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Note

An automatic sample loader for column chromatography

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An automatic sample loader has been designed and constructed and has been in continuous operation on a Beckman-Spinco Model 120 C amino acid analyser for 3 years. It was developed from the design of Dus *et al.*[1], which had the disadvantage of a complicated loading procedure and was suitable only for a single-column system.

Sample loaders to cope with single- or dual-column systems have subsequently been developed by Gerding and Peters[2].

The improved loader described incorporates a number of desirable features; single- or dual-column loading, the ability to add or subtract samples easily from the loader during operation of the machine and the introduction of samples automatically into the sample loops, thereby eliminating tedious loading procedures or the possibility of leaks resulting from the manipulation of fittings.

DESCRIPTION

The loader is controlled by an electronic programmer, developed in these laboratories, which also controls all the timed sequences, a six-position ten-channel buffer flow valve and the pumps.

Basically the loader consists of two Delrin discs each with four pairs of holes through which the samples are loaded. The two discs are held together by spring pressure and are sealed by neoprene "O"-rings recessed into the top disc. Connection of the buffer lines to the discs is made generally as shown in Fig. 1.

The top disc, which remains stationary, holds the connections for the buffer lines to the columns, the lines from the buffer pumps and the lines from the sample cups to the sample loading pump. The bottom disc rotates and holds the sample coils which have identical volumes. We routinely employ sample coils holding 0.39 ml and a sample volume of 0.40 ml in the sample cup. This allows for a small overlap of sample at each end of the sample coil preventing air from being loaded.

The sample loading pump used in our system consists of two Metrohm AG Herisau Piston Burettes (E274) coupled in parallel to a single precision screw shaft driven by a Phillips Synchronous Motor and gear box. The pumping time is controlled by an Omron Subminy Timer (Type STPYM) and is adjusted so as to give a slight overlap of sample at each end of the sample coil. Under our operating conditions it takes approximately 3 min to load the sample.

Fig. 2 shows a part section of the disc system and a diagrammatic arrangement of the driving mechanism.

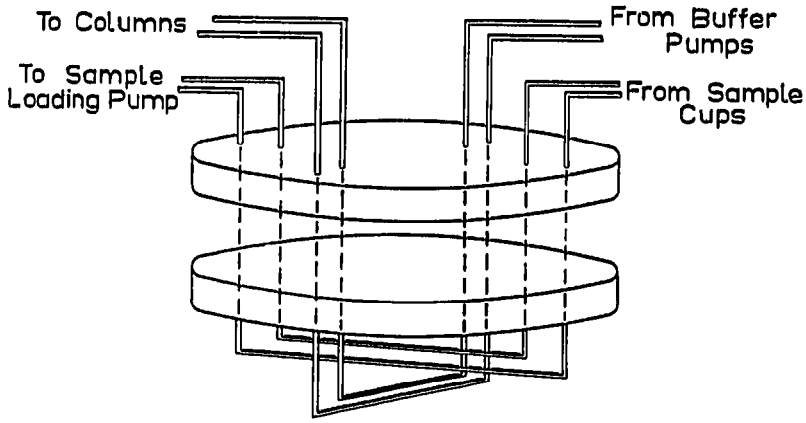

Fig. 1. Schematic representation showing the general manner of connection of the loader to the analyzer and the sample load pump. In the single-column system only one of each pair of lines is used.

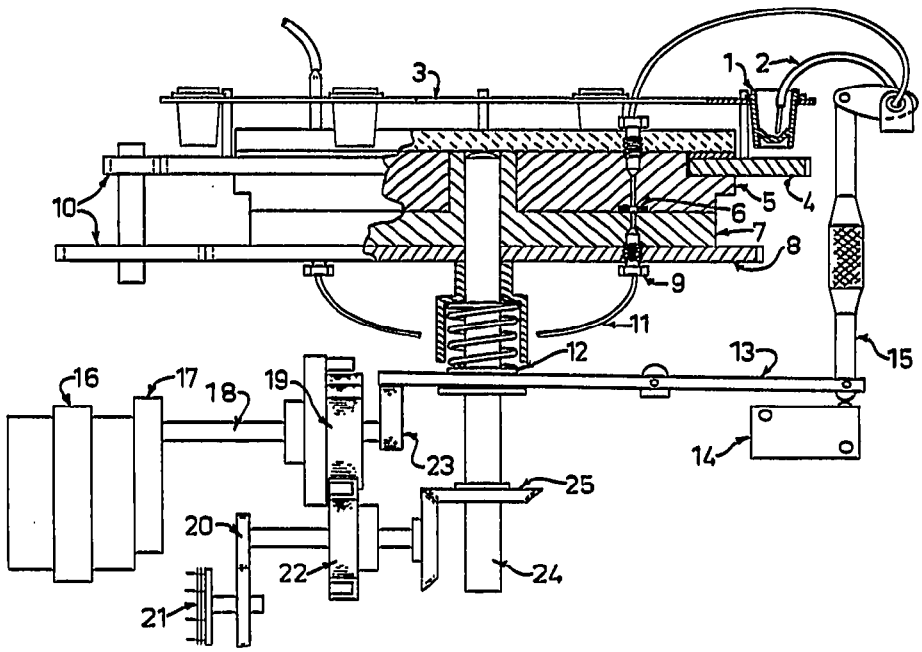

Fig. 2. Partial cross-section of the discs, the manner in which they are sealed, and a diagrammatic representation of the drive mechanism. Only one of each pair of sample cups is shown (see Fig. 3).

The motor (16) drives through a reduction gear box (17) to a four point Geneva system (19, 22) coupled to an eccentric cam (23). The movement of the Geneva mechanism can be considered in three stages and is directly related to the movement of the eccentric cam.

Stage 1. The driven wheel (22) is stationary while the driver wheel (19) rotates. The cam (23) lowers the lever (13) thereby raising the arm (15).

Stage 2. The driver (19) operates the driven wheel (22). The cam (23) is at its dwell point and the lever (13) remains stationary.

Stage 3. The driver (19) locks the driven wheel (22) while the cam (23) raises the lever (13) to its former position.

Stages 1, 2 and 3 are over 135°, 90° and 135° rotation of the shaft (18), respectively.

The driven wheel (22) operates the six-position switch (21) via the gears (20) and also the main shaft (24) through the bevel gears (25). The gear plate (8) is attached to the disc (7) and the shaft (24). The gear (8) drives the gear (4) through step down the gears (10). The ratio between the gears (4) and (8) is 2–3 for six samples. The sample cups (1) (Technicon AutoAnalyzer cups) are held on a ring (3) which is mounted on the gear (4), and this assembly rotates free of the disc (5). Pressure between the discs (5) and (7) is produced by a spring (12) and the seal between the discs is made by neoprene "O"-rings (6) partly recessed into the disc (5).

OPERATION

When the motor (16) is activated by the programmer, the cam (23) raises the arm (15) by way of the lever (13), thereby pivoting the sampling tube (2) and withdrawing it clear of the sample cup (1). In addition, as the lever (13) moves, it releases the pressure created by the spring (12) sufficiently to enable relatively free movement between the discs to occur. Contact with the microswitch (14) is also broken.

On completion of this movement the Geneva mechanism operates and the main shaft (24) is rotated through 90°, while the switch (21) is advanced on one place indicating the new sample number to the programmer.

The coils (11) (which are connected to the gear (8) and the disc (7) by Beckman type swivel fittings (9)) have now been rotated 90° to align with the main buffer flow to the column, and the next sample cup has been rotated through 60° to align with the sampling tube (2).

The cam (23) now lowers the arm (15) via the lever (13) and the sampling tube (2) into the next sample cup. Pressure is reapplied to the discs and the microswitch is activated. The programmer is thereby instructed to begin the analysis and also to pump the next sample into the sample coil. At the termination of each analysis the programmer switches off the pumps and delays the next sample change for a time, sufficient for the pressure to return to atmospheric. In this way the loader is not under any pressure during the sample change.

A greater number of sample cups could have been used, but 6 (pairs of) cups were found to be quite adequate for our particular application.

Further construction details are shown in Fig. 3.

PERFORMANCE CHARACTERISTICS

Data showing the reproducibility of this loader and the lack of significant carry-over are presented in Fig. 4. These data relate to the comparatively stringent operating conditions we normally use *i.e.* loading a 0.39-ml sample from a total sample volume of 0.40 ml. Obviously if one were to use a total sample volume greater than 0.40 ml and suitably adjust the timer so that the larger overlap was at the exit

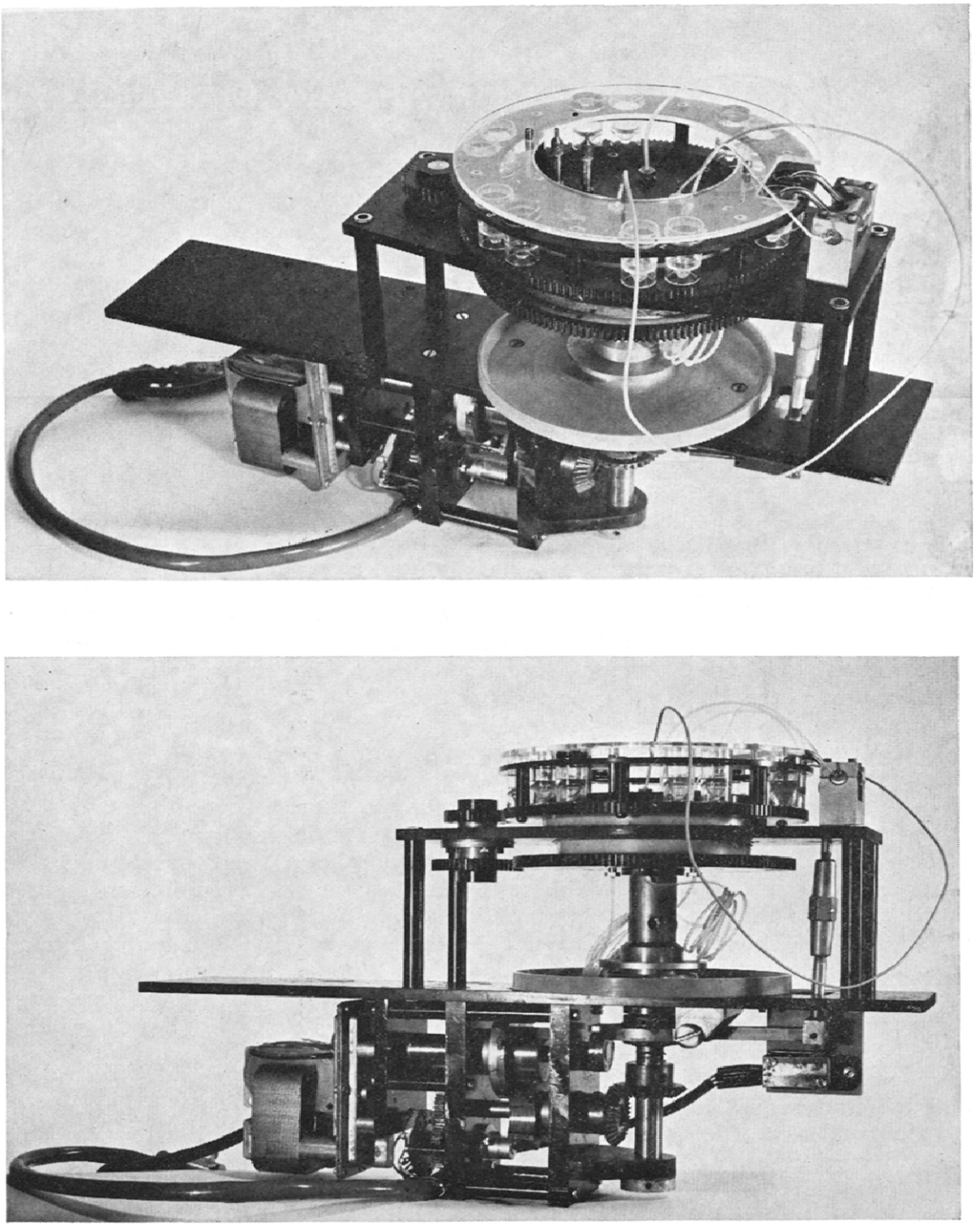

Fig. 3. The working construction. Note that only one of each pair of sample storage coils is connected.

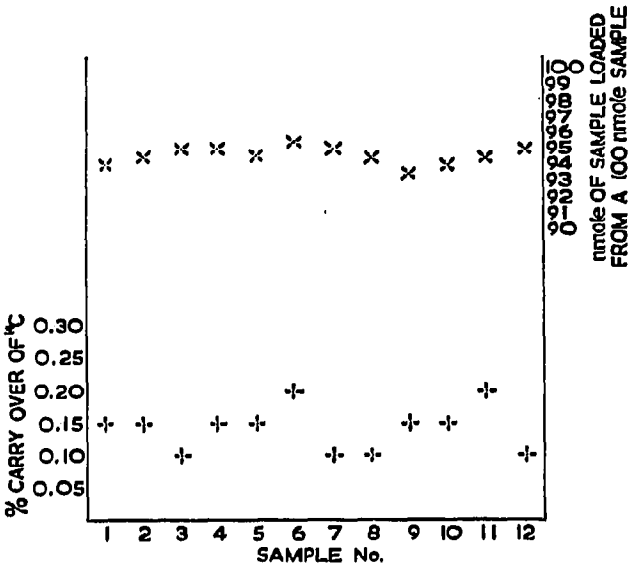

Fig. 4. Percentage carry-over of a sample to the following sample, and reproducibility of loading as estimated from the amount of sample analysed from known amino acid mixtures. The percentage carry-over was estimated from counting ^{14}C-S-carboxymethyl-L-cysteine samples and following blank water samples.

(waste) end of the coil, then even this small carry-over could be eliminated. The errors illustrated in Fig. 4 are well within the quoted accuracy of amino acid analysis (±3%) and hence are acceptable for our purpose.

To us the device seems to have three major advantages when compared to commercial loaders of which we have knowledge, *viz.*

(1) There are a number of loaders which contain the sample in PTFE loops which are then connected into the analyser buffer line. To the best of our knowledge however, these all require the analyst to fill the loops manually, a time-consuming and tedious procedure. Additionally the automatic loading, because of the slow uniform flow, is less likely to lead to trouble due to entrainment of air bubbles or traces of particulate matter than the less reproducible manual loading[1] procedure.

(2) It is a simple matter with this device to insert an important sample into the system at any time. All other commercial devices of which we are aware do not permit this, with the exception of the Technicon loader which is based on a completely different principle. The device described by Gerding and Peters[2] also has this facility.

(3) Finally the device is much cheaper to make than any commercial equivalent. The cost of materials was less than Au$ 200 and approximately 120 man-hours of labour were additionally involved. The precise cost to be placed on this latter factor will of course vary widely, but at any realistic estimate the total cost does not approach that of commercial loaders, which in Australia are currently priced from Au$ 1680 upwards. At the time the project was initiated the cost margin was in fact very much greater even than this.

A possible criticism of our design is that although a Perspex cover plate rests on the sample cups, they are not completely sealed, and hence evaporation and

possibly contamination may occur. There are a number of points which can be made against this criticism.

Firstly it should be made clear that the sample is loaded into the coil immediately after the sample cup has rotated into loading position. The sample is thus protected by the cover, except for the actual 3 min loading period. Moreover, the cups being machine-moulded are quite uniform, so contact between them and the cover plate is extremely good, evaporation loss is slight, and the chance of contamination negligible. (In any case, for amino acid analysis the pH of the sample is too low to permit bacterial growth.) The apparatus has been in use for 3 years in a laboratory which is only partially air-conditioned and where outside summer temperatures may exceed 40° and be accompanied by very low humidity. Nevertheless the maximum variation due to evaporation loss has not exceeded 4–5% between samples 1 and 6 using a 5-h programme per sample. With an overall experimental error of $\pm 3\%$ it is even doubtful whether this is really significant.

The point should also be made that routinely we use norleucine as an internal standard which automatically circumvents this problem.

In conclusion we feel that this design of sample loader has a number of technical advantages over many commercial models and, moreover, can be fabricated much more cheaply by any reasonably equipped instrument workshop.

REFERENCES

1 K. Dus, D. Lindroth, R. Pabst and R. M. Smith, *Anal. Biochem.*, 18 (1967) 532.
2 J. J. T. Gerding and K. A. Peters, *J. Chromatogr.*, 56 (1971) 357.