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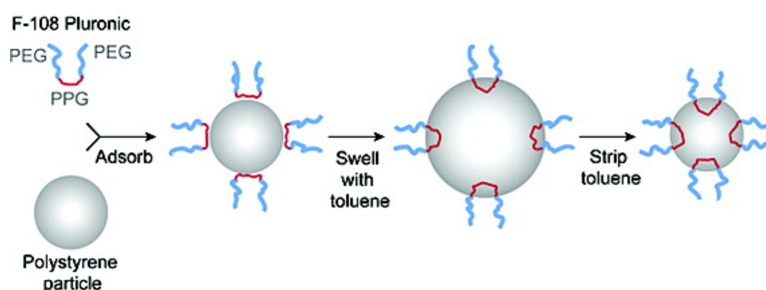

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Swelling-Based Method for Preparing Stable, Functionalized Polymer Colloids

Anthony J. Kim, Vinothan N. Manoharan, and John C. Crocker*

Department of Chemical and Biomolecular Engineering, University of Pennsylvania, Philadelphia, Pennsylvania 19104

Received August 18, 2004; E-mail: jcrocker@seas.upenn.edu

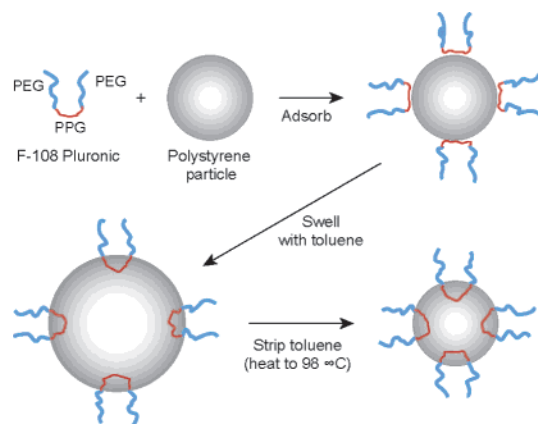
The varied applications of polymer microspheres benefit from precise control over surface chemistry. Grafted molecules or functional groups determine the rheology[1] and stability[2] of the colloids used in pastes and coatings, influence the self-organization of the dense suspensions used in materials synthesis,[3] and provide specific biomolecular binding sites for biological assays and cell labeling.[4] Here, we describe a straightforward method for attaching molecules to polymer microparticles that, at the same time, enhances colloidal stability. This is accomplished by trapping a water-soluble, functionalized polymer surfactant on the microparticle surface via swelling with an organic solvent followed by deswelling.

Usually, desirable surface groups are not present on polymer particles as synthesized, but are attached afterward. Emulsion and dispersion polymerizations,[5] the techniques used to prepare most commercial monodisperse polymer particles, allow only limited control over surface chemistry and are ill-suited for preparing particles grafted with sensitive or expensive reagents. Methods have emerged for preparing the specialized (generally polydisperse) particles used in drug delivery,[6] but in general, the simplest and most versatile approach has been to attach molecules to preformed, well-characterized, monodisperse particles. In most biological applications, the attachment is made by either physical adsorption[7] or covalent coupling.[8] Both techniques have limitations. Adsorbed species, for example, will desorb if the dispersion is diluted. Species grafted by avidin−biotin binding[9] or common covalent coupling procedures, such as the carbodiimide[10] method, do not desorb, but the reaction can render the particles unstable in commonly used molecular biology buffers (∼140 mmol/L NaCl, often with several mmol/L of divalent ions, e.g., Mg^{2+} or Ca^{2+}). Such particles usually require surfactants or "blockers" to reduce nonspecific binding and remain dispersed.

The first step of our method is to adsorb block copolymer surfactants onto the surfaces of the particles. Then, we swell the particle with a small amount of organic solvent, liquefying the polymer and allowing the hydrophobic block of the surfactant to penetrate the surface.[11] Finally, we deswell the particle by stripping the solvent. We find that many surfactants become permanently anchored to the surface after deswelling. The exposed hydrophilic blocks of these surfactants can readily be labeled with other molecules, providing long spacers for displaying proteins, antibodies, or DNA, for example.[12]

In the basic procedure illustrated in Scheme 1, we mix 0.1 mL of 10% (w/w) polystyrene particles (1 μm diameter, sulfate surface groups, Seradyn) in deionized water with 0.9 mL of 1% (w/w) of the triblock copolymer, poly(ethylene glycol)-*b*-poly(propylene glycol)-*b*-poly(ethylene glycol) [Pluronic F108: $(PEG)_{129}$-$(PPG)_{43}$-$(PEG)_{129}$, BASF], in deionized water. We then add toluene (99.8%, Aldrich) to the dispersion in a 1:1 toluene/polymer volume ratio and gently mix it for 12 h at room temperature. Toluene is a good solvent for polystyrene and is slightly soluble in water. As it diffuses into the polymer particles, they swell and the matrix becomes

Scheme 1



liquid.[13] Without a surfactant, the swollen particles coalesce, but when F108 is present, they remain stable, indicating that the surfactant adsorbs to the interface. The toluene can be removed by steam stripping the dispersion, a common method for removing organic solvents from polymer dispersions.[14] We simply heat an open container of the sample to 98 °C in a fume hood. (**Caution:** avoid combustion of the toluene vapor.) Because toluene and water have a large miscibility gap, this procedure removes nearly all the toluene. Finally, to remove excess F108, we wash the particles five times by centrifugation and redispersion in deionized water.

Figure 1 shows micrographs of the original polystyrene sulfate spheres and the F108-grafted particles prepared by this procedure. While the original sulfate particles aggregate rapidly in 1X phosphate-buffered saline (PBS) at 140 mmol/L NaCl, washed F108-grafted particles are stable for days even after the addition of 10 mmol/L $MgCl_2$ to the PBS. This stability was not reduced even after three additional cycles of heating to 98 °C for 5 min, followed by centrifugation/resuspension washing. If the initial swelling step is omitted, the particles lose stability after two room-temperature washes, indicating that the physical adsorption of F108 is reversible.

Comparable stability was seen after swelling particles were mixed with a chemically similar triblock, Pluronic F127 [$(PEG)_{97}$-$(PPG)_{56}$-

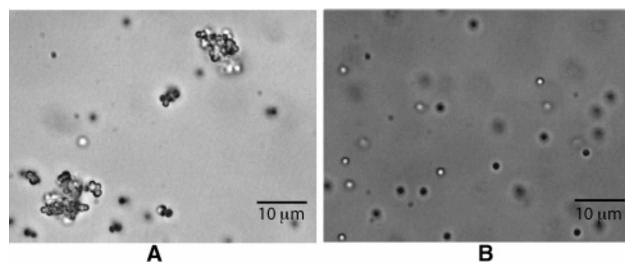


Figure 1. Optical microscope images of 1 μm polystyrene sulfate particles in high ionic strength solution: (A) bare polystyrene sulfate particles in phosphate-buffered saline (140 mmol/L NaCl); (B) Pluronic F108-grafted polystyrene sulfate particles in PBS with 10 mmol/L $MgCl_2$.

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$(PEG)_{97}$, BASF] and diblock [$(PEG)_{148}$-$(PPG)_{52}$, Polymer Source, Inc.]. The polymers, Pluronic P105 and F68, resulted in particles that were only marginally stable at 140 mmol/L NaCl (but still more stable than sulfate colloids), presumably due to the lower MW of their PEO blocks. Interestingly, substituting poly(butadiene)-*b*-poly(ethylene glycol) diblocks[15] [$(PB)_{102}$-$(PEG)_{110}$ or $(PB)_{93}$-$(PEG)_{442}$, Polymer Source] results in particles that rapidly aggregate after three washes.

A simple explanation for our observations is that during swelling, the PPG hydrophobic blocks mix with the polystyrene core, becoming trapped in the glassy polystyrene matrix upon deswelling. The reversibility of the PEG-PB polymer adsorption is presumably due to the lower miscibility of the PB block in PS preventing mixing or entrapment.

Flow cytometry allows us to quantify the number of polymer molecules grafted onto the particles. We attach the fluorescent label 4-(aminomethyl) fluorescein (Molecular Probes) to the hydroxyl end groups on the PEG blocks of F108 using the method of Monfardini et al.[15] Briefly, 0.5 g of F108, 40 mg of 4-nitrophenol chloroformate (4-NPCF), and 40 μL of triethylamine are added to methylene chloride to make up a final volume of 2 mL. The reaction, which activates the hydroxyl end groups of the PEG chains, proceeds at room temperature for 12 h. Then, 65 μL of 250 mmol/L 4-(aminomethyl) fluorescein is added to 20 μL of 1% (w/w) activated F108 in 50 mmol/L carbonate buffer at pH 9.5 to make up a final volume of 100 μL. The mixture is allowed to react for 4 h at room temperature with constant mixing. Once the 4-(aminomethyl) fluorescein is attached, 100 μL of 10% (w/w) PS particles, 100 μL of 1% (w/w) 4-(aminomethyl) fluorescein-attached F108, and 10 μL of toluene are added in 1X Tris/EDTA (TE) buffer at pH 8.0 to make up a final volume of 1 mL. We then follow the same swelling/deswelling procedure outlined above with TE buffer substituted for deionized water. After eight washes, flow cytometry indicates roughly 500 000 fluoresceins per particle, or about 1 PEG chain per 5 nm^2 of surface, comparable to equilibrium adsorption.[17]

Thus far, we have described what amounts to a simple method of preparing densely PEGylated, sterically stable particles. However, we can also label the hydroxyl end groups of the PEG with biomolecules via the same chemistry used to attach the 4-(aminomethyl) fluorescein. We demonstrate this by attaching 65 base-long single-stranded DNA[18] (ssDNA) onto two different kinds of polymer-based colloids, polystyrene sulfate and carboxylate-modified latex (CML) particles. We use the same coupling procedure described above, with 320 μmol/L 5′-amine-modified ssDNA substituted for 250 mmol/L 4-(aminomethyl) fluorescein. For the CML beads, we use three times as much ssDNA. Once the ssDNA/Pluronic is grafted onto the particles by swelling and deswelling, we hybridize a 28 base-long, fluorescent 5′-FITC-modified target ssDNA[19] to it. Three microliters of 300 μmol/L target ssDNA is mixed with 10 μL of 1% (w/w) DNA-functionalized particles in TE buffer at pH 8.0 (200 mmol/L NaCl) to make up a final volume of 100 μL. This mixture is incubated for 12 h at room temperature in the dark, then washed three times with TE buffer, pH 8.0, to remove excess fluorescent ssDNA. Flow cytometry indicates roughly 1500 DNA molecules per polystyrene sulfate particle and 4000 DNA molecules per CML particle. Since three times as much DNA was used to prepare the DNA/Pluronic complex for the CML beads, the final labeling yield appears limited by the coupling reaction, rather than the swelling procedure. While both particle types are stable in high salt buffers, the CML particles are more convenient for most applications because they are stable under high-shear mixing (e.g., vortexing).

Proteins or other molecules that cannot tolerate the elevated temperatures during steam stripping can be attached to the F108 after particle swelling and deswelling. We first graft F108, preactivated by 4-NPCF, onto the particles using a 10 mmol/L citric acid buffer at pH 2.5 to minimize hydrolysis of the activated end groups.[20] The amine-modified ssDNA is attached after increasing the pH to 9.5, and the FITC-modified target DNA is hybridized using the same procedure outlined above. After labeling, the particles do not aggregate, but tend to stick to the polyethylene centrifuge tubes used for washing, due perhaps to residual activated chloroformate groups. Using DNA concentrations similar to the first labeling reaction, this reverse method also yields 1500 DNA molecules per particle.

We have demonstrated a simple method for grafting molecules onto polymer microspheres. Unlike physical adsorption, the swelling method permanently anchors the molecules to the polymer surfaces. Unlike covalent coupling methods, the swelling method generally *increases* the stability of the microspheres, as the PEG chains of the anchored polymer provide a steric barrier to aggregation, even at high ionic strength. Our experiments suggest that the anchoring mechanism is simple entanglement, so that this method should be applicable to a wide variety of polymer colloids and polymer surfactants. Although here we have focused on the attachment of biomolecules, the method is scalable and could be used to modify the surfaces of industrial polymer colloids.

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