

# MEASURING PERCENT SOLIDS

## Introduction

Two methods of measurement are described: gravimetric and turbidimetric. Percent solids are usually measured by the reference gravimetric (w/w) method which requires drying of particles. In a large scale manufacturing environment, drying particles is inconvenient. Therefore, we have also described a turbidimetric percent solids method that will speed up the measurement.

For a complete guide to our basic strategy, protocols, tips, and techniques for coupling proteins to microparticles (MPs) and immunological reagent development, order our laboratory reference manual, *Microparticle Reagent Optimization*.

## Benefits

It is necessary to know both % solids and MP diameter for determination of surface area available for the coupling of proteins. In addition, % solids is usually controlled throughout the processing of microparticle reagents. These techniques are therefore useful for:

- Optimization of MP reagents
- Processing/manufacturing
- Quality control
- Gravimetric and turbidimetric methods given
- Plain or protein-coated particle techniques given

Following are the principles, tips and protocols that we use in our laboratories.

## Uncoated Microparticle Procedures

### GRAVIMETRIC METHOD FOR UNCOATED MICROPARTICLES

Dry to constant weight a preweighed volume of MP suspension in a convection oven. For accuracy use a volume that will yield 1-2 gms dry weight. We have found 80 °C for 2 hours to overnight works well. Use the formula below to calculate the percent solids (w/w):

T = Tare weight of pan

MP = Weight of MP solution

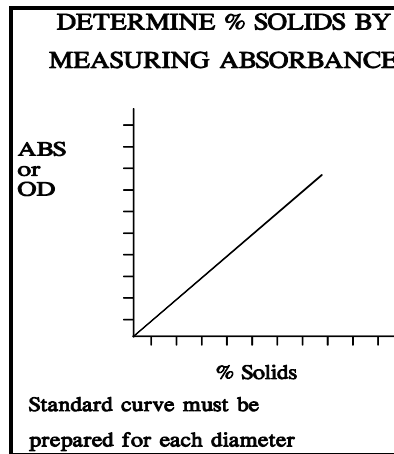
D = Weight of dried MPs in pan

% solids (w/w) = 
$$\frac{(D-T)}{MP} (100)$$

## Uncoated Microparticle Procedures

### TURBIDIMETRIC METHOD FOR UNCOATED MICROPARTICLES

This method is quicker and easier and requires much less of the MP suspension. It can be used to monitor % solids during manufacturing processing steps. Absorbance of the unknown MP suspension (or a dilution thereof) is read on a spectrophotometer. This reading is compared to a standard curve prepared with a MP suspension of known concentration (by the gravimetric method).



#### Standard curve:

Obtain the absorbance readings of serial dilutions of known MP suspension in water (for example, 1%, 0.5%, 0.25%, 0.125%, etc.). From these readings, construct a standard curve of known MP concentration vs. absorbance. Absorbance is a linear function of MP concentration over a range which varies with the instrument used. Obviously, a high precision spectrophotometer will give a wider linear range than an inexpensive one. Do the standard curve at several wavelengths (400 to 800 nm) and choose the one that gives the maximum linear range for that diameter MP (see sample curves, Figures 1-6).

#### Unknown Microparticle Suspension:

Use a dilution of the unknown MP suspension which gives readings in the linear portion of the previously constructed standard curve. The following factors may affect the absorbance:

- **Diameter:** a different standard curve must be made for each diameter particle used.
- **Dispersity:** it is very important to mix or sonicate the microparticles completely during the various processing steps before making an absorbance measurement. Any clumping or aggregates formed during MP processing will affect the absorbance and hence, accurate % solids measurements. It would be ideal to confirm monodispersity (separate particles) by microscopic examination or particle sizing techniques.
- **Buffer constituents:** if the MPs are suspended in buffers, proteins, or other ingredients, you should determine the effect of these on absorbance.
- **Adsorbed protein:** protein adsorbed onto the MP surface may affect the way the particle scatters light. Use the following procedure to run the standard curve with protein-coated MPs.



## **Protein-Coated Microparticle Procedures**

### **GRAVIMETRIC METHOD FOR PROTEIN-COATED MICROPARTICLES:**

Protein-coated microparticles (protein-MP) must be washed free of buffer components before drying to obtain an accurate dry weight. Below is a procedure for accomplishing this using only 2 mLs of protein-MP suspension (at least ~1% solids):

1. Mark two 1.5 mL microcentrifuge tubes #1 and #2.
2. Obtain the weights of the tubes to the fourth decimal place.
3. Pipet 1 mL protein-MP suspension into each tube.
4. Cap the tubes snugly and obtain the weights to the fourth decimal place.
5. Spin in microcentrifuge at top speed to pellet.
6. Remove supernatant, taking care not to aspirate any MPs (it is better to leave a little liquid if you have to).
7. Add 1 mL DI water to each tube.
8. Sonicate to resuspend, rinsing probe with a few drops of DI water into each tube, so as not to lose any MPs.
9. Spin in microcentrifuge at top speed to pellet.
10. Repeat steps 6 to 9 one more time.
11. Remove supernatant, taking care not to aspirate any MPs (again, it is better to leave a little liquid).
12. Add 0.5 mL DI water to each tube.
13. Sonicate to resuspend, rinsing probe with a few drops of DI water into each tube, so as not to lose any MPs.
14. Mark two aluminum weigh boats #1 and #2.
15. Obtain the weights of the boats to the fourth decimal place.
16. Carefully pour the contents of the tubes into the like-numbered weigh boats.
17. Use two successive 0.5 mL portions of DI water to rinse all of the MP into the boats.
18. Place the boats in the convection oven set at 80 °C.
19. Allow to dry for 1 hour; cool 5 minutes.
20. Obtain the weights of the boats to the fourth decimal place.
21. Calculation:  
A = Weight of filled tube – tare weight of tube  
B = Weight of boat with dried MP – tare weight of boat  
% Solids = B/A x 100

### **TURBIDIMETRIC METHOD FOR PROTEIN-COATED MICROPARTICLES:**

Once an accurate gravimetric % solids has been obtained for the protein-MP suspension, a standard curve of absorbance vs. % solids can be made as described previously. This standard curve can then be used to determine the % solids of unknown protein-MP suspensions.

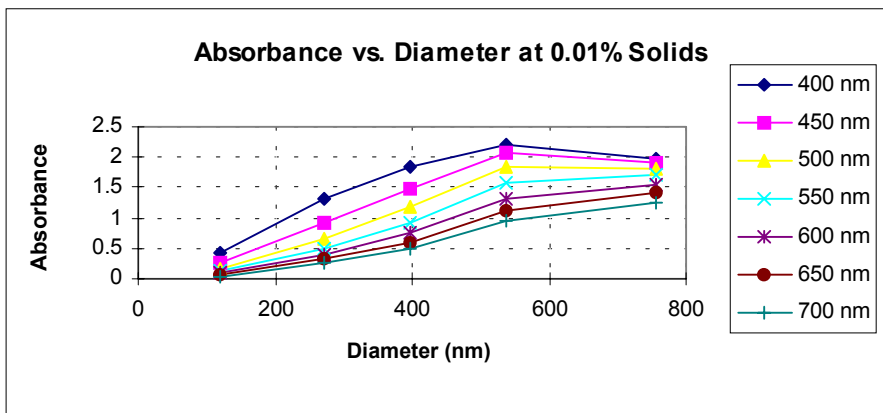


Figure 1. Polystyrene MPs of five diameters were diluted to 0.01% solids in water. The absorbance at various wavelengths was measured against water in a Shimadzu UV160U recording spectrophotometer.

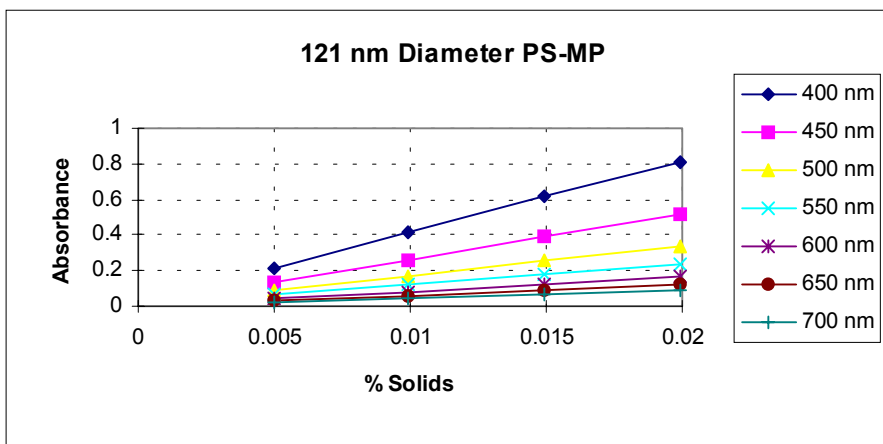


Figure 2. A polystyrene MP of diameter 121 nm was diluted in water to various % solids. The absorbance of each dilution was read at wavelengths from 400 to 700 nm.

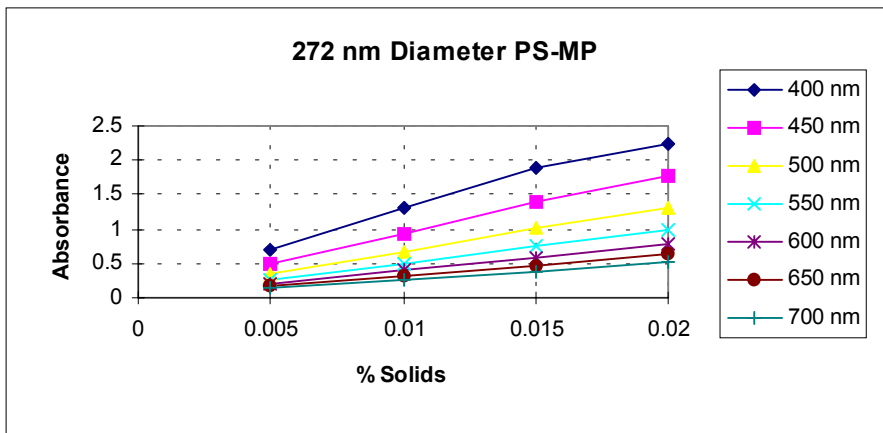


Figure 3. A polystyrene MP of diameter 272 nm was diluted in water to various % solids. The absorbance of each dilution was read at wavelengths from 400 to 700 nm.

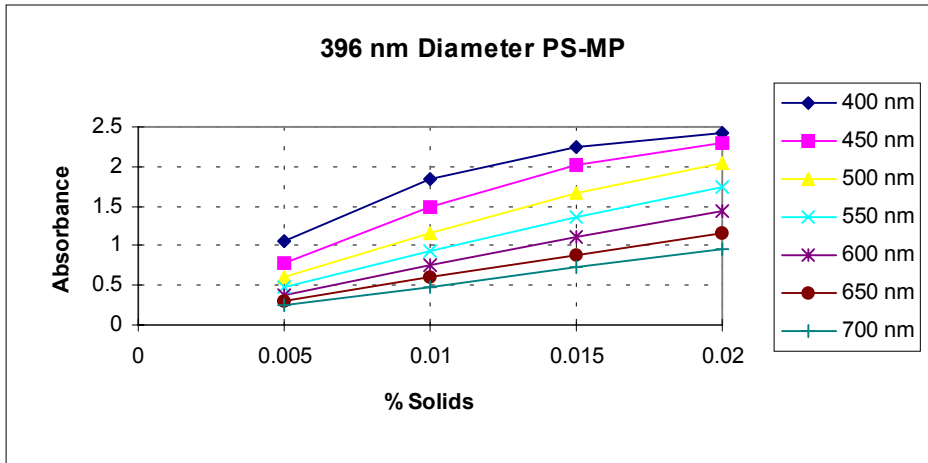


Figure 4. A polystyrene MP of diameter 396 nm was diluted in water to various % solids. The absorbance of each dilution was read at wavelengths from 400 to 700 nm.

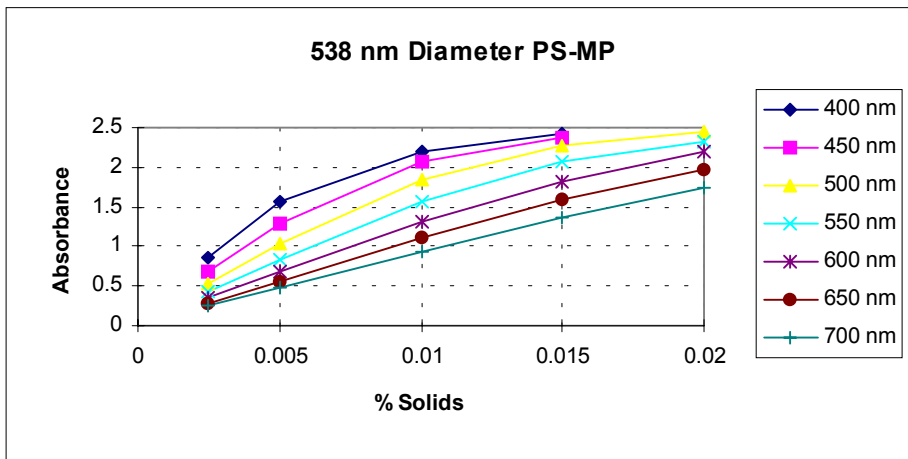


Figure 5. A polystyrene MP of diameter 538 nm was diluted in water to various % solids. The absorbance of each dilution was read at wavelengths from 400 to 700 nm.

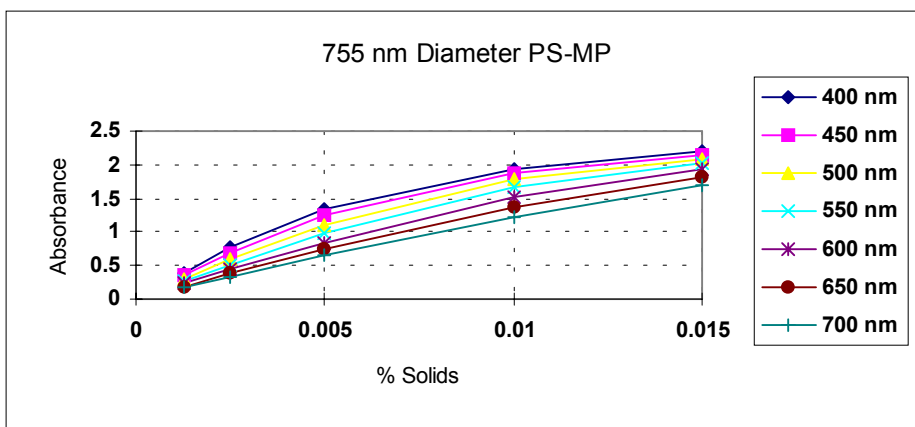


Figure 6. A polystyrene MP of diameter 755 nm was diluted in water to various % solids. The absorbance of each dilution was read at wavelengths from 400 to 700 nm.

## 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 other 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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