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Note

Three-buffer single column analyses on two-buffer amino acid analyzers

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Although protein hydrolysates can be analysed on a single long column on conventional amino acid analyzers which have provision for only one buffer change, such two-buffer analysis programs require approx. 300 min for completion (for a review, see ref. 1). Accelerated, single column analyses can shorten this time to 200 min, but require the use of three buffers (two automated buffer changes)[2]. Unfortunately, the modification of conventional two-buffer instruments such as the Beckman 120 C to provide facilities for an additional automated buffer change normally requires the addition of a special timer, valve, and valve motor. These items, though commercially available, are expensive and may not appear warranted to some users.

We report here a simple and inexpensive method for three-buffer single column analysis on two-buffer analyzers. Analysis time is complete in 195 min, and the method gives satisfactorily reproducible retention times. The only modification required is the insertion of a small easily manufactured glass reservoir, with manually operated valves, into the second buffer line in series with buffer 3. This small reservoir is manually rinsed and recharged with the second buffer prior to each new analysis, an operation which takes less than 1 min.

MATERIALS AND METHODS

Amino acid analysis was performed on a Beckman 120 C amino acid analyzer using a 55 × 0.9 cm I.D. column of Beckman UR-30 resin. The formulae for the buffers used are listed in Table I. Trisodium citrate was Baker reagent grade; all other reagents for analyses were purchased from Pierce (Rockford, Ill., U.S.A.).

TABLE I

BUFFERS USED FOR SINGLE COLUMN ANALYSIS

Buffer	*pH*	*Sodium citrate*	*Thiodiglycol (25% solution)*	*Brij-35 (20% solution)*
1	3.24	0.2 *N*	10 ml/l	2 ml/l
2	4.12	0.2 *N* (+ 0.2 *N* NaCl)	10 ml/l	2 ml/l
3	7.10	0.2 *N* (+ 1.0 *N* NaCl)	—	2 ml/l

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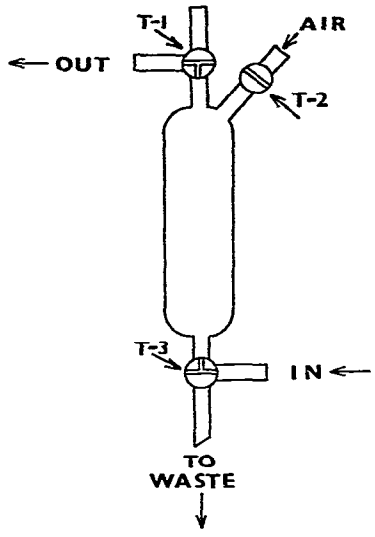

Fig. 1. Design of buffer 2 reservoir and connection scheme. The reservoir is constructed of glass (approx. 2.5 cm O.D. × 7 cm) with PTFE valves and ball-socket ground glass joints for easy connection and disconnection.

The modification for three-buffer analysis is shown in Fig. 1, and consists of a small, in our case 27-ml, reservoir which is inserted into the "buffer 2" line between the normal "buffer 2" reservoir, and the buffer selector. A flow diagram is given in Fig. 2. This reservoir must be manually rinsed free of buffer 3 and refilled with buffer 2 before each new analysis as follows.

(1) Open the three-way tap T-3 counterclockwise 90° to drain the small reservoir to waste: buffer 3 line is closed.

(2) Open the two-way tap T-2 to admit air into the reservoir for draining.

(3) Open tap T-1 counterclockwise 90° to rinse the reservoir with approximately 10 ml buffer 2; the outlet to buffer pump 1 is in the closed position.

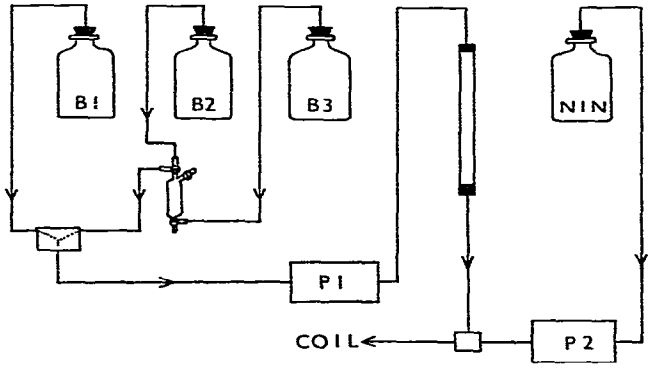

Fig. 2. Flow diagram of buffer reservoir connection.

(4) Close tap T-3 at a 45° angle so that all outlets are closed. Allow the reservoir to fill with buffer 2. When filled close tap T-2.

(5) Rotate tap T-1 counterclockwise 90° again, and with the buffer selector valve on "buffer 2", pump buffer until the line from the small reservoir to the valve is filled with second buffer. Return the buffer selector valve to "buffer 1" and flush the line from valve to column inlet with buffer.

(6) Return tap T-1 to the position in Fig. 1; the large buffer 2 reservoir is closed, and buffer pump 2 line is open to the 27-ml reservoir.

(7) return tap T-3 to the position shown in Fig. 1; buffer 3 line is open to the 27-ml reservoir, and the waste drain is closed. The run is ready to be started.

Note: the reservoir for buffer 2, and preferably for buffer 3, should be positioned higher than the 27-ml reservoir for proper rinsing and filling. The 27-ml reservoir can be conveniently mounted by clamping to a small stand (outside the instrument if desired).

This procedure takes less than 1 min to accomplish, and loads the reservoir in the buffer 3 line with a set volume of buffer 2. The exact amount of buffer 2 required for the particular analysis conditions must be determined experimentally by varying the effective volume of the small reservoir.

The small volumes of buffer 2 and 3 lost each cycle during re-loading of the reservoir are negligible in cost. This entire system can be inserted or removed at any time through the glass ball joints that normally are used to connect the buffer reservoir and buffer pumps in the Beckman 120 C or similar analyzers.

RESULTS AND DISCUSSION

The result of a typical analysis of a protein hydrolysate is shown in Fig. 3. The timing of the automated buffer selector change is chosen so that buffer 2 takes effect

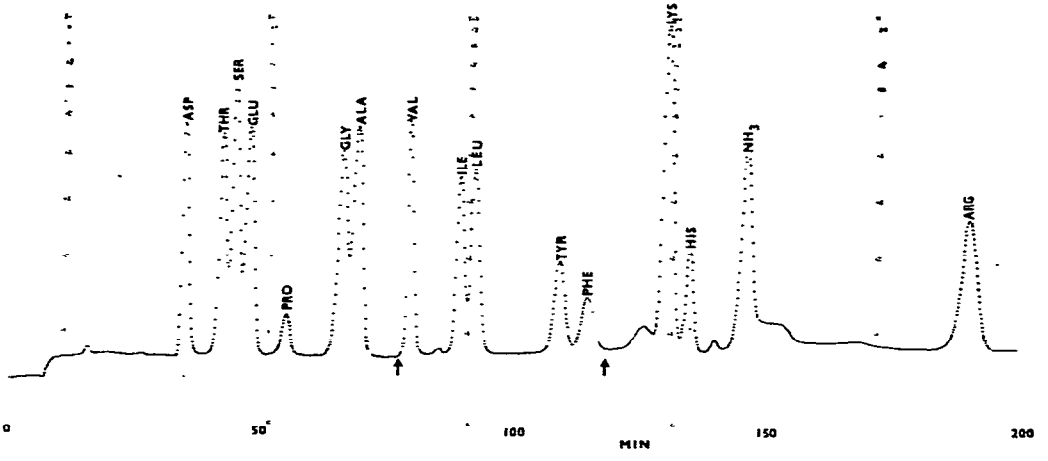

Fig. 3. Three-buffer amino acid analysis on a 2-buffer analyzer. The 55 × 0.9 cm I.D. UR-30 resin column was run isothermally at 54°, at a flow-rate of 68 ml/h. The buffer change time was set at 40 min. The sample applied was a hydrolysate of trout testis histones. For standard amino acid mixtures, cysteine elutes between proline and glycine, and methionine elutes after valine.

just before valine (first arrow). After the 27 ml of buffer 2 in the small reservoir is exhausted, the third buffer comes into effect gradually and is complete before the elution of lysine (second arrow). Note that the amount of buffer 2 can be easily altered for particular requirements by having on hand several small reservoirs of varying volume, by partially filling a standard small reservoir with varying volumes of clean glass beads, etc. The pH value, ionic strength, flow-rates and flow times of the buffer can be varied to suit particular separations.

In our experience this system of effecting three-buffer single column analysis on a two-buffer instrument is very reproducible and requires little manipulation. It is, of course, not a fully automated procedure, but the two-buffer analyzers such as the Beckman 120 C for which this procedure was designed require manual operation (column regeneration, sample injection) each cycle anyway. The small reservoir can be quickly re-loaded with buffer during sample application or column regeneration. The savings in time per analysis is comparable to that obtainable on fully automated 3-buffer analyzers and is effected at virtually no cost to users of older design 2-buffer instruments, of which there are many still in operation. Although we have used a 3-buffer system here which requires a small volume of second buffer (27 ml) there is no reason why this system should not in principle be suitable for somewhat larger volumes of second buffer, so long as the reservoir has a large enough height/diameter ratio to prevent excessive buffer 2–buffer 3 admixture during the analysis. Other applications can be envisioned, such as the insertion of two such small reservoirs in series in the second buffer line to provide four-buffer capability on a two-buffer instrument.

ACKNOWLEDGEMENT

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