Expanded Bed Adsorption

The first new unit process operation in decades

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People of a certain age (and a certain difficult disposition) may remember the old cylindrical vacuum cleaners—the reversible ones that looked like jet engine nacelles and blew a strong column of air out the back through an aperture tantalizingly similar to the one designed for the hose fitting. It was child's play (quite literally) to plug the hose into the wrong end and wreak havoc with the strong stream of warmed air. One of the least destructive games was to balance a purloined ping-pong ball atop the stream. The ball would bob to a height determined by its weight, diameter, and the force of the air-jet.

Now, Pharmacia (Uppsala, Sweden) has exploited the same suspension effect to introduce the first new unit process operation in decades—a method that offers to consolidate particulate separation (via, say, centrifugation), clarification (via filtration), and initial volume reduction (via chromatography) into a single step.

Last spring, Pharmacia invited a group of industry journalists out to their Uppsala research headquarters for the unveiling of their soon-to-be introduced Streamline—what they call an “expanded bed adsorption” (EBA) separation system, using slight modifications of the company's familiar ion-exchange column media. Expanded bed adsorption, company spokesmen said, was intended to be not only economical but precisely scalable. They introduced a 50-mm-diameter, 6-liter-per-hour column following that meeting, and followed up this month with a 200-mm-diameter, 100-liter-per-hour pilot-scale column.

The concept, which has been tried before, is to combine chromatography's specificity and volume reduction with centrifugation's throughput and ability to remove gross cellular debris—with some of filtration's advantages thrown in. Past efforts, however, have used fluidized beds of adsorption media—and, as anyone who has watched the roiling eddies in a fluidized bed can readily attest, the flow is turbulent. Turbulence, with accompanying backflow and dead spaces, increases the amount of time needed to accomplish a separation and also increases the remnant load of residual debris.

EBA, on the other hand, follows a slow-but-steady approach, based on the reproducible characteristics of highly uniform 100-300-micron-diameter cross-linked agarose beads (with quartz cores to increase density and support higher flow rates) bonded to sulphopropyl and diethylaminoethyl functional groups. The column fea-

tures an inlet manifold that entrains the feed stream to produce a smooth, laminar flow upward through the column over the entire face of the baseplate, and a headplate that just barely touches the top of the (expanded) medium column, drawing off the feedstream (now stripped of the target compound) while preventing turbulence in the top of the column.

In practice, a buffer is first pumped in through the bottom of the column. This causes the bed to expand under hydrodynamic pressure—like the ping-pong ball over the vacuum hose—until it reaches equilibrium. The feed stream is then added, and the column expands still further (because of the feedstock's higher density and viscosity) until the top of the medium column just touches the headplate. The distance between the beads is large enough to permit the passage of large pieces of junk, and small enough to ensure that nearly all of the target compound binds to the ion-exchange medium.

A wash cycle follows, sweeping upward through the still-expanded medium bed, removing any lingering debris and compounds weakly bound to the medium. Finally, the expanded bed is drawn down, and eluent is passed through from the headpiece. As it flows through the column, it elutes the target compound (as in conventional chromatographic separations) and concentrates it in a volume one or two times that of the separation medium.

In tests, Pharmacia engineers have isolated soluble intracellular annexin V from *Escherichia coli* homogenate, obtaining 95 percent yield in 10 liters of eluate from a 50-liter fermentation in two-and-a-half hours. The company also claims substantial potential cost savings—about 40 percent, in the case of a test-run of 8,000 liters of a periplasmic bacterial lysate, according to a poster prepared by company scientists.

Two Synergen (Boulder, CO) engineers report that their own first-stage feasibility tests (on an unspecified soluble intracellular protein produced in *E. coli*) show results that are intriguing but less startling than the manufacturer's. The Synergen researchers replaced centrifugation, filtration, and packed-bed chromatography steps with a single EBA step. The unoptimized EBA separations were certainly faster (taking 56 percent of the time required by the three conventional steps); the cost was about the same (109 percent of conventional costs; Pharmacia engineers are quick to point out considerations of accounting and method that would substantially reduce that figure); and the yield was comparable (equivalent to 93±6 percent of the yield from packed-bed chromatography alone; comparison with the net yield from the three-step conventional purification was not available). Process developers at the Boulder-based drug-maker were emphatic that they consider the preliminary results quite encouraging, and that EBA remains a strong candidate in their process development. ///

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