Plasmid DNA Purification Possibilities Using the Disposable Rotary Drum Filter

John Kossik

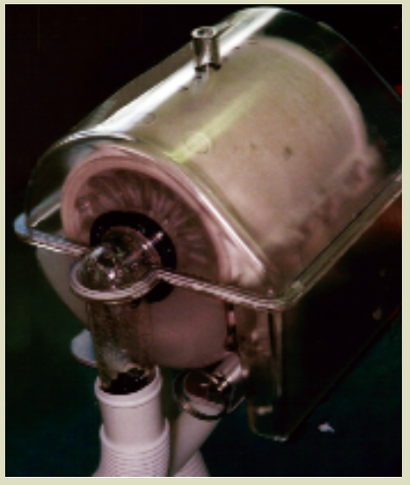
As progress is made in the development of gene therapy and DNA vaccines, emphasis starts shifting to methods by which to produce such materials economically on a production scale. Purification of plasmid DNA for therapeutic efforts becomes of great concern. A larger body of work is being developed to approach this challenge, but producing production scale quantities currently requires extensive processing, and some methods used could prove difficult to scale up (**1**).

Recent work has examined the ability of silicon dioxide materials to facilitate separation of components from the crude DNA mixture (**2**). Of specific interest is the purported ability of gyrolite (hydrated calcium silicate) to separate supercoiled plasmid, open-circular plasmid, and genomic DNA (**3**). Here I explore the use of these separation capabilities combined with a new device, the disposable rotary drum filter, to produce a method analogous to continuous affinity chromatography that could economically purify plasmid DNA with ease and scalability.

Purification of plasmid DNA is currently an extensive process involving a number of techniques: precipitation, depth filtration, tangential flow filtration, and chromatography (**1**). Use of gyrolite provides a method to simplify that process because the material has been shown to selectively absorb open-circular plasmid and genomic DNA from supercoiled plasmid (**3**). That ability alone could be exploited by using this material in a traditional affinity chromatography-type column application. A simpler approach may be mixing followed by filtration to accomplish the same result. What if that is taken a step further, with the simple batch mixing-filtration system extended into a continuous process? Large quantities of material could be purified using small, self-contained equipment. If this could be done, you have essentially developed a continuous affinity chromatography system like those simulated by expensive moving-bed column equipment. Enter the disposable rotary drum filter (Picture 1).

How It Works: The disposable rotary drum filter is a small, disposable, continuous cake filtration device normally used for a variety of solid–liquid separation applications: starch purification, fine chemical synthesis, and diatomaceous earth filtration. The function of the filter is based on the same principles as a traditional rotary drum vacuum filter. As with all such filters, a drum covered with filter media is positioned on its side and partially submerged in solid–liquid slurry. A vacuum on the inside of the drum pulls liquid through the filter media to become the filtrate, leaving solids from the slurry deposited on the filter media as a cake. Rotation of the drum along its axis causes sections of it to move out of the areas of submergence. At that time, the vacuum on the inside of the drum dries the deposited solid cake. As the drum rotates further, the cake comes in contact with a device that removes all or part of it from the filter media. The drum continues rotating past that point, and the whole process repeats itself.

Picture 1: The disposable rotary drum filter can process slurry at a rate of 1–3 L/min.



Product Focus: DNA vaccines and therapeutics

Process Focus: Downstream processing

Who Should Read: Project managers, process developers

Keywords: Filtration, continuous processing

Level: Intermediate

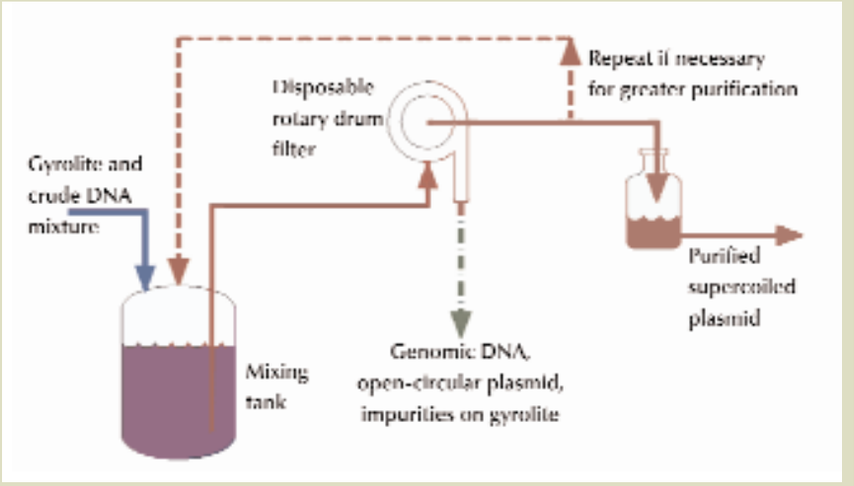
Continuous operation creates a piece of equipment with a theoretically infinite or regenerated surface area for filtration (**4**).

Using the disposable rotary drum filter, you can conduct continuous cake filtration on a scale more in line with the quantities involved in clinical- and commercial-scale DNA purification. To feed the continuous filtration device, you will of course need a mixing tank and pump (Figure 1).

Along with hydrated calcium silicate, the DNA mixture to be purified is added continuously to the mixing tank, from which it is pumped directly to the disposable rotary drum filter. The flow rates of solutions (Picture 2) and the resulting slurry in and out of the mixing tank, as well as the size of this tank, can be adjusted to facilitate the time in which the DNA solution is exposed to the hydrated calcium silicate, thus controlling the degree of absorption and separation that occurs.

If residence time in the mixing tank cannot be made long enough by use of tank sizing and flow rate control, two other options are available. First, the mixing tank could be operated in batch mode, allowing as much contact time as needed. Otherwise, a recycle loop could be added that returns filtrate from the filter back to the mixing tank, thus allowing more time for the materials of interest to be adsorbed onto the hydrated calcium silicate.

Figure 1: Continuous DNA purification system centered on the use of a disposable rotary drum filter

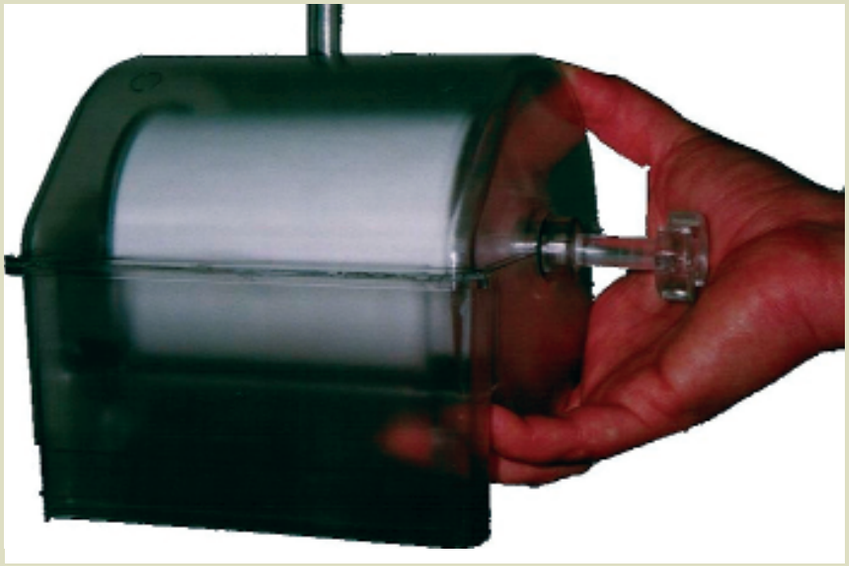


The resulting process creates an analog of a continuous chromatography system without the capital, labor, and resin expenses associated with column operation. As an added benefit, the equipment involved provides a method of processing material in an enclosed apparatus with a small footprint. And the disposability of the device reduces initial capital investment as well as the operating costs associated with equipment cleaning, validation, and such.

An Alternative with Potential

The advent of hydrated calcium silicate and other inexpensive silicon dioxide materials to facilitate purification of DNA for gene therapy and DNA vaccination applications could be of great use in bringing these potentially life-saving technologies to the marketplace. That separation capability and the use of continuous equipment like the disposable rotary drum filter could provide a method of minimizing or eliminating the need for costly downstream processes, especially those involving chromatography. It could allow the simplified scale-up of those processes as DNA products move forward into the commercial phase of their development.

Picture 2: Only 7 in. long by 6 in. in diameter, the filter can process up to 1000 L of slurry.



References

1 Eon-Duval, A. Large-Scale Manufacturing of Plasmid DNA for Gene Therapy and DNA Vaccination. *BioPharm International* 2002, 16, 9: 26–31.

2 Koo, K; Foegeding, PM; Swalsgood, HE. Isolation of RNA and DNA Fragments Using Diatomaceous Earth. *Biotechnol. Tech.* 1998, 12: 549–552.

3 Winters, MA; et al. Plasmid DNA Purification by Selective Calcium Silicate Adsorption of Closely Related Impurities. *Biotechnol. Prog.* 2003, 19, 2: 440–447.

4 Kossik, JM. Operation of a Disposable Rotary Drum Filter. AIChE Annual Meeting: Pharmaceutical and Biotechnology Pilot Plants; 4–9 November 2001, Reno, NV.

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