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Note

Sodium chloride in buffers for amino acid analysis

Application to the analysis of lysine in maize samples

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Although manual photometric methods of analysis for lysine or tryptophan[1] are possibly most suitable for screening the large number of samples associated with maize-breeding programs, the accuracy of an automatic amino acid analyser is invaluable for a more subtle distinction between selected samples of high lysine maize. Nevertheless, as the number of such selected samples can be large it is essential that the accuracy of the automatic analyser be coupled with a high speed of operation. In a previous communication[2] a method was described employing L-2,4-diaminobutyric acid (DAB) as an internal standard to minimise errors and using an overlapping system of elution from two short columns, each operated with the conventional short column buffer, to reduce the time of analysis. By this means a sample could be analysed in 40 min.

A further reduction in the time of analysis has now been achieved by the inclusion of sodium chloride in the elution buffer. As reported by Moore and Stein[3], a decrease in the elution time can be effected by an increase in either the pH or the ionic strength of the buffer. An increase in pH, however, is attended by a relatively poorer separation between lysine and the internal standard, DAB, and between histidine and ammonia. Similarly, if the ionic strength is increased by increasing the buffer concentration, lysine and DAB tend to be eluted together (*e.g.* Fig. 1a). However, we have observed that, in general, substitution of part of the sodium citrate by sodium chloride or addition of extra sodium chloride causes the DAB to be eluted later relative to lysine. Consequently, if the buffer concentration is kept constant and the ionic strength is increased by adding sodium chloride, a decrease in the elution time is effected without a concomitant deterioration in the resolution between lysine and DAB (*e.g.* Fig. 1b). Addition of sodium chloride to the conventional pH 5.28 buffer is attended by a decrease in pH which causes histidine to be eluted together with ammonia. For the analysis of lysine this is no disadvantage but should a separation between histidine and ammonia be required, this can be effected simply by increasing the pH slightly.

The buffer system described above has an advantage in common with the conventional short-column buffer[3] in that a single buffer can be used both for lysine analysis and for complete basic amino acid analysis. However, a buffer suitable specifically for lysine analysis can be prepared by further increasing the proportion

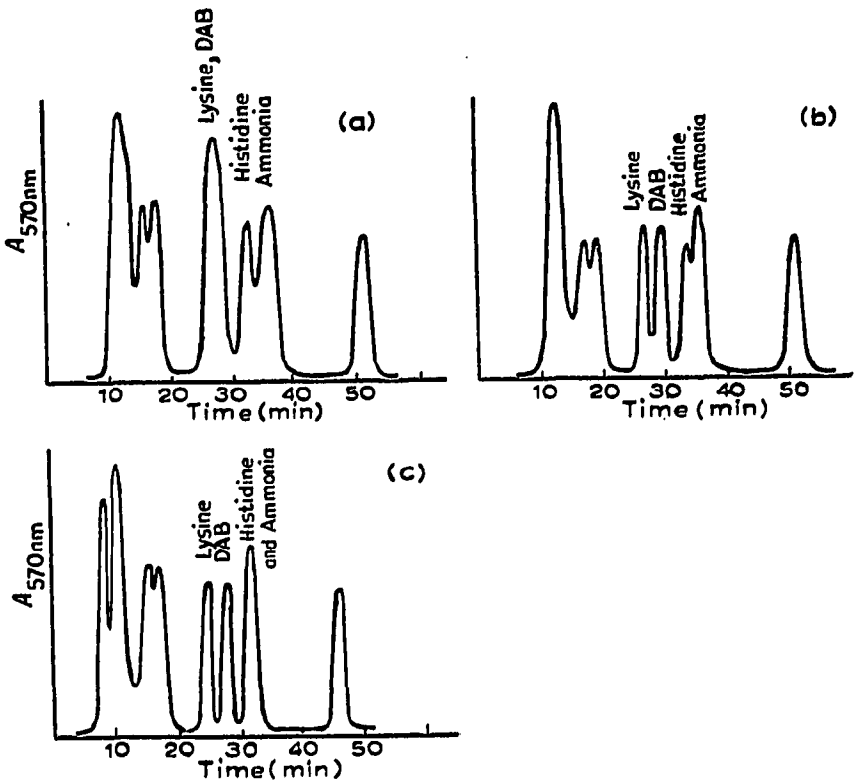

Fig. 1. The effect of substituting sodium chloride for sodium citrate on the elution of basic amino acids in buffers of elevated ionic strength. Buffers, (a) 0.7 *N* sodium citrate, pH 4.95; (b) 0.35 *N* sodium citrate–0.35 *M* sodium chloride, pH 4.95; (c) 0.1 *N* sodium citrate–0.6 *M* NaCl, pH 4.95; temperature, 58°; resin, 10.5 × 0.9 cm, Beckman type PA-35.

of sodium chloride, at the expense of sodium citrate, thus further improving the separation between lysine and DAB and by simultaneously lowering the pH to retard histidine. Lowering of the pH causes histidine to be co-eluted with ammonia but eliminates the overlap between DAB and histidine which results from the increased concentration of sodium chloride. For the routine analysis of lysine in maize samples we have used a buffer of pH 4.95 containing 0.1 *N* sodium citrate and 0.6 *M* sodium chloride, in addition to the usual proportions of detergent and preservative. Using this buffer system and a bed of PA-35 resin (Beckman, Palo Alto, Calif., U.S.A.; 10.5 × 0.9 cm), lysine and DAB are eluted at approximately 25 min and 28 min, respectively, while arginine is eluted at 47 min (Fig. 1c). In a two-column overlapping system[2] using this buffer, it is therefore possible to analyse a lysine sample, with internal standard, every 20 min. Twenty or more samples can thus be analysed per 8-h day.

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