TechNote 100

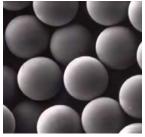


Figure 1: SEM image of polystyrene microspheres

INTRODUCTION

Bangs Laboratories supplies a full range of uniformly sized polymeric microspheres that support a variety of applications in the life sciences. Available in diameters ranging from 20nm to 20µm, products exhibit excellent size uniformity.

Most of our microspheres are polystyrene-based, though some other base polymers are available on a limitied basis. Plain (non-functionalized) polymer microspheres are ideal for protein adsorption applications, while surface modified microspheres (COOH or NH₂) are used for covalent ligand attachment. Cross-linked polymer microspheres are available for improved solvent, heat, and pressure resistance. Polymer microspheres are also available with impregnated visible or fluorescent dyes. (See dye colors and fluorescence spectra in the tech support section of our website.

POLYMER BEAD SYNTHESIS AND CHARACTERISTICS

Our polymer microspheres are synthesized via emulsion polymerization process in which surfactant as is dispersed in water to form an emulsion. The hydrophobic tail and hydrophilic head of the surfactant molecules permit the formation of micelles (Figure 2a) above the surfactant's critical micelle concentration (CMC).

When hydrophobic monomers (e.g. styrene or methyl methacrylate) are added to the aqueous dispersion, they enter the hydrophobic interior of the micelle (Figure 2b). The addition of an initiator induces polymerization of the monomer, and the resulting polymer chains form the uniform bead matrix. Sulfate ions from the initiator terminate the ends of the polymer chains at the bead surface (Figure 2c).

At neutral pH, negatively-charged sulfate groups (from the initiator) populate the surface of plain and functionalized polystyrene beads. Functionalized polystyrene spheres (e.g. COOH- or NH $_2$ -modified) also have surface groups from respective co-monomers used during synthesis. When introduced, the density of the surface carboxyl groups is determined post-synthesis via conductometric titration and reported in units of $\mu eq/gram$. For amine-modified microspheres, the presence of NH $_2$ groups is confirmed using a ninhydrin test. A number of our larger spheres contain the crosslinker divinylbenzene (DVB), which offers enhanced heat and solvent resistance. Some general characteristics from the literature for bulk polymers may be found in Table 1. See also Additional Reading.

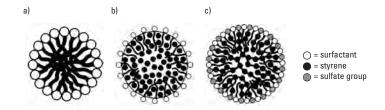


Figure 2: Emulsion polymerization

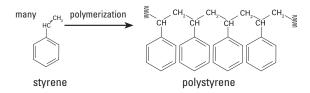


Figure 3: Structure of polystyrene

 Table 1: Polymer Microsphere Characteristics - Nominal values

Composition	Abbreviation	Density (g/cm³)	Refractive Index (at 589nm)	T _g (°C)
Polystyrene	PS	1.05	1.59	95
Carboxyl-modified polystyrene	P(S/V-COOH)	1.06	>1.59	95
Polystyrene/2% divinylbenzene	P(S/2%DVB)	1.062	n/a	99
Polystyrene/10% divinylbenzene	P(S/10%DVB)	1.067	n/a	114
Polystyrene/55% divinylbenzene	P(S/55%DVB)	1.097	n/a	250
Polymethyl methacrylate PMMA	1.19	1.49	1.49	105

Polystyrene microspheres generally have highly hydrophobic surfaces and aqueous suspensions are stabilized (colloidally) by the inclusion of wetting agents known as surfactants. Unless designated as surfactant-free, bead suspensions will contain surfactant. Surfactants routinely added to bead suspensions include Tween® 20 (non-ionic) and sodium dodecyl sulfate (SDS), an anionic surfactant that provides charge stabilization in addition to the wetting function.

Our synthesis processes typically produce monodisperse bead populations with CVs \leq 10%. Sizing results, i.e., mean diameter and standard deviation when available, are provided in our online catalog.

Our general QC program includes sizing (see TechNote 208), microscopic examination (400X), and bioburden. Standard microscopy is used to assess general surface properties and morphology of our polystyrene microspheres. Though we do not measure surface roughness, we do expect beads to be spherical with smooth surfaces via light microscopy.

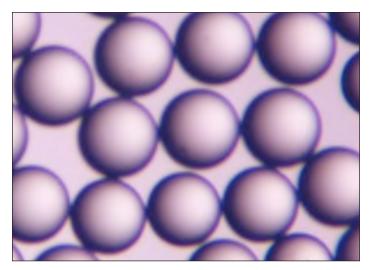


Figure 4: 10µm polystyrene microspheres at 400X magnification

COATING MICROSPHERES

Polymer microspheres present a flexible platform for applications in diagnostics and bioseparations. They may be coated with ligands such as antibodies, antigens, peptides, or nucleic acid probes, and can be loaded with hydrophobic dyes and other compounds. Unmodified polymer spheres also find extensive use as standards for instrument set-up and calibration.

Protein Adsorption

Polystyrene microspheres are ideal for protein adsorption and have been utilized in a range of diagnostic tests and assays. Reference our TechNote 204, *Adsorption to Microspheres*, for protein adsorption guidelines, information on the use of blockers, and further references.

Covalent Ligand Attachment

Surface modified microspheres are available with carboxyl or primary amine groups for covalent ligand attachment. Reference our TechNote 205, *Covalent Coupling*, which provides a basic foundation for successful attachment of a variety of ligands through coupling protocols, buffer recipes, blockers, and references.

Affinity Binding

Affinity binding systems offer simple and efficient ligand attachment. Coatings of Fc-binding proteins are able to orient antibodies for optimal activity, and streptavidin offers extremely stable attachment of biotinylated molecules such as proteins, peptides, and oligonucleotides. See TechNote 101, *Affinity Ligand Microspheres*, for basic attachment protocols.

ADDITIONAL READING

Technical Support Doc 0021, *Material Properties of Polystyrene*, Technical Support Doc 0022, *General Physical Properties of Polymer and Silica*, Technical Support Doc 0023, *Common Solvents and Non-solvents of Polystyrene*.

REFERENCES

- 1. Bangs, L.B. 1987. *Uniform Latex Particles*. Indianapolis: Seragen Diagnostics, Inc.
- 2. Brandrup J. Immergut EH, and Grufke EA. (Eds) (1975) Polymer Handbook, 2nd edition New york, New York: John Wiley

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