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Accelerated chromatographic analysis of primary mono- and diamines in an amino acid analyzer

The chromatographic analysis of primary mono- and diamines in an amino acid analyzer has been reported previously[1-3]. In the earlier papers, the chromatographic procedure for mono- and diamines[2] and the relationship between retention volumes and dissociation constants of monoamines[3] were described. In the previous elution system, about 8 h was required for the analysis of sixteen amine compounds through to hexamethylenediamine on a 12 cm Aminex A-4 resin (BioRad Laboratories) column. This report describes an accelerated chromatographic procedure with high flow rates for the analysis of primary amines and the revision of the components of elution buffers on a cation exchange resin column.

Materials and methods

Authentic amines were purchased from Nakarai Chemicals Ltd., Kyoto and Tokyo Chemicals Co., Tokyo; all these amines were reagent grade except for pyrrolidine. The other chemicals employed in this experiment were obtained from Nakarai Chemicals Ltd. and Wako Pure Chemical Industries, Ltd., Osaka. A ninhydrin reagent was prepared according to the usual methods for amino acid analysis.

A Hitachi amino acid analyzer model KLA-3B (Hitachi Ltd., Tokyo) was employed. To improve the colour yield from the ninhydrin reaction with amines the reaction bath coil was extended in length to 20 m from 8.5 m as prepared by the maker for the analysis of amino acids. Hitachi custom spherical resin No. 2611 (supplied for chromatography of basic amino acids) was packed into a 0.6 × 10 cm column and equilibrated with a citrate buffer pH 5.28. Two mm flow cuvettes were employed and the absorbance of the ninhydrin colour developed was measured at 440, 570 and 640 mμ, and recorded on a three-point current recorder. An elution pump was adjusted to supply the elution buffers to the column at a rate of 120 ml/h, and the ninhydrin reagent was applied at the same rate as that of the buffers. The ninhydrin colour development was carried out in a 20-m length heating bath coil of I.D. 0.65 mm at 115° for 1.7 min. The elution was carried out by the combination of two stepwise and one gradient elutions. A three chamber gradient device was used to produce the gradient buffer, each chamber was charged with 120 ml of the buffer, and had a rubber stopper which connected with a trap filled with sodalime to prevent carbon dioxide uptake by the buffers. The buffer in the tube from the outlet of the gradient elution device to the buffer selector valve was replaced with the same buffer as that in the first chamber of the gradient system. The temperature of the column was maintained at 55° for the first 120 ml and at 60° for the remainder of the elutions. After 70 ml of the first buffer was pumped out, the buffer selector valve was changed to the second buffer, and at 150 ml from the start of the analysis, the eluant was changed to a three-component gradient buffer throughout the analysis. The buffer compositions used in the chromatography are shown in Table I.

Results and discussion

A chromatogram of a synthetic mixture of 0.25–1.0 μmole of amines on a

TABLE I

COMPOSITION OF THE BUFFER SYSTEM

Buffer	*I*	*II*	*III (gradient elution[a])*		
			1	*2*	*3*
Sodium citrate (*N*)	0.116				
Sodium borate (*N*)		0.025	0.050	0.050	0.050
Sodium salicylate (*N*)				0.100	0.200
Sodium hydroxide (*N*)			0.05	0.05	0.15
Sodium ion concn.[b] (*M*)	0.35	0.60	0.20	0.25	1.50
pH[c]	5.28	8.02	10.80	10.80	12.50
Benzylalcohol concn. (%)	0.5	1.0		1.0	1.0

[a] Each chamber contained 120 ml of the buffer solution.
[b] Adjusted with sodium chloride.
[c] Adjusted with conc. HCl or 6 *N* NaOH.

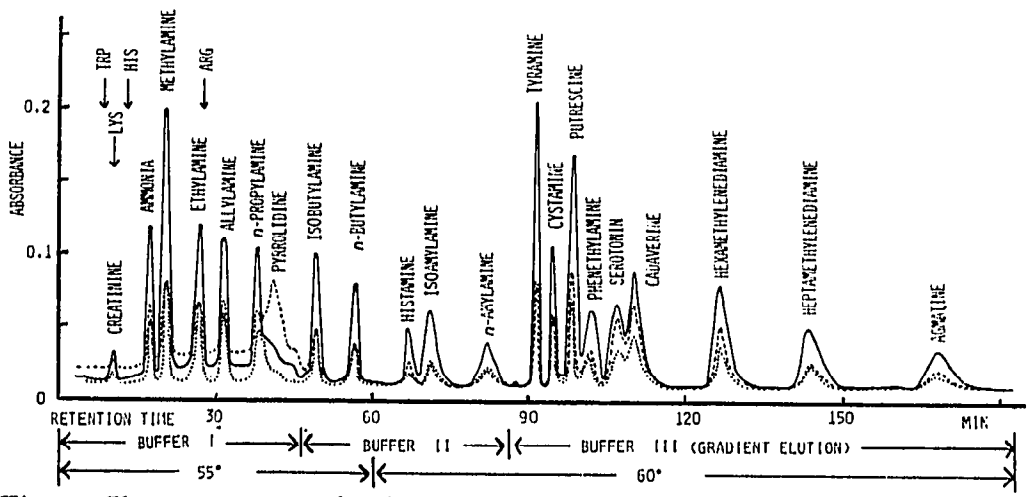

Fig. 1. Chromatogram of primary amines with 120 ml/h of buffer flow rate. 0.25 μmole of each amine was added except in the case of: agmatine 0.5; serotonin 1.0 μmole (as creatinine sulfate salt) and pyrrolidine *ca.* 1.0 μmole. Ninhydrin colour was detected in 2 mm cuvettes and recorded on a three-point current recorder at 440 (– – – –), 570 (———) and 640 mμ (· · · · · ·). The elution positions of the basic amino acids are indicated with arrows.

cation exchange resin column at a buffer flow rate of 120 ml/h is shown in Fig. 1. Several components were added to the previous system and the buffer composition was modified to reduce the time for analysis. In this system hexamethylenediamine and agmatine were eluted at 110 and 165 retention minutes, respectively, although the former had been eluted at 8 retention hours previously and the latter not eluted from a column. The retention times and peak areas calculated by the $H \times W$ method (net peak height $\times$ width of peak at half net height) of the amines in this system are shown in Table II, and the reproducibility of these values is also presented as coefficients of variation. The incomplete separation of serotonin and cadaverine was the reason for the considerable fluctuation of the $H \times W$ values of these two amines. If putrescine or phenethylamine do not occur in the sample, it is advantageous to add 0.5% of benzyl alcohol to buffer III-1 for a good resolution of serotonin and cadaverine. A better resolution of putrescine and phenethylamine was obtained when

TABLE II

REPRODUCIBILITY OF RETENTION TIMES AND $H \times W$ VALUES OF AMINES[a]

Amine	*Retention time (min)*	*C.V.[b] of retention time (%)*	$H \times W$ *value*[c]	*C.V.[b] of* $H \times W$ *value (%)*
Methylamine	20.1	1.1	1.83	2.4
Ethylamine	25.1	1.4	1.36	3.9
Allylamine	30.4	1.3	1.46	2.1
n-Propylamine	37.1	1.5	1.57	2.4
Pyrrolidine	39.5	1.5	—	—
Isobutylamine	48.2	1.0	1.49	3.6
n-Butylamine	55.5	0.9	1.34	4.0
Histamine	65.5	1.1	0.75	5.7
Isoamylamine	69.4	1.2	1.30	5.1
n-Amylamine	80.0	1.3	1.27	3.2
Tyramine	91.0	0.9	1.50	2.5
Cystamine	94.2	0.9	1.05	1.9
Putrescine	97.3	1.1	1.67	3.6
Phenethylamine	100.9	1.1	1.25	4.4
Serotonin	107.1	2.1	0.81[d]	6.8
Cadaverine	110.1	2.6	1.52	5.6
Hexamethylenediamine	125.6	5.0	1.62	1.6
Heptamethylenediamine	140.0	4.8	1.35	3.9
Agmatine	164.7	3.5	1.34[e]	3.9

[a] Each value is average of 5 experiments.
[b] Coefficient of variation.
[c] The values of 0.25 μmole were calculated.
[d] Value for 1.0 μmole.
[e] Value for 0.5 μmole.

a flow rate of 60 ml/h was chosen. When the analysis was carried out at the buffer flow rate of 120 ml/h, histamine was sometimes observed as two unresolved peaks. This phenomenon has never been observed at lower flow rates of elution buffers, 60 or 30 ml/h.

The pH of the mixture of equal aliquots of ninhydrin reagent and eluant was in the range 5.5–6.0 which was sufficient for the colour development.

This procedure requires no equipment other than a commercially available amino acid analyzer[4] and a specially designed simple gradient elution device. The resin, the column and the buffer I used for this analysis were the same as those employed for the analysis of basic amino acids. Therefore basic amino acids and primary amines can be simultaneously analysed except for arginine and ethylamine which are eluted at the same retention volume.

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