CHROM. 4146

CONTINUOUS CHROMATOGRAPHY APPARATUS

I. CONSTRUCTION

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SUMMARY

A description is given of the details of construction of an apparatus for continuous liquid–solid phase chromatography.

INTRODUCTION

The underlying principle of continuous chromatography was first mentioned in print as early as 1949 (ref. 1) although, as noted in the paper, earlier discussions on the subject had been held. In the mid-1950's interest in the subject led to the development of a continuous system composed of a Gatling-gun arrangement of tubes[2] and a paper cylinder device[3], both liquid–solid phase devices. Latest developments in the field have been restricted to gas–solid phase devices, operating as cylinders[4–8], and at least one disc device[9]. Recent activity in continuous liquid phase chromatography has not been as great. Perhaps one of the difficulties has been that the materials available for the fixed phase have not been of a quality good enough for continuous chromatography, for as will be pointed out later in this paper, a uniform flow rate throughout the column is an absolute requirement for successful operation of a continuous chromatography column. It has also been suggested that the lack of application was due to the failure of mixtures of substances differing greatly in polarity to separate[10]. Here again, advances in the art and technique of the making of fixed phase materials has led to materials with higher resolving power and although the separations we have achieved are not especially difficult, there appears to be no reason to believe the technique cannot be applied to more complex mixtures, even in the relatively simple device we have designed.

DESCRIPTION

Principle

Reference to Fig. 1 will explain the principle. The solution is applied continu-

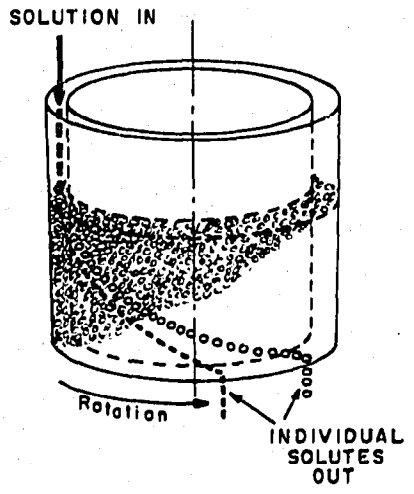

Fig. 1. The principle of continuous partition chromatography. —, faster moving component A; ○, slower moving B.

ously to the top of a bed of fixed phase which is contained between two concentric cylinders (the chromatographic column is in the shape of a cylindrical annulus). A head of eluting solvent is maintained above the fixed phase bed. As the solution and solvent move down the column, component A (dashes) moves faster than component B (circles). The column is slowly rotated under the point of application of solution. Component A is transported a distance, S_A, around the circumference of the column

$$S_A = \text{r.p.m.} \times \pi d t_A$$

where t_A is the time in minutes component A is on the column; similarly for component B. Since $t_B > t_A$, $S_B > S_A$, *i.e.*, component B comes off the column at a distance $S_B - S_A$ farther around the circumference than component A. And of course, since the solution goes on the column continuously, the components come off continuously.

Apparatus

The continuous chromatography apparatus described herein is shown in Fig. 2. The device consists of two acrylic cylinders, clamped concentrically to a Plexiglas* base plate. The outer cylinder was cut from a 12 in. O.D. × 11 1/2 in. I.D. cast acrylic tube and the inner cylinder was machined down to 10 3/4 in. O.D. from an 11 in. O.D. × 10 in. I.D. tube, both 12 in. high. The width of the annulus is therefore 3/8 in., and the total volume of the column space is 2.58 l. These particular dimensions were chosen so that the column capacity would be comparable to an 8.3 × 30.0 cm (1.6 l) column used in this laboratory so that yield results could be compared for the two. The outer cylinder is clamped to the base plate by means of a stainless steel ring, 11 3/4 in. I.D., set into a 1/8 in. groove cut into the outer wall of the cylinder. The inner cylinder is clamped by means of a plate which rests on a shoulder machined into the inner wall. Twenty machine screws are spaced equidistant around the cir-

* Mention of commercial names does not imply endorsement by the U.S. Department of Agriculture.

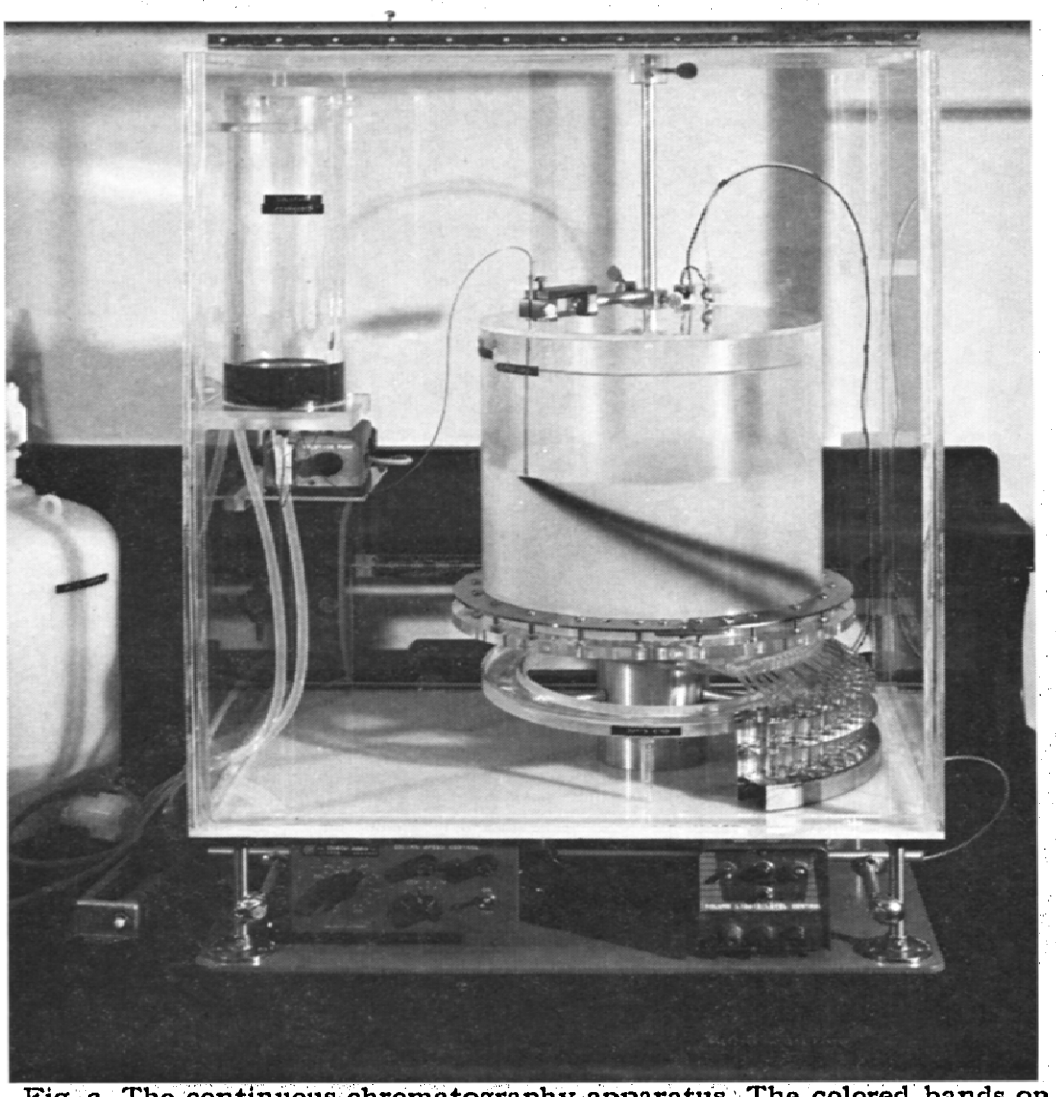

Fig. 2. The continuous chromatography apparatus. The colored bands on the column are hemoglobin (lower band) and myoglobin. Note multiple sample collector in place under column at right.

cumference on both ring and plate, with the heads up. Twenty is the minimum number necessary to ensure a tight seal between the cylinders and the bottom plate, the spacing being a maximum of 2 in. between screws in the outer ring, and 1/4 in. between the screws and the center of the clamping surface. In order to support the fixed phase media in the column, a ring of coarse grade filter paper is clamped between the bottom edges of the two cylinders and the base plate, compressed tightly so as to prevent leakage. The column is assembled upside down. The support filter paper is centered on the clamping surface of the inner cylinder and the base plate placed on top, drip tips up. The screws, which are permanently mounted in the inner clamping plate, suffice to guide and center the base plate into position. The nuts are placed on the screws, finger tightened, and the assembly is then placed in the center of the outer ring, again guided by the machine screws. The nuts are placed on the outer cylinder screws, and all the nuts tightened with a torque wrench to 15 inch-pounds.

The exit orifices are 100 in number, drilled equidistant on a circumference in

the center of the columnar space between the two cylinders. On the bottom side the exit orifices terminate in drip tips 1/4 in. in length, made of 0.032 in. I.D. × 0.052 in. O.D. polyethylene tubing. The holes in the base plate were drilled 0.050 in., the short sections of polyethylene tubing pressed into the holes and cemented in place. The top of the base plate was left planar, so that no solvent interflow between tips could occur with the filter paper support medium in place. The paper has a fine enough capillary structure to prevent solvent interflow between tips, but during assembly of the column care must be exercised to prevent wrinkling of the paper, which might provide inter-tip channels. With wrinkles, or very slow flow rates (below 500 ml/h) with the rayon filter paper we used, interflow could occur, which resulted in preferential flow of eluant through some tips and not through others. If this occurred for two or three tips, it made no important difference in the collection of fractions, but if more tips were involved, the bands were smeared and displaced.

The column assembly rests on a turntable, which rests on the vertical low-speed shaft of a 1:300 gear reductor (Boston Gear Works No. VLW 13-300). The latter is driven by a variable (2 to 10 r.p.m.) speed motor (Chemical Rubber Co. Model No. SCPVBK), thus giving speeds of column rotation from 0.4 to 2 r.p.h. The turntable has a 1 in. diameter stud machined in the center, and the column base plate has a centered 1 in. hole to fit closely over the stud. A teflon sheet is placed between the column and the turntable to allow for easy rotation, and the two are not fastened together.

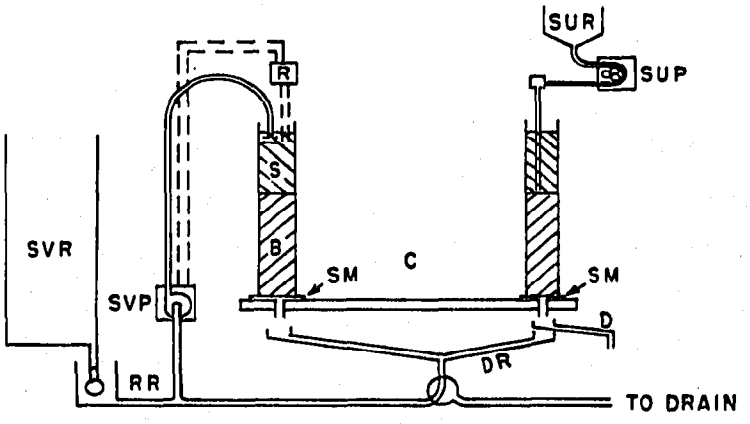

Fig. 3. Schematic diagram of the plumbing and electrical connections required to operate the apparatus. B = bed; C = column; D = diverter; DR = drain ring; R = relay; RR = recirculating reservoir; S = solvent; SM = support medium; SUP = solution pump; SUR = solution reservoir; SVP = solvent pump; SVR = solvent reservoir; — — —, electrical.

The plumbing and electrical connections needed to operate the column are shown in Fig. 3. The buffer supply is fed into the column plumbing through a constant level device into the recirculating reservoir. From there buffer is pumped into the column on demand. When the liquid level in the column drops, an electrical circuit is broken between two platinum electrodes immersed in the buffer. A sensitive relay (Fisher Transistor Model 30) closes a pump circuit, and the solvent is pumped into the column through a discharge head that directs the liquid flow horizontally so as not to disturb the surface of the fixed phase. In our apparatus this was accomplished

by drilling two holes in the side of a 13 gauge needle near the tip, and then plugging the tip by using the needle to bore through a piece of polyethylene and leaving the plug thus formed in the tip. When the liquid level rises the liquid contacts the platinum electrodes and the relay shuts off the pump. The relay is sensitive enough to respond to distilled water in the column.

The effluent from the bottom of the column drips into a drain ring under the column. The liquid in the ring may be directed either to the drain or back to the recirculating reservoir to be pumped back onto the column. The apparatus described herein was built with no facility for shutting off the column flow, partly for simplicity of design. However, for reasons which will be gone into in the next paper, continuous flow through the column is probably the best method of operation and column shut-off may be neither necessary nor desirable. Continuous flow through the column presents no problems in the operation thereof, and in fact, most operations must be carried out with solvent flowing through the column. For our recent work we have not used the recirculating system but have connected the solvent pump directly to the reservoir and the drain ring to the drain, since we only run the column while purifying proteins.

The solution pump is a commercially available pump of the peristaltic type (Holter Model RD174), chosen for low and adjustable flow rates, without pulsation, since the liquid should flow onto the surface of the fixed phase as smoothly as possible to avoid solution–solvent mixing. The solvent pump is a commercially available

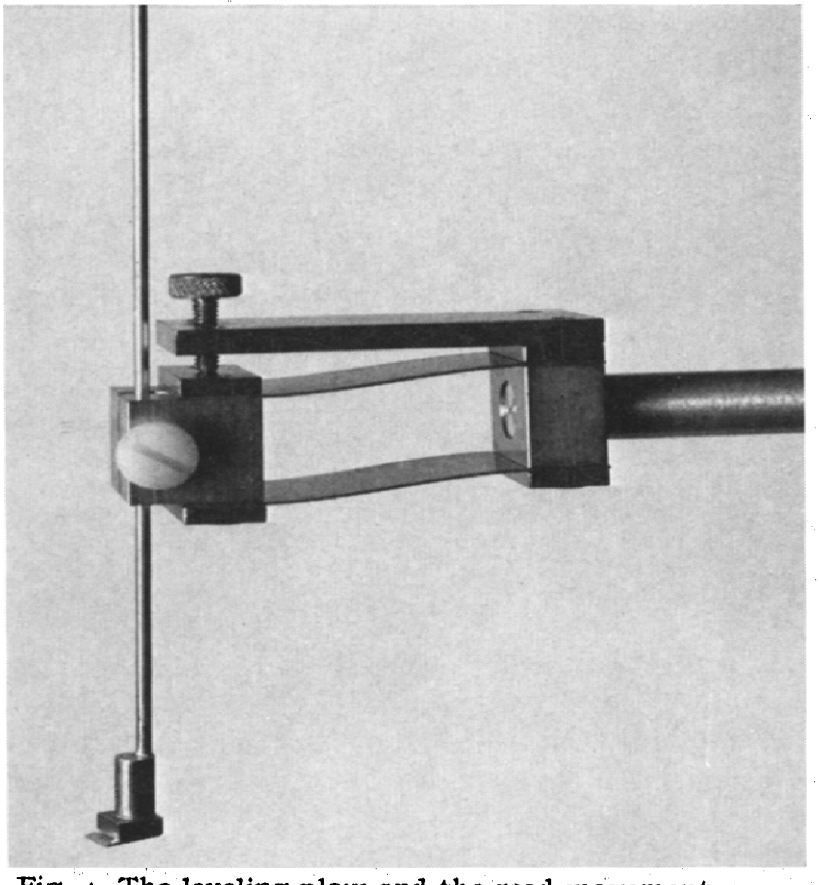

Fig. 4. The leveling plow and the reed movement.

pump, in this case of the "peristaltic" type although any pump with a pumping rate faster than the column flow rate would be suitable.

Leveling plow

Since it is necessary to have an absolutely flat fixed phase bed surface for even layering of the solution and uniform entry of solution into the fixed phase, a special device, the leveling plow, was developed. This device is shown in Fig. 4, held in place in the reed movement (see below). The plow consists of a shoe-like attachment on the end of a 13 gauge hypodermic needle. A flat plate, sharpened on the leading edge, is soldered to the bottom of the shoe, the leading edge extending past the "toe" of the shoe. A channel, extending almost the width of the shoe and 0.005 in. deep, connects to the bore of the needle and opens out toward the sharpened edge of the plow blade. The plow blade itself is tilted downwards 10° out of a plane perpendicular to the needle. The shoe and blade are 5/16 in. wide so that the assembly will fit inside the column. The needle terminates at the other end in a standard female Luer fitting. How this assembly is used will be described in the following paper[11].

Solution needle

In order to prevent solution–solvent intermixing while pumping solution on the column it is desirable to have as small an orifice as possible. On the other hand, too small an orifice results in high solution velocities which can cause stirring of the column bed surface. The solution to the proper balance between orifice size and solution volume is to flatten the orifice, which has the added advantage of spreading the solution across the width of the column bed if the long dimension of the orifice is aligned with the radius of the column. For the flow rates used in this laboratory it was found sufficient to flatten a 10 gauge hypodermic needle to give an orifice 0.004–0.005 × 5/32 in.

Reed movement

In order to make micro-adjustments of the height of both the leveling plow and the solution needle, the reed movement shown in Fig. 4 was made. The slot in the front block will accommodate needles from 9 gauge up. The front block is connected to the back block by two phosphor bronze spring strips. The thumb screw on top pushes the front block down away from the plate on top, the front block remaining parallel to the back block at all times. This type of device has an advantage in that there is no play in the operation of the movement.

High-speed column rotator

In order to achieve even speed of rotation, an additional rotation device was built, consisting of a 10 r.p.m. reversible synchronous motor mounted on a base in such a manner that the motor could be raised or lowered. Two drive wheels were made for the motor, one to accommodate a 2 in. "O" ring, the other a 6 in. "O" ring, fitted into grooves in the rims of the wheels. The appropriate wheel is mounted on the motor and the wheel positioned beneath the outer edge of the base plate of the column. The motor is then raised until the "O" ring contacts the bottom side of the base plate with sufficient force to rotate the column. Since the column rests on a teflon sheet on the turntable, very little force is necessary, and just sufficient force is

applied to keep the column rotating so that should the column jam, either the column will slip on the drive wheel or the motor will stall.

Fraction collection

Once the input conditions are established and even and continuous flow rates established for both solution and solvent, a fraction-collecting device is placed in position under the drip tips. The particular device used for the described apparatus consists of a quarter circle Plexiglas plate, mounted in a groove in the fixed column under the turntable, rotatable about the axis of the column, and which has a spring clip and two pins for purposes of locating and holding the fraction collector. This positioning plate does not extend out as far as the drip tips. In order to collect individual fractions, a second plate is placed under the drip tips of the column to divert the fraction drops away from the drain ring. This fraction diverter has a groove and slots to engage the clip and pins, respectively, of the positioning plate and is in position when seated against them. The diverter is a quadrant, with 25 collecting positions. The last are conical depressions drilled on the same radius as the drip tips. At the bottom they connect with holes drilled at an angle, outward and downward. These holes are of a size to fit snugly around 3/16 in. polycarbonate tubing. For collecting fractions, polycarbonate tubes, of appropriate heights and/or configurations, are pressed into the holes. The fractions dripping into depressions with tubes are diverted to the appropriate accumulation devices, test tubes, beakers, etc. Liquid dripping into depressions without tubes drips into the drain ring. Finally, the whole column is enclosed in a Plexiglas case to protect the column against air currents and ambient temperatures.

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