Continuous chromatography using time-varying eluant flow
by Mark David Swanson

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Chemical Engineering
Montana State University
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Abstract:
This research was conducted to develop a method to increase the volumetric rate of flow of material that can be separated using chromatography. This was done by developing a continuous chromatography system. In this research, a square plate, 18.3 cm by 18.3 cm by 0.25 cm, was filled with Sephadex G-25, a chromatographic packing. This plate, or bed, was built so that eluent could be introduced on either of two adjacent sides, and withdrawn from the opposite sides of the bed. A dye mixture was introduced in the bed, and would flow with the eluent. By alternating the direction of eluent flow, the dyes were made to flow to characteristic points in the bed, and were extracted at these points. After the initial building of the bed, the procedure was to fix problems as they arose. Proper eluent flow proved to be very important, and specialized apparatus, "spider feeders", were developed for this. From computer simulation and observation, it was found that the fewest eluent flow direction changes possible should be used, because the flow in the bed was slow to respond to changes made at the spider feeders. The points where the dye was extracted from the bed are very interdependent, so that slight alterations in one may have large effects on the operation of the other. The system investigated did work, because a continuous separation was demonstrated, but the operation of the bed was not as consistent as desired.

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A thesis submitted in partial fulfillment
of the requirements for the degree

of

Master of Science

in

Chemical Engineering

MONTANA STATE UNIVERSITY
Bozeman, Montana

March, 1989

N378
Sw245

APPROVAL

of a thesis submitted by

Mark David Swanson

This thesis has been read by each member of the thesis committee and has been found to be satisfactory regarding content, English usage, format, citations, bibliographic style, and consistency, and is ready for submission to the College of Graduate Studies.

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ABSTRACT

This research was conducted to develop a method to increase the volumetric rate of flow of material that can be separated using chromatography. This was done by developing a continuous chromatography system. In this research, a square plate, 18.3 cm by 18.3 cm by 0.25 cm, was filled with Sephadex G-25, a chromatographic packing. This plate, or bed, was built so that eluent could be introduced on either of two adjacent sides, and withdrawn from the opposite sides of the bed. A dye mixture was introduced in the bed, and would flow with the eluent. By alternating the direction of eluent flow, the dyes were made to flow to characteristic points in the bed, and were extracted at these points. After the initial building of the bed, the procedure was to fix problems as they arose. Proper eluent flow proved to be very important, and specialized apparatus, "spider feeders", were developed for this. From computer simulation and observation, it was found that the fewest eluent flow direction changes possible should be used, because the flow in the bed was slow to respond to changes made at the spider feeders. The points where the dye was extracted from the bed are very interdependent, so that slight alterations in one may have large effects on the operation of the other. The system investigated did work, because a continuous separation was demonstrated, but the operation of the bed was not as consistent as desired.

INTRODUCTION

Chromatography is a very useful tool in the separation of many hard to separate components. The basic theory is that a mix of the components to be separated is carried through a packed bed of some kind by a solvent or eluent. Because each component has a different affinity for the packing, each component has a different residence time in the packed bed, and hence the time for each component to travel through the bed is different. By collection of the eluent at different times, the various components can be separated. This is usually a batch process. For chromatography to be more useful to industry as a separation tool, it should be capable of separating large quantities of material. According to C. D. Scott,

> Simply scaling up a batch type column is not very desirable, because after a column has been scaled up to a 2.5 cm or more diameter, a measurable loss of resolution is noticed.[1]

Although scaling up is not very desirable, chromatography is still one of the best separation processes available. "In recent years, the purification of enzymes by chromatography on columns has become the most effective of all separation methods."[2] This use of column chromatography makes more products available, but it increases the price noticeably. S. H. Elwan says,

> We estimate the total cost of an industrial-type gel chromatography operation to be on the order of $5/gram or less. The major costs are labor (40%), gel (25%), filters (20%), chemicals (10%), and capital (5%).[3]

This chromatography expense is a factor in many of the products available today. According to J. J. Kelly,

> We surveyed the 1982 volume of the journal of Biological Chemistry and found that 60% of the published laboratory-scale protein purification schemes included at least one gel chromatography step.[4]

A continuous flow chromatography system would be capable of separating larger quantities of material, and several systems have been developed. According to P. C. Wankat,

> Although extensively studied, including industrial prototypes and pilot-plant studies, the continuous two-dimensional chromatographs have not been accepted as standard separation methods.[5]

Of the continuous chromatography systems now available, the packed annular bed seems to have the best chance of becoming accepted by industry. This system uses a packed annular space which is slowly rotated, while the feed point and extraction points are fixed.[6] This system can utilize a gradient elution technique, and has even been modified to produce three dimensional separations.[7] The problem with this technique is that it has moving parts, and needs seals where the rotating drum meets the fixed feed and withdrawal sites.

Another system is a magnetically stabilized fluidized bed, which requires the packing be a magnetic material, and

can only be used for the separation of gaseous feed products.[8] The fluidized bed is shaped like a box, with the fluidizing medium coming from the bottom. A slow gaseous flow across the bed moves the entire contents at a fixed rate. The product is introduced at the bottom, near the side where the cross flow enters the bed. The rate at which the product moves upward is determined by the affinity that the product has for the chromatographic packing, and the rate at which it moves across the bed is determined strictly by the flow across the bed. The products are then collected on the top of the fluidized bed, with those with a greater affinity for the packing, and thus a longer residence time in the bed, being further from the side where the cross flow entered.

There is a proposal for a system that uses a rectangular plate with eluent introduced on two adjacent sides, while the temperature of the system changes each time the direction of flow changes.[9] The different relative velocities of the products at the different temperatures results in the two products exiting the plate at different points.

Another system uses a slanting triangular plate, which is equilateral, with the horizontal side of the triangle at the bottom.[10] Two different eluent streams are introduced on the two upper adjacent sides, while the feed is introduced at a point on the center line of the triangle

near the top. The triangle is set at an angle so the eluents and the feed flow toward the third side of the triangle by gravity. This system has been demonstrated of being capable of continuously separating Fe^{+3} and Co^{+2}.

Although there are continuous chromatography systems which have been developed, none have been accepted as a standard method. If an effective, cheap, low maintenance system were to be developed, it would give an attractive option to anyone considering using chromatography as a separation tool.

The purpose of this research is to develop an operational continuous chromatography system using time-varying eluent flow in a square plate. The purpose is not to demonstrate any particular separation, but to show that the technique is workable.

Most chromatographic columns use position and time to achieve a separation. As the components move through the column, they become separated spatially, and thus each component exits the column at a different time. By collecting the eluent when each component is leaving the column, a separation is achieved. If a continuous system is to be operated at steady state, time cannot be used as an aspect in the separation, so something must replace it. In all the continuous chromatography systems so far developed, a second spatial dimension is used to replace time.[11] This is also true for this research project.

It is desired to achieve a separation by setting up a square plate, or bed, with the capability of introducing eluent on two adjacent sides, and allowing the eluent to exit the bed on the other two sides (see Fig. 1). This eluent could be introduced entirely on one side of the bed for a fixed amount of time, then entirely on the adjacent side for the same amount of time, thus producing a square wave introduction technique. It could also be introduced entirely on one side, and then have a gradual pressure change, so that after a fixed time it would be entirely introduced on the adjacent side, creating a sinusoidal eluent introduction. Thus, time would be used as a method of operation of the bed, but not as an aspect of the actual separation.

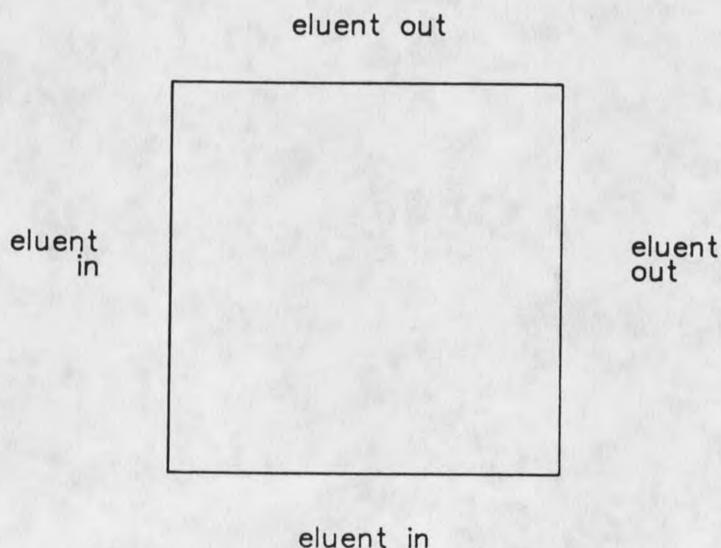

Fig. 1. Eluent flow

Because of the different distances traveled by the various components, there should be points in the bed to which each component, or dye in this case, characteristically moves. If the carrier fluid could be extracted at these points without significantly disturbing the flow pattern, a continuous separation would be achieved (see Fig. 2). As can be seen from the figure, there would be a trace of the large molecules collected with the small molecules. The large molecules collected should be free of any small molecule contamination. The optimum location for the feed would also be a consideration.

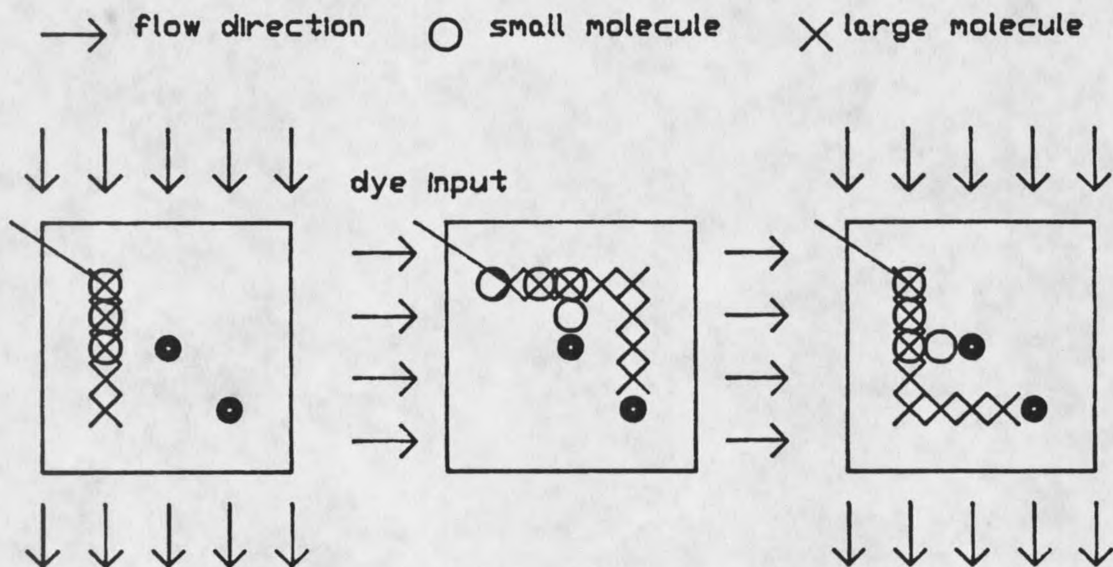

Fig. 2. Dye flow

EXPERIMENTAL

The basic experimental plan was to determine the flow characteristics in the bed, then to put in the product extraction holes, and finally to demonstrate a separation. Since this is a new technique, most of the experimental procedure was to find a problem with the system, and then fix the problem. For this reason, the experimental and the results sections of this thesis are not really separable. The results from one experiment were needed to determine the next experiment. There was an initial design, so that will be presented here in the experimental section.

The experiments were conducted using gel permeation chromatography, which separates components on the basis of molecular size. Sephadex G-25 was used as the chromatographic packing material, and the molecules which were separated were vitamin B-12 and blue dextran. Vitamin B-12 is red, and has a molecular weight of 1355 atomic mass units, while blue dextran is blue, and has an average molecular weight of two million atomic mass units.

The bed shape was square, with internal dimensions of 18.3 cm by 18.3 cm by approx. 0.25 cm. The basic bed was constructed of closed cell foam, which allowed no eluent to flow through it. The inside surfaces were notched, and open cell foam was affixed in each of these notches (see Fig. 3). The eluent could flow through the open cell foam with little resistance, and thus bypass the bed packing.

The notches prevented this. Eluent needles introduced the eluent to the bed through the closed cell foam. The open cell foam served as a filter, preventing the chromatographic packing (Sephadex) from clogging the eluent needles. The closed cell foam frame was attached with silicone sealant to a Plexiglas plate on the bottom, and there was another Plexiglas plate that was placed on top of the foam frame. This created an empty space between the Plexiglas plates and inside the foam frame. This open space was entirely enclosed by Plexiglas and foam, and was where the Sephadex was placed.

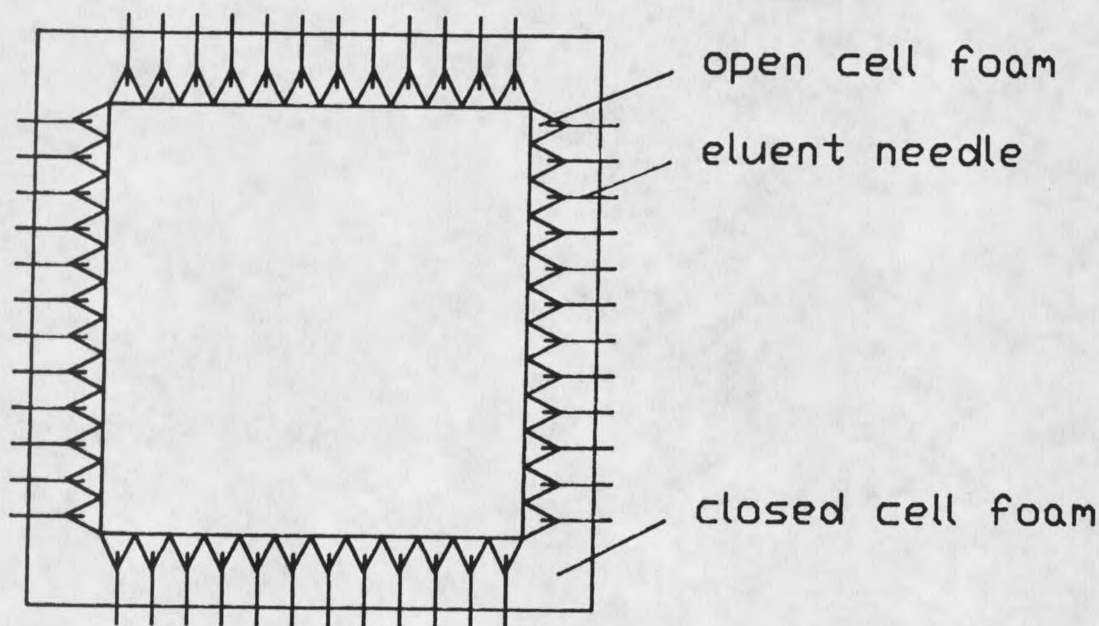

Fig. 3. Basic bed construction

There was an attempt to construct the bed out of silicone, so it would be thinner. It was found that the silicone was not compressible enough, making the bed packing process almost impossible. The wet Sephadex is a compressible mixture, and had to be compacted some in the bed packing process so it would maintain its shape while in use. The foam was compressible, hence after filling the open bed and placing the top Plexiglas plate in place, the volume of the bed would decrease in the clamping process. This bed volume change was not attainable in the silicone bed. Because the Sephadex was not compressed, it would slip and form open void spots when the eluent was introduced on a side. These open void spots disrupted the flow, and would not give reproducible results with different bed packings, so the use of the silicone bed was discontinued.

It was thought that a thinner bed would give a better separation. A thinner bed would allow the eluent less vertical motion, giving it a more constant path, and thus produce better resolution. Since the silicone did not work in making the bed thinner, a Plexiglas spacer plate was inserted into the bed to reduce the packing thickness (see Fig. 4). This Plexiglas plate had to be fixed in position, using silicone sealant, with the sealant around the outer edges of the plate, to prevent eluent from flowing under the plate, and bypassing the Sephadex. To keep the packing

a uniform thickness, it was important that this plate be very flat. It was discovered that placing a lamp close to the plate when the bed was empty and dismantled caused the plate to "lift up" and become separated from the rest of the apparatus. This proved helpful in relocating the plate if it had been positioned poorly.

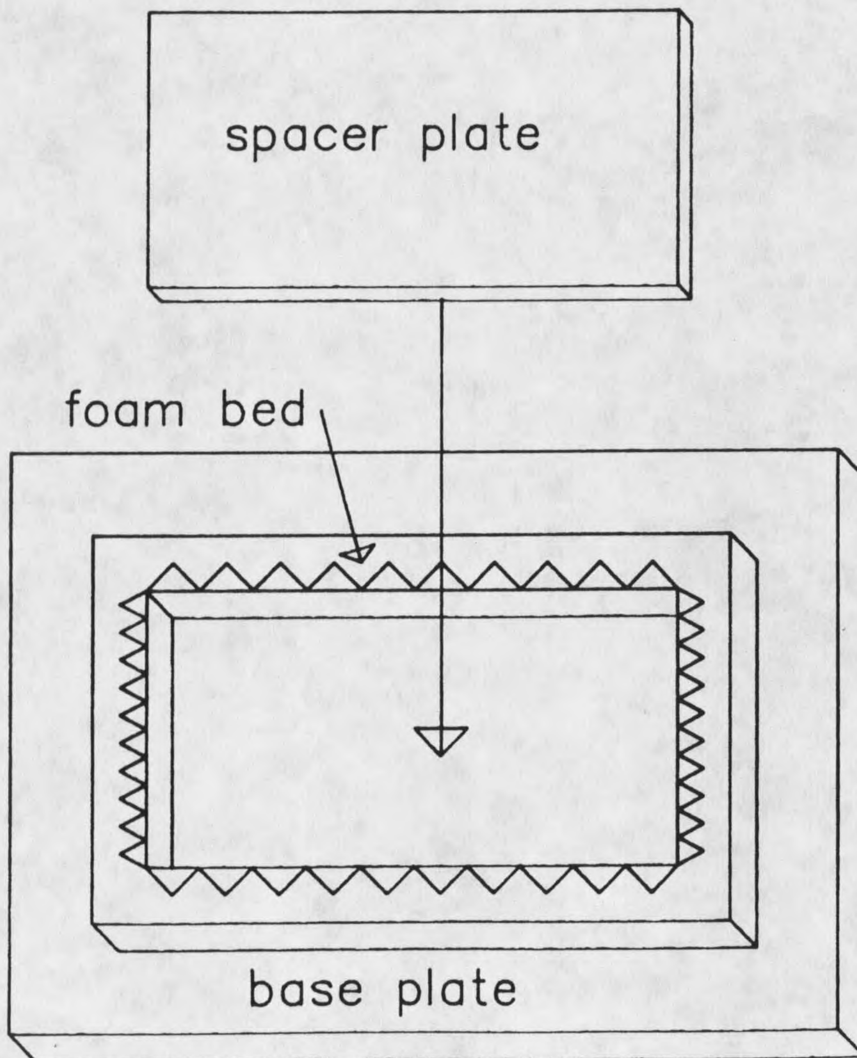

Fig. 4. Spacer plate

Because of the spacer plate, the eluent needles had to be placed high in the closed cell foam so they did not inject fluid directly into the spacer plate. To prevent leaks around these needles, they were sealed into place.

Two clamps held the upper Plexiglas plate, or cover plate, onto the base plate, which had the foam bed on it. These two clamps were flat metal bars welded into a rectangular frame, so that part of the metal bars were touching the edges of the Plexiglas plates, and the rest of the metal bars overhung the Plexiglas plates (see Fig. 5). There were six holes in the overhanging part of the metal bars, so that screws could be put in and the metal clamps could be drawn together, compressing the packing media and the bed apparatus.

An eluent feed tank with a drain hole near its base was kept on a shelf over the bed apparatus. This tank was filled with water, which was the eluent, and provided the head pressure to drive the eluent through the bed. Latex tubing connected the eluent feed tank with the bed apparatus via the "spider feeders", which will be explained later. There was also a drain tank, which was connected to the bed apparatus via latex tubing and the spider feeders.

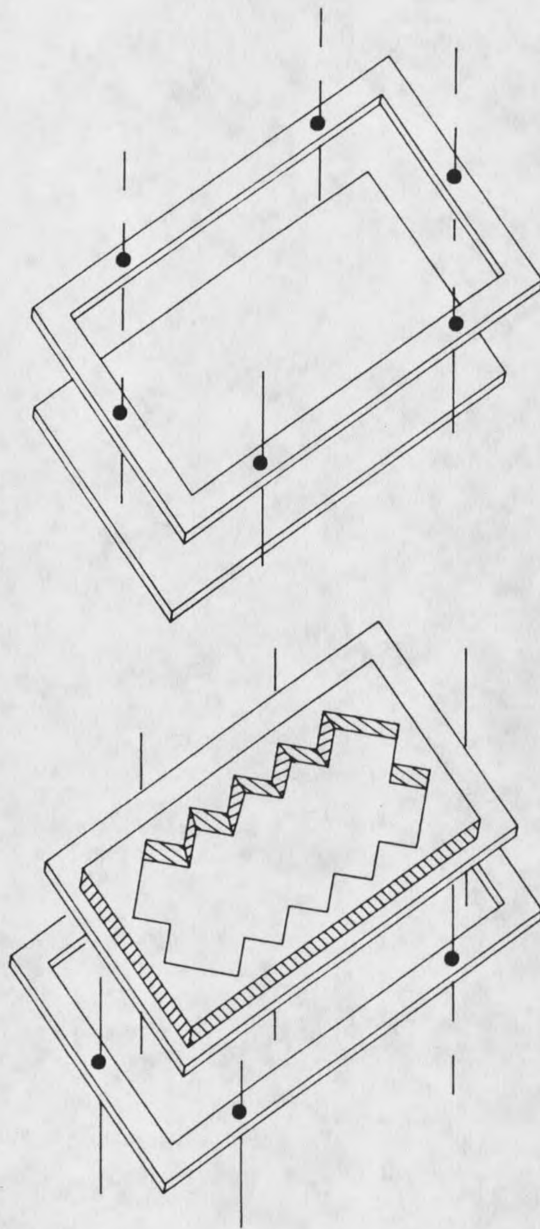

Fig. 5. Clamps