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The chromatofuge, an apparatus for preparative rapid radial column chromatography

Liquid chromatography in columns has many advantages over other chromatographic techniques[1], notably simplicity, flexibility, and capacity, but solvent flow through columns packed with fine or gelatinous sorbents may be very slow. The use of high pressure to force the solvent through the sorbent introduces several complications, such as safety hazards, changes in flow rate, and high cost. Recently, RIBI *et al.*[2] have substituted centrifugal force for pressure, but their apparatus does not permit fractional elution.

These problems may be eliminated by developing chromatographic columns, not from one end to the other, but from the center to the periphery. Twenty-five years ago HOPF[3] coined the term chromatofuge for this type of equipment and described its operation. Radial flow of the solvent in the column is achieved by rotating it around its axis. Such an arrangement not only accelerates the solvent flow, but also simplifies packing, loading, and eluting the column. Apart from a suggested use for gel filtration[4], the chromatofuge has not received the attention which we feel it deserves. We are reporting our experiences with a modified chromatofuge.

Experimental

The top of Fig. 1 shows a cross section of the essential parts of the apparatus. An aluminum delivery tube, D, $\frac{5}{16}$ in. O.D. and $\frac{4}{16}$ in. I.D., has an opening at the top and a row of perforations, 0.025 in. in diameter, at one side. A basket, B, made of 0.0187 in.-thick stainless steel, is 4 in. in diameter and $1\frac{1}{2}$ in. high and has 14 perforations per in., 0.032 in. in diameter, in the wall and an opening, 1 in. in diameter, on top. The open bottom of the basket fits over an aluminum rotor, R. The rotor is mounted on the spindle of an air-driven filtration apparatus (Filtermatic Type III, Chemical Rubber Co., Cleveland, Ohio)*.

The bottom of Fig. 1 shows the inside of the basket, as seen from the bottom when it is detached from the rotor. It is lined with a $1\frac{1}{4}$-in. wide strip of $\frac{1}{16}$-in. thick polypropylene felt, F, (Pacific States Felt & Mfg. Co., San Francisco, Calif.) which overlaps as shown for the basket rotation in the direction indicated. The basket and rotor spin in a stationary Teflon-lined cup, C, which drains through a tube and sits inside a cylindrical shield (not shown). The delivery tube is stationary, while the basket spins on a turbine driven by compressed air. We have used a pressure of 10 lbs./sq. in., which drives the basket at 1950 r.p.m. and produces a centrifugal force of $210 \times g$.

To pack the basket the felt strip is first moistened with solvent and the delivery tube is removed. While the basket is rotating, a slurry of sorbent in solvent is poured in a slow stream into the hole on top of the basket. The solvent leaves the basket through the holes in the side wall, while the solid, S, retained by the filter, forms a very uniform chromatographic column with a hollow core.

* Reference to a company or product name does not imply approval or recommendation of the product by the U.S. Department of Agriculture to the exclusion of others that may be suitable.

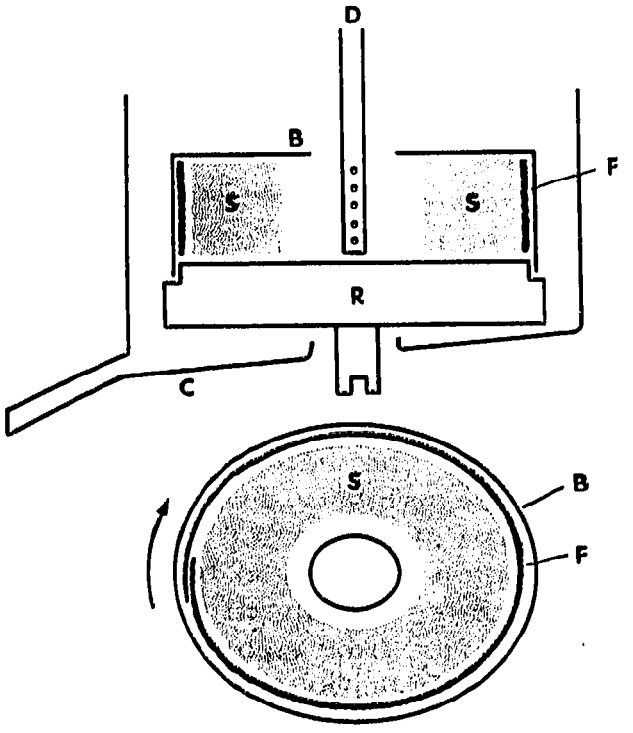

Fig. 1. Apparatus for rapid radial column chromatography. B, basket; C, cup; D, delivery tube; F, filter; R, rotor; S, sorbent.

The delivery tube is placed back into position, and the solution containing the sample is delivered from a height of about 80 cm so as to spray out of the holes in the side of the tube and deposit the sample evenly over the exposed surface of the core. Solvents are now delivered through the tube in the same manner. They penetrate the spinning column evenly from the center to the periphery to produce a radial development of the chromatogram. The individual components of the sample form concentric cylinders that gradually expand until each component leaves the spinning basket. The effluent collects in the cup and drains out of the apparatus.

Fig. 2 illustrates the functioning of the apparatus. For this experiment a solution of test dyes (Testgemisch, Desaga GmbH., Heidelberg, G.F.R.) was diluted 1:10 with benzene and 6 ml of this was applied to a column prepared from 80 g silica gel (Kieselgel zur Säulen-Chromatographie, feiner als 0.08 mm, M. Woelm, Eschwege, G.F.R.), slurried in benzene. After 2 min of development with benzene, delivered at a rate of 375 ml/min, the machine was stopped and the basket was detached from the rotor in order to take this photograph. Three concentric rings may be seen with the yellow dye moving faster than the red dye, and the blue dye not yet migrating from the origin of the chromatogram.

The performance of the apparatus was further illustrated by the separation of four closely related purines: caffeine (1,3,7-trimethylxanthine), theobromine (3,7-dimethylxanthine), theophylline (1,3-dimethylxanthine), and xanthine. Solutions[5] containing 5 mg of each of the four purines were mixed and the mixture was applied to a silica gel column prepared as before. In this experiment a mixture of ethyl acetate, methanol, and concentrated ammonium hydroxide (8:1:1) was used for packing and development. With solvent flow at a rate of 325 ml/min, forty 25-ml

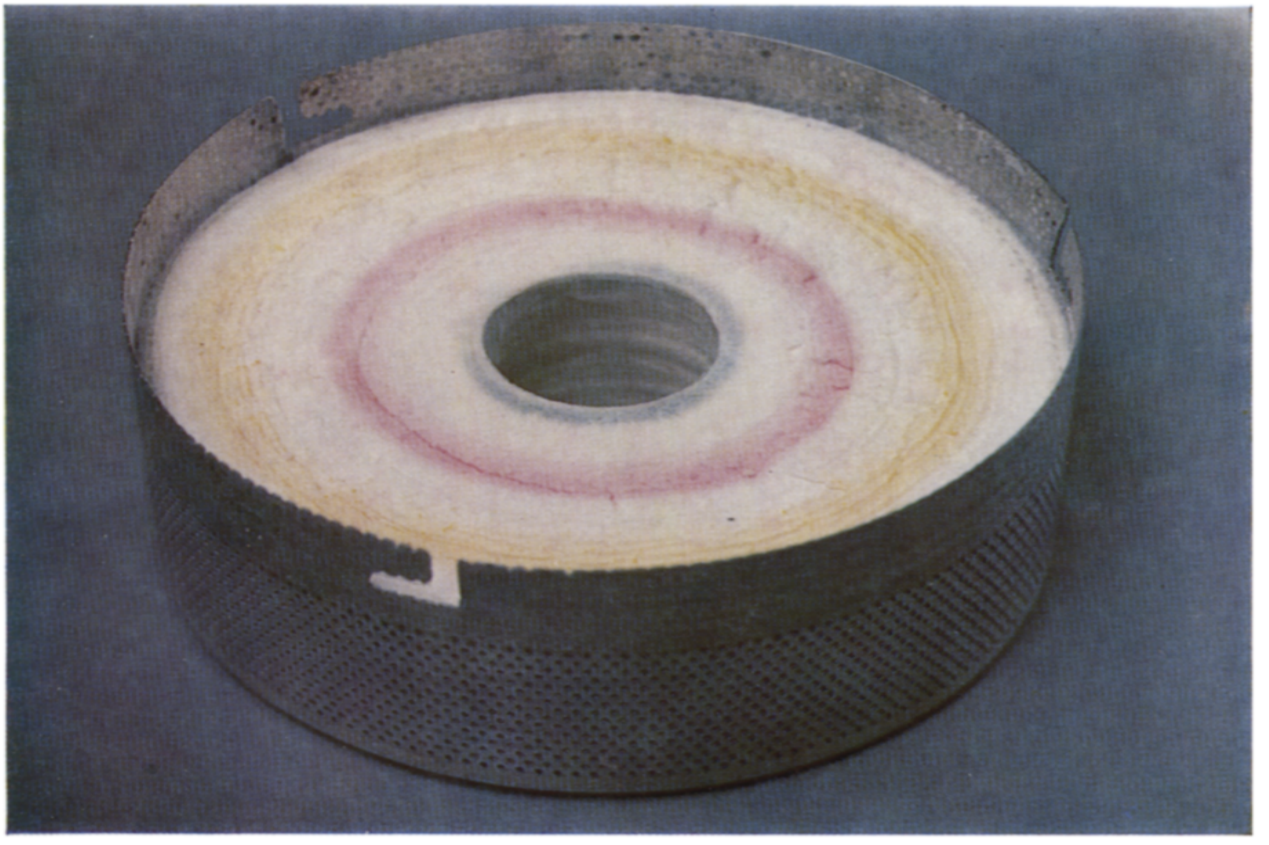

Fig. 2. Separation of dyes by rapid radial column chromatography. The rotor has been detached to show the migrating dyes from the bottom of the basket.

fractions were collected at the rate of 4 sec per fraction. The purines were detected in the eluate fractions by their UV absorption at 275 nm and identified in the peak tubes by TLC[5]. The analyses indicated that caffeine was eluted in fractions 2–8, theobromine in fractions 10–17, and theophylline in fractions 30–35, while xanthine remained on the column. Thus, complete separation took less than 3 min.

Discussion

Preliminary work with our modification of the chromatofuge has indicated the possibilities and limitations of this technique. The apparatus is simple to construct, explosion-proof, and easy to operate. The sorbent is evenly packed by pouring a slurry into the rotating centrifuge basket. The column is conveniently and rapidly loaded and eluted through an axial delivery tube. Eluents of various kinds can be used in sequence or in gradient elution.

Accelerated development often increases resolution by decreasing zone diffusion. One of the advantages of radial development is the sharpening effect due to expansion of the zones as they migrate from the center to the periphery. However, the shape of the collection cup shown in Fig. 1 allows some hold-up and remixing of fractions. The present design of the apparatus makes it more suitable for preparative work than for analysis. The capacity of the system will depend on its size, particularly the height of the cylinder, while the resolution will depend on its diameter.

Although we have only used it for adsorption chromatography, a similar arrangement has been used for gel filtration[4], and we believe that our apparatus and technique should also be applicable to other sorptive processes (*e.g.*, ion exchange and solvent partition) as well as to a number of non-chromatographic processes (*e.g.*, reverse osmosis and immobilized enzyme reactions). We hope that our work will revive interest in HOPF's chromatofuge technique and rescue it from undeserved obscurity.

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