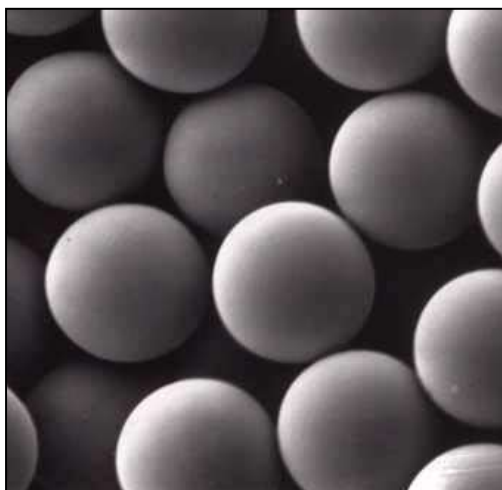


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**B E A D S • A B O V E T H E R E S T™**



*Figure 1: SEM image of polystyrene microspheres*

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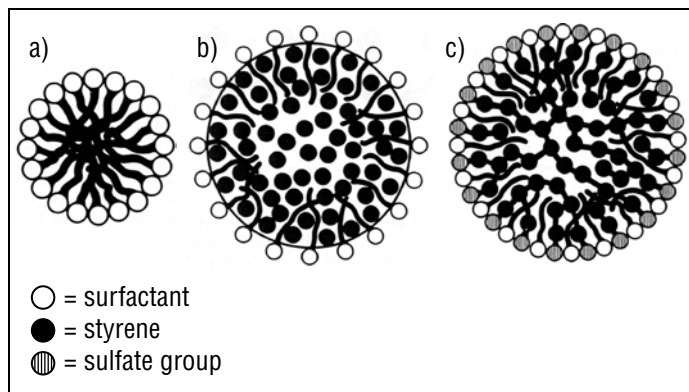
### I. Introduction

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, although some other base polymers are also offered. Plain (non-functionalized) polymer microspheres are ideal for protein adsorption applications, while surface modified microspheres (COOH or NH<sub>2</sub>) 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.

### II. Polymer Bead Synthesis and Characteristics

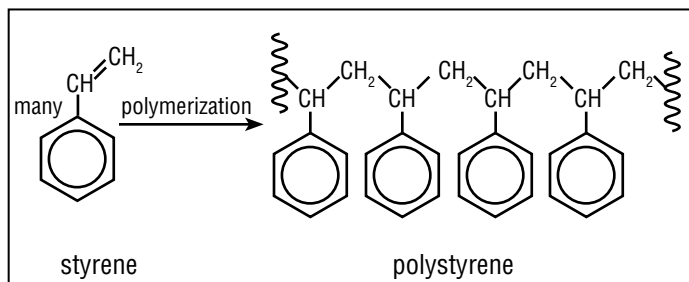
Bangs Laboratories' polymer microspheres are synthesized via an emulsion polymerization process in which surfactant is dispersed in water to form an emulsion. The hydrophobic tail and hydrophilic head of the surfactant molecules permit the formation of micelles (Figure 1a) above the surfactant's critical micelle concentration (CMC).



*Figure 2: Emulsion polymerization<sup>1</sup>*

When hydrophobic monomers (e.g. styrene or methyl methacrylate) are added to the aqueous dispersion, they enter the

hydrophobic interior of the micelle (Figure 1b). 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 1c).



**Figure 3:** Structure of polystyrene

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<sub>2</sub>-modified) also have surface charge 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 µeq/gram. For amine-modified microspheres, the presence of NH<sub>2</sub> 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, i.e., density, refractive index, and glass transition temperature ( $T_g$ ).

**Table 1:** Polymer Microsphere Characteristics

Composition	Abbreviation	Density (g/cm <sup>3</sup> )	Refractive Index (at 589nm)	$T_g$ (°C)
Polystyrene	PS	1.05	1.59	95
Carboxyl-modified polystyrene	P(S/V-COOH)	1.06	n/a	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

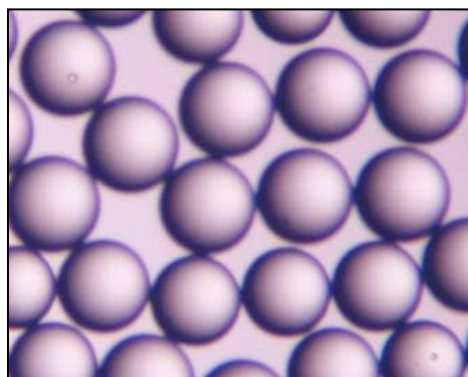
**Table 1:** Polymer Microsphere Characteristics, cont.

Composition	Abbreviation	Density (g/cm <sup>3</sup> )	Refractive Index (at 589nm)	$T_g$ (°C)
Polystyrene/ 55% divinylbenzene	P(S/55%DVB)	1.097	n/a	250
Polymethyl methacrylate	PMMA	1.19	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 ≤10%. Sizing results, i.e., mean diameter and standard deviation when available, are provided in our online catalog.

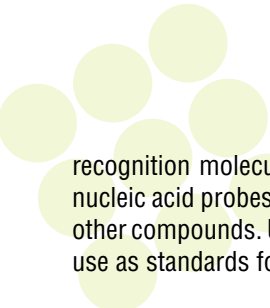
Our general QC program includes sizing (see TechNote 208), microscopic examination (400X), and bioburden. Standard microscopy is used to assess general surface properties and morphology of our polystyrene microspheres. Though we do not measure surface roughness, we do expect beads to be spherical with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



**Figure 4:** 10µm polystyrene microspheres at 400X magnification

### III. Coating Microspheres

Polymer microspheres present a flexible platform for applications in diagnostics and bioseparations. They may be coated with



recognition molecules 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.

#### **A. 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.

#### **B. 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.

#### **C. 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, *ProActive® Microspheres*, for basic attachment protocols.

### **VI. References**

1. **Bangs, L.B.** 1987. Uniform Latex Particles. Indianapolis: Seragen Diagnostics, Inc.

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