MATERIALS AND METHODS

COMPOSITE FILTERS FOR THE LAB

Filtration is a universal step for cleaning up samples before chromatography or other analytical procedures. An effective filtration process improves the accuracy of subsequent steps and extends the lifetime of more expensive separation media. But filtration is difficult with protein-rich fluids, particularly those containing serum, which tend to clog and foul filters rapidly. Serial, or composite, filters have been one approach for improving overall performance.

While serial filtration cartridges for large-volume use, such as Pall's (East Hills, NY) Profile™—consisting of a series of outer prefilters and inner constant-pore-diameter microfilters—have long been available, combination filters for laboratory-scale filtration have only recently appeared on the market.

Small-scale serial, or composite, filtration devices generally combine a primary non-selective, gross filtration medium with a precise membrane medium, in a disk or syringe format. By merging two filtration steps in a single device, composites offer ease of use and help prevent contamination that can occur when samples are transferred from one device to another.

In most cases, the first filter is glass fiber, which holds lots of dirt and is inherently low in protein binding ability, but is inefficient at removing small particles. The second is most often a membrane, efficient at particle removal, not at holding dirt. While in large-volume applications such sandwich structures present a potential self-fouling problem—caused by glass fibers sloughing off onto the membrane filter—there is little danger of sloughing in low-pressure, laboratory-scale sample preparation, assuming the glass fibers are well made.

New Sources of Composites

Schleicher & Schuell (Keene, NH) is one supplier of such a composite device. Its Uniflow™ syringe filter, designed mainly for cell culture media, performs both clarification and membrane filtration. A sample loaded onto the syringe first encounters a glass fiber depth filter that serves to

trap gross particulates. The permeate from the first stage then reaches a cellulose acetate membrane (pore size 0.2 or 0.45 microns). Schleicher & Schuell is developing additional types of composites to fill other needs.

Anotec (Banbury, U.K. and New York, NY) also offers a composite filter. According to sales and marketing vice president Jeff Hoffman, the composite is designed specifically for biotech applications such as purifying fermentation broths and serum, which clog a normal microfilter quickly.

Anotec's composite, the Anotop™ Plus, consists of a sandwich of a binderless glass fiber prefilter and the company's inorganic (aluminum oxide) membrane, made by a patented electrochemical anodization process. The prefilter extends the useful life of the membrane, which is available in pore sizes of 0.2, 0.1, and 0.02 microns, and increases flowthrough ten times over non-pre-filtered membranes.

According to Hoffman, the composite filter (Anotop 25 Plus, 0.1 micron) removes 99.99 percent or more of Mycoplasma from tissue culture media and sera. An independent test lab reported on the results of filtering 10-ml aliquots from cultures of three common human Mycoplasma (*M. hominis*, *M. salivarium*, and *M. fermentans*) and two common contaminants of fetal calf serum (*M. arginini* and *Acholeplasma laidlawii*) at a concentration of 10^4 organisms/ml, pressure 75 psi. No Mycoplasma could be cultured from any of the filtrates.

Hoffman credits the inorganic membrane's defect-free structure with these results: "The filter has no holes, which Mycoplasmas are good at finding and getting through."

Genex (Gaithersburg, MD) also manufactures a device that combines pre-filtration and microfiltration in one step. Its UniPrep™ syringeless filter contains glass microfiber filters with a choice of polytetrafluoroethylene (PTFE), low-protein-binding polyvinylidene fluoride (PVDF), or Nylon 66 membranes.

Millipore (Bedford, MA) does not yet offer a single-device composite for lab-scale work, but its Millex® syringe-operated filter units may be

connected in series, so that one acts as a prefilter for the second.

Porous Polymers

Another interesting development offering a dual filtration mechanism in one device uses an inert, temperature-stable, durable class of porous PTFE. Sold by Norton-Performance Plastics (Wayne, NJ) under the tradename Zitex™, the material operates at temperatures from -450-+550° F, and handles corrosive, acidic, and basic fluids. It works as an absolute screen filter down to 2 microns, and withstands cleaning by steam, ethylene oxide (ETO), or other chemical sterilizers. The material filters both liquids and gases.

PTFE is chemically inert and does not retain contaminants or support the growth of bacteria or fungi. As adapted by Norton, it boasts an exceptionally high pore volume. In addition to being chemically inert, PTFE is strong, so it doesn't require special support media in most filtration applications. Sample biotech applications include DNA synthesis, monoclonal antibody purification, and sterile liquid handling.

These porous PTFE filter membranes can replace polypropylene, polyvinylidene chloride, glass, nylon, cellulose, vinyl, and paper filters in many demanding lab applications involving sensitive or reactive fluids.

Norton's basic Zitex design—class A—acts as a combination labyrinthian (depth) and absolute screen (membrane) filter because it contains the same tortuous paths as standard filters. Unlike standard materials, however, Zitex interstices are bonded together. This lends it pore size stability, creating a defined screening size. These two mechanisms in combination give high retention efficiency without clogging. Compared with other high-temperature, chemically resistant membranes with similar pore size ratings, Zitex filters give tighter pore size control. Class A, with filter porosities ranging from 2-60 microns, suits operations with high flow rates; Class G materials are thicker and stronger than Class A, with small (1-6 micron) pore sizes, and better suited to lower flow rate procedures.

—Pamela Knight