Technical Supplement:
Microparticle Reagent Optimization
Microparticle Sonication & Mixing

September 2007

Introduction

Processing microparticles is one of the most critical phases in particle technology. Guidance on use of sonication will make your life easier. At Thermo Fisher Scientific, our strength is offering you a complete microparticle technology. We give you simple validated protocols for coupling proteins and processing the Microparticles (MPs). We take the mystery out of working with MPs by giving you concrete data, backed by years of applications research in our own labs.

Use our experience, read our communications and save yourself months of aggravation. We have beneficial new ways to utilize our microparticle products and services. They have all been designed and engineered to meet the productivity requirements of multiple industries such as diagnostics, genomics, and proteomics.

Sonication

Sonication provides a way to re-suspend the particles thoroughly and efficiently without harm to the reagents. After centrifugation, processing steps, and coupling reactions, difficulties that arise from improper particle resuspension can be avoided by using sonication.

We routinely sonicate our protein-coated MP preparations with a probe-type ultrasonicator to re-suspend pellets after centrifugation, and to reverse mild aggregation induced by coupling proteins. We have not found this to be detrimental to sensitized MPs in any way, and have even seen improvement in agglutination sensitivity after sonication. It is advised to guard against temperature rise during sonication in sensitive systems.

Using HSA/anti-HSA as a model system, we tested whether sonication caused desorption of proteins or loss of functional activity. We subjected particle reagents to full power sonication. Prolonged sonication did not result in measurable loss of HSA from the particle surface.

From our experience it has proven impossible to damage our plain Polystyrene (PS) and magnetic beads with sonication or heat. In certain instances we take the plain PS to boiling with no

ill effect. This is **not** true if you have ligands bound to the surface of the particle. The particle will survive, but surface ligands may be lost. In the process of optimization of the following procedure, one should consider the characteristics of one's ligand and adjust the time and handling to ensure ligand activity.

Materials

1. Probe sonicator: An immersible ultrasonic probe is the ideal tool for efficient resuspension of MP pellets. Vortex mixing and bath-type sonicators are not effective for resuspending most pellets.
   Note: proper performance of the sonicator is ensured when one performs appropriate maintenance of the probe.

2. Appropriate sonicator probes: A key factor that affects optimal performance of sonication is the sonication probe. The volume to be sonicated is considered when selecting the proper probe: i.e. for samples with volumes of 500 mL or less, or samples in a 1 Liter (L) narrow-mouth container we typically use a tapered micro-tip (diameter 1/8 inch) and use a "macro"-tip probe (diameter 1/2 inch) for samples greater than 500 mL that are not in a narrow mouth container.

3. Container for sonication: If the volume of material is 1 L or less, then the material may be sonicated in the bottle or transferred to a beaker. If a sample is larger than 1 L and in a narrow-mouth container, it will have to be transferred to an appropriate-size beaker before sonication. Typically, sonication is more effective in glass container than plastic.

4. Optical microscope and necessary supplies: Capable of use at 400X magnification

Procedure

1. Handling particles before sonication: For efficiency, the material should be thoroughly mixed before sonication begins. This can be done by rolling the bottles of material using a mechanical roller or with an overhead mixer for bulk material.

2. Select sonication intensity: For volumes using the micro-tip probe, the intensity is set between 30% and 40%, or a setting from 3 to 4 on a scale of 10. For volumes using the "macro"-tip probe, the intensity is set to 50%, or 5 on a scale of 10.

3. Select sonication time: Material being sonicated with the micro-tip probe is exposed for the following times according to fluid volume:

Up to 10 mL	15-20 seconds
10 to 50 mL	20-30 seconds
50 to 100 mL	30-45 seconds
100 to 1000 mL	a minimum of 60-90 seconds

NOTE: When sonicating smaller samples the solution heats more quickly due to less volume available to disperse the heat.

Material being sonicated with the "macro"-tip probe is exposed for the following times according to fluid volume:

1 L	5 minutes
3 L	5 to 10 minutes
Greater than 3 L	Up to 20 minutes

4. Mixing particles during sonication process: It may be necessary either to mix the larger samples as they sonicate or to sonicate them using more repetitions of shorter times, if the material tends to settle out of solution quickly. For example, when working with large particles (greater than 1 micron) or if the material is excessively clumped:
   - If sonication of material is in a 1L bottle, in between sonication increments of 10 minutes, the bottle with material is rolled for five minutes.
   - If sonication of nonmagnetic material is performed in a beaker, a magnetic stirrer is recommended to keep any aggregates in solution during sonication.

5. Observe dispersity of particles: After a set amount of sonication time, the material should be mixed thoroughly and observed under a microscope at 400X. When in focus, one should not see clumps but a nice uniform field. If you see

aggregates, then the material is not monodispersed. Repeat sonication and observation until you do not see clumps.

Mixing

When handling Microparticles it is best to mix the material to ensure the material is monodispersed and uniformly distributed. Microparticles may be mixed according to the type of particle and volumes by the use of various equipment including overhead mixer, magnetic stirrer, vortex mixer and roller mixer. An overhead mixer is typically used for pooling, diluting, and handling large batches. The use of a vortex mixer can be used for mixing product stored in small containers such as 15 mL bottles, or other applications where the container is of similar size. The roller mixer is used to resuspend if necessary and mix uniformly the microparticles. Magnetic stirrers are used for the purpose of making a uniform mixture rather than resuspending.

Before you Begin

- If higher than normal levels of surfactant are in the solution or if excessive foaming is observed in any of the mixing techniques, reduce the speed and time of mixing accordingly to minimize the impact on the product.

- When resuspending material, visually confirm if possible that resuspension is complete by checking the bottom of the container for unsuspended material.

- Magnetic material can be mixed using all of the above methods except for the magnetic stirrers. Rolling and/or mixing times are the same for the magnetic particles as they are for any other particle of similar size and percent solids.

Mixing by Roller

- **Roller Mixer**

The roller mixer has a motor-driven horizontal cylinder adjacent to a free-turning horizontal cylinder that together form a cradle on which containers of product can be placed. The placement of the free-turning cylinder can be adjusted as necessary to accommodate different-size containers. Use a roller mixer of sufficient size and speed for the container being mixed. The roller for smaller bottles such as 100 mL can be found in general small lab supply catalogs.

- **Mixing Time**

Since the speed of the mixer is constant, mixing time is the way to control sufficient mixing. This mixing time can also vary based on the diameter of the container; smaller diameter containers rotate faster than large diameter containers, therefore mixing is accomplished more quickly. Mixing time can also vary based on the size of the particles; larger particles may take more time to resuspend. Higher concentrations of particles also require more mixing time.

Note: Containers must be at least 50% and less than 90% full to have enough material covering the bottom of the container when rolling, yet not too full to prevent insufficient mixing.

Extending the mixing time is acceptable, however nothing should need to mix for longer than 72 hours.

The following guidance is for minimum mixing time according to microparticle and container size.

MINIMUM MIXING TIMES USING THE ROLLER MIXER		
Container Size	Particle Size (μm)	
	≤ 0.4 μm	> 0.4
≤1 L	10 min	40 min
>1 L	30 min	60 min

Mixing by Vortex

- **Vortex Mixer**

A mixer used for mixing small volumes by holding the container of solution in a rubber holder and allowing a motor to rotate the shaft in an oscillating motion causing the solution to be mixed. Different models of vortex mixers have different methods of being activated. Most have a continuous action and a manual pressure activated system. Either may be used as is convenient with the continuous mode preferred for longer vortexing times and the manual mode preferred for shorter mixing times. A vortex mixer with adjustable speed setting is preferred.

- **Mixing Speed**

Using the controller on the mixer, adjust the speed of the mixer to a speed sufficient to cause good mixing (usually around 80% of full speed). Going too fast makes the container difficult to control.

- **Mixing Time**

Mixing usually can be completed in 30 seconds, however larger particles such as 0.8 and 1.0 µm require longer mixing to resuspend, at least one minute or longer, especially if the product has been stored for an extended period of time.

- **Verification of Mixing**

Visually verify mixing is completed by observing the product during mixing to ensure adequate agitation. After mixing, make sure no product remains settled on the bottom of the container. Clumps should not be observed in the suspension under a microscope at 400X.

Mixing by Overhead Mixer

- **Overhead Mixer**

An overhead mixer consists of an electrical or air-driven motor whose speed can be controlled, to which an agitator blade and shaft is attached. Choose an overhead mixer of sufficient capability to mix the volume required. The range of volumes is dependent on the proper agitator (use short-shafted agitator for smaller volumes).

- Container: Ensure that the container is such that the blade will be covered with enough product to prevent splashing.

- Stand: Position the blade high enough on a stand to allow clearance of the container but not so high as to prevent sufficient submersion of the agitator. Best results are usually obtained when the agitator blade can be placed at a position in the lower one-third of the container.

- **Mixing Speed**

The proper mixing speed can be determined by observing the action of the solution. If there is no visible movement of the product, increase the speed of the mixer until there is visible movement. In most circumstances, overhead stirring is used to achieve or maintain a uniform mixture; therefore mixing speed is not critical as long as it maintains sufficient motion. If one is removing aliquots from the mixture, then care should be taken to monitor the level of product being mixed, in order to periodically reduce the speed of the mixing so that product does not splash on the side of the container as the volume changes.

- **Mixing Time**

If mixing is for the purpose of resuspension, then follow the Table for Minimum Mixing Times Using a Roller Mixer.

Mixing by Magnetic Stirrer

- **Magnetic Stirrer**

A mixer that consists of a variable speed motor with an attached magnetic rotor encased in a platform. The rotor causes a magnetic stir bar placed in the solution to spin, thereby causing mixing. Select a stirrer that has sufficient power to move the volume of solution and hold the container on the stirrer.

NOTE: Magnetic stirrers are not used for the mixing of magnetic particles.

- Container: Effective mixing requires matching the size of the container with the volume of the solution and selecting a suitable stir bar that will be large enough to effectively move the solution but not so large as to cause splashing. The size of the container is determined by the size of the batch taking into consideration several factors. Too large a container may cause splashing and loss of yield due to increased surface area. Containers should have a volumetric working range of 20 to 80%; use a flat bottom in order for the stir bar to spin freely.

- Select an appropriate sized magnetic stir bar which will fit the container and thoroughly move the volume to be stirred.

- **Mixing Speed**

Adjust the speed of the stirring to create enough movement of the suspension to adequately mix. Sufficient movement ranges from creating a "dimple" ¼ inch into the surface to a funnel shape extending approximately one-fourth of the way into the suspension. Because the purpose of the mixing is to evenly distribute the material in the suspension, it is not necessary to rapidly mix the suspension; however, slightly faster mixing may be required for large particles. Avoid splashing the material. If the volume decreases, then mixing is to decrease; slow the speed of the mixing as the volume decreases to reduce splashing.

- **Mixing Time**

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The length of time for mixing will vary with the size of the batch; the larger the batch the longer the mixing time. However, mixing should not require longer than 30 minutes unless resuspension is the purpose of the mixing. If mixing is for the purpose of resuspension, then follow the Table for Minimum Mixing Times Using a Roller Mixer.

Other Products:
You are cordially invited to visit our facilities any time you are in the Indianapolis area. We are just minutes from the Indianapolis International Airport. Please inquire about our exceptional microparticle products. We have a complete range of microparticles to suit your individual requirements.

Technical Help/Ordering Information

For technical help, call the Particle Technology Division or write, e-mail or fax to the address below. We'll be glad to help you in any way we can.

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