Journal of Chromatography, 136 (1977) 417–419

CHROM. 9997

Note

Amino acid analysis. A novel reaction chamber

L. B. JAMES

Department of Biochemistry, John Curtin School of Medical Research, Australian National University, Canberra A.C.T. (Australia)

(First received November 15th, 1976; revised manuscript received February 9th, 1977)

The heating bath supplied with the Technicon AutoAnalyzer (Model AAA-1) has many undesirable features.

These features include: thermostat malfunction, periodic replacement of a special oil, removal of the oil's decomposition products which coat the glass coil with an insulating layer, manipulations with the glass coil can lead to fractures that are difficult to repair, continuous operation of a stirrer motor and heating element over long periods resulting in energy wastage, and replacement parts for the oil bath being sometimes difficult to obtain. Hence with these problems in mind an alternative method for heating was sought.

MATERIALS AND METHODS

Fig. 1 is a sketch of the new reaction chamber. To the stainless steel base plate ($7\frac{1}{2} \times 7\frac{1}{2} \times \frac{7}{8}$ in.) has been spot welded 4 brackets. The brackets support the glass coil and the metal cylinder cover. The cover is 8 in. long and has a 7 in. diameter.

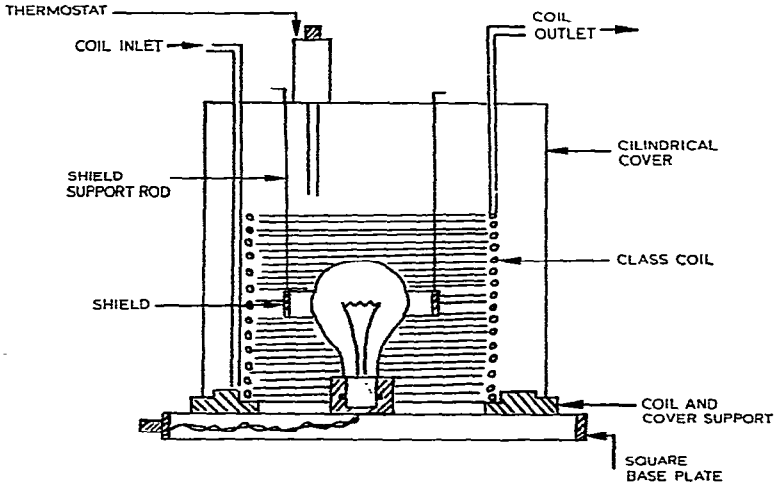

Fig. 1. Sectional view of the reaction chamber.

The inner surface of the cover is reflecting and the outer is painted black. There is a gap of $\frac{1}{2}$ in. between the cover and the base plate. The top of the cover has 5 holes drilled into it. Two holes (5/8 in. diameter) at $6\frac{1}{2}$ in. centres through which approximately 3 in. of coil glass tubing projects; a 5/16-in. hole to allow insertion of the thermostat probe and two holes (1/8 in. diameter) at $3\frac{3}{4}$ in. centres for the positioning of a circular shield around the light globe filament. The width of the shield is 3/8 in. and the thickness 1/32 in. There is a space of 3/4 in. between the shield and the globe. A 100-W clear light globe was located centrally on the base plate and push-in type electrical connections, through which power was supplied to the thermostat, were also fitted to the base plate. A type T.S. 2 N.C. thermostat from Associate Electrical was used in the construction of the reaction chamber. The thermostat can be adjusted by rotation of a cam to obtain the desired temperature of 80°.

The Technicon AutoAnalyzer had previously been converted from a single to a dual column instrument with increased sensitivity[1]. Colour development was obtained by the reaction of amino acids with ninhydrin reduced with titanous chloride[2].

RESULTS

Table I contains the results of the analysis of a standard mixture of amino acids with the new reaction chamber or the commercial oil bath installed in the Technicon. 100 nmoles of each amino acid were present in the standard mixture. Under the conditions of AutoAnalyzer operation given previously, the use of a double

TABLE I

COMPARISON OF CONSTANTS OBTAINED WHEN SYNTHETIC MIXTURES OF AMINO ACIDS (100 nmoles) WERE ANALYSED UNDER VARIED CONDITIONS

Constants shown are average of 3 determinations and the variation for most amino acids in all the analyses was within ±2%.

Amino acid	*Double glass coil*		*Single glass coil*	
	in oil bath at 96°	*with reaction chamber*	*in oil bath at 96°*	*with reaction chamber*
Lys	60	63	62	50
His	57	61	56	50
Arg	54	60	52	50
Asp	55	54	51	47
Thr	58	59	60	50
Ser	58	61	60	50
Glu	63	60	60	51
Pro	16	12	12	10
Gly	61	60	58	52
Ala	63	58	58	50
Hcy	32	29	29	29
Val	61	53	60	50
Met	57	61	55	56
Ile	60	61	56	50
Leu	62	64	56	55
Tyr	60	60	58	55
Phe	60	58	56	50

glass coil allows the reactants to be heated for a period of 17 min with the given flow-rate[1]. As can be seen from Table I there is not much variation in the value for a constant when using the double glass coil in oil bath or reaction chamber but with the single glass coil in the reaction chamber there is a consistent decrease in the value comparative with that obtained using the oil bath. However, this decrease in sensitivity comes about partly from the particular coil used in the reaction chamber. In order to speed the analysis only the inner glass coil was used. The inner coil diameter is approximately 1 in. smaller than that of the outer coil and is consequently about 3 ft. shorter in length. Another contributing factor is that for these analyses the thermostat was set at 80°.

Thus, samples containing as little as 40 nmoles of most amino acids can be analysed satisfactorily with the single coil installed in the reaction chamber without resorting to electronic amplification of the recorder print-out. Although obviously the ninhydrin reaction with amino acids has not gone to completion when using the single coil and reaction chamber, duplication of analyses have shown that the results obtained are accurate and reproducible.

Finally, the desirable features of installing the reaction chamber are: (1) a considerable saving in time and fuel consumption is achieved, as it is only necessary to switch on the light globe at commencement of an analysis; (2) it is speedier to flush out the coil at the termination of an analysis, especially if the proportioning pump is equipped with a two-speed motor, a 14 min flush at high speed is sufficient (Beckman analyzers require 60 min for this operation); (3) no longer will it be necessary to replace oil in the heating bath and descale the glass coil to obtain maximum efficiency of heat transfer; (4) back pressure from the shorter coil length does not overtax the peristaltic operation of the proportioning pump, thus, a smoother liquid flow through the colorimeter cuvettes is achieved without the necessity of installing in-line pulse suppressors and (5) there is a slight improvement in the resolution of threonine and serine, again possibly due to the smoother liquid flow and the shorter coil preventing prolonged mixing of the column effluent containing these two amino acids.

ACKNOWLEDGEMENT

I wish to thank Mr. G. W. McLennan for assembly of the reaction chamber.

REFERENCES

1 L. B. James, *Lab. Pract.*, 21 No. 9 (1972) 639.
2 L. B. James, *J. Chromatogr.*, 59 (1971) 178.