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Note

Simple buffer gradient for the chromatography of amino acids, including tryptophan, on a single-column Technicon analyser

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The simplified buffer system described previously[1] for gradient elution on a Technicon analyser resolves all common amino acids found in acid hydrolysates of proteins. Histidine and tryptophan are not resolved in this system, but the latter is destroyed during established acid hydrolysis procedures. However, the introduction[2,3] of acid hydrolysis conditions under which tryptophan is stable makes amino acid analysis the most suitable method for its determination. This note describes a modification to the buffer gradient such that histidine and tryptophan are resolved.

EXPERIMENTAL

The only change to the Autograd described previously[1] is that 120 ml of a third buffer replaces pH 5.00 buffer in chambers 8 and 9. This new buffer is prepared by adding sodium chloride (25.00 g) to 1 l of pH 5.00 buffer.

RESULTS

Increasing the sodium chloride concentration in the final stages of elution completely resolves histidine from tryptophan. The same effect was reported by Wells[4] using a more complicated system. If the increase is made uniform throughout chambers 6–9, phenylalanine is not resolved from the "ammonia baseline rise". By adding the extra salt only to chambers 8 and 9, satisfactory resolution of all the common amino acids is achieved. In this case tryptophan emerges 20 min after histidine, as measured by the time between peak maxima.

The inclusion of a third buffer makes the system slightly less simple. However, it still requires only buffers of two pH values and requires fewer operations than in the conditions recommended by the manufacturer, thereby reducing the possibility of error. Moreover, the addition of salt considerably shortens total analysis time as arginine is eluted approximately 75 min earlier. To avoid the Autograd running dry, a change from elution with the Autograd to elution with an alternative buffer still has to be made. However, if this alteration is made at 17.5 h it will be late enough to have no effect on arginine. Alternatively the change may be to sodium hydroxide for regeneration, again without risk of affecting the elution of arginine.

The presence of *p*-toluenesulphonic acid in the sample, as used in one method of tryptophan determination[3], has no effect on resolution.

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REFERENCES

1 D. G. Redman, *J. Chromatogr.*, 46 (1970) 107.
2 H. Matsubara and R. M. Sasaki, *Biochem. Biophys. Res. Commun.*, 35 (1969) 175.
3 T. Y. Liu and Y. H. Chang, *J. Biol. Chem.*, 246 (1971) 2842.
4 J. R. E. Wells, *Anal. Biochem.*, 19 (1967) 448.