Bangs Laboratories, Inc.
BEADS ABOVE THE REST

Washing Microspheres

TechNote 203

INTRODUCTION

Washing is an important first step in using most microsphere products prior to coating or use in an assay as it serves to remove stabilizers, antimicrobials, and other residuals that may impact performance. This technote will overview the most common washing methods, as well as considerations for washing microspheres of varying types, and for different applications. The specific method(s) used will vary depending on the type and size of sphere, volume being processed, and other considerations such as the final intended application. Users should be aware that process scale up and/or changes in washing methods are expected to necessitate the same level of re-optimization.

QUICK REFERENCE GUIDE

Below is a table listing each method, and can be used as a quick selection guide for users. More discussion/overview on each method is included below.

Method	Bead type	Benefits	Limitations	Time required
Centrifugation	all types 0.5µm+	economical easily accessible fast	lower sizes are impractical scalability is limited over-pelleting or resuspension concerns	~5 minutes per run
Vivaspin	all types 20nm-500nm	eases centrifugation of very small particles	some product loss scalability is limited	5 - 10 minutes per run
Dialysis	all types 20nm-500nm	economical accommodates many different beads resuspension isn't a concern	much longer times required (upwards of a day) labor intensive scalability is limited	will vary, can take hours or days
Cross-Flow Filtration	all types 20nm-500nm	highly scalable for any volumes	some product loss in the filters will occur filters can be more expensive compared to centrifugation	30 minutes - 6 hours
Mixed-Bed Ion Exchange	functionalized (COOH or NH_2) - all sizes	effective at removing charged species	some product loss can cause notable clumping	~ 2 hours
Magnetic	superparamagnetic 0.35µm+	easy, fast, suitable for automation	requires rare earth or electromagnet polydisperse size populations may have heterogeneous separation rates	~30 seconds - 5+ minutes (depends on bead size & magnetic content)

CENTRIFUGATION, 0.5µm

Centrifugation is often the simplest method available to many users, and most applicable to smaller volumes due to minimal product loss between washes. Factors that can impact centrifugation are particle density and size, as well as the force applied and viscosity of the solution. Sedimentation rates rapidly decrease with lower sizes, and generally it becomes more challenging for sizes below 1 µm (for a general polystyrene sphere). With large beads (e.g. 10µm+), buoyancy forces may come into play, making centrifugation a bit more challenging. If polymer spheres stick to the sides of the tube rather than pelleting, they can be centrifuged for a longer time with more force, or a bit of surfactant (0.0005%) may be used in the wash buffer to aid in bead wetting so that they spin down more effeiciently. Silica and other denser materials will be easier to centrifuge in general, and lower sizes may be used than what is traditionally practical with polystyrene.

Bead Type	Diameter Range	Relative Centrifugal Force Range (×G)	Speed Range (rpm)
polymer	> 0.5µm	6500 - 14000	8925 - 13100
	> 1.0µm	3000 - 5500	6060 - 8210
	> 5µm	1300 - 3000	3990 - 6060
silica	> 0.5µm	3000 - 5500	6060 - 8210
	> 1.0µm	1300 - 3000	3990 - 6060
	> 5.0µm	750 - 1300	3030 - 3990
protein/Ab-coated	> 0.5µm	8000 - 11000	9900 - 11610
	> 1.0µm	5500 - 8000	8210 - 9900
	> 5.0µm	2000 - 5500	4950 - 8210

Table 1: *Sample protocols for benchtop (7.3 cm rotation radius) centrifuge, all ~ 5 min.*

SPIN FILTERS/VIVASPIN, 20nm - 500nm

Spin filters are specialized centrifuge tubes that contain a membrane with an appropriate MWCO to retain smallest particles (e.g. 20nm - 500nm) while allowing the filtrate through. They ultimately result in reduced spin forces as well as time compared to conventional centrifugation for such small spheres. Spin filters also produce a concentrated suspension rather then a true pellet (due to a dead-stop volume), which is ideal for resuspension. Bangs carries Vivaspin ultracentrifugation spin filters for use with swing bucket or fixed angle centrifuges that can accommodate 15 mL tubes (can be found in the Equipment section of our website, catalog code AA022).

DIALYSIS, 20nm - 500nm

Dialysis is a diffusion-based method that produces an equilibrium state between two solutions due to the net movement of molecules from areas of high concentration to areas of low concentration. This process is inherently much slower than centrifugation, and more time-consuming to carry out; it is typically used to process larger volumes of submicron spheres. The microspheres suspension will become more dilute during dialysis and may be concentrated by laying the tubing on a desiccant bed (silica gel). Limited process scaling is supported through the use of larger pumps, tubing, and reservoirs.

Newer dialysis methods employ flow-through technology - termed dynamic dialysis. The flow of the dialysate and/or sample constantly maximizes the concentration differential, markedly increasing the rate of molecular exchange, and dramatically reducing process times (see Tube-A-Lyzer by Spectrum).

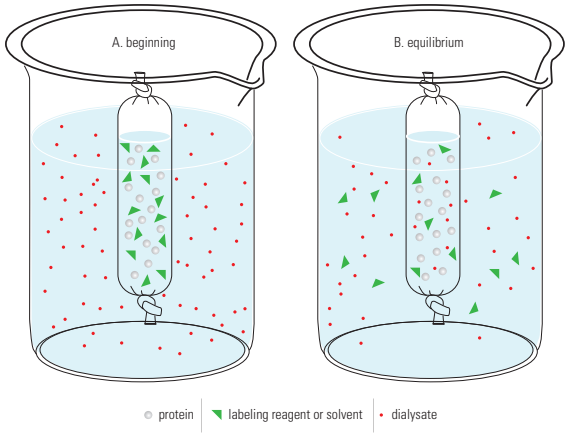


Figure 1: *Example of a dialysis setup.*

Considerations

1. Concentration Differential
   The driving force in dialysis is the concentration differential between the two solutions on the opposite sides of the membrane. Maximum efficiency occurs when the membrane is thin and the concentration differential is large.
2. Molecular Weight Cut Off (MWCO)
   Dialysis membrane performance is characterized by the molecular weight at which 90% of the solute will be retained by the membrane. In addition to the MW, the exact permeability of a solute is dependent on the shape of the molecule, its degree of hydration, and its charge. Each of these may be influenced by the nature of the solvent. Extreme pH, ionic strength, or non-aqueous solvents may cause a deviation in the MWCO. Because of this, the MWCO should be used as a guide, and not an absolute prediction of performance. In the case of microspheres, a pore size close to the mean microsphere diameter will ensure a rapid exchange, but also a potential loss of 10% of the material. A narrow pore size distribution is also important. The pores should be very uniform and very close to reported size. Check with the membrane supplier for more info.

MWCO	Diameter	
300,000	0.0110µm	110Å
100,000	0.0100µm	100Å
50,000	0.0040µm	40Å
10,000	0.0025µm	25Å
5,000	0.0015µm	15Å

Table 2: *Pore Sizes*

4. Hydrodynamic Flow
   Flow is not a molecular process, but includes bulk movement of the fluid through a porous medium. The flow rate of the fluid through the medium is influenced by pressure, porosity, and the viscosity of the fluid.

	Increased Flow	Decreased Flow
Membrane Porosity	high	low
Pressure Difference	large	small
Fluid Viscosity	low	high

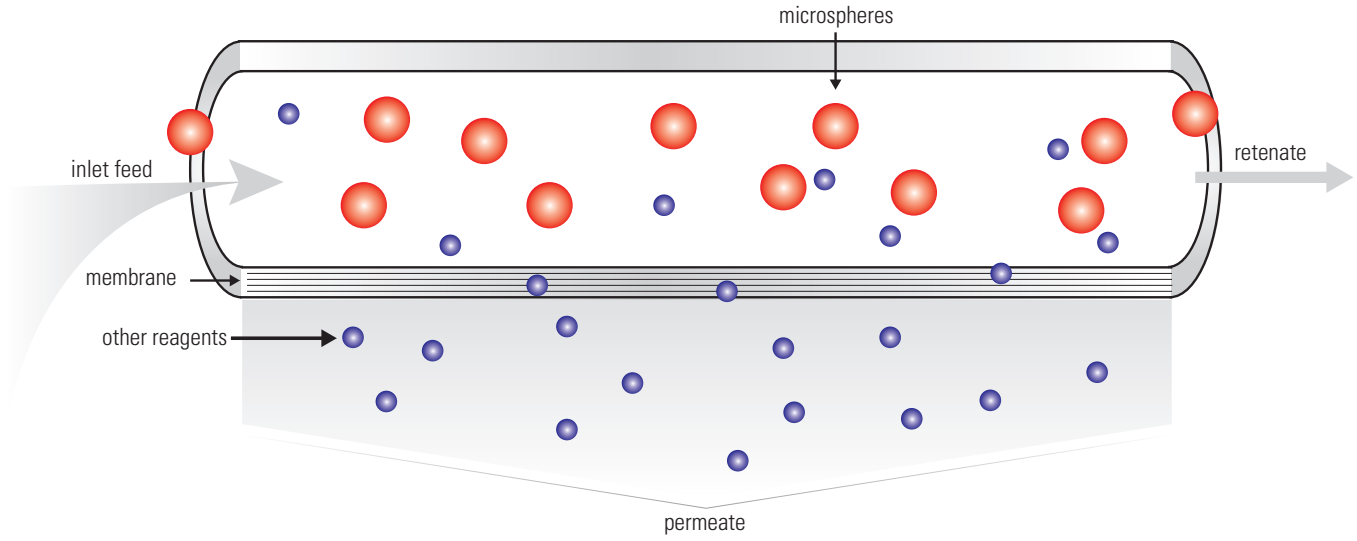
Table 3: *Hydrodynamic Flow*

5. Sample Volume
   Tubing size should be selected to ensure that the sample can be contained within 5-20cm of tubing. The flat width of the tubing should provide the greatest surface area to volume ratio to enhance the rate of dialysis. There should also be an allowance for increased volume to retentate (2X).
6. Solvent Concentration
   The solvent volume should be maintained at least 10X the sample volume, the larger the solvent volume the quicker the procedure will take place. The solvent should be changed frequently to ensure that diffusion takes place across the membrane against essentially zero concentration.

Dialysis Equipment Suppliers

Spectrum	http://www.spectrumlabs.com/
EMD Millipore	http://www.emdmillipore.com/
Pall	http://www.pall.com/

CROSS-FLOW FILTRATION, 20nm - 500nm



General Information

Cross-flow filtration, also known as tangential-flow filtration (TFF), is another method that can be used to clean microspheres. In this process, the sample passes across the membrane tangentially, allowing solutes that are small enough to pass through the membrane, while retaining the microspheres. Making the flow run tangential to the membrane reduces the chance of clogging the pores and developing a filter cake. This method is effective for small microspheres as well as large ones, and may be a nice alternative to centrifugation, especially with larger volumes. One major advantage of cross-flow filtration is scalability, as conditions are able to be kept relatively similar across a wide range of sample processing volumes, allowing better predictability on the method's suitability in future scale up. Filter manufacturers also offer distinct filtration units for various volumes as well as applications, such as washing diagnostic/polymer particles. In comparison, dialysis and centrifugation become impractical at larger pilot/production scales (lower throughput). While cross-flow filtration is an effective method for cleaning microspheres, there is some loss of sample with this method due to material being incompletely removed from the filtration unit and this method will also have a tendency to concentrate the microspheres.

We have listed several suppliers of this equipment below. If decided that this is the proper method, seek technical help and protocols from the supplier. Both EMD Millipore and Spectrum offer dedicated resources and technical pieces in this regard.

Cross-Flow Filtration Suppliers

Spectrum	http://www.spectrumlabs.com/
EMD Millipore	http://www.emdmillipore.com/
Pall	http://www.pall.com/

MIXED BED ION-EXCHANGE

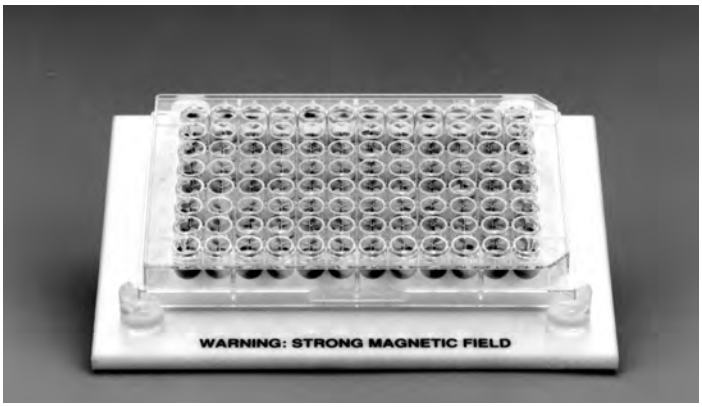
General Information

Ion-exchange cleaning will remove all electrolytes from the suspension, including charged surfactants such as sodium dodecyl sulfate (SDS). This is a more specialized form of washing, and is usually performed before a titration (e.g. for determination of surface COOH groups), or before studies that focus on surface charge (zeta potential, electrophoresis). A mix of positive (cation) and negative (anion) exchange resins, typically composed of porous or non-porous beads of larger diameters (0.5 – 1 mm), are used in this process. The sample containing the microspheres is added to the mixed resins and agitated over a period of time (either through rotation or rolling). Any charged material on the particle or in the solution is sequestered by the exchange resins. Following mixing, the resin can be removed by filtration, although a small percentage of unrecoverable product will be retained in the resin volume.

Ion - Exchange Resin Suppliers

Dow Chemical	http://www.dow.com/
Bio-Rad	http://www.bio-rad.com/

MAGNETIC



Washing of (Superpara)Magnetic Microspheres

Magnetic microspheres offer advantages in washing, as they offer rapid separation from solution and minimized handling. There are many suppliers of magnetic separation racks specialized for test tubes and centrifuge/microfuge tubes, and we offer our own in the equipment section of our website. Scalable large volume magnetic separation equipment is available through SepMag and Dexter Magnetic Technologies, although users will need to optimize the process, and both companies can offer technical support in this regard. Some magnetic spheres can also be centrifuged if desired, although this is not recommended for our BioMag® or COMPEL™ product lines.

Magnetic Separator Suppliers

Bangs Laboratories	https://www.bangslabs.com
SepMag	http://www.sepmag.eu
Dexter Magnetic	https://www.dextermag.com

REFERENCES

1. Ahmed, S.M., M.S. El-Aasser, G.H. Pauli, G.W. Poehlein, J.W. Vanderhoff. 1980. *Cleaning latexes for surface characterization by serum replacement.* J Coll Interface Sci, 73(2): 338-406.
2. Wilkinson, M. C., Hearn, J., & Steward, P. A. (1999). *The cleaning of polymer colloids.* J Coll Interface Sci, 81(2), 77-165.
3. Goodall, A. R., Hearn, J., & Wilkinson, M. C. (1979). *Problems associated with the surface characterization of polystyrene latices.* J Polym Sci: Polym Chem Ed, 17(4), 1019-1037.
4. Dalwadi, G., Benson, H. A., & Chen, Y. (2005). *Comparison of diafiltration and tangential flow filtration for purification of nanoparticle suspensions.* Pharm Res, 22(12), 2152-2162.
5. Cappione, A., Mabuchi, M., Briggs, D., & Nadler, T. (2015). *Optimized small molecule antibody labeling efficiency through continuous flow centrifugal diafiltration.* J Immunol Methods, 419, 48-56.
6. Tsao, T. M., Wang, Y. N., Chen, Y. M., Chou, Y. M., & Wang, M. K. (2013). *Automated ultrafiltration device for environmental nanoparticle research and implications: a review.* Micromachines, 4(2), 215-231.
7. Borlido, L., Azevedo, A. M., Roque, A. C. A., & Aires-Barros, M. R. (2013). *Magnetic separations in biotechnology.* Biotechnol Adv, 31(8), 1374-1385.
8. Sweeney, S. F., Woehrle, G. H., & Hutchison, J. E. (2006). *Rapid purification and size separation of gold nanoparticles via diafiltration.* J Am Chem Soc, 128(10), 3190-3197.