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Simplification of sample loading in continuous automatic amino acid analysis

Some recent papers on automatic amino acid analysis are concerned with acceleration and simplification of the analysis. DUS *et al.*[1] carried out 24 analyses continuously on one single column of a modified Beckman/Spinco analyser. The Technicon Corporation have developed an instrument where even 40 samples can be analysed continuously according to the method of EVELEIGH AND THOMSON[2]. In the meantime in our laboratory we finished the construction of an automatic amino acid analyser capable of chromatographing six preloaded sample injectors, successively, on the same column.

Because the basic principles of the instrument are very similar to commercial ones only a brief description of the complete instrument will be given. Those parts and methods which are essentially different will be described in more detail. The sample loading device in particular is remarkably different from other data published and this device appears to be a very important simplification.

Description of the instrument

Flow scheme. A flow scheme for the automatic amino acid analyser is shown in Fig. 1. One of six buffer solutions, selected by automatically or manually setting the rotary valve, is pumped to the sample injector unit. By means of a double rotary valve, the buffer flows through one of the sample tubes to the column. The eluent is mixed with ninhydrin, supplied by the ninhydrin pump, then heated in the reaction bath, the light absorption is measured in the colorimeter (Multi-Channel Absorptiometer LKB 5900 A), and after passing a flow meter, it flows to a drain tube.

Sample selector valves. The sample selector valves are 6-port valves and they can be automatically or manually turned to any port signalled by a timer or push button, respectively.

The Delrin plugs in the valve housings (Carpenter Stainless Steel Cb.) are

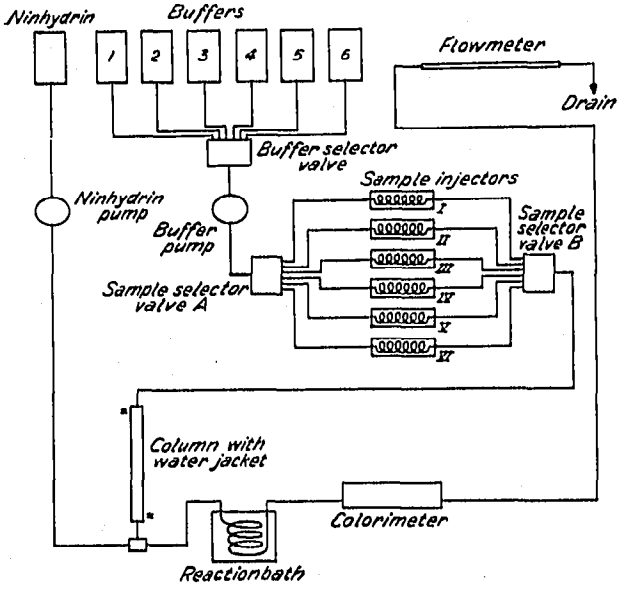

Fig. 1. Flow scheme.

connected by one drive shaft in order to rotate the plugs simultaneously when the motor is started.

The central port of the rotary valve A is connected by teflon tubing (I.D. 0.7 mm, O.D. 1.5 mm) to the buffer pump, the central port of the rotary valve B is connected by teflon tubing to the column plug. The other ports of the valves are connected with inlet and outlet tubing of sample injectors, respectively. All the seals are made with silicone rubber O rings.

Sample injectors. The six sample injectors of the amino acid analyser can be seen in Fig. 2. A cross section of the sample injector is shown in Fig. 3. The main parts are a plunger, a plunger housing (Carpenter Stainless Steel Cb.) and a teflon coil containing the sample to be analysed. The plunger has two longitudinal bores and at the end of each a transverse bore, as is seen in the figure. The plunger is closed at one end with a serum cap. The plunger housing is built up from six different segments connected by two bolts and nuts. To assure tight sealing under high pressure, silicone rubber O rings were used.

All bores in the segments and plunger have diameters of 1 mm. The teflon coil has an inner diameter of 0.7 mm, and an outer diameter of 1.5 mm. The length of the coil is dependent on the amount of sample to be loaded. About 2.67 m has a content of 1 ml. When the sample injector is in the line of the buffer flow the buffer pushes the sample into the column by way of the small space left between plunger and plunger housing. At the end of the analysis the rotary valves are turned to another sample injector and the first one can be reloaded.

Fig. 2. CIVO automatic amino analyser.

Loading of sample injectors. The loading of sample injectors is very easily carried out by pushing in the plunger so that the transverse bores are in line with the teflon coil of the sample injector. By means of a syringe, the needle of which is pushed through the serum cap into the plunger bore, the sample, preceded by a small air bubble, is pressed into the coil, pushing the buffer present into the drain tubing. As soon as the air bubble reaches the drain tubing, the sample tube is completely filled with sample solution and the plunger is drawn out again. When the syringe is now filled with water, the bores of plunger and drain tubes can be washed without removing the sample solution. The sample injectors were tested under pressures up to 500 p.s.i. The serum cap has only to resist the pressure required to put the sample into the teflon coil, which is sufficiently low.

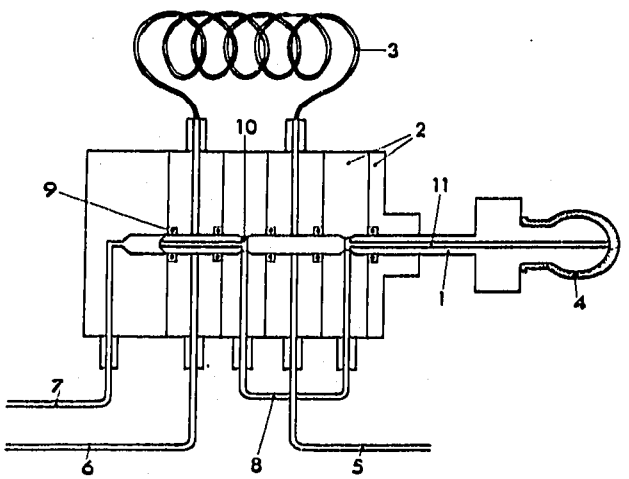

Fig. 3. Sample injector. 1 = Injector plunger (Carpenter Steel Cb); 2 = plunger housing segments; 3 = sample coil (Teflon I.D. 0.7 mm, O.D. 1.5 mm); 4 = serum cap; 5 = outlet tubing to rotary valve B (Teflon I.D. 0.7 mm, O.D. 1.5 mm); 6 = inlet tubing to rotary valve A; 7 = drain tubing; 8 = connection tube to plunger bores; 9 = silicone rubber O ring; 10 = transverse bore of plunger; 11 = longitudinal bore of plunger.

Adjustable column plug. The adjustable column plug (applied to LKB high pressure columns) is somewhat different from that used by DUS *et al.*[1] In contrast to the method used by these authors, we fitted the teflon filter into the plug. This filter is only necessary to prevent resin from entering the teflon tubing. Impurities from buffer and sample are filtered by suitable filters placed in the buffer line to the adjustable plug.

Electrical control system. Because it is beyond the scope of this paper to give a detailed description of the programmer unit composed of 40 relays, we will confine ourselves to remarking that 15 different functions can be independently programmed by means of a programming switch board (Fig. 2). For instance each port of the six-port buffer-change rotary valve represents one strip on the timer, thus enabling the buffer to be changed in any sequence at any selected time. At the end of each run the same programme will be repeated as many times as sample injectors are loaded.

Elution programme. As a consequence of the flexibility of the analyser it is possible, for example, to analyse protein hydrolysates with the two-column system described by BENSON *et al.*[3] as well as by the one-column system of DUS *et al.*[1]. The

column panel has places for two columns, and it is possible to switch manually, by means of a rotary valve, so that each one in turn is in the line of the buffer and reaction coil.

BENSON's method[3] was used with some modification. The regeneration and equilibration of long columns are integrated steps in the elution programme. With the same buffer pump, citrate buffers pH 3.25 and pH 4.25, and 0.2 *N* NaOH and finally citrate buffer pH 3.25 can be pumped in this sequence. Six short column runs can be completed during the day, five long ones overnight.

Discussion

Volume of sample injectors. It is possible to calculate from the internal diameter of the tubes the approximate length required for a desired volume, *e.g.* 1 ml. The coil is filled with a calibration mixture of amino acids and the amino acid constants are calculated from the chromatogram. A small deviation of the volume from 1 ml is not important because unknown samples are analysed with the same sample injector for which the calibration was carried out. Coils of equal length have not exactly the same volume, therefore in order to avoid different amino acid constants for each sample injector coil, a correction factor was calculated for each coil of the same length. The correction factors ranged from 0.99 to 1.01, the same deviation as found by MURDOCK[4] between volumes of equal lengths of teflon tubing.

Applicability of the sample loading system. First we followed the 4 buffer elution programme of DUS *et al.*[5] on 55 cm columns. Unfortunately we did not have the opportunity of testing the system with AA 15 resin of Beckman/Spinco. Only the PA 28 resin was used, and a steadily increasing back pressure and shrinkage of the resins occurred. It was almost impossible to maintain a small dead space between resin and plug without manual correction by turning the adjustable plug. Buffers 3 and 4 (normalities 1.4 and 0.7 respectively) in particular, cause shrinkage of the resin. A further disadvantage of the method is the jump in the base line occurring after phenylalanine.

The automatic sample injector appeared to be very successful in the two column programme, using Bio Rad[6] Aminex A4 spherical resin for the long column and Beckman/Spinco AA 27 spherical resin for the short column.

Separation and determination of ε-dinitrophenyl-lysine on Amberlite IR 120 columns according to SLUMP[7] and tryptophan determinations with Sephadex G 25 columns appeared to be possible (to be published).

Comparison with other sample injectors. DUS *et al.*[1] have used sample tubes which are connected directly with the ports of the rotary valves. The disadvantage of this method is the necessity of disconnecting the Swagelok union every time the samples have to be loaded. The same disadvantage probably occurs with the device described by MURDOCK *et al.*[4]. Moreover the last mentioned sample injectors cannot resist back pressures of more than 100 p.s.i. A completely different sample loading system was recently developed by EVELEIGH[2] and has been used in the commercial instruments of the Technicon Corporation. Here the buffer lines are automatically disconnected before loading of samples.

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Chromatography of isomeric methylene interrupted methyl *cis,cis*-octadecadienoates

I. Argentation thin layer chromatography

Argentation chromatography (recently reviewed[1]) on thin layers or columns has become one of the more valuable separation techniques available to lipid chemists. Though a wide variety of applications in the lipid field have been described, comparatively few reports of studies with model compounds are available and the full potential of the method has not been determined. Two reports of the chromatographic behaviour of isomeric methyl octadecenoates on thin layers of silica gel impregnated with silver nitrate have recently appeared[2,3]. The isomeric methylene-interrupted methyl *cis,cis*-octadecadienoates[4] (*i.e.*, methyl *cis,cis*-2,5-octadecadienoate to methyl *cis*-14,17-octadecadienoate) have now been examined in a similar manner.

Experimental

Silica Gel G (Merck) layers (0.25 mm) containing silver nitrate (10%) were activated by heating at 110° for 2 h. Plates were developed in unlined tanks with hexane–diethyl ether (9:1) as solvent system and visualised by charring with sulphuric acid (10%) at 150°.

Results and discussion

As might be expected, the migration pattern of the isomers conforms to a sinusoidal curve (Fig. 1). The 2,5-isomer has a remarkably high R_F value in comparison to other members of the series. The 3,6- to 6,9-isomers can be separated but the