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Note

Automatic sample introduction system for gas–liquid chromatographic analysis of amino acids

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In earlier papers[1,2] the design and performance of an automatic sampler for solvent-free introduction of samples into a gas chromatograph was reported. Since then there has been an increasing demand for such a device and, as a result, there are now several models commercially available. Most of them are simply an automated version of the manual procedure of using a syringe to inject a sample in solution. The automatic samplers have a tray where the samples are arranged in a circular disc, and also a pneumatically operated syringe for taking up the sample from the sample vial, puncturing a septum and injecting the sample into the gas chromatograph. The Barber-Coleman (now Nuclear-Chicago, Des Plaines, Ill., U.S.A.) apparatus has a facility for handling both dry samples in capillaries and fluid samples in "Hot-pop" capsules. The capsules are made of PTFE and explode to open when dropped into the flash heater.

In this paper, we report the changes made in order to improve the performance of the automatic sampler described earlier[1,2] and some results obtained for its utilization in amino acid analysis.

MECHANICAL DESIGN

In the original design, the sample storage disc was placed in a gas-tight casing under an atmosphere of carrier gas (argon or nitrogen), in order to reduce the rate of auto-oxidation of polyunsaturated fatty acid esters while stored as dry samples. However, loss of short-chain fatty acid methyl esters by evaporation occurred during overnight storage[2] and some kind of cooling system was therefore deemed necessary. This cooling was achieved by circulating chilled water around the sample storage unit, which maintained the temperature inside the unit below 10°. If necessary, it was possible to decrease the temperature further by changing the chilling medium.

To accommodate the special PTFE capsules (2.5 mm O.D., 6.3 mm long), the

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storage chambers were made larger (4 × 4 mm instead of 3 × 3 mm) without reducing the number of chambers or their depth.

As the operational reliability had to be improved while running the gas chromatograph at higher than 1.5 kg/cm^2 gas pressures, the connection between the storage unit and the vaporizer unit had to be redesigned. In the new design, the vaporizer unit is mechanically fitted to the storage unit by using a disc with slots. The storage unit is firmly pressed against the head of the vaporizer and secured with bolts and nuts. Heat-resistant Viton washers give a gas-tight seal between the cylindrical hole of the storage unit and the piston-like head of the vaporizer.

Vaporizer

A complete redesign of the vaporizer unit was desirable as the efficiency of the original version[1] decreased after prolonged use. In the new design (Fig. 1), the sample container falls by force of gravity into the vaporizer (2), where it is held for a predetermined period by a piston (7). The vaporizer is heated by two thermocouple elements placed into the cylindrical bores (3) on each side of the sample. Owing to the sudden evaporation of the sample in the capsule, the vapour pressure increases and the capsule explodes to open, releasing the sample instantaneously. The effluent is swept by the carrier gas, which enters the vaporizer through the inlet (4), into the outlet (5) connected to the GLC column. At a pre-determined time, the electronic controller (Fig. 2) activates the electromagnet (6) for a short time and the piston, which is normally held in position to block the passage of the capsules by a spring (8), is drawn

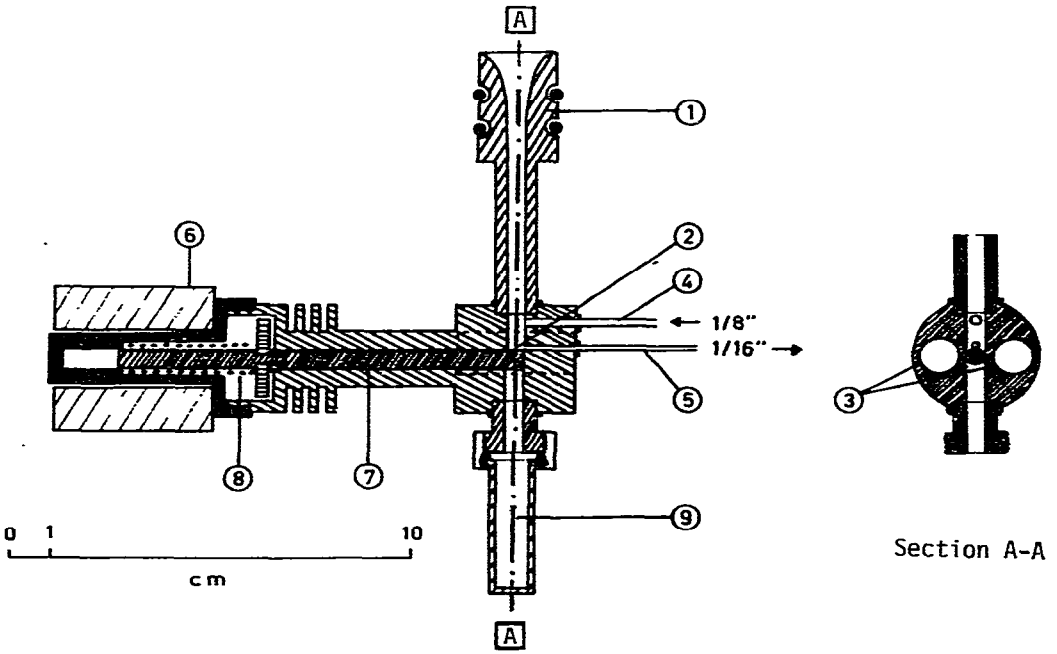

Fig. 1. Section through the vaporizer assembly. All parts are made of stainless steel except for No. 9, the empty capsule containers, which are made of glass, the portion of the piston proximal to the magnet, which is made of iron, and the bush (black shading) on which the magnet rests, which is made of brass. For identification of components, see legend to Fig. 2.

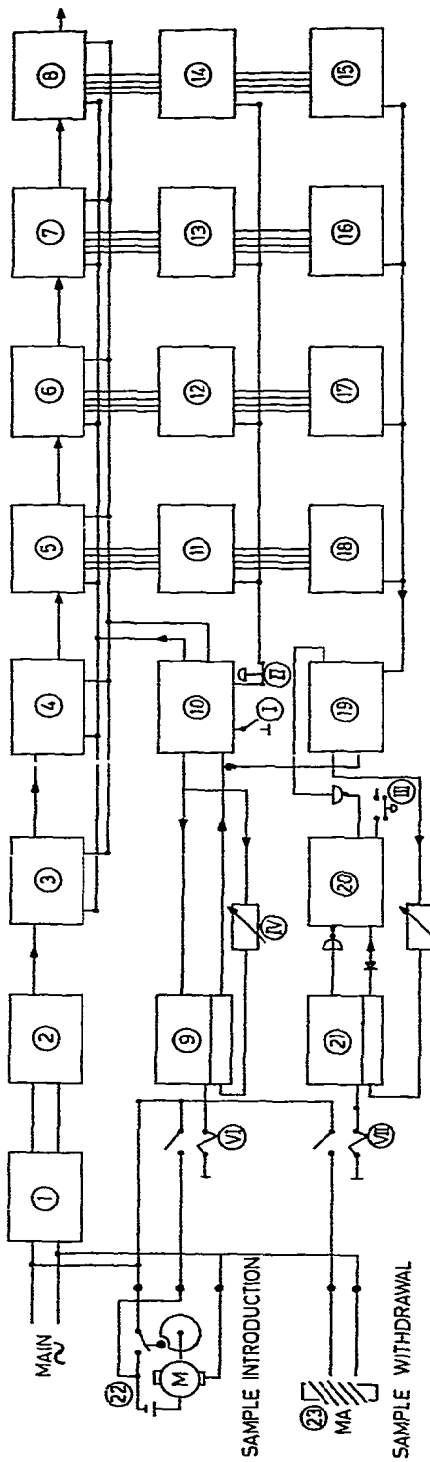

Fig. 2. Time controller for the automatic sampler. 1 = Power supply; 2 = pulse generator on double mains frequency (100 Hz); 3 = frequency divider 100/100 Hz; 4 = frequency divider, 10/6 Hz; 5–8 = decade counters; 9 = driver for output relay for "sample introduction"; 10 = coincidence and zero position unit; 11–14 = pre-selector for "sample introduction interval"; 15–18 = pre-selector for sample withdrawal-relay pulse interval; 19–21 = driver and flip-flop for output relay "sample withdrawal unit"; 22 = motor, cam and micro-switch for sample introduction-unit; 23 = electromagnet for "sample withdrawal". Function controls: I = automatic stop after a complete cycle; II = manual sample introduction; III = manual sample withdrawal; IV = pulse duration control for relay VI; V = pulse duration control for relay VII.

back for a few seconds so as to allow the empty capsules to fall into the empty capsule collector (9).

Time controller

Fig. 2 shows the time controller, the operation of which is as follows. At zero time the motor (22) of the sample-introduction unit is activated so that the sample disc is moved 1/60th of a turn and a sample-containing capsule falls into the vaporizer by force of gravity. At the same time, a micro-switch placed behind the storage unit is closed by a cam to re-set the integrator. The sample is evaporated at once and, at a time that can be determined previously, the electromagnet is activated to draw the piston back and allow the empty capsule fall into the collector. The duration of the pulses and the interval between them can be adjusted. There are also facilities for manual operation through push-buttons.

EXPERIMENTAL

Reagents

The standard amino acid solution (2.5 μmole/ml) was obtained from Bio-Rad Labs. (Richmond, Calif., U.S.A.). Trifluoroacetic anhydride was purchased from Eastman-Kodak (Rochester, N.Y., U.S.A.), *n*-butanol and methylene chloride from BDH (Poole, Great Britain) and hydrogen chloride gas from Mathesson & Co. (Joliet, Ill., U.S.A.).

Apparatus

The automatic sampler was used in connection with a Varian Aerograph Model 2100-20 gas chromatograph with a flame-ionization detector, a dual differential electrometer and a linear temperature programmer unit. The gas chromatograph was connected to a dual-pen recorder (Varian 480) and a teletype printer (TT4-33-TBM).

Preparation of derivatives

Aliquots of the standard amino acid solution were evaporated to dryness and the N-trifluoroacetyl-*n*-butyl (TAB) derivatives were prepared according to Roach and Gehrke[3].

Chromatographic conditions

The column (1500 × 4 mm I.D.) was prepared by filling the glass U-tube with the packing material prepared and conditioned as described by Gehrke *et al.*[4]. The packing material contained 0.65% (w/w) of stabilized ethylene glycol adipate (EGA) on 80–100-mesh Chromosorb W, HP.

Experiments were conducted only on the EGA column and the results refer only to those amino acids which are separated from this material. The vaporizer has a temperature of 250° as measured with a pyrometer connected to the walls as near to the position of evaporation as possible. The sample storage disc was chilled by circulation of cold water and the internal temperature was 12°.

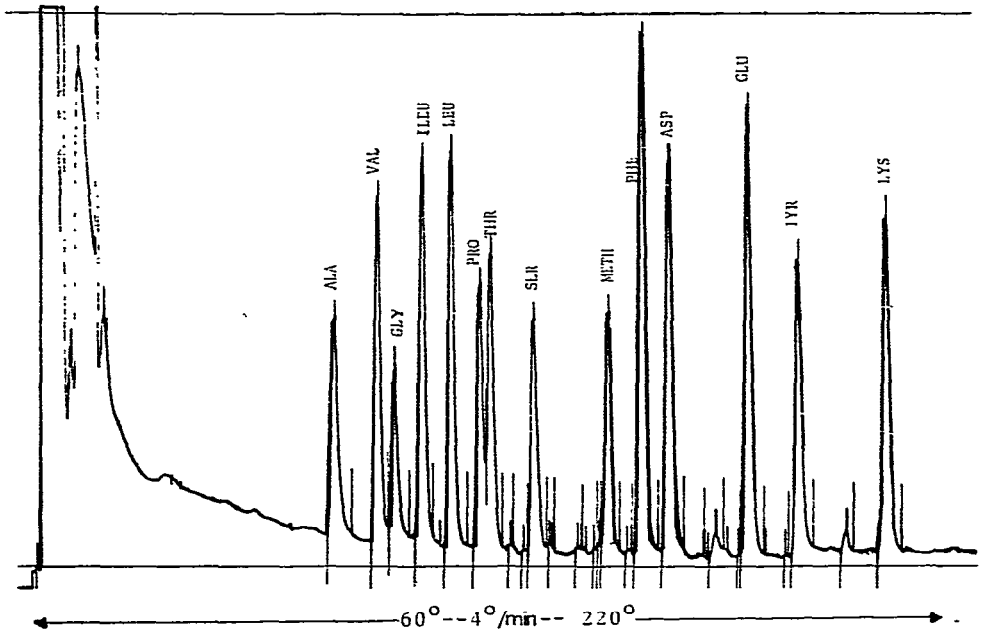

Fig. 3. Separation of TAB-amino acids from an EGA column after manual injection.

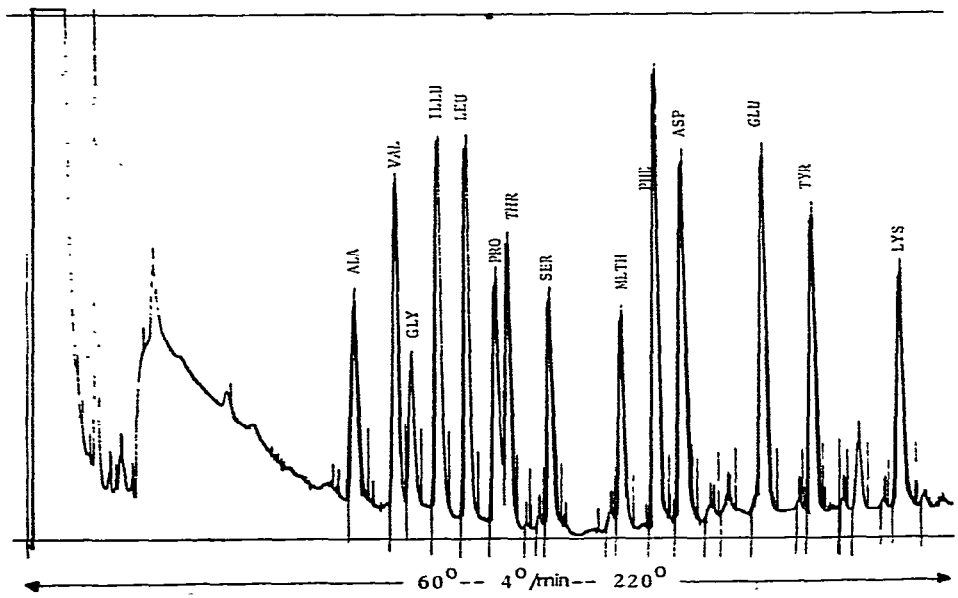

Fig. 4. Separation of TAB-amino acids from an EGA column after introducing the sample using "hot-pop" capsules through the automatic sampler. The samples were stored in the apparatus for 2–4 h before the run.

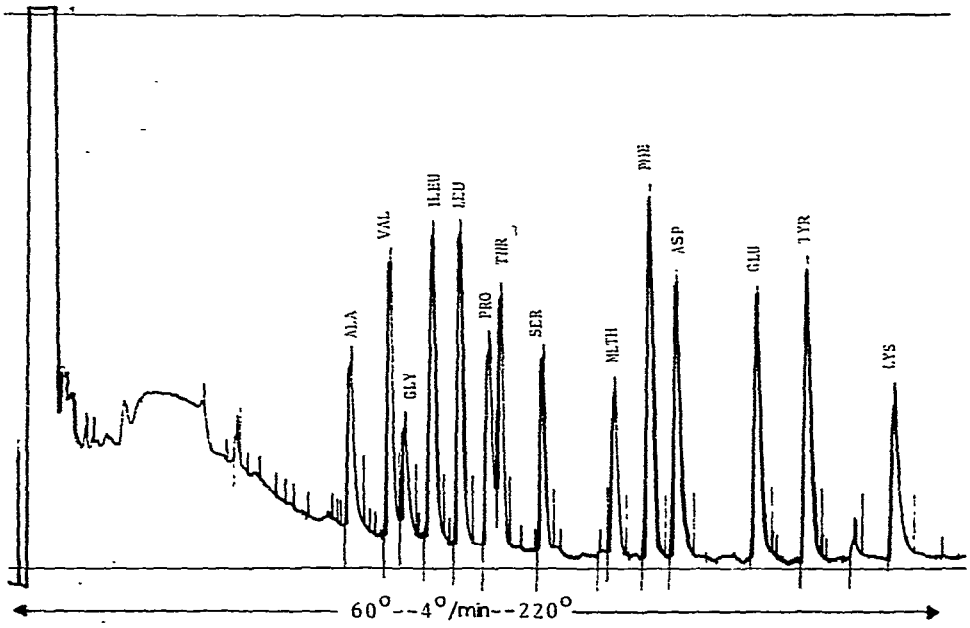

Fig. 5. Separation of TAB-amino acids from an EGA column after introducing the samples through the automatic sampler after overnight storage.

TABLE I

COMPARISON OF THE RELATIVE MOLAR RESPONSE RATIOS OF THE TAB-AMINO ACIDS SEPARATED ON AN EGA COLUMN AFTER MANUAL INJECTION, 2–4 h STORAGE AND OVERNIGHT STORAGE

Amino acid	*Manual injection, Aaa/Aglu*			*Automatic sampler*					
				Aaa/Aglu (2–4 h storage)			*Aaa/Aglu (overnight storage)*		
	*Mean**	*S.D.*	*Rel. S.D. (%)*	*Mean**	*S.D.*	*Rel. S.D. (%)*	*Mean**	*S.D.*	*Rel. S.D. (%)*
Alanine	0.548	0.023	4.19	0.570	0.032	5.31	0.575	0.050	8.69
Valine	0.781	0.036	4.61	0.783	0.013	1.66	0.905	0.088	9.26
Glycine	0.429	0.017	3.96	0.373	0.038	10.88	0.421	0.058	13.77
Isoleucine	0.831	0.036	4.33	0.944	0.046	4.87	1.005	0.084	8.35
Leucine	0.865	0.034	3.93	0.963	0.052	5.39	1.038	0.086	8.29
Proline	0.610	0.019	3.11	0.773	0.042	3.50	0.792	0.072	9.09
Threonine	0.715	0.095	13.28	0.685	0.024	3.50	0.762	0.073	9.58
Serine	0.575	0.022	3.82	0.573	0.021	3.66	0.650	0.072	11.07
Phenylalanine	1.164	0.016	1.37	1.284	0.030	2.33	1.355	0.061	4.50
Aspartic acid	0.956	0.018	1.88	1.069	0.065	6.08	1.114	0.093	8.35
Glutamic acid	1.000	—	—	1.000	—	—	1.000	—	—
Tyrosine	0.731	0.013	1.78	0.814	0.047	5.77	1.070	0.090	8.41
Lysine	0.788	0.031	3.93	0.779	0.068	8.73	0.696	0.056	8.04
		Mean:	4.18		Mean:	5.10		Mean:	8.95

* Mean of four sample injections.

RESULTS AND DISCUSSION

Fig. 3 shows a chromatogram obtained after manual injection, while the chromatograms in Fig. 4 and Fig. 5 were obtained after automatic sample introduction. The sample in the case of Fig. 4 was stored for a period of 2–4 h and that in Fig. 5 was stored overnight in the storage unit.

In Table I, the molar responses for all of the amino acids relative to glutamic acid are given.

From a comparison of the peaks, it appears that the automatic sampler is applicable to amino acid analysis by chromatography. The mean relative standard deviation for all of the amino acids increases with storage from 4.18% for fresh samples to 8.95% for samples stored overnight.

In our experiments we used a 10-cm long stainless-steel tube (diameter 1/16 in.) between the vaporizer and the column. This may not be desirable as tyrosine and probably threonine and serine derivatives are destroyed to some extent. However, for routine screening of large numbers of samples, the arrangement described here should be of great value.

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