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MULTI-COLUMN LIQUID CHROMATOGRAPHY

I. A NEW MULTI-COLLECTOR FOR SIMULTANEOUS FRACTION COLLECTION FROM A NUMBER OF CHROMATOGRAPHIC COLUMNS

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SUMMARY

A new type multi-collector for the simultaneous collection of fractions from a number of chromatographic columns is described. It allows collection directly in multi-cuvettes for direct quantitation or in PTFE collection modules for initial reaction and transfer to multi-cuvettes for quantitation.

INTRODUCTION

Fifteen years ago we described a special fraction collector for multi-column liquid chromatography[1]. The capacity of the system has later been expanded from the original six-column system to a design for simultaneous chromatography from twenty-five columns at a time[2]. The basic principle of construction remained unchanged, however, in this expansion of capacity. The collection was performed by a stop/go procedure in which test tubes carried in holders on rails moved by gravity past a timer-activated latch that made the test tube carriers advance one row of tubes at a time down a slope when activated by the timer. This design has proven exceptionally reliable because of its basic simplicity. It depends only on gravity, a highly reliable industrial type timer and a latch for its performance. Some of these collectors have been in daily use in our laboratory for more than fifteen years and have required only occasional routine maintenance.

Nevertheless some shortcomings have been found for some applications of these collectors. In the collection of radioactive material from chromatographic columns drops of radioactive material might fall between adjacent test tubes as the collector moved forward one tube. This would give contamination of the test tube holders unless every second test tube was fitted with a small funnel overlapping the gap between the tubes or special tubes were made with a funnel-like upper section. This was found to be inconvenient and not very practical for routine use. Also if very small amounts of liquid are collected in chromatography not involving radioisotopes a loss of even a drop of liquid can introduce a significant error.

In the new multi-collector described herein this shortcoming in design has been overcome. Although the new multi-collector has been built primarily for incorporation into our integrated new system for multi-column liquid chromatography we believe that it may be useful also for other applications where large scale multi-column chromatography is contemplated.

DETAILS OF CONSTRUCTION

Our older collectors featured a stepwise collection of fractions. The current design uses a variable speed motor that at a preset speed drives a wheeled carrier that holds the special forty-compartment collecting modules machined from PTFE or the forty-section multi-cuvettes[3] on its flat surface (Fig. 1). Since each section of the compartmented PTFE collectors and the multi-cuvette have the same width (within the machining tolerances of the milling machine used in the construction) approximately the same amount of liquid will be collected in each section.

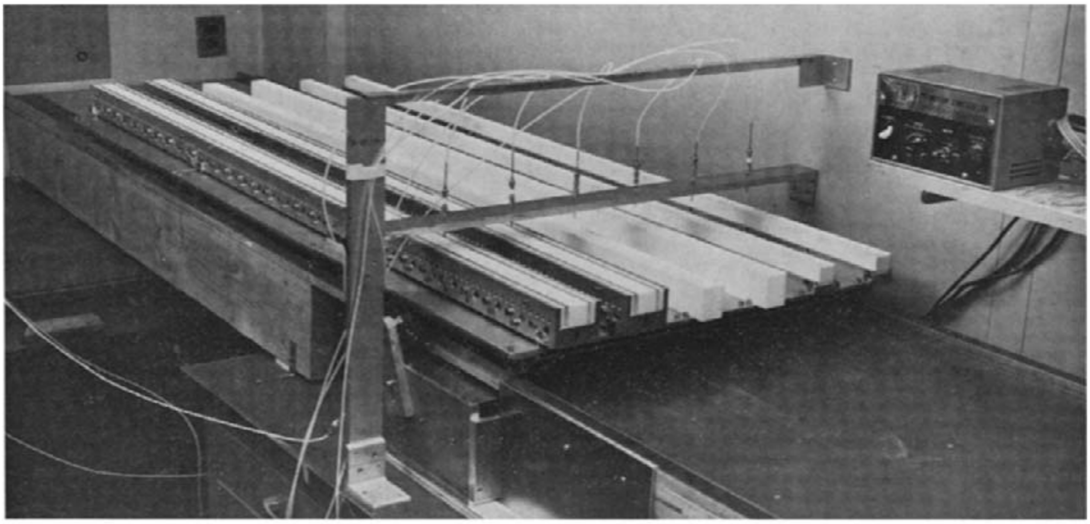

Fig. 1. The multi-collector demonstrating collection directly in multi-cuvettes and PTFE collector modules of different capacity.

One of several versions of the drive mechanism is shown in Fig. 2. A 1/8-hp variable speed motor (No. 12R-254, G. K. Heller, New York, U.S.A.) is controlled by a solid state variable speed motor controller (No. S20, G. K. Heller). The movement is transferred to the carrier holding the cuvettes via a gearbox and a rack-and-pinion transfer with the rack attached to the bottom of the supporting metal plate on the carrier. The carrier itself is basically a flat plate of aluminum mounted on two L-shaped bars of aluminum to which are attached two sets of wheels at front and back. The wheels are made of brass with wheel bearings. They are grooved and run on aluminum tracks that are parallel and are spaced about 24 in. apart in a twenty-five-column collector, but this width obviously depends on the number of columns needed at one time for simultaneous collection.

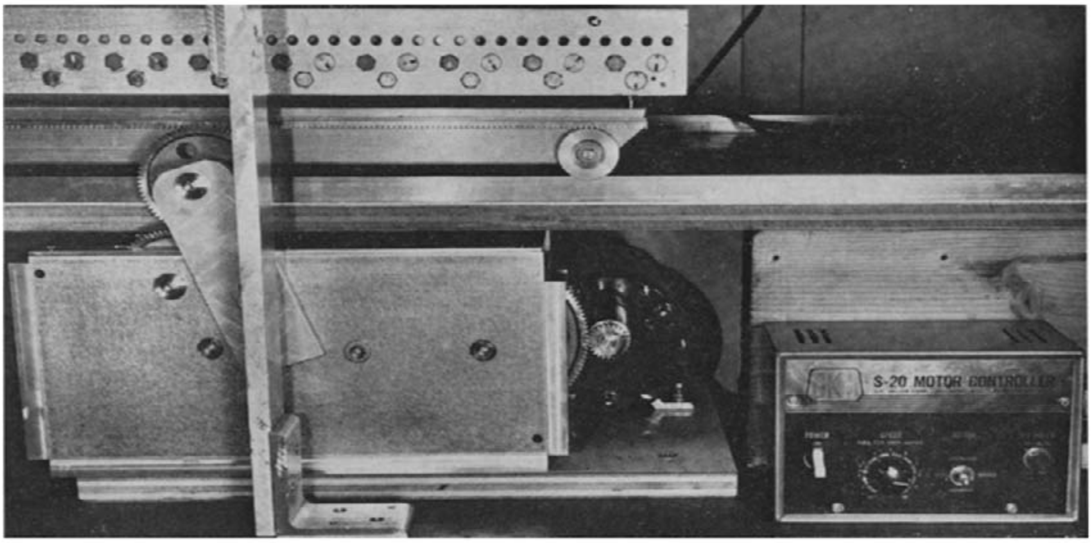

Fig. 2. The drive mechanism. A variable-speed motor transfers power through a gear box and a rack-and-pinion drive to the rack mounted underneath the wheeled carriers supporting the multi-cuvettes and PTFE collector modules.

We have, in another design of the collector, replaced the fixed-rate gear box with a gear box with a number of interchangeable gear ratios, varying from 1:1 to 1:1000 (Apcor No. 2411, B & B Motors, New York, N.Y., U.S.A.).

This together with the variable speed of the motor allows complete flexibility of the speed adjustment with collection rates variable from a fraction collected in a few seconds as may be needed in high pressure small-particle chromatography to a rate of a fraction collected in 60 min as may be needed in very high resolution liquid chromatography.

The different ways the collector can be used is shown in Fig. 1 illustrating collection directly in a glass multi-cuvette[3] for direct quantitation of colored compounds or for reaction directly in the cuvette prior to quantitation. The standard 6-ml volume per compartment PTFE collectors are also shown together with a set of 2.5-ml volume microcollectors. The 6 ml per compartment PTFE module shown in Fig. 3 is the collector module we have standardized on in our routine work. It consists of forty compartments each milled so that they are 25 mm wide, 9 mm across and 32 mm deep. The top few millimeters of the cuvette sections are cut a few mullimeters wider, so that only an inversed V-shaped piece of wall remains between collector compartments at the top. This type of collector module has stood up well in daily routine use over a two-year span in the laboratory. Care must be taken when it is put in an automatic laboratory glassware washer so that the collector is not supported on the between-compartment ridges but it has otherwise proven a rugged design compatible with all chemicals in use in the laboratory. A small tent-roof shaped PTFE clip is used to connect each forty-column collector module with the next when several collectors are used sequentially. We have constructed different sized PTFE collectors for different applications: larger 15-ml compartment collectors for larger volumes, smaller 2.5- and 1-ml compartment collectors for smaller volumes. For some applications

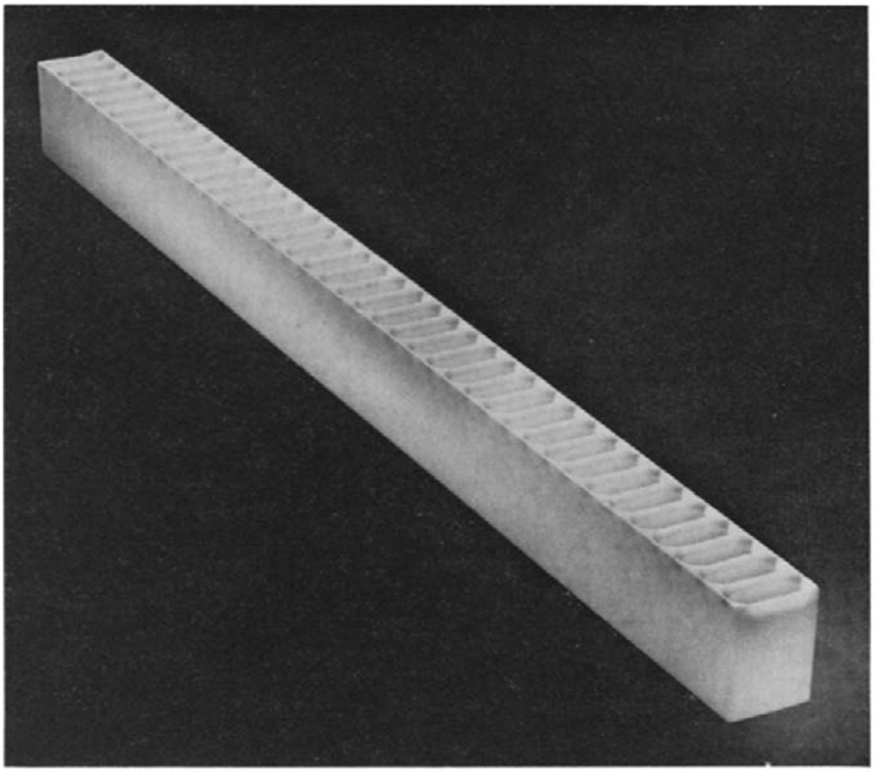

Fig. 3. The standard 6-ml capacity PTFE collector module. It has 40 sections each 12.7 mm (0.5 in.) wide. Collection and subsequent chemical reactions before quantitation are usually performed in these modules.

PTFE is not necessary. We have found polypropylene which is considerably cheaper, useful for collector construction when compatible with the solvents and chemicals used.

PERFORMANCE

This new type multi-collector has been used for more than two years in daily routine operation in our laboratory. We have had no difficulties of any kind with the collectors. They have often been left overnight for collections in high-resolution chromatography and no problems have so far been encountered.

In most of our applications the question of how accurately each fraction has been collected has not been a problem. We have in most of our routine work evaporated the organic solvents used in the chromatography and re-dissolved each fraction in a known amount of reagent solution before quantitation.

The reproducibility with which individual fractions can be collected clearly depends on two factors: the constancy of the speed with which the collector is advanced on the track and the precision with which individual sections can be machined. Stop-watch measurements of carrier speed has shown the speed to be constant within 0.5% at a given motor controller setting. The milling machine has a rated tolerance of 0.001 in. for machining. Since each section is 0.500 in. wide this gives a precision of 0.2% on the machining of sections. This has been confirmed by precision measurement of the machined collectors.

We have found the 0.5% precision on the collections set by the constancy of the motor speed adequate for biological analyses. Higher constancy of speed can be obtained by using stepping motors in the design but at a considerable increase in cost.

ACKNOWLEDGEMENTS

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REFERENCES

1 P. Vestergaard, *J. Chromatogr.*, 3 (1960) 554.
2 P. Vestergaard and E. Jacobsen, *J. Chromatogr.*, 50 (1970) 239.
3 P. Vestergaard and T. Piti, *J. Chromatogr.*, 111 (1975) 63.