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Note

Buffer system for ion-exchange chromatography of amino acid mixtures containing methionine sulphone

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In a study of sulphur nutrition of ryegrass at this laboratory, it was necessary to determine (a) free amino acids, extracted from the leaves by 80% aqueous ethanol, and (b) the amino acid composition of the leaf protein (in the residue after ethanol extraction), especially the proportions of cystine and methionine. To minimize the losses of the sulphur amino acids during hydrolysis of the leaf protein, the vacuum-dried samples were treated with performic acid to convert cystine and methionine residues to cysteic acid and methionine sulphone, respectively[1]. Analyses were by ion-exchange chromatography. However, when the buffer system developed for the analysis of the free amino acids[2] was used for the hydrolysates, methionine sulphone was incompletely separated from aspartic acid and neither could be determined accurately (Fig. 1A). It would have been possible to use the systems of Thomas[3] or Redman[4], but this would have required two sets of buffers, one for the free amino acids[2] and the other for the protein hydrolysates, which would be inconvenient and uneconomical. The effects of starting the chromatography with a buffer of lower pH than 2.875 were therefore studied.

EXPERIMENTAL AND RESULTS

A Technicon NC-1 single-column amino acid analyser equipped with a nine-chamber Autograd was used. The 140 × 0.6 cm I.D. column was packed to a height of 126 cm with "Chromobeads" Type A and the column temperature was 60°. As both Thomas[3] and Redman[4] used a starting buffer of pH 2.5, this was tried, but in the first chamber only, the other buffers remaining the same as in the earlier work[2]. The modified buffer gradient increased the time interval between the methionine sulphone and aspartic acid peaks from 6 to 21 min but the peaks were broadened and skewed. The threonine, serine and glutamic acid peaks were similarly distorted. Obviously the reduction of pH in the starting buffer was too great. An adequate separation of 11 min between methionine sulphone and aspartic acid was obtained when the pH in the first chamber was adjusted to 2.72, peak broadening being slight (Fig. 1B). Furthermore, an analysis of a standard mixture of amino acids found in ethanolic plant extracts, including phenylalanine, ethanolamine, γ-aminobutyric acid and ammonium, showed that the resolution of all peaks was still perfect. The modified buffer system

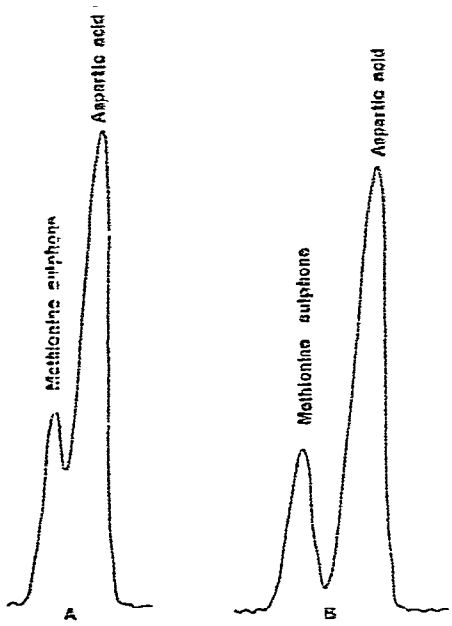

Fig. 1. Effect of pH of starting buffer on the separation of methionine sulphone and aspartic acid. (A) pH 2.875, (B) pH 2.72.

was therefore satisfactory for the determination of amino acids in both oxidised protein hydrolysates and non-protein ethanolic extracts.

It was not found necessary to equilibrate the resin with pH 2.72 buffer before loading the sample on the column and starting the analysis; pH 2.875 buffer could be used for this as previously. The pH 2.72 buffer was conveniently prepared by readjusting some of the pH 2.875 solution with 5 *N* hydrochloric acid.

The new system is given in Table I. Buffer compositions are detailed in the earlier publication[2].

TABLE I

BUFFER GRADIENT

Autograd chamber No.	*Buffer pH*	*Amount of buffer (ml)*	*Methanol (ml)*
1	2.72	70	5
2	2.875	72	3
3	2.875	75	—
4	2.875	75	—
5	4.20	75	—
6	5.00	75	—
7	5.00	75	—
8	5.00	75	—
9	5.00	75	—

REFERENCES

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