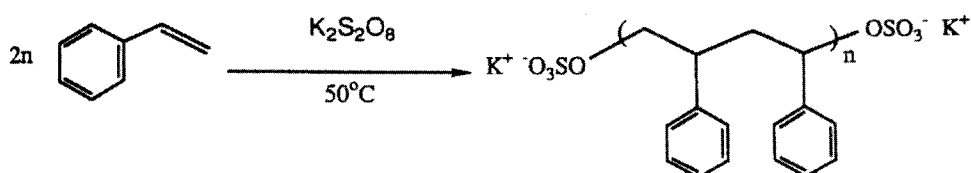


III. Product and Price Information

1. SPHERO™ Polystyrene Particles

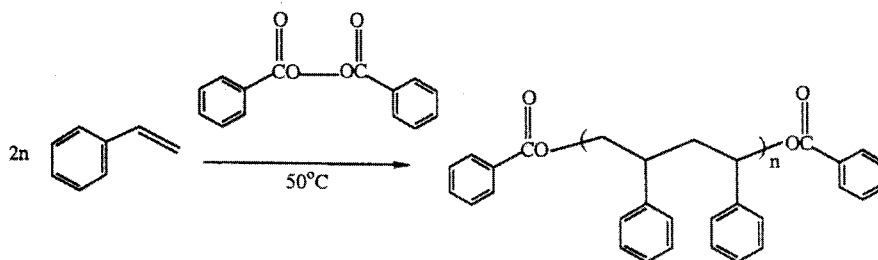
The SPHERO™ polystyrene particles are prepared by conventional emulsion polymerization with styrene as monomer and potassium persulfate or benzoyl peroxide as polymerization initiator. In general, microparticles < 0.5 μm are prepared in one step followed by a cleaning step on mixed bed ion exchange resin to remove detergent and inorganic salts. Larger particles are prepared by stepwise growing of smaller particles with the addition of styrene monomer and initiator without any additional detergent. The microparticles are cleaned by repeated centrifugation. Cleaned microparticles are resuspended in deionized water. Sodium azide (0.02%) is added as bacteriostatic. The SPHERO™ microparticles, in general, can be coated with proteins without further cleaning.

Microparticles made using potassium persulfate as initiator have sulfate groups on their surface. As a result these particles are negatively charged and are hydrophilic, as shown in the following equations (1):



(1)

The SPHERO™ polystyrene particles > 3 μm are usually prepared using benzoyl peroxide as the initiator. These particles are relatively more hydrophobic, as shown in the following equations (2):



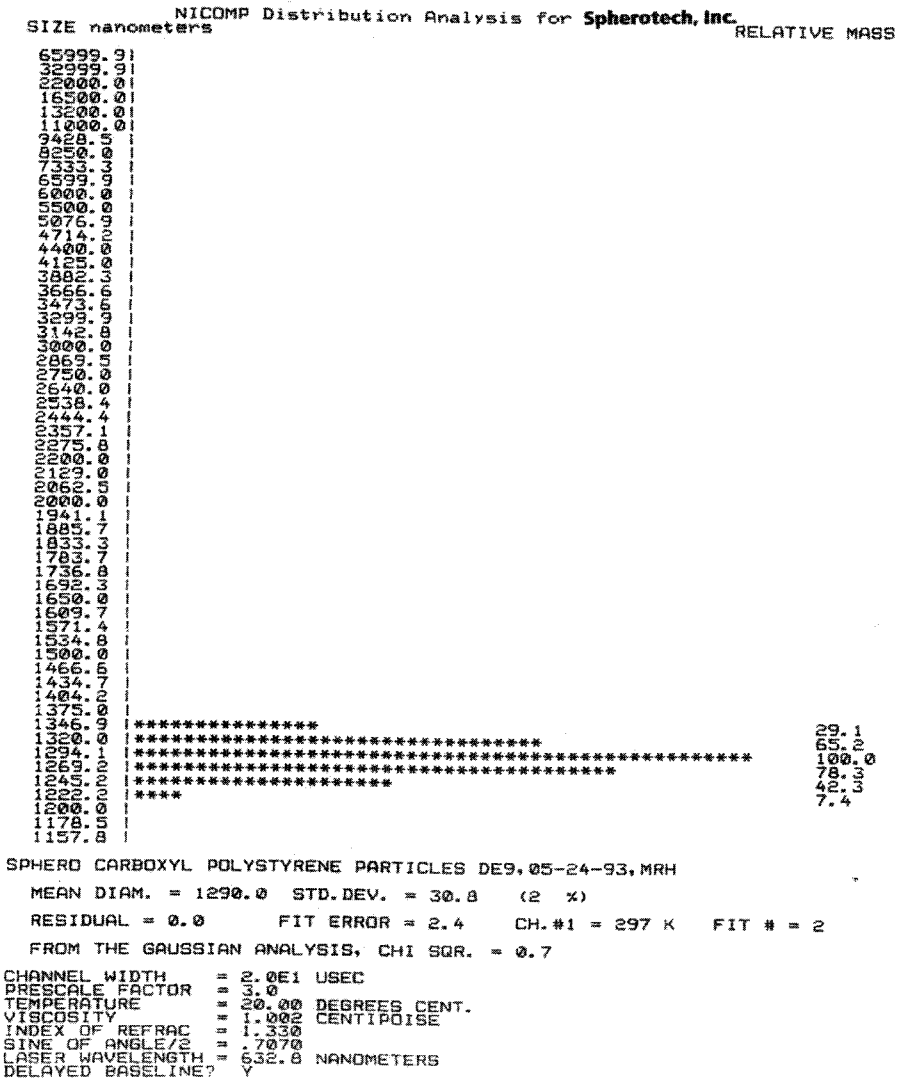
(2)

SPHERO™ polystyrene particles are composed of linear polystyrene without any cross-linking agent. These particles cannot tolerate organic solvents such as toluene, xylene, chloroform, methylene chloride, acetonitrile, dimethyl formamide or acetone. However, SPHERO™ polystyrene particles are stable in the presence of some water miscible solvents such as dimethyl sulfoxide and alcohols.

Uniform size cross-linked polystyrene particles that are stable in the presence of organic solvents are available as custom products. Please inquire.

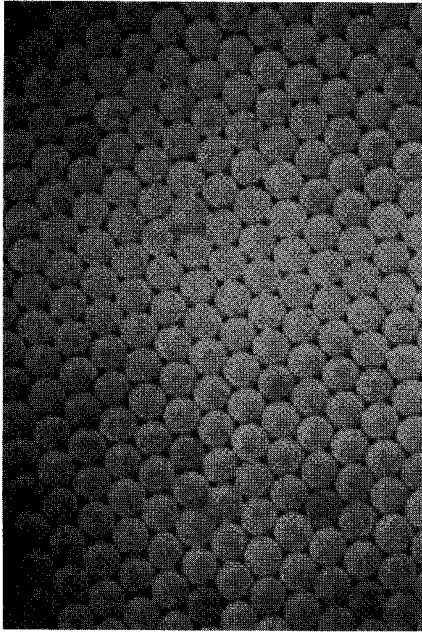
Uniform SPHERO™ polystyrene particles are ideal for use in immunoassays such as latex agglutination, particle base enzyme immunoassays and fluorescence immunoassays. Tight size range of SPHERO™ polystyrene particles is maintained by monitoring size by NICOMP Laser Particle Sizer (for particles < 3 μm) and using a Scanning Electron Microscope for larger particles. Although the size measurements are accurate, these particles are not certified for use as calibration standard for size measurements or pore size analysis.

Histogram of SPHERO™ polystyrene particles from the NICOMP Laser Particle Sizer

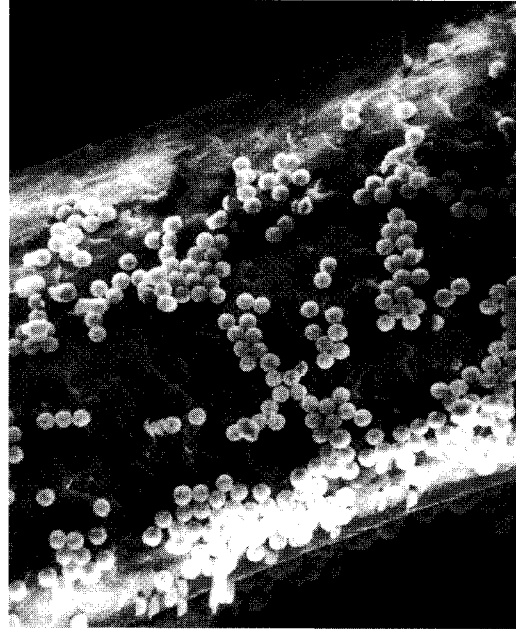


The Scanning Electron Microscope (SEM) photos of polystyrene particles are shown below to illustrate the uniformity of the size and the comparison of the size of polystyrene particle to the human hair. (a) Single sheet of 0.8 μm polystyrene particles. (b) Face-centered-cubic packing of 0.86 μm particles (note that theoretically particles should fill $\sim 74\%$ of the space regardless of the size of the particles). (c) 3.4 μm polystyrene particles on the surface of a human hair which is about 100 μm in diameter. (d) A NIST particle size standard (NIST #1692, 2.98 μm) used for SEM calibration and as a reference for size analysis.

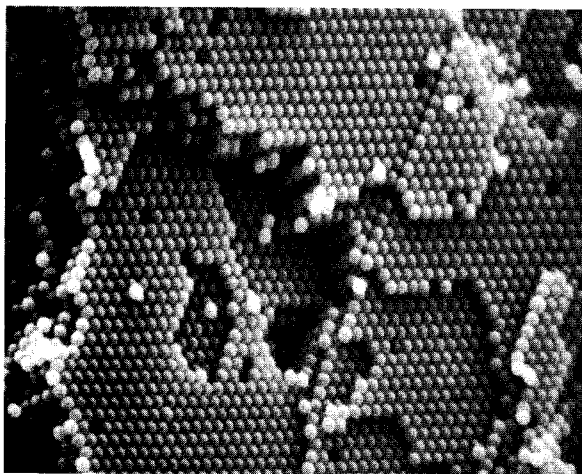
(a)



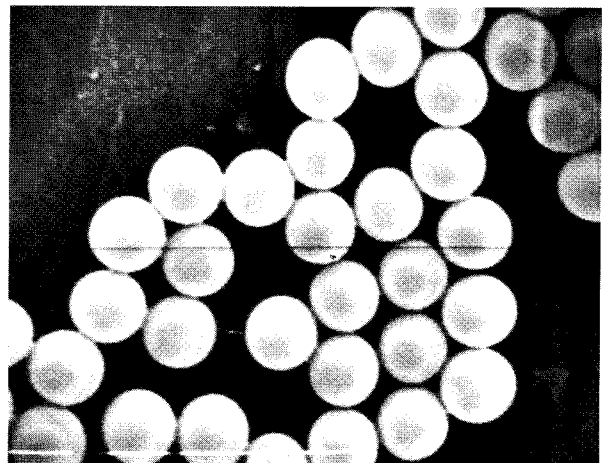
(c)



(b)



(d)



There are several ways to prepare particles with functionalized surface. As mentioned previously, if potassium persulfate is used as an initiator for polymerization, the surface of the particles will have sulfate groups present. Similarly, other functional groups can be introduced on the surface of the particles by using other functionalized initiators. The surface functional groups can also be introduced by chemical modification of the existing surface functional groups by using functionalized monomer either during the particle formation step or during the final coating step.

Most of the **SPHERO**TM functionalized polystyrene particles are prepared by coating a thin layer of functionalized polymer onto the surface of plain particles. As a result, all of the functional groups are on the surface of the particles. The functional groups are attached to the surface of the particles by alkyl chains of two to eight carbons in length depending upon the type of functionalized monomer used. The number of functional groups on the surface can be adjusted by varying the amount of functionalized monomer used for coating. The 0.8 μm **SPHERO**TM carboxyl polystyrene particles typically contain about 50 $\mu\text{eq/g}$ of carboxyl groups on the surface of the particles and the 0.8 μm **SPHERO**TM amino polystyrene particles typically contain about 15~20 $\mu\text{eq/g}$ of amino groups on the surface of the particles. These two types of the functionalized particles are very useful for covalent coupling of proteins, antibodies or antigens to the surface of the microparticles using water soluble carbodiimide methods. Other types of functionalized particles such as hydroxyl, sulfate and dimethylamino particles offer different surface charges on the particles. These particles can be used to manipulate the orientation of the coated material by passive adsorption.

In general, polyclonal antibodies can be coated to polystyrene particles by passive adsorption. According to our experience, the optimal amount of antibody to particles ratio is ~100 μg of antibody per mL of 0.5% w/v (5 mg solid per mL) of 0.8 μm polystyrene. Since the total surface area of the particles is inversely proportional to the diameter of the particles, the amount of antibody to particles ratio needs to be adjusted accordingly. If needed, the proteins or other Ligand can also be coated to carboxyl or amino polystyrene using water soluble carbodiimide as the coupling agent. Please refer to SpheroTECHNICAL NOTES-1 (STN-1) for more information on particle coating.

The washing of polystyrene particles to remove unbound proteins or ligands during coating can be accomplished by centrifugation or cross flow filtration for particle with size of 0.4 μm or larger. For smaller size particles, gel filtration or other means of washing should be used. The choice of particle size and type is dependent upon the intended application. For example, particles with size of 0.4 to 2.0 μm are suitable for latex agglutination assay, solid phase enzyme immunoassay or solid phase fluorescence immunoassay, while particles with size of 2.0 μm or larger are preferred for flow cytometry applications.

We list a wide variety of microparticles in our catalog. If you cannot find a particular product that you need in our catalog, please feel free to contact us by phone or E-mail.



Our Pledge:



Spherotech shall meet or exceed customer expectations by delivering products of highest quality at competitive prices on time and every time.

