ditions described above for the direct method. Calibration curves for thiamphenicol and for its trimethylsilyl ether, based on measurement of peak area by planimeter, were linear throughout the range of 0.05 to 0.2 μg and the range of 0 to 0.02 μg, respectively. The results of recovery experiments of thiamphenicol added to human heparinized plasma and urine are presented in Tables I and II.

The direct method is recommended when the concentration is over 10 μg/ml, but below this concentration, the trimethylsilyl ether method seems to be superior.

Faculty of Pharmaceutical Science, Kyushu University, Fukuoka (Japan)

TOSHINOBU AOYAMA
SADAO IGUCHI

1 M. F. SHAFFER, K. C. MILNER, D. I. CLEMMER, L. POTASH AND L. S. SHAFFER, *Antibiot. Chemotherapia*, 4 (1954) 992.
2 Y. CHABBERT AND H. BOULINGRE, *Rev. Franc. Etudes. Clin. Biol.*, 2 (1957) 636.
3 E. W. MCCHESNEY, J. M. SHEKOSKY, H. W. ECKERT AND R. F. KOSS, *J. Am. Pharm. Assoc.*, 49 (1960) 28.
4 P. D. SHAE, *Anal. Chem.*, 35 (1963) 1580.
5 M. YAMAMOTO, S. IGUCHI AND T. AOYAMA, *Chem. Pharm. Bull.*, 15 (1967) 123.

Received May 12th, 1969

J. Chromatog., 43 (1969) 253–256

CHROM. 4177

A new sampling apparatus for amino acid analysis

The method of SPACKMAN *et al.*[1] for amino acid analysis has been speeded up[2,3] and automated[4–9]. In addition, several methods for automatic sampling have been used. These methods are based on the use of either a number of columns and the elution of these columns, one after another, with the sample[5] or on the use of a number of sample loops or sample tubes as was done by MURDOCK *et al.*[5], DUS *et al.*[7], ALONZO AND HIRS[8] and by SLUMP AND VERBEEK[9]. A sample loop is a teflon or nylon tube in which a sample can be stored. Both ends of the loop are connected to two corresponding openings of a double rotary valve. The difficulty with this system is the necessity of connecting and disconnecting the sample loop for every new sample. A special valve has been designed by SLUMP AND VERBEEK[9] in order to overcome this problem.

A different system was developed by EVELEIGH AND THOMPSON[4] in which samples are loaded into small tubes, and are adsorbed on an ion-exchange resin.

The automatic sample loader described here is a simplification of the system as reported by SLUMP AND VERBEEK[9]. An extra valve is not neccessary, and furthermore, the problem of connecting and disconnecting the sample tube (DUS *et al.*[7]) is solved. The whole apparatus can easily be connected to a commercial amino acid analyzer provided it is coupled to a suitable timing system.

Description of the apparatus (Figs. 1–4)

The whole apparatus is fitted into a steel box with perspex on one of its long sides which can be removed by sliding sideways. In this particular case the sampler is constructed so that it has twelve loops, but this number can easily be changed. The sample loops (teflon) are rolled around perspex rods (Fig. 2). Perspex cylinders are placed around the rods to keep the loops in place. The rods fit into a perspex base (the rod holder) which is attached to a stainless steel (AISI 316) plate which hangs in the box. The stainless steel plate moves along a rail and can be set in motion either by a clock system or manually.

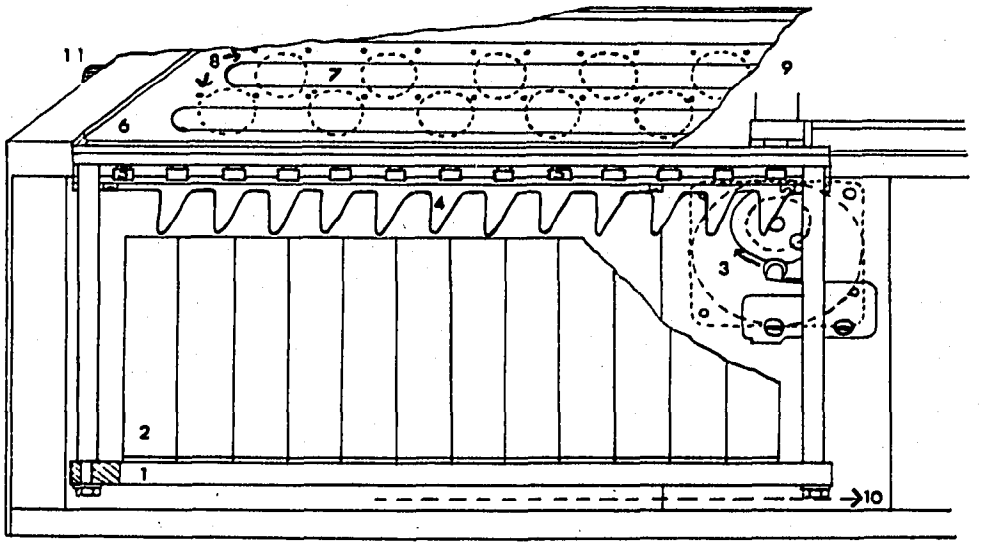
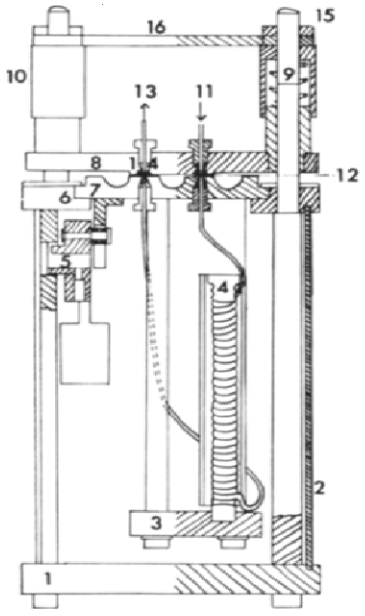


Fig. 1. 1, rod holder; 2, rods for loops; 3, ratchet mechanism; 4, cam; 5, thirteen connections; 6, stainless steel sample plate; 7, grooves; 8, holes; 9, crossbeam; 10, direction of movement; 11, set screw.

Both ends of the teflon loop (Fig. 2) are connected to the stainless steel plate. Thirteen pairs of holes were drilled in this plate (Fig. 1). The plate is moved by a ratchet mechanism which consists of a pawl and pinion, and a cam. Every time the pinion is turned the cam makes one step. A stainless steel (AISI 316) crossbeam is fixed in the middle of the box (Fig. 2), and this crossbeam contains the connections to the column and to the chromatographic pump.

Two small O-rings (Fig. 3) ensure the connection from the column and the chromatographic pump to the sample loops (Fig. 2). The ratchet mechanism is constructed so that for every step taken by the loop holder, a new loop is connected with precision to the pump and to the column. In order to avoid excessive wear of the two O-rings, the face of the stainless steel plate making contact with the rings should be polished smoothly. The O-rings are kept tightly pressed to the stainless steel plate by means of two springs (Fig. 2, 9) which can be adjusted by the adjustable spring holder (Fig. 2, 10). The correct spring pressure was measured empirically. A small crossbeam is placed above the spring holder (Fig. 2, 15) and this is fastened by two nuts (Fig. 2, 16).

When the O-rings need replacing this can be performed in less than 10 min by unscrewing the nuts and the spring holder and disconnecting the crossbeam and the springs.



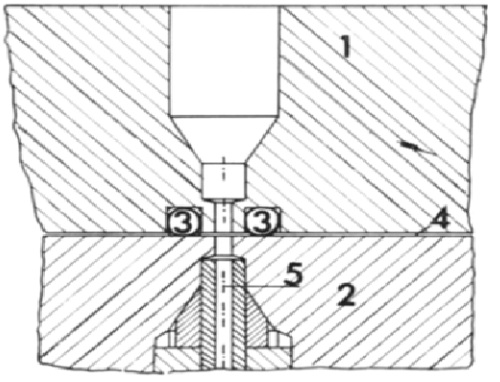


Fig. 2. 1, bottom; 2, perspex front cover; 3, rod holder; 4, sample loop rod; 5, ratchet mechanism; 6, rail; 7, stainless steel plate with 3 grooves for sample overflow; 8, crossbeam; 9, spring pressing crossbeam on underlying plate; 10, adjustable springholder; 11, inlet for developing reagent; 12, end of sample loop; 13, outlet to column; 14, O-rings; 15, nuts; 16, small crossbeam.

Fig. 3. 1, crossbeam; 2, stainless steel sample plate; 3, viton O-rings (parker size 003, viton or ethylene propylene); 4, small split; 5, sample loop.

The first pair of holes in the stainless steel plate are directly connected to each other. At the start the pump and the column are connected to these holes. The following twelve pairs of holes are connected to the two ends the twelve sample loops.

The drilled plate also contains three longitudinal grooves to take any possible overflow of the samples. The loops with the sample cannot drain because their openings are horizontal. The whole box is covered by two perspex plates that can easily be removed.

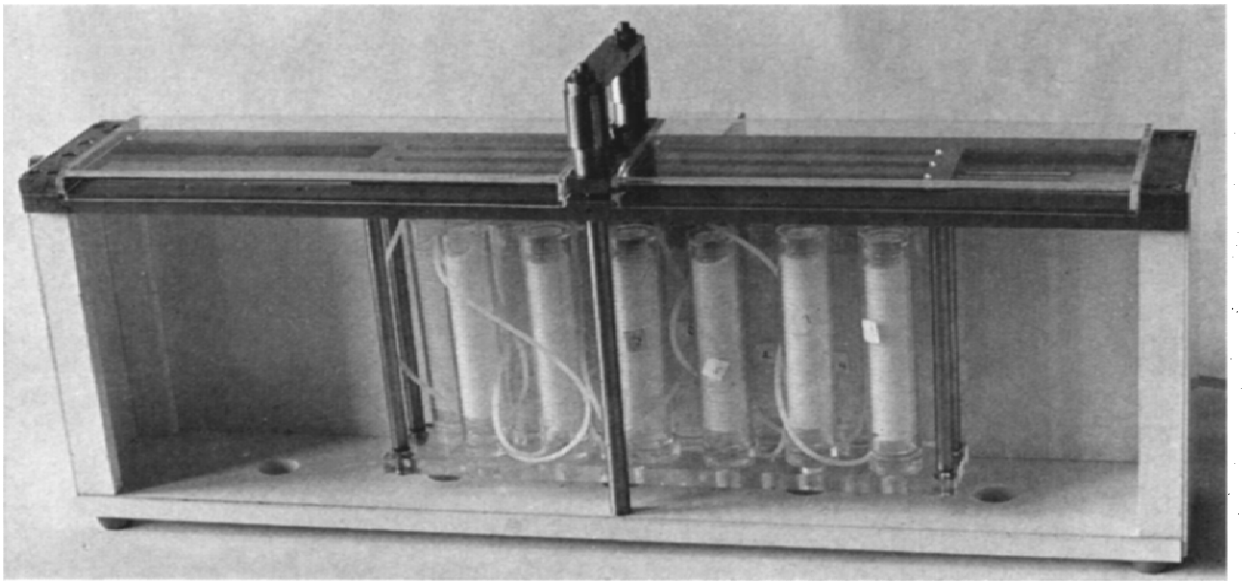


Fig. 4. Photograph of the apparatus.

Sample injection

When twelve samples have to be injected the apparatus is set manually in the start position. An adjusting screw (Fig. 1, 11) fixes the correct position of the sample plate. The samples are applied by means of a syringe and injection needle. The sample is pushed into the sample loop which is entirely filled and the overflow on the other side of the loop is removed by suction with a laboratory air pump.

It is not necessary to introduce all the samples at the beginning, *e.g.* when sample number 5 is running on the analyzer, sample number 6 can be introduced.

Discussion

The apparatus was designed for the simple application of samples on the amino acid analyzer. The system consists of sample loops which can be eluted one after another. Two kinds of teflon tubing were used for the sample loops: (a) tubing of I.D. 0.85 mm, O.D. 1.6 mm, and (b) tubing of I.D. 0.8 mm and O.D. 2.8 mm. The second is recommended because thinner tubing sometimes breaks at a weak spot with the high pressures that are used for the accelerated system in practice in our laboratory (long column $>$ 20 atm).

Only two O-rings were necessary to seal the system and these could easily be replaced. The whole system could be cleaned easily because the smooth part of the sample plate could be reached immediately simply taking away the perspex cover. The diameter of the outlets of the sample loops was very small (0.6 mm) and the uptake of ammonia was slight; accurate analyses of ammonia were possible.

The thicker tubing sample loops gave good reproducibility. The calculation of the analyses by automatic peak integration and computer data evaluation[10] together with the automatic sample loading systems have considerably reduced the time of analysis.

The authors wish to thank Prof. H. BLOEMENDAL for his continuous interest in this work, and Mr. W. J. NAGELVOORT and Mr. H. T. J. SMITS for their part in the construction of the apparatus described in this paper.

Department of Biochemistry, University of Nijmegen, Nijmegen (The Netherlands)

J. J. T. GERDING
K. A. PETERS

1 D. H. SPACKMAN, W. H. STEIN AND S. MOORE, *Anal Chem.*, 30 (1958) 1190.
2 P. B. HAMILTON, *Anal. Chem.*, 32 (1960) 1779.
3 D. H. SPACKMAN, *Federation Proc.*, 22 (1963) 244.
4 J. W. EVELEIGH AND A. R. THOMSON, *Biochem. J.*, 99 (1966) 49.
5 A. L. MURDOCK, K. L. GRIST AND C. H. W. HIRS, *Arch. Biochem. Biophys.*, 114 (1966) 375.
6 K. DUS, S. LINDROTH, R. PABST AND R. M. SMITH, *Anal. Biochem.*, 14 (1966) 41.
7 K. DUS, S. LINDROTH, R. PABST AND R. M. SMITH, *Anal. Biochem.*, 18 (1967) 532.
8 N. ALONZO AND C. H. W. HIRS, *Anal. Biochem.*, 23 (1968) 272.
9 P. SLUMP AND A. M. VERBEEK, *J. Chromatog.*, 34 (1968) 401.
10 J. J. T. GERDING, *Intern. J. Protein. Res.*, II (1969) 169.

Received May 8th, 1969