New Continuous Chromatography Options

Why continuous chromagraphic processes should be explored

One of the most powerful and increasingly more relied upon purification methods used in pharmaceuticals and biotechnology is chromatography. This application has also acquired added significance as regulatory bodies have required higher and higher purity and reproducibility in the new products being developed. This is especially true in the field of biologics where the use of chromagraphic methods is paramount in producing products of acceptable purity. Since starting out in its infancy as an analytical method, one of chromatography's biggest challenges has been in scale up. As processes move out of the lab and into a production setting, the batch nature of traditional column-based systems starts showing their significant constrains. As the material to be processed becomes larger, the labor-intensive requirements of column-based chromagraphic applications have effects that increase costs and the potential for production errors and contamination. This can easily result in this section of the process becoming a significant bottleneck.

In traditional Chemical Process Industries (CPI), processes are also developed in the lab in a batch-mode, but as these processes emerge and are moved to the production phase, great emphasis is put on converting them from batch to continuous operation. This conversion is paramount in these industries due to the fact that the quantities eventually handled in order to make them economically viable are so large that batch methods are usually not be feasible. Thus, concentration on how these processes will be converted into a continuous mode are on the minds of the developers far back into the research stage.

Profit margins, regulatory requirements, and processing quantities of the pharmaceutical industry have insulated them from these pressures to immediately push towards continuous processes options for the products they are involved with. Even though this may be true, the push towards continuous processes in the pharmaceutical industry is still present, and will be more so in the future. Granted, when we talk of continuous operations in the pharmaceutical industry we are not taking about them in the same sense as you would in say an oil refinery where you operate the process continuously for months at a time. The requirements for lot separation, monitoring, and control, necessitate that pharmaceutical processes cannot take advantage of pure, long term continuous operation. As an intermediate alternative though, they can be run in a semi-continuous mode. That is to say, a lot or batch of material can be processed through a device operating in a continuous fashion for the duration of the batch. In this manner, the process can take advantage of the smaller equipment size and more stable operation of a continuous system during this processing time.

Continuous Chromatography Operations

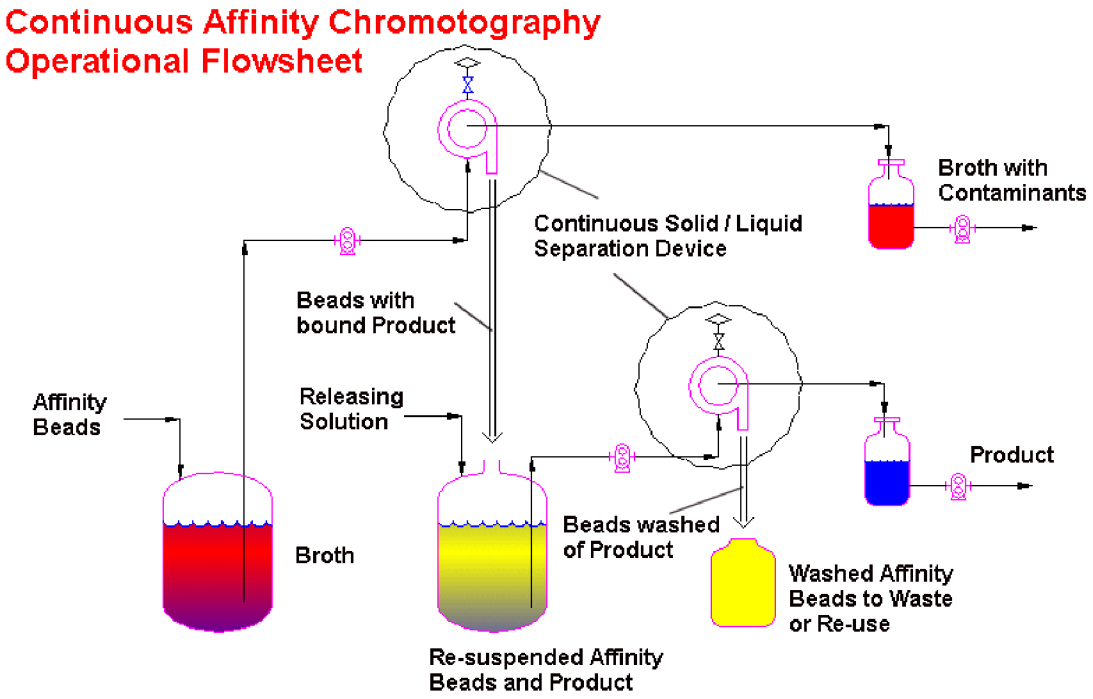
Enter now continuous chromatography systems. For some time now attempts at developing continuous chromatography systems have been limited to devices that try to generate or approximate "moving" column beds. When concentrating on creating these continuous systems on traditional column chromatography, these "moving" bed systems, essentially adding a third dimension to the two-dimensional column chromatography operation, are the only option. As you may know, these "moving" bed systems come in various forms and have varying degrees of acceptable operation, but since they are based on column operation at their core, they are usually intensely complicated and expensive in both capital cost and operations cost. What may be needed to move continuous chromatography to the next level is an entirely new approach.

The approach to be examined here is one based on the ability to facilitate a continuous, low cost, easily reproducible, easily controlled solid-liquid separation step. In applications where the height of the chromatography column is not a factor, that is the number of mass transfer units in the column is not important, as in affinity chromatography, the use of columns is essentially a "carryover" from traditional methods like reverse phase chromatography. In affinity-type operations the contact time between the resin and the materials to be removed from the solution is usually the critical factor, not the fact that this contact time is being facilitated by its residence time of the material traveling through a column. With that in mind, what if a totally different approach to developing continuous chromatography operations was explored? In traditional column chromatography once the liquid is pumped into the column the

subsequent separation of this liquid from the resin material is facilitated by the fact that the resin is a solid that is held in the structure of the column and the liquid is allowed to flow through this material, facilitating the contact time, then out the bottom of the column. This is still a mechanical separation, the resin being "held" in place while the liquid is allowed to flow through it. Looking at this from afar, this solid-liquid separation it is not much different that a traditional cake filtration application. What if both the resin and liquid are allowed to "flow" freely with the two process concerns being the ability to control the time that they are in contact with one another and after this contact is complete, being able to separate them. Contacting them with one another can be done by simply mixing them together for a controlled amount of time. Separation at the end of this contact time can be simply facilitated by a cake filtration step.

These two actions of our new system need to be made continuous to facilitate a continuous process. The mixing part is simple. In a method analogous to a continuous stirred tank reactor (CSTR), a contact time can be held constant by simply sizing the mixing tank and the flows in and out of the vessel such that the residence time (size of vessel / flow through vessel) meets the required contact time needed to allow the materials of interest to adhere to the resin using the forces that are applicable for the materials at hand. The second, and more critical action, is the ability to separate this solid and liquid in an applicable and continuous fashion. Figure 1 illustrates a basic flow diagram of the system described here.

FIGURE 1



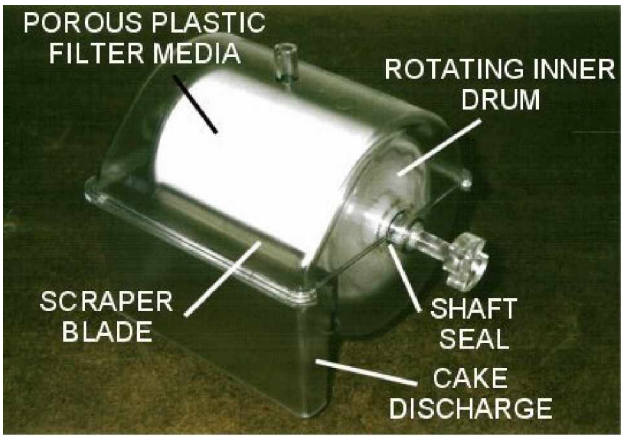
But how to facilitate this solid-liquid separation in a manner that is continuous yet small enough to accommodate the production quantities involved? Enter the Disposable Rotary Drum Filter.

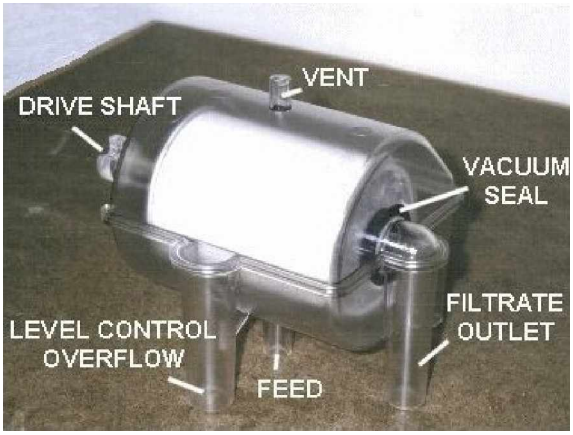
Traditional solid-liquid separation techniques normally seen in biotech and pharmaceutical applications are predominantly batch in nature, using equipment from simple Buchner funnels and Nutsche-like filters, to plate and frame and disc filters. This is primarily due to the fact that the volumes usually being processed are too small to accommodate feasible continuous solid-liquid separation equipment, or unit operations. To make the above continuous chromatography scheme feasible, some type of small-scale

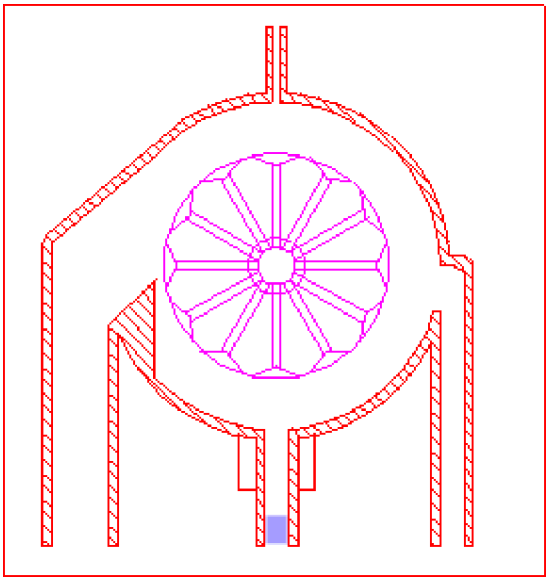
continuous solid-liquid apparatus is needed. One such device recently available is the Disposable Rotary Drum Filter.

The Disposable Rotary Drum Filter is based on the principles of large scale rotary vacuum filters that have been in operation in various CPI's for years. The challenge in developing this device was making it small enough to accommodate the normally smaller volumes and thus flow rates that would be present in a continuous chromatography system of this type.

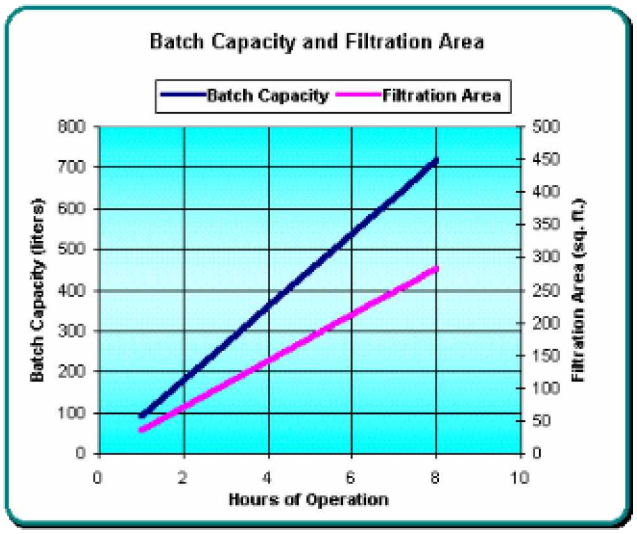
The operation of the Disposable Rotary Drum Filter is analogous to all rotary vacuum filters. It is made up of an inner rotating drum encased in an outer cylinder or half-cylinder (trough). The slurry enters the outer cylinder or trough and settles in the bottom. The level is controlled so that the inner drum's surface is covered to 30% - 35%. The surface of the drum is covered with a filter fabric. A vacuum is pulled on the inside of the drum, pulling filtrate into the inside and depositing the solids on the fabric. The filtrate is pulled into channels in the interior of the drum and routed out of the device. The system is made continuous by rotating the drum. As it rotates out of the liquid, the solids start to dry as air passes though them via the vacuum on the drum. When the drum completes its revolution the surface comes in contact with a scraper or knife blade, which removes the solids that have accumulated. The drum continues rotating back into the slurry and the process starts all over again. Once the device has reached steady state, the drum is fully covered with the solids cake for the first time, then the device can operate continuously taking in slurry and discharging clarified filtrate and dried solids.







The key with the Disposable Rotary Drum Filter is that this whole device is made of injection molded plastics and only measures about 6 inches in diameter and 7 inches long. Now there is other continuous solid-liquid separation equipment that could also do this job, like continuous centrifuges, but these are usually much more expensive to purchase originally and maintain in operation. Because this equipment operates in a continuous mode while processing a lot or batch of material, this small device can process large quantities of material, with this total quantity being primarily determined by the length of time that the device is operated. Depending on the solids, in this case affinity chromatography resin, being processed, and the type of cake it forms, this device can process from 1 to 3 liters/minute of slurry.

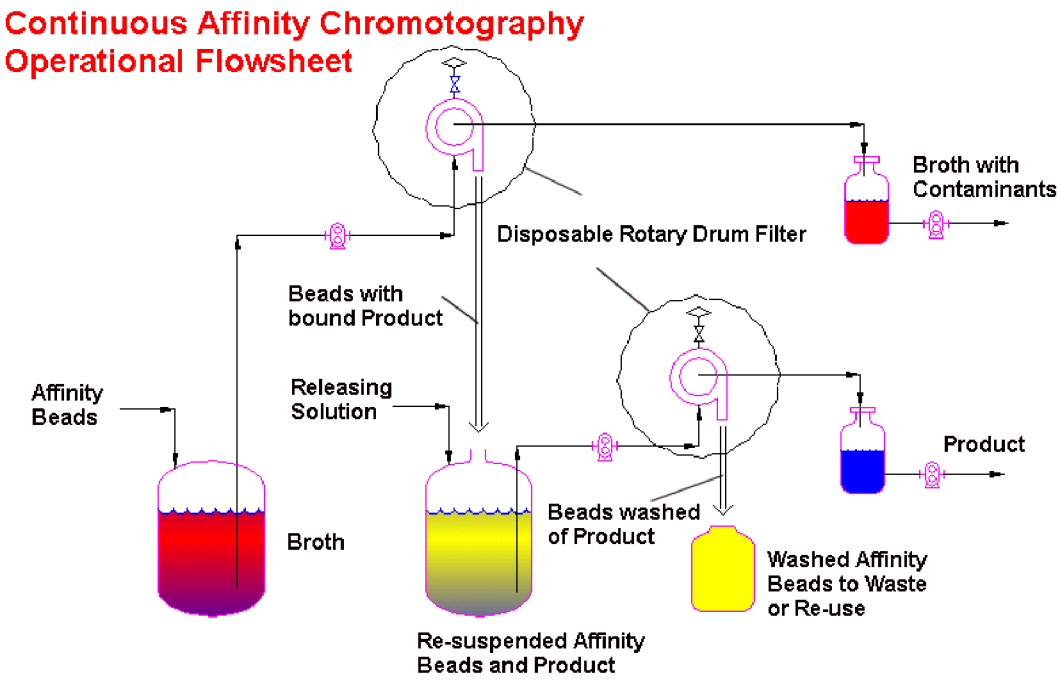


Imagine the size, complexity, and cost, both capital and operational, of a “traditional” continuous chromatography systems that could process at a rate of 1 to 3 liters/minute.

As implied from Figure 1, this type of continuous chromatography system can come in two forms:

The first would be that shown explicitly in this figure with the product or material to be recovered being initially bound to the resin. As can be seen from this figure this requires a second step in which the “bound” material is released from the resin and then separated from is by processing it over the Disposable Rotary Drum Filter again. This process can be made purely continuous by using two filters or can be conducted in two distinct continuous steps by using the same filter in one location and then the next.

FIGURE 2



As an analog of this process, if the material of interest in not bound to the resin, as in the case when the resin is used to remove impurities from the solution, then only the first step is needed and the product will come off in the filtrate of the first filter. In this case, a second filter step is only needed if the resin is to be recovered in its “clean” form.

In order for a process like this to be feasible, the first thing to consider is the properties of the resin itself, and what properties the cake it forms on the filtration drum of the Disposable Rotary Drum Filter will have.

First, the resin particles must be large enough to be stopped by the filter media or septum on the surface of the rotating drum. The Disposable Rotary Drum Filter does not act as a depth filter, thus it functions best if none of the solids to be removed from the solution enter and/or pass through this septum. Once this basic point is met, the next thing to consider is the type of cake formed by the solids dewatered on the filter drum surface. The more rigid, non-compressible, and dry this cake is the better the operation of the filter. If the cake is rigid and dry it will provide the least resistance to flow thus increasing the unit’s processing capacity. These properties will also allow it to be more easily removed by the knife or scraper blade that it comes in contact with at the end of the rotation of the drum. If the resin to be used forms a cake that is compressible and wet on the drum, then this compression will reduce the flow rate through the unit and make it more difficult to remove the cake from the drum with the knife blade. If the resin to be used forms a cake with too much of these latter qualities it may not be a feasible candidate for this technology.

If these initial considerations on the properties of the resin cake formed are met, then the filtrate to be created should be investigated. The major item to remember in aspect to the properties of the filtrate to be processed through this device is fact that the motive force for this device is vacuum. Thus, once the filtrate passes through the filter septum on he drum it is exposed to vacuum. Because of this fact and the small pores that the filtrate must pass through to get to this point, some organic materials may be prone to foaming in this situation. While some foaming may be acceptable, excessive amounts of foaming may cause problems in the downstream processing of the filtrate leaving this device.

The last aspect to consider in using this solid-liquid separation device in the application proposed, is the shear experienced by molecules present in the filtrate as it passes through the cake and septum of the device. There will be some amount of shear as the liquid passes through small pores of the device and is thus separated from the solids that were originally present. In addition, some biological solutions may experience foaming tendencies on the vacuum side of this device. Both of these effects may adversely affect some shear sensitive biological molecules processed in this fashion.

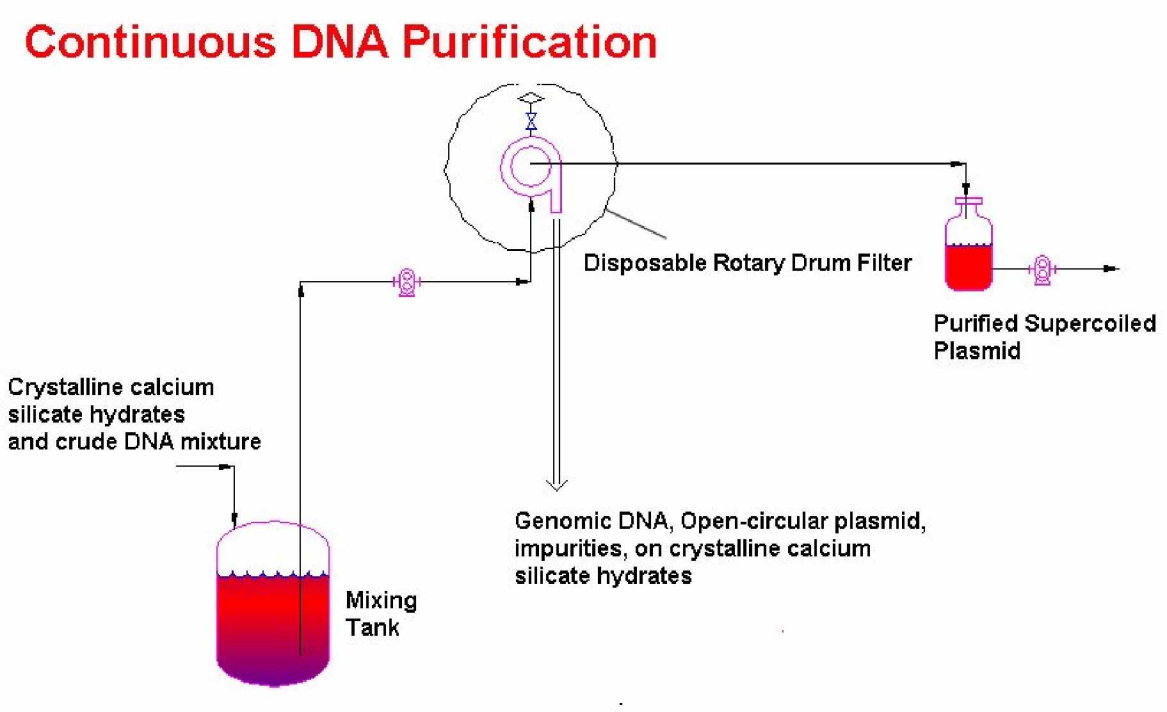
Even though there are a number of considerations for using this new technology to develop new continuous chromatography systems, the potential of having a feasible continuous chromatography system that can process at a rate of 1 to 3 liters per minute for a few thousand dollars is very exciting.

Applications in DNA Purification

In addition to the affinity chromatography applications outlined above, this same processing method could be applied to other difficult pharmaceutical purifications in analogous ways. One such application is DNA purification (DNA vaccine production and for gene therapies) using the properties of crystalline calcium silicate hydrates and their abilities to bind to different degrees, Genomic DNA, open-circular plasmid, and conformationally constrained supercoiled plasmid.

A number of recent studies have concentrated on the abilities of hydrated calcium silicates to preferential absorb Genomic DNA, open-circular plasmid, or conformationally constrained supercoiled plasmid. These materials were also shown to absorb impurities from upstream processing without significant absorption of supercoiled plasmid under certain conditions (1). This referenced paper illustrates a new and reviews existing information on the purification of nucleic acids using these types of materials instead of traditional chromagraphic media. Beyond this, it also notes the ability of these materials to perform separation on lipoproteins, triglycerides, cholesterols, phospholipids, glycolipids, and glycoproteins from mammalian blood, plasma fluids. Of course the advantage of using materials instead of traditional chromagraphic materials is their inherent lower costs.

The properties of crystalline calcium silicate hydrates used in this manner could improve the cost effectiveness of purifications if done in a traditional column mode, but even more efficient and scaleable operations could be envisioned if these are used in a mixing and filtration mode as we illustrated earlier. Although studies have been done on these materials in a batch mixing and filtration operations, it is feasible that a continuous mixing and filtration system could be devised, as outlined above, producing cost effective, high volume purifications. The key element here would be replacing the batch mixing, or absorption, time in the tank with the residence time in the tank as mentioned earlier.



Conclusion

Chromatography and related techniques are of ever growing importance in the development and manufacture of new drugs especially those dependent on biological production processes. Growing out of tedious batch methods, this powerful separation technique is ideal for research work, but can facilitate significant bottlenecks when applied to larger scale production processes. The key in capitalizing on the purification possibilities of this method, yet insuring that its use does not insert unwanted cost and complexity into the final production process, is to implement a continuous version of this technique into operation. The use of the Disposable Rotary Drum Filter in affinity and analogous chromatography applications is one such step in this direction.

References

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