Application Brief

MAR COR PURIFICATION
A Cantel Medical Company

Increasing Microsphere Processing Yields 30-50% with Crossflow Filters

Introduction

Microsphere-based diagnostic tests are used in many biomedical applications. Particles from 0.1 to 10 micrometers may be used suspended in fluids, used dry in test strips, or even dried and then re-suspended. While "plain" microspheres may be used in some situations, more commonly, the particles are coated with materials for specific applications. Examples of coatings include proteins and dyes. Particles may be made of polystyrene latex or other polymers, magnetic materials, or even silica.

Coating of the microspheres is a vital step in manufacturing diagnostic tests. The particles must be cleaned, coated, and washed before further processing. Components can be expensive and high yield is critical to a cost-effective process. Hollow fiber crossflow filtration can make all of these steps easier and more cost-effective.

Effective Processing. Figure 1 illustrates a basic microsphere treatment process. First, particles are "washed" to remove surfactants that might be present on the microspheres as received from the manufacturer. At the next step, particles are mixed with a solution containing the coating material of interest and allowed to "react" for a sufficient amount of time to assure thorough coating of all particle surfaces. The particles are then concentrated, coating solution is recovered, and the microspheres are moved on to the third step where excess coating material is washed from the particles.

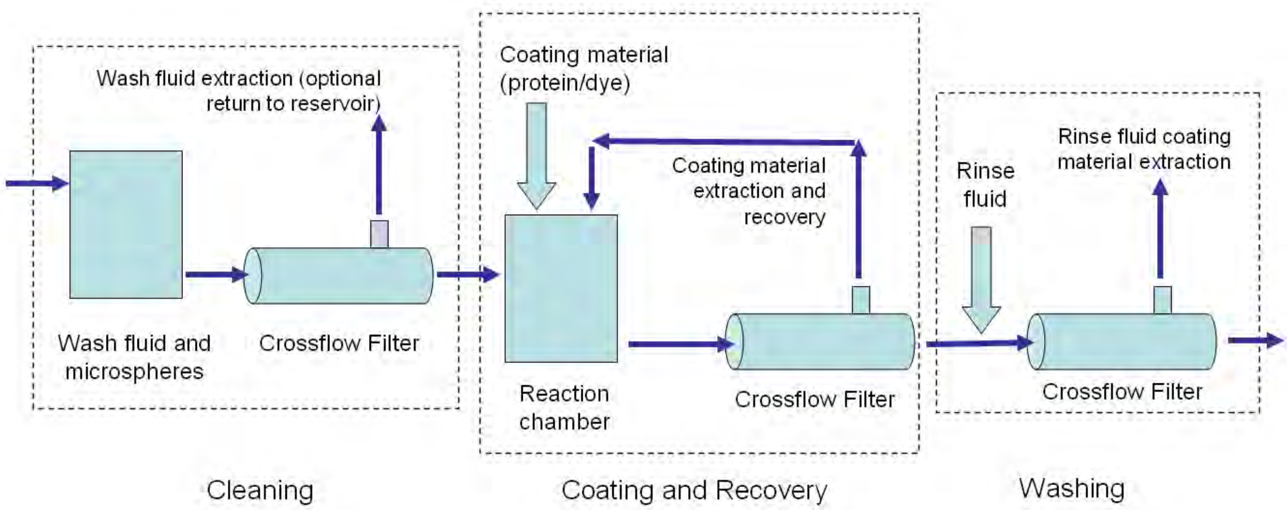

Figure 1. Basics of Microsphere Processing

Each of the three steps described above can be performed by crossflow filtration devices. In fact, if the process can be performed in a series of independent steps, the same crossflow device can potentially be used at each step.

Hollow fiber crossflow filters are ideal for use in the coating and cleaning of polystyrene latex and other microspheres and are frequently recommended for solid particle processing. Their design allows microspheres to be contained within the center or lumen of a hollow fiber while the washing or treating fluid can be extracted through the walls of the fiber. Figure 2 illustrates the basic operation of a crossflow hollow fiber membrane filter.

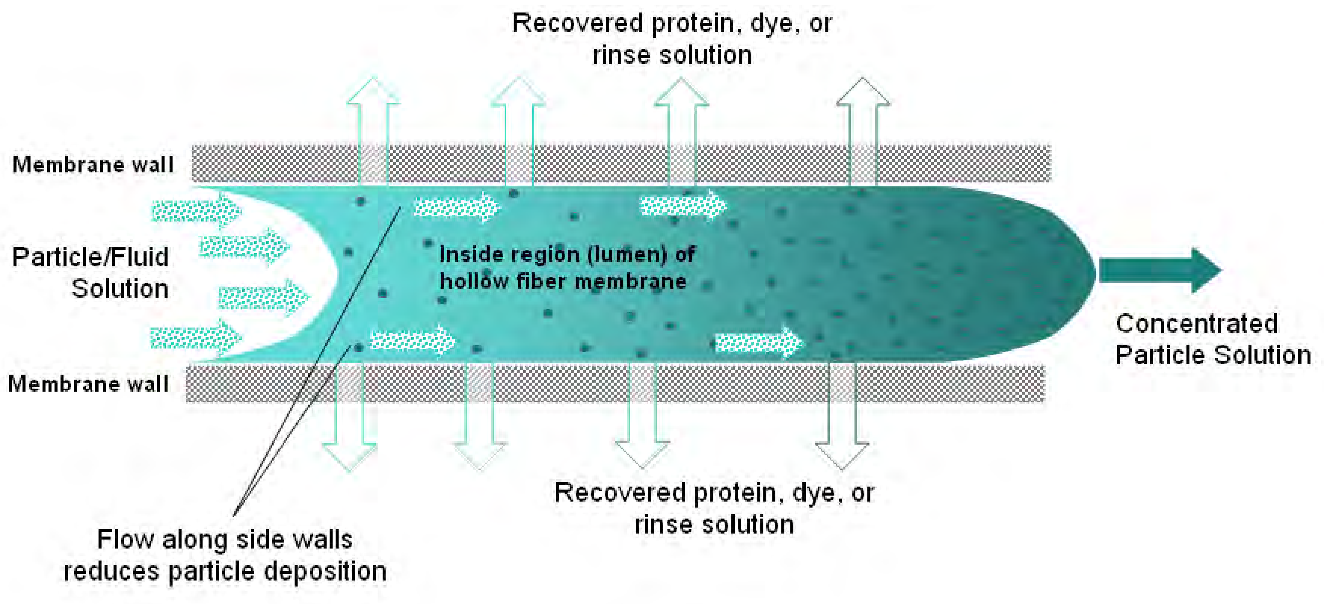

Figure 2. Basic Operation of Hollow Fiber Membrane (single fiber)

Proper Selection of a Crossflow Filter

For proper selection of a filter for processing microparticles, several characteristics should be evaluated. These include:

- Membrane Pore Size
- Pore Size Distribution
- Membrane Material
- Sieving Coefficient

Pore Size And Yield. Pores are the openings in a membrane filter through which fluid and possibly particles can flow. We want to select a pore size that holds back but doesn't trap the microspheres. If particles are trapped, yield will be reduced. For microsphere treatment, we want the spheres to stay on the surface of the membrane where they won't be trapped, so we want the surface pores to be smaller than the particles of interest.

That should be easy; right? Membrane filters come with a micron rating; so it should be a simple thing to choose a filter with a micron rating smaller than the particle that is being treated. And yes, that would be a simple choice. As we discuss below, however, the pore size rating of the filter is not the only pore size we need to be interested in.

What happens in the depth of the membrane? Virtually all filter media contains a range of pore sizes within their structure. In the case of the membrane pictured in Figure 3, a particle traveling into the depth of the filter will encounter increasingly smaller pores until the smallest pore size is reached. The pores in this membrane reach their minimum size within 1 to 2 microns of the surface of the inside fiber wall. It is at this point that the membrane reaches its "absolute" rating or the pore size at which at least 99.999% of particles are stopped. Other hollow fiber or flat membranes may reach their minimum pore size at different depths of the membrane.

But for microsphere processing we don't want the particles to be stopped 2 microns into the membrane. We want them to be stopped before they enter the membrane wall. So let's look more critically at the inner wall of the membrane.

Figure 3 shows the cross-section of a MarCor hollow fiber membrane. The inner surface of the hollow fiber membrane is the one that microspheres will be exposed to during processing. Note the smoothness of that surface. What isn't so easily seen is that not all the pores on the surface are the same size and not all of them are equal to or smaller than the pore size rating of this membrane.

Figure 4 illustrates the range of pore sizes that might be found on the inner surface of a hollow fiber membrane. The chart reflects cumulative frequency. As you follow a curve from left to right (in order of increasing pore size), the portion of pores smaller than that size increases. For example, for the 0.1 micron membrane (FF100) shown, about 90% of the pores are 0.1 microns or smaller at the inside surface. The steepness of the curves indicates a sharp cut-off in the size of the pores. In filtration of uniformly sized particles, this is generally considered a good thing.

In order to maximize yield while maintaining processing rate, select a filter with few pores greater than the size of the particles being processed. For example, if the particles being processed are 0.1 microns in diameter, it may be appropriate to select a 0.05 micron membrane, as over 98% of surface pores are smaller than 0.1 micron. The 0.1 micron membrane, in turn, may be a good selection for processing microspheres of 0.2 microns and larger.

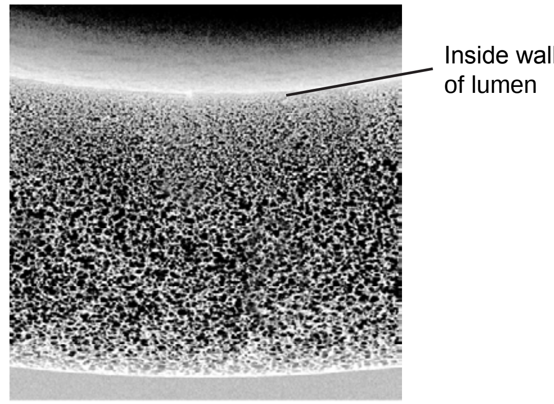

Figure 3. Cut away view of 40µ wall of hollow fiber membrane

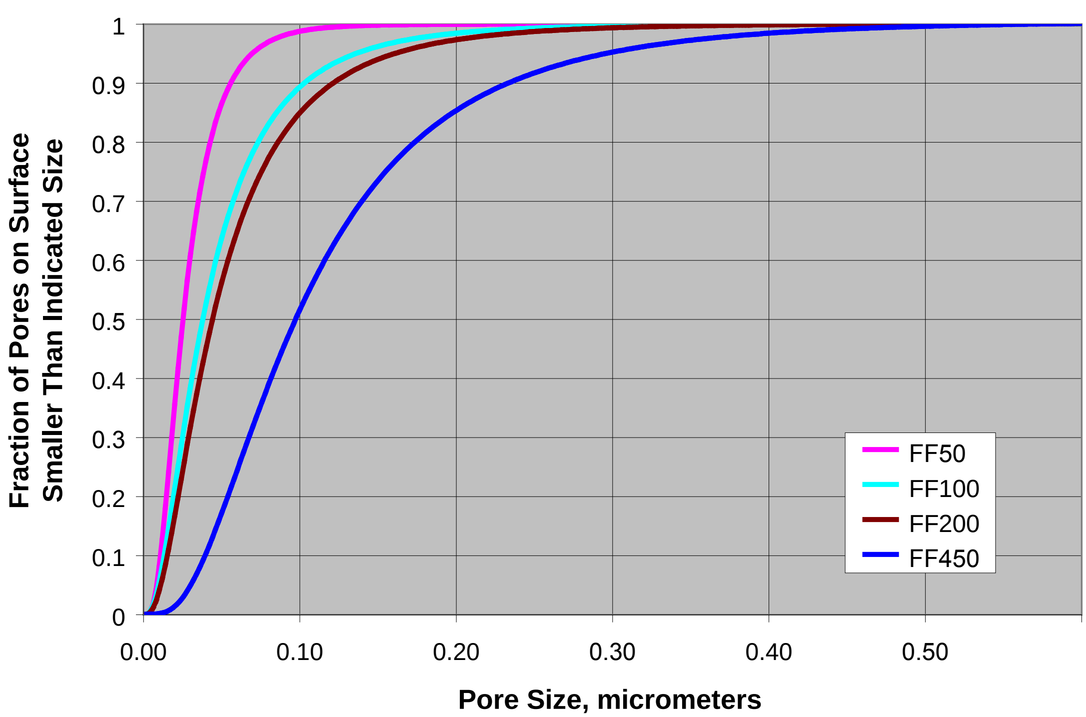

Figure 4. Fractional Distribution (Cumulative) of Pores on Inner Surface of Several MarCor Hollow Fiber Membranes

Membrane Material. Different types of membrane materials are available in crossflow devices. Examples include polysulfone, polyethersulfone, and cellulose acetate. Each type of material has its own unique characteristics in terms of wettability and affinity for particles and coating materials. The polysulfone membrane discussed here has shown excellent capability for processing microspheres during coating with protein.

Sieving Coefficient. Let's now look at the specific material being used to coat the latex beads. In most cases of particles used in diagnostic tests, the beads are being coated with a protein. After the particles are washed, the coating process begins. If the coating material is expensive, we may wish to recover unused protein solution for continued use. Referring back to Figure 2, we note that the protein solution flows from inside the hollow fiber membrane to the outside from where it can be collected or returned to the coating material. In order to maximize recovery of the protein solution, we want to be sure that the membrane is removing minimal amounts of protein through adsorption. This is done through proper selection of membrane media and filter. In Figure 5 we show examples of protein adsorption by a polysulfone hollow fiber filter. Adjustment of pH and flow rate for a specific process can optimize a sieving coefficient for a particular protein, if needed.

Protein Solution (in 0.9% Saline)	Molecular Weight	Initial Concentration (mg/dl)	Amount of Protein Infused (mg/60 min)	Protein Binding (mg/60 min)	Average Sieving Coefficient (1 = 100% passage)
Bovine Serum Albumin (BSA)	66,000	5200	31,200	0	1.00
Cytochrome-C (Horse Heart)	12,384	30	180	0	1.00

Figure 5. Sieving coefficients of a polysulfone filter

Example Application

A major manufacturer of diagnostic equipment for blood and urine testing came to Mar Cor Purification because they were not getting satisfactory performance from their existing hollow fiber cross flow filter. At issue was a loss of product; up to 30-50% loss per batch. In addition, a filter was being used and discarded at each step of the build.

The manufacturer washes and coats as many as 16 different polystyrene latex bead products, building a variety of coatings on the microparticles. The hollow fiber filter is used to keep the microparticles suspended in the buffer solutions as they bleed off one solution and introduce another. The coatings vary depending upon the type of test being produced. The microparticles are then put into solution and into a vial that will react in the diagnostic machine with the patients' sample. This is not a sterile application as the sample has already been extracted from the patient.

The manufacturer looked first at the processing of 0.12 - 0.24 micron particles using a variety of buffer solutions. The filter they had been using contained 3.9 m^2 of filtering surface and was discarded after a single step in the process. From start to finish, overall yield was only 50 to 70% because the microparticles became trapped in the hollow fiber. When bead solutions can cost thousands of dollars per liter, yield becomes a critical issue.

Solution:

The customer selected a 0.1 micron hollow fiber crossflow filter from MarCor Purification. MarCor's disposable cartridge and cleanable, re-usable stainless steel housing provided a higher processing rate with 1.6 m^2 of filter surface than did the other hollow fiber 3.9 m^2 filter. The Mar Cor filter was successfully re-used at each step in the process. The filter performed better, even with less filter surface area, because of a smoother lumen (fig. 3) and a sharper retention cut off (fig. 4). The result was fewer trapped microspheres and less rinse time which caused the yield to skyrocket to nearly 100%. The manufacturer is very pleased and continues to evaluate MarCor's devices for use in other processes.

Conclusion

Hollow fiber membrane filters have been shown to be an important step in microsphere washing and coating processes. Proper selection of a filter based on pore size, surface structure, pore size distribution and sieving coefficient can increase higher yields.

High value microparticle solutions demand cost-effective processing. MarCor Purification's Crossflow flow cartridge has demonstrated improved yield and greater processing capacity when compared with another hollow fiber filter.

Free Trial Filter

Try it for yourself. Contact John Spung (jspung@mcpur.com)

MarCor Purification (435-649-6376) for a free trial filter so you can demonstrate for yourself the increased performance.

References

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www.mcpur.com

Mar Cor Purification
4450 Township Line Road
Skippack, PA 19474-1429
U.S.A.
Tel: (484) 991-0220
Toll Free: (800) 346-0365
Fax: (484) 991-0230

Mar Cor Purification
14550 28th Avenue North
Plymouth, MN 55447
U.S.A.
Toll Free: (800) 633-3080
Fax: (763) 210-3868

Mar Cor Purification
3250 Harvester Road - Unit 6
Burlington, ON L7N 3W9
Canada
Tel: (905) 639-7025
Toll Free: (800) 268-5035
Fax: (905) 639-0425

Minntech B.V.
Sourethweg 11
6422 PC Heerlen
The Netherlands
Tel: (+31) 45 5471 471
Fax: (+31) 45 5429 695

Minntech Asia/Pacific Pte. Ltd.
438 Alexandra Road
#03-01 Alexandra Point
Singapore 119958
Tel: (+65) 6227 9698
Fax: (+65) 6225 6848