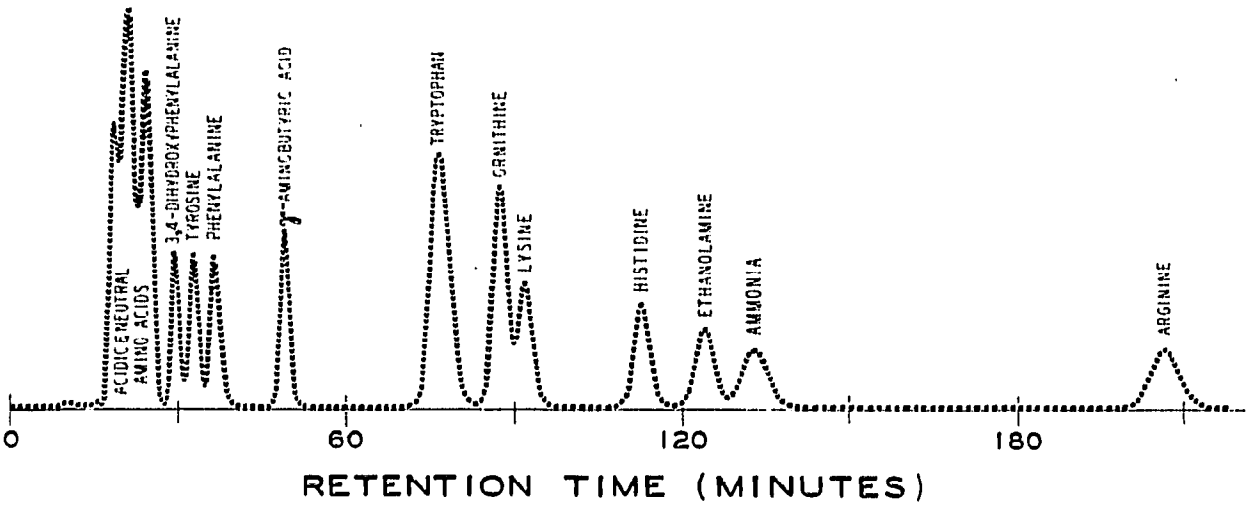

Fig. 2. Chromatogram of a synthetic mixture of basic amino acids. 0.125 μmole of each compound was used except for tryptophan (0.50 μmole) and ornithine (0.30 μmole).

In the analysis of basic amino acids, 3,4-dihydroxyphenylalanine, tyrosine and phenylalanine were separated with the pH 4.62 first buffer. Ethanolamine, ornithine and hydroxylysine can interfere with ammonia, lysine and tryptophan, respectively. However, the concentrations of the former group in most plant samples are such that they are unlikely to cause serious problems. This schedule is particularly useful for studies on aromatic amino acid metabolism.

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