TechNote 202

Microsphere Aggregation

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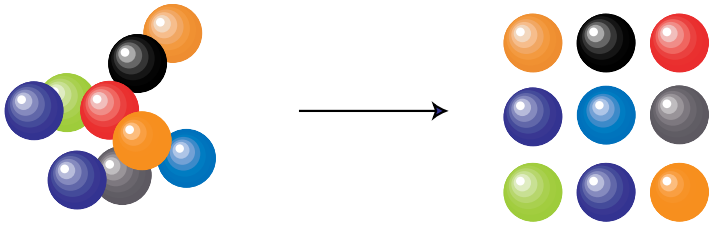
B E A D S • A B O V E T H E R E S T ™



Contents:

I. Introduction

Our microspheres are made from a variety of materials, including polystyrene and other hydrophobic polymers, silica, and superparamagnetic polystyrene (or polystyrene infused with varying amounts of iron oxide). These materials can sometimes encounter environments that cause unwanted aggregation. Some factors influencing aggregation are microsphere size and concentration, surface charge level, and the nature and concentration of ions in the suspending medium. Where the mechanism of microsphere aggregation is surface charge reduction, the resultant formation of aggregates is termed coagulation. If the aggregation arises from interparticle bridging by ligands or macromolecules, the process is termed flocculation. If the interparticle bridging is specific, as in the case of an antigen-antibody interaction, then the process of aggregation is called agglutination.[1]

The following guidelines first present general handling conditions that are the least likely to cause microsphere aggregation. Next, should aggregation be suspected, verification methods are reviewed. Finally, once aggregation has been confirmed by one of these methods, ideas and methods for reversing this aggregation are discussed.

II. Preventing Aggregation

Microspheres undergo quality control inspection prior to shipment to ensure that they are monodisperse. However, adverse conditions, either during shipment or when transferred to a working medium, can cause aggregation. The most important factors influencing aggregation are:

A. Microsphere Size

The likelihood of aggregation decreases as the mean diameter of the microspheres increases, because Brownian motion of smaller microspheres makes hydrophobic interactions more likely. For very small microspheres (sub-micron), maintaining a monodisperse suspension might not be possible without adding a surfactant, lowering the microsphere concentration, or both.

B. Surface Charge

Few of our microspheres carry a neutral charge. Their charge, positive or negative, causes repulsion between microspheres in solution, thereby increasing colloidal stability. The greater the amount of charge, the greater the colloidal stability of the suspension.

C. Temperature

An increase in temperature causes an increase in the kinetics of the microspheres in suspension, thereby increasing their likelihood of coming into contact with one another. As many of our microspheres are hydrophobic, increased contact with one another translates into increased likelihood of the hydrophobic interactions that are a leading cause of aggregation. Also, many polymeric microspheres will irreversibly aggregate if their aqueous suspending medium is frozen.

D. Concentration

As the concentration of the microspheres in the suspension increases, so does the likelihood of collisions and of the hydrophobic interactions which cause aggregation.

E. Counterions

Buffer salts that exist as individual ions in solution will bind to either positively or negatively charged microspheres, decreasing their surface charge. Similarly, divalent cations (with negatively charged microspheres) or divalent anions (with positively charged microspheres) can cause bridging between microspheres, and hence aggregation.

F. Protein Coupling

Many microspheres are offered with some type of surface functional group, to which proteins can be covalently attached. If the amount of protein added is miscalculated, so that some of these functional groups are left free, multiple attachment sites on the protein are likely to cause cross-linking between the microspheres. This type of aggregation is irreversible.

III. Methods to Detect Aggregation

Depending on the size of the microspheres and the degree of aggregation, it is sometimes possible to detect aggregation visually (large clumps throughout the suspension). This is normally the case when the microspheres have been frozen, or clumped by incorrect reagent addition in a protein attachment protocol. A degree of aggregation below the visual threshold can still cause problems with the application in which the microsphere suspension is used, and therefore can be examined instrumentally to make a more sensitive assessment of aggregation (doublets, triplets, etc.). The instrumental method used will depend on the characteristics of the microspheres, primarily their size. Five commonly used methods of analysis are as follows:

A. Light Microscope

If the microspheres are large enough (>0.5µm), they may be observed in the light microscope, and approximate counts of singlets, doublets, etc. may be made. This method must be used with caution, because the necessity for sample dilution before observation may introduce an artifact (the act of dilution may cause or eliminate the particle aggregation that you are trying to observe).

B. Spectrophotometer

The light scattered by single small particles will change if the same number (or weight) of particles are partially aggregated. The scattered light may be read as 'absorbance' on any spectrophotometer. Reading absorbance at wavelengths in the visible spectrum is best for most sizes of microspheres. If absorbance changes with time or differs from batch to batch, one can make inferences about the aggregation state. It may, however, be difficult to quantify the exact numbers of doublets, triplets, etc. Again, dilution may cause changes in the state of aggregation.

C. Dynamic Light Scattering

This type of measurement is accurate for microspheres with mean diameters of up to ~1µm. This is especially useful for smaller microspheres, because in addition to indicating whether aggregation is present or not, it also indicates the degree of aggregation (based on the size of the peaks at diameters other than the expected mean diameter).

D. Electrophoretic Mobility

When an electrical field is applied across a suspension of polymeric microspheres, the microspheres migrate toward the electrode of opposite charge sign. The electrophoretic mobility (particle velocity per unit of applied electrical field) is a measure of the surface charge of the microsphere.[2] A monodisperse microsphere suspension should show a uniform electrophoretic mobility, and variations can be used to quantitate the degree of aggregation present.

E. Field-Flow Fractionation

Field-flow fractionation (FFF) is a family of analytical separation techniques[3,4] used to characterize particulate and polymeric materials. FFF is an evaluation technique, in which physiochemical variables, such as particle diameter, density, molar mass, and diffusion coefficient, can be determined from the retention time.

IV. Methods for Reversing Aggregation

Although some types of aggregation are irreversible, in many cases there are procedures, either physical or chemical, to reverse unwanted aggregation. The approach taken will depend on the physical characteristics of the microspheres. Some approaches for different materials are as follows:

A. Polymeric Microspheres

Aggregation involving polymeric microspheres can be due to a number of causes, perhaps the most common being hydrophobic interactions. Although these can be a challenge to prevent, they are not difficult to overcome. Normally, a good first step is to try one of the physical means of reversing the aggregation listed below.

1. Sonication

Although a probe sonicator will provide the most ultrasonic energy, we advise being cautious about using this. An unclean probe tip can contaminate the microsphere suspension, and an old tip can shed metal, even if it is clean. A better option might be a bath type sonicator. To achieve maximum efficiency, it is best to work with a small volume (or a large volume dispersed in a larger container), so that the relative microsphere concentration is reduced as much as possible. This allows the ultrasonic energy to reach all of the microspheres effectively, increasing the likelihood of breaking the aggregates. Also, glass seems to be a better conductor of ultrasonic energy than plastic, and therefore working in glass is recommended if at all possible. It should be noted that extended exposure, even in a mild ultrasonic bath, can heat the microspheres to an undesirable level. Therefore, the temperature should be monitored, and ice can be added to the bath to ensure that the microspheres do not overheat.

2. Vortexing

The ruggedness of all of our microspheres makes vortexing a suitable approach to reducing aggregation. Working with small volumes and vortexing in repeated short pulses (perhaps 5 seconds) seems to be the most effective. A case in which this is not recommended is when unwanted aggregation is present after passively adsorbing ligand to the surface of non-functionalized microspheres. The shear forces involved in vortexing can dislodge some of the protein adsorbed at the microsphere surface.

3. Pipetting

Much like vortexing, the shear forces created by rapid pipetting of a suspension of microspheres through a fine tip pipet are often enough to reduce or eliminate aggregation caused by hydrophobic interactions, and should be avoided if ligands have been adsorbed at the microsphere surface.

4. Combined Approaches

Should the above approaches be ineffective on their own, a 'cocktail' of approaches might prove to be more effective. In addition, as there is always the possibility that charge interactions are causing the aggregation, the method of reversing the aggregation might be as simple as raising or lowering the pH of the suspension.

If a physical means is not effective, or cannot be used for some reason, aggregation can often be reversed by the addition of a molecule that will make the surface of the microspheres less hydrophobic.

'Blockers' are commonly used for this application. These can be inert proteins, such as bovine serum albumin, casein, or 'irrelevant' IgG's (IgG's that won't cross-react with the specific IgG conjugated). Alternatively, or in conjunction with one of these inert proteins, surfactant can be used to make the microsphere surface more hydrophilic. Cationic or non-ionic surfactants work best with positively charged microspheres, while anionic or non-ionic surfactants are preferred when working with negatively charged microspheres. Also, you can increase the effectiveness by vortexing or sonicating the suspension after adding the blocker. Our TechNotes 204 and 205, for passive adsorption and covalent coupling protocols, respectively, give ideas for optimizing the amount of blocker added to reduce the amount of aggregation most efficiently.

B. Silica Microspheres

These microspheres are much more hydrophilic than polymeric beads, reducing the likelihood for hydrophobic interactions as a potential cause for aggregation. More often, aggregation is a result of charge interactions between microspheres, or a remnant of incomplete separation during the microsphere formation process. For this reason, the physical means of redispersion mentioned previously (e.g., sonication, vortexing, pipetting) should suffice. These microspheres are more rugged and less susceptible to physical deformation than polymeric microspheres. Therefore, more aggressive approaches to breaking up aggregates, such as grinding with a mortar and pestle, can be taken without causing damage to the microspheres. In certain instances, our experience has shown that addition of an anionic surfactant can be effective. Additional information regarding handling of silica microspheres, including a drying procedure, can be found in our TechNote 104.

V. List of Manufacturers/Vendors

Note: This list of vendors is intended to help you find the appropriate reagents for carrying out your covalent coupling procedures, and does not constitute a product endorsement on our part. The list is not all-encompassing and we encourage you to explore several reagent vendors before committing your time and resources.

A. Reagents (Blockers, Surfactants, etc.)

- Sigma Chemical Company
  P.O. Box 14508
  St. Louis, MO 63178
  Telephone: 800-325-3010
  Fax: 800-325-5052
  e-mail: custserv@sial.com

- Calbiochem
  P.O. Box 12087
  La Jolla, CA 92039-2087
  Telephone: 800-854-3417
  Fax: 800-776-0999
  www.emdbiosciences.com

- Pierce Chemical (U.S.)
  3747 North Meridian Road
  P.O. Box 117
  Rockford, IL 61105
  Telephone: 800-874-3723
  Fax: 800-842-5007

- Pierce Chemical (Europe)
  Pierce Europe B.V.
  P.O. Box 1512
  3260 BA Oud Beijerland
  The Netherlands
  Telephone: 31-1860-19277

- Pragmatics, Inc.
  29477 County Road 16 W
  Elkhart, IN 46516
  Telephone: 800-213-1293
  Fax: 219-262-3911

- Fitzgerald Industries, Int'l
  34 Junction Square Drive
  Concord, MA 01742
  Telephone: 800-370-2222
  Fax: 978-371-2266
  e-mail: antibodies@fitzgerald-ffi.com

B. Particle Counting / Electrophoretic Mobility Instruments

- Beckman-Couter
  P.O. Box 3100
  Fullerton, CA 92834-3100
  Telephone: 800-742-2345
  Fax: 800-643-4366

- Micrometrics Analytical Svcs.
  One Micrometrics Drive
  Norcross, GA 30093-1877
  Telephone: 770-662-3630
  Fax: 770-662-3653

- Brookhaven Instruments
  750 Blue Point Road
  Holtsville, NY 11742
  Telephone: 631-758-3200
  Fax: 631-758-3255

- Nicomp/Particle Sizing Systems
  75 Aero Camino, Suite B
  Santa Barbara, CA 93117
  Telephone: 805-968-1497
  Fax: 805-968-0361

- Malvern Instruments, Inc.
  10 Southville Road
  Southborough, MA 01772
  Telephone: 508-480-0200
  Fax: 508-460-9692

- Wyatt Technologies
  6300 Hollister Avenue
  Santa Barbara, CA 93117
  Telephone: 805-681-9009
  Fax: 805-681-0123

C. Field-Flow Fractionation

- FFFractionation / Postnova Analytics
  230 South 500 East, Suite 120
  Salt Lake City, UT 84102
  Telephone: 801-521-2004
  Fax: 801-521-2884

VI. References

1. **Corry, W.D., G.V.F. Seaman.** 1978. On the interaction of polystyrene latices and poly (L-lsyine): Characterization of the polystyrene latices and the poly (L-lysine). *J Coll Interface Sci,* 63: 136-150.
2. **Abramson, H.A.** 1934. *Electrokinetic phenomena and their application to biology and medicine.* New York: The Chemical Catalog Company.
3. **Giddings, J.C.** 1993. Field-flow fractionation: Analysis of macromolecular, colloidal, and particulate materials. *Science,* 260: 1456.
4. **Myers, M.N.** 1997. *J Micro,* 9: 151.

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