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Accelerated analysis of some amines and amino acids

Quantitative analyses for *p*-tyramine, cadaverine, putrescine, histamine, and tryptamine were found to be necessary in the course of some studies of the amino acid metabolism of grass silage on an automatic amino acid analyzer. Estimations of these silage components have been carried out by quantitative paper chromatography[1–3], but a method which would require the minimum of desalting or other pretreatment was considered to be desirable.

The studies of PERRY AND SCHROEDER[4] on the range of amines found in urine samples indicated that an extension of automatic amino acid analysis[5] to amine analysis was possible if weak (carboxylic acid) cation exchangers were used in place of the strong (sulphonic acid) cation exchange resins in the chromatographic columns.

Methods and materials

Samples of the polymethacrylic acid exchanger used by PERRY AND SCHROEDER[4], Amberlite CG-50 (Rohm and Haas Co., Philadelphia, Pa., U.S.A.), and the polyacrylic acid exchanger, Zeo Karb 226 (2.5 % and 4.5 % DVB grades, Permutit Co., Ltd., London), were fractionated in the sodium salt form by the method of HAMILTON[6]. Those particles which were washed through a 2 l separating funnel at flow rates between 50 and 200 ml/min were found to be most suitable for the chromatographic studies described in this paper.

Sample volumes were 0.5 ml or less and were loaded on the columns by application of gas pressure; the inside of the chromatographic tube was carefully washed with three 0.2 ml portions of the eluting buffer before the column was filled with buffer to begin an analytical run.

The pyridine–acetic acid buffers used by PERRY AND SCHROEDER[4] were found to swell the resins and soften them in such a way as to prevent adequate flow rates to be used with the desired particle sizes. Since the purpose of the separations was purely analytical, there was no objection to inorganic buffer salts, and the method was accordingly developed with sodium and potassium salt buffer systems.

Amberlite CG-50 resins are only available as ground materials in the particle sizes used, but these irregular particles were found to be too fragile to allow a fixed bead size to be maintained in use in small columns. The bead form resin, Zeo Karb 226 × 4.5 (Permutit SRC-48, batch 4) was the only commercially available product which could be made to give adequate separating power with reproducible hydraulic properties.

Basic amino acids. It was soon obvious that the weak cation exchange columns were just as efficient for separations of basic amino acids as the conventional stronger acid exchangers, with the proviso that very weakly basic amino acids such as 4-aminobutanoic acid and 3-aminopropanoic acid were not differentiated from the neutral and acidic amino acids. Table I gives the elution times of the (completely separated) basic amino acids on a column (14 by 0.5 cm) of Zeo Karb 226 × 4.5 (20 ± 8 μ bead diameter) at a flow rate of 31 ml/h of sodium maleate (0.15 *M*) buffer (pH 6.83) at 48°.

Amines. Unfortunately, silage extracts contain significant quantities of 4-amino-

TABLE I

ELUTION TIMES OF THE BASIC AMINO ACIDS

Amino acid	*Elution time (min)*
Lysine	25
Ornithine	32
Histidine	38
Arginine	46
Ammonia	55

butanoic acid, so further development of the weak acid resin columns was limited to the amine components alone. Amberlite CG-50 was the most efficient resin tried for the separation and at 48° complete resolution of all the amines present in an extract was readily achieved on a column (16 by 0.5 cm) of CG-50 (average particle diameter 20 μ) at a flow rate of 31 ml/h of sodium maleate buffer (pH 6.14, 0.12 *M* disodium maleate plus 0.18 *M* sodium chloride, 1.04 *M* in sodium) in rather less than 2 h, but the continuous fragmentation already mentioned prevented use of this granular resin. The Zeo Karb 226 × 2.5 resin was also very efficient, but it was so soft that even moderate operating pressures of 50 p.s.i. caused bead deformation and consequent rapid increase of the operating pressure.

TABLE II

ELUTION TIMES OF THE PHYSIOLOGICAL AMINES

Amine	*Elution time (min)*
Ammonia	31
2-Amino-3-guanidino-propanoic acid	49
p-Tyramine	81
Cadaverine	95
Putrescine	104
Histamine	140
Tryptamine	165

Satisfactory results were obtained for amine analyses with the Zeo Karb 226 × 4.5 exchanger only after a switch from sodium to potassium buffer salts. Table II gives the elution times of the amines (and the 2-amino-3-guanidinopropanoic acid used as internal standard) on a column (22 by 0.5 cm) of this latter resin (20 ± 8 μ bead diameter) at 52°, and a flow rate of 28.5 ml/h of potassium citrate buffer (pH 6.15, 1.1 *M* in potassium, composition as given in Table III). Back pressure with this system is about 80 p.s.i. and the column can be used (without regeneration) for at least a dozen analyses before deterioration of the resolving power becomes noticeable.

The separations were monitored by continuous colorimetry using a standard Technicon (Technicon Instruments Co. Ltd., Chertsey, Surrey, England) amino acid analytical system with 1.5 and 0.8 cm path length flow cuvettes. With this system

TABLE III

POTASSIUM CITRATE BUFFER FOR AMINE SEPARATION

Component[a]	*Amount*
Potassium concentration (*M*)	1.10
Citrate concentration (*M*)	0.033
Potassium citrate (g)	21.6
Potassium chloride (g)	149.1
Brij 35 (ml) (50 g/100 ml)	20
Octanoic acid (ml)	0.20
Final volume (l)	2
Concentrated hydrochloric acid	to pH 6.15 ± 0.02

[a] All reagents except Brij 35 are "Analar" grade from British Drug Houses Ltd., Poole, Dorset, England. Brij 35 (polyoxyethylene lauryl ether) was obtained from Koch-Light Laboratories Ltd., Colnbrook, Buckinghamshire, England.

the diamines, cadaverine and putrescine, gave ninhydrin colour yields equivalent to those of their parent amino acids, lysine and ornithine; the three monoamines, *p*-tyramine, histamine, and tryptamine, were slightly less reactive and gave about two-thirds of the colour yield of lysine. The complete analytical system allows reasonably rapid determination of samples containing 0.25 to 0.01 μmoles of each amine component with a reproducibility of ± 3 %.

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