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Note

Modifications in gradient elution of amino acids

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Gradient elution of amino acids from ion-exchange resins, introduced by Piez and Morris[1] in 1960, is still commonly used in amino acid analysis. Many gradients have been devised and adapted to particular ion-exchange resins, column lengths, analysis times and other special requirements[2–8]. Improved resins and 50- to 75-cm columns permit two or three analyses per day with gradient systems, which is sufficient for many laboratories.

This communication describes modifications in a commonly used system for the Technicon Model NC-1 (ref. 8) that simplifies operation and increases sensitivity of the instrument. Changes in buffers, gradient, manifold tubing and ninhydrin reagent are presented.

MATERIALS AND METHODS

Basic system

A Technicon Model NC-1 amino acid analyzer is used in this work (Technicon, Tarrytown, N.Y., U.S.A.). Amino acids are resolved on a column, 0.63 × 60 cm, containing Type C-2 Chromobeads. The column is kept at 55°, and buffer is pumped through the column at 0.80 ml/min. A complete analysis takes about 7 h.

Gradient system

The new gradient and buffers are derived from a system designed for analysis of protein hydrolysates in 5.5 h on 65-cm columns with Type C-2 Chromobeads[8]. Buffers of pH 2.875, 3.80 and 6.10 are prepared as described[8] except that they are made from sodium citrate alone and contain 1 ml liquefied phenol per liter. A new pH 2.85 buffer, which is prepared in the same way as the pH 2.875 buffer[8], contains 10% *n*-propanol which is added prior to pH adjustment and final dilution.

A nine-chambered gradient device is used with 40 ml of buffer in each chamber. Chamber 1 contains pH 2.85 buffer; chambers 2 and 3, pH 2.875; chambers 4 and 5, pH 3.80; and chambers 6–9, pH 6.10. The resin is regenerated after each analysis with 0.2 *N* sodium hydroxide that contains 1 m*M* ethylenediamine tetraacetate and 0.3% Brij, and then the resin is equilibrated with buffer of pH 2.875.

Manifold

The proportioning pump is fitted with the following manifold tubings: sample,

0.04 in. I.D., 0.6 ml/min; nitrogen, 0.04 in. I.D., 0.6 ml/min; ninhydrin, 0.045 in. I.D. (Solvaflex), 0.7 ml/min; and colorimeters, 0.056 in. I.D. (Solvaflex), 1.06 ml/min.

Ninhydrin reagent

The usual stock ninhydrin–hydrindantin reagent[8] is diluted 1:1 with 50% methyl cellosolve, and the final concentration of ninhydrin is 10 g/l. A 4- or 6-liter bottle of the diluted reagent is prepared and stored under nitrogen by the technique of Spackman *et al.*[9].

RESULTS AND DISCUSSION

Fig. 1 shows a typical analysis of 50 nmoles of the common amino acids found in acid hydrolysates using the described buffers and analytical system.

The small amount of *n*-propanol introduced in the buffer in the first gradient chamber improves resolution of threonine and serine without adversely affecting other parts of the chromatogram. If pH 2.875 buffer is placed in the first chamber instead of the 10% propanol buffer, the valley between these residues changes from 34% to 74% of the peak height of serine. Others have observed the effects of *n*-propanol on the resolution of acidic and neutral amino acids[10–12].

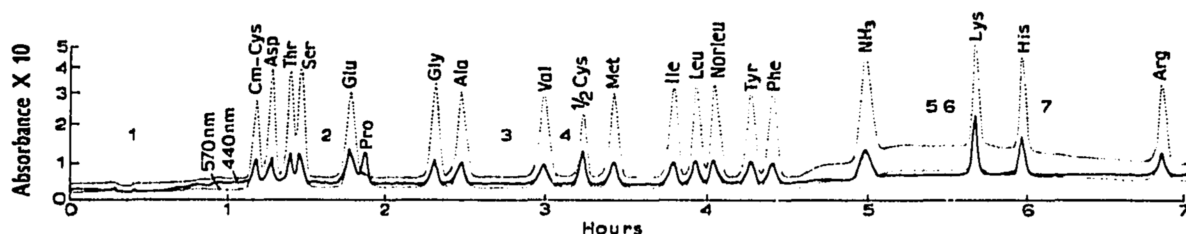

Fig. 1. Chromatogram of amino acid standard. Analysis of a solution containing 50 nmoles of each common amino acid plus S-carboxymethylcysteine and norleucine using the system described in the text. Numbers indicate the elution position of other compounds of interest: 1 = cysteic acid; 2 = homoserine; 3 = glucosamine; 4 = galactosamine; 5 = ornithine; 6 = S-aminoethylcysteine; 7 = tryptophan.

The new gradient is made with a single stock buffer in each chamber and does not require multiple measurements to fill a single chamber as in the system designed for 5.5-h analysis on 65-cm columns[8]. Also, adjacent chambers of the mixer which contain the same buffer can be filled with a single measurement of a graduated cylinder. Equilibration of the column with a pH 3.10 buffer, as also recommended, is unnecessary; and the pH 2.875 buffer can be used. Other simplified gradients, adapted to the flow-rate and column size of this system, do not resolve the acidic and neutral amino acids as well as the described system[13,14].

The new manifold analyzes 62% of the sample through the colorimeters compared to 32% with the manifold designed for the 5.5-h system[8]. Another column (0.63 × 10 cm) for analyzing only the basic amino acids using a single buffer (0.35 *N* sodium citrate, pH 5.28) is easily attached to the described manifold and is used to measure protein concentration or to resolve just the basic amino acids. This analysis requires about 75 min, and a two-column system utilizing this short column and

overlapping of short and long column elutions takes about 5 h for a complete analysis. A system that runs all the column effluent into a segmented ninhydrin stream could increase sensitivity even further[15], but these manifolds are not readily interchangeable with different columns.

Linearity in the production of ninhydrin color was observed with standard solutions of leucine up to 0.1 m*M*, which gives an absorbancy of 1.31.

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