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Note

Rapid preparative separation of amino acids with the chromatofuge

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Ion-exchange chromatography is the preferred analytical method for resolving mixtures of amino acids[1]. Applications of this method to preparative work are hampered by high cost, low flow-rates, or the need for high pressures. In the chromatofuge[2], centrifugal force replaces pressure and effects rapid fractionation of relatively large amounts of material by radial migration through a hollow column rotating around its axis. A larger version of the original chromatofuge was constructed and its applicability to the isolation of amino acids on a preparative scale was tested.

EXPERIMENTAL

The present version of the chromatofuge is basically a modified basket centrifuge. It is schematically represented by Fig. 1. An International centrifuge, size 2 (International Equipment, Boston, Mass., U.S.A.)* was fitted with a modified 3-1 perforated 4 × 11 in. diameter cylindrical basket (G), and with a custom-made collector (K), designed to drain without appreciable hold-up into an outlet tube (L). The 2-in. top ledge of the basket was widened to 3.5 in. in order to accommodate a sorbent layer 3.2 in. in thickness. The delivery tube (F), made of 0.25-in. I.D. pipe, had a nozzle (Floodjet Nozzle 1/8 KSS 2.5; Spraying Systems, Wheaton, Ill., U.S.A.) attached to its end, which produced, in operation, a vertical sheet of liquid.

The ion-exchange resin (Dow Chem., Midland, Mich., U.S.A.; 4.5 kg of Dowex 50W-X8, finer than 400 mesh) was suspended in an equal weight of water. After standing at room temperature for 150 min, the water was decanted and replaced by an equal volume of fresh water. After 30 min, the water and fines were decanted and replaced by an equal volume of 0.1 *N* NaOH. After another 30 min, the NaOH solution and fines were decanted, and the resin was washed with three portions of water. It was allowed to stand in the last water wash overnight and was subsequently washed twice with 0.2 *N* HCl and three times with water, being kept in the last water wash overnight. Next, the resin was treated twice with an equal volume of 0.2 *N*

* Reference to a company and/or product named by the Department is only for the purpose of information and does not imply approval or recommendation of the product named to the exclusion of others which may also be suitable.

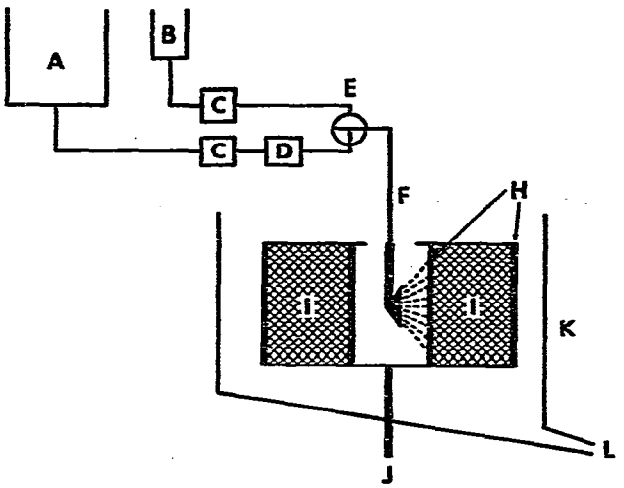

Fig. 1. Schematic representation of the chromatofuge. A = Eluent reservoir; B = sample reservoir; C = pump; D = flow-meter; E = 3-way valve; F = delivery tube; G = centrifuge basket; H = felt lining; I = ion exchanger; J = drive shaft; K = collector; L = effluent outlet. All pipes are made of 1/4-in. stainless-steel tubing.

NaOH and washed three times with water. Finally, a slurry of resin with an equal weight of water was prepared for packing the basket.

The delivery tube was taken out of the chromatofuge and the basket was lined with a previously moistened strip of polypropylene F felt (Pacific States Felt Mfg., San Francisco, Calif., U.S.A.) (H), 4 in. wide. Then, with the centrifuge spinning at 1000 rpm (200 *g*), the slurry was slowly poured into the basket, forming a uniform column with a hollow core. The centrifuge was stopped and the inner wall of the hollow cylinder was lined with another moistened 4-in. strip of felt to protect it from spray damage. With the delivery tube placed in position, the chromatofuge was now ready for operation.

The amino acid samples were dissolved in 250 ml of a buffer solution, prepared by making 19.6 g sodium citrate·$2H_2O$ and 16.5 ml conc. HCl to 1 l with water (pH 2.2, 0.2 *N* Na^+ conc.). For elution, a buffer, prepared by making 3432.5 g sodium citrate·$2H_2O$, 655 ml conc. HCl and 5 ml octanoic acid to 100 l with water (pH 5.29, 0.35 *N* Na^+) was used.

The eluent was transferred to Reservoir A (Fig. 1), which had a capacity over 50 l, and the sample was introduced into Reservoir B. While the centrifuge was running at 1000 rpm (200 *g*), the resin was first flushed with 10 l of eluent by turning the 3-way valve (Three-Way Ball Valve SS 44 X S6; Whitey, Oakland, Calif., U.S.A.) (Fig. 1) to connect the delivery tube with Reservoir A through a metering pump (All-Chem Rotary Pump, Model PP2; ECO Pump, South Plainfield, N.J., U.S.A.; driven with a Master Motor, Style 208270, with Master Speed Ranger, Style 3035850, Size VD 15, Master Electric, Dayton, Ohio, U.S.A.) and flow-meter (Precision Bore Flowrator Tube No. 4-19-10/77; Fischer and Porter, Hatboro, Penn., U.S.A.). The valve was switched to Reservoir B, effecting sample delivery through a metering pump (All-Chem Rotary Pump, Model PP1, ECO Pump; driven with a Master Motor,

Style 209470, with Master Speed Ranger, Style 3035850, Size VD 15, Master Electric) within 30 sec. Switching back to Reservoir A, eluent was then delivered to the column at a flow-rate of 1 l/min. The effluent from the chromatofuge was collected in 500-ml fractions for subsequent analysis.

For qualitative analysis the thin-layer chromatographic method of Von Arx and Neher[3] was used. Samples of effluent were spotted on Eastman Chromagram Sheets (Sheet 13255; Eastman-Kodak, Rochester, N.Y., U.S.A.), precoated with a 160-μm layer of microcrystalline cellulose. The sheets were developed with *n*-butanol–acetone–diethylamine–water (10:10:2:5). The amino acids were detected by spraying with 0.2% ninhydrin in acetone, followed by heating the sheets over a steam bath.

For quantitative analysis a Technicon Autoanalyzer (Technicon Instruments, Tarrytown, N.J., U.S.A.) was used according to the method of Quinn *et al.*[4]. The details of operation are evident from the left side of Fig. 2.

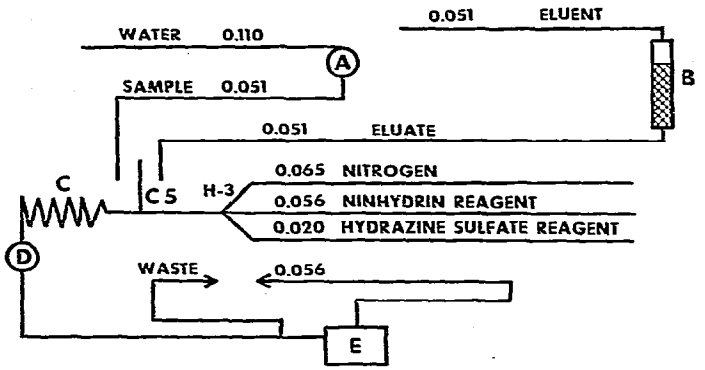

Fig. 2. AutoAnalyzer connections for amino acid analysis of samples of the fractions from the chromatofuge (left) or of the eluate from the ion-exchange column (right). A = Sampler, furnishing 40 samples/h; B = ion-exchange column; C = double heating coil; D = heating bath at 95°, containing a 40-ft. glass coil; E = colorimeter at 570 nm; F = recorder. The figures denote the internal diameter of plastic tubing, in mm.

For comparison, the same eluent was used in a Dowex 50W-X8 column (8 × 0.9 cm I.D.) to separate the same amino acid mixtures as the chromatofuge. The column was always loaded with 0.25 ml of sample in the sample buffer (see above) and eluted with the eluent buffer (see above) at a flow-rate of 1 ml/min. The column effluent was monitored by replacing the sampler (see left side of Fig. 2) with a 0.051-mm tube, connected to the column outlet (see right side of Fig. 2).

RESULTS AND DISCUSSION

The selection of amino acids for this feasibility study was based partly on our ability to separate them by analogous conventional column chromatography and partly on the practical significance of the isolation of the nutritionally important ones. The purification of amino acids, whether they are obtained by synthesis or isolation from natural products, is a major factor in their cost and availability. We have tested

the performance of our chromatofuge by comparison with the short-column analytical separation of basic amino acids reported by Spackman *et al.*[5].

Fig. 3a represents the chromatogram we obtained with the conventional ion-exchange column. With 50 μg of each of the five amino acids shown, the results were similar to those in ref. 5, except for the arginine peak, which was somewhat broader in our system. Because the elution of arginine was expected to require large amounts of eluent, this amino acid was omitted from the chromatofuge tests. Fig. 3b represents the chromatogram obtained with up to 5 g of each of the remaining four amino acids. The less complete separation with the chromatofuge compared to the conventional column reflects the much greater sample load and the much more rapid flow-rate. The flow-rate was arbitrarily selected to determine the resolving efficiency of the chromatofuge at different sample loads. The flow-rate through the ion-exchanger is controlled by the speed of rotation rather than the eluent feed rate. The efficiency of the chromatofuge will be constant at constant speed of rotation as long as the eluent is not fed at a rate that causes flooding of the basket. More rapid separation could have been accomplished by increasing the speed of rotation. While the separations are not as complete as in conventional column chromatography, they are adequate for preparative purposes. Greater purification and concentration is attainable by rechromatographing the effluent in the chromatofuge.

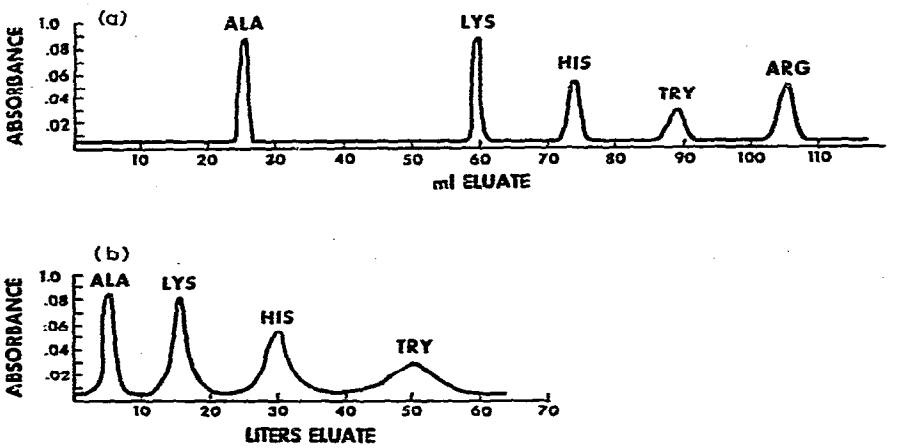

Fig. 3. Chromatogram of amino acids from a conventional column (a) and from the chromatofuge (b). ALA = Alanine; LYS = lysine; HIS = histidine; TRY = tryptophan; ARG = arginine.

The recovery of amino acids from the chromatofuge was just as good as that from conventional chromatography (Table I). Moreover, it was independent of sample load up to 5 g per amino acid.

Our results indicate that rapid and efficient separations by ion-exchange are feasible with the aid of the chromatofuge. Relatively large quantities of amino acids can thus be prepared in pure form without recourse to expensive high-pressure equipment. Further work will be required to delineate optimal conditions of rotational and pumping speeds as well as basket and spray configurations.

TABLE I

PERCENT RECOVERY OF AMINO ACIDS FROM 50 μg OF EACH AMINO ACID, SEPARATED BY A CONVENTIONAL ION-EXCHANGE COLUMN, AND 1, 2, OR 5 g OF EACH AMINO ACID, SEPARATED BY THE CHROMATOFUGE

Amino acid	*Recovery (%)*			
	Amount of each amino acid			
	50 μg	*1 g*	*2 g*	*5 g*
Alanine	93	94	91	92
Lysine	91	90	91	91
Histidine	90	89	87	91
Tryptophan	85	83	84	85

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