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Use of Tartrate Buffers for the Preparative Elution of Amino Acids from Ion-Exchange Columns[1]

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Sulfonic acid resin columns are readily used for the separation of even large quantities of amino acids. Such chromatography is especially useful if the physical and chemical properties of the amino acids in question prevent their efficient separation by other means. The removal of organic buffer from the column eluate by the use of volatile buffer systems (1) or ion-exchange resins (2) is, however, not easily handled on the large, preparative scale. Our need for gram quantities of the separated geometric isomers of 2-aminobicyclo[2,2,1]heptane-2-carboxylic acid (BCH) (3) led us to the use of a tartrate buffer system from which amino acids with apolar side chains are recovered by extraction from the dehydrated buffer.

Preparative separations were made on a 3.8×120 cm column jacketed at 50°C. The resin was AG 50W-X8, −400 mesh (Bio-Rad) from which fines had been removed. Two pumping systems were used. In the first, a flow rate of 280 ml/hr was maintained by a Milton Roy Minipump. The column effluent was led directly to a fraction collector by which 25 ml aliquots were collected. A drop of each fraction was spotted on Whatman No. 1 paper which had been freshly sprayed with 0.3% ninhydrin in acetone and the color was allowed to develop for 15 min at 110°C. The second system employed a Spinco amino acid analyzer (model 120 C) equipped with pumps geared to deliver 400 ml/hr and with a stream splitter for the continuous analysis of the column effluent

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through the amino acid analyzer. Analytical parts of the study were performed with a Phoenix amino acid analyzer (model K-8200-B).

Sodium citrate and sodium tartrate buffers (0.2 N in Na^+) with pH 3.25 or 4.25 were prepared in deionized water using trisodium citrate and citric acid or disodium tartrate and D-tartaric acid. All buffer chemicals were reagent grade and were obtained from the J. T. Baker Chemical Co. No mold inhibitors, reducing agents, or surfactants were added. Fresh buffer was prepared frequently to minimize microbial contamination.

In order to determine the applicability of tartrate buffers for use in amino acid separations, 0.2 ml of the Calbiochem standard solution of amino acids (catalog No. 89300) was applied to the 60 cm column of the amino acid analyzer and the amino acids were eluted using either the citrate or the tartrate buffers under accelerated conditions. All 14 neutral and acidic amino acids of the standard were slightly retarded when the citrate buffers were replaced by tartrate buffers. The retardation, however, did not result in any change in the sequence of elution, or in any distortion in the shapes of the chromatographic peaks. The color obtained with ninhydrin per mole of amino acid was only 68–94% as great using the tartrate buffers as it was using the citrate buffers.

Table 1 shows the extent to which various amino acids were extracted from dried tartrate buffer by hot ethanol. Extraction of the dried buffer itself led to the dissolution of no more than 5 mg/hr of its components, whereas extraction of sodium citrate buffer under similar conditions yielded over 10 times that quantity. The tartrate residue must be dried carefully and stored in a desiccator over KOH to minimize extraction of its components. The pH of the column eluate before dehydration is not critical so long as there is little or no free tartaric acid present and no free strong base present. Buffer-amino acid solutions dehydrated at pH 4.25–6.5 gave acceptable results. Commercial absolute ethanol proved suitable as the solvent without further purification. We consider the procedure to be particularly useful for the isolation of amino acids found to be extracted by 50% or more during the interval selected.

For the large-scale separation of the isomers of BCH a 0.5 gm sample of the mixed synthetic product [from either the Bucherer or the Strecker procedure (3)] was dissolved in 50 ml of tartrate buffer, pH 4.25, and the solution was applied to the preparative column previously equilibrated with the same buffer.

Using the 280 ml/hr pump, the column was eluted for 24 hr using the pH 4.25 tartrate buffer. Sequential samples containing significant amounts of the desired isomers were combined and the solutions were evaporated nearly to dryness over an open flame. The residue was

TABLE 1

Extent of Amino Acid Extraction from the Buffer Residue

To mimic the eluate from a preparative column, a solution was prepared containing 0.50 mmole of each of the amino acids listed, in 1.00 liter of sodium tartrate buffer at pH 4.25. The solution was evaporated to near dryness over an open flame, dried at 110°C for 18 hr, and ground to a fine powder using a mortar and pestle. The powder was placed inside a Whatman No. 1 filter paper cylinder and extracted for 12 hr in a Soxhlet extractor (Kontes, catalog No. K-585000, size 23) using absolute ethanol. The solvent was refluxed at a rate such that the extraction reservoir emptied approximately 12 times/hr. The extract was brought to dryness on a rotary evaporator and dissolved in 1.00 liter of the same buffer. The amounts of the amino acids present before and after extraction were determined by the amino acid analyzer, and the percentage of each extracted was calculated.

Amino acid	Per cent extracted
L-Cysteic acid	10.0
L-Aspartic acid	11.5
L-Glutamic acid	4.0
L-Serine	13.6
L-Proline	35.0
Glycine	17.7
L-Alanine	36.4
L-Valine	58.5
L-Norleucine	60.5
L-Isoleucine	88.5
L-Phenylalanine	76.5
β-Alanine	20.6
L-Histidine	12.6
L-Lysine	8.6
L-Arginine	12.3

further dried and extracted with ethanol as described in Table 1. The ethanol extract was taken to dryness, the residue taken up in a minimal amount of water, and the solution decolorized with norite. The aqueous solution was then brought to 70% in ethanol at the boiling point and the amino acid allowed to crystallize at 2°C for 48 hr.

If the dried buffer powder contained as much as 200 mmoles of amino acid/kg, we found it advantageous to discontinue the extraction after 12 hr, then to dissolve the buffer residue in water and dry it again, before continuing the extraction for another 12 hr. The procedure was followed until 4 extractions had been made; in this way recoveries of 85% were routinely obtained. If no more than 20 mmoles of BCH was present per kg dried buffer, a single extraction for 12 hr recovered 90% of the amino acid. The absorbance per mg BCH after ninhydrin treatment was the same before and after the extraction procedure. Rechromatography of the two isomeric preparations using the amino acid analyzer showed that their separation from each other was complete.

The results in Table 1 show a wide range in the rate at which amino acids are extracted by ethanol under the conditions described. Clearly those amino acids with apolar side chains as large as or larger than the isopropyl group are extracted rather well. This method seems suitable for the isolation of such amino acids.

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