Fluorescent Reactive Core–Shell Composite Nanoparticles with A High Surface Concentration of Epoxy Functionalities

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A method for the synthesis of fluorescent core–shell nanoparticles with reactive epoxide functional groups on the surface has been developed as a platform for the generation of functional and labeled nanoparticles for diverse applications. Namely, poly(glycidyl methacrylate), PGMA, was used to form a reactive and fluorescent shell on the nanoparticle surface. Prior to deposition on the silica nanoparticle, the PGMA was fluorescently labeled with Rhodamine B via a reaction between the epoxy groups of the polymer and carboxyl groups of the dye. A strong fluorescence emission was observed for the colloidal suspension of the modified nanoparticles. Atomic force microscopy, dynamic light scattering, and thermogravimetric and elemental analysis were used to characterize the nanoparticles. The reactive nanoparticles are a convenient and straightforward platform to generate fluorescent particles with various molecules attached to their exterior, because epoxy groups (located on the particle exterior) are highly active in various chemical reactions. Reactivity of the particles was confirmed via modification of the surface of polyester fibers with the nanoparticles and synthesis of polymer grafted layers on their surface using "grafting to" and "grafting from" approaches. The newly designed technique will be applicable toward particles made from a wide variety of organic and inorganic materials, as long as the surface of material contains functional groups reactive with epoxy moiety.

Introduction

Increasing demand for the development of electronic components, optical detectors, and biochemical sensors with nanodimensions has initiated theoretical and experimental investigations into the area of nanoobjects. To this end, nanoparticles have attracted a great deal of attention because of their intriguing chemical, electronic, optical, and mechanical properties.[1–6] Significant efforts have been directed toward the preparation of (re)active fluorescent nanoparticles, which can serve as fluorescent bio/chemosensors.[7–12] The particulates recognize a selected substrate and signal via anchoring or variation of their fluorescence emission. Such particles are being actively developed for a wide range of applications, such as labeling, sensing, in vivo imaging, the biomolecular profiling of cancer biomarkers, and targeted drug delivery.[13–16]

To use fluorescent nanoparticles in a specific application, it is vital to functionalize them with organic ligands to achieve target specificity. Weissleder et al.[17,18] proposed a series of methods to modify the surface of aminated iron oxide nanoparticles with small molecules. Their strategy explores complex synthetic routes to attach small molecules with a number of reactive functional groups (anhydride, amine, hydroxyl, carboxyl, thiol, and epoxy) to the nanoparticle surface. A strategy based on silane chemistry to modify the surface of silica particles with a fluorescent label and functional groups has been developed.[12] Also, the modification of gold nanoparticles employing thiol-containing molecules has been extensively studied.[19]

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The modification of the particles with functional polymer layers has been attempted as well. The importance of this binding system is connected to its multifunctionality, where the same macromolecule has functional groups reactive with the nanoparticle surface and fluorescent dye, and functional groups available for further chemical reactions. In one instance, semiconductor fluorescent nanocrystals have been modified with multifunctional polymer ligands carrying reactive ester groups, which could be substituted with multiple anchors and functionalities.[20] Tremel et al. have also used reactive (via ester group) polymers to modify the surface of TiO_2 nanoparticles.[21] The developed strategy allows the functionalization of the nanoparticles by a polymer carrying functional groups capable of reacting with the nanoparticle surface and various molecules (fluorescent dyes and/or molecules possessing reactive functional groups). The polymer with necessary functions is prepared before the nanoparticle modification. The method, successfully used for TiO_2 nanoparticles, is expected to be effective for the modification of various metal oxides.

In the present communication, we report yet another original and effective method for generating fluorescent reactive (epoxydized) nanoparticles with a high surface concentration of epoxy functionalities. The method is developed as a robust platform for generation of functional and labeled nanoparticles for diverse applications. Specifically, a proven method of permanent surface modification of inorganic and polymeric (organic) surfaces with a dense polymer layer containing epoxide groups was transferred to the surface modification of nanoparticles.[22–25] This method is distinctive in that it can be applied to a variety of particles. The obtained epoxydized and fluorescent nanoparticles can react with a range of substrates/surfaces to create fluorescent objects. Additionally, the reactive nanoparticles are a convenient and straightforward platform to generate fluorescent particles with various molecules attached to their exterior, because epoxy groups are highly active in various chemical reactions. In the work presented, we demonstrate the modification of commercially available silica nanoparticles with the fluorescent epoxydized shell. In fact, the particulates obtained were fluorescent and reactive. To confirm the reactivity of the particles, we modified the surface of polyester fibers with the nanoparticles and synthesized the polymer grafted layers (brushes) on their surface using "grafting to" and "grafting from" approaches.[26–29]

Experimental Section

Materials. Bare silica particles in the form of nonporous, spherical beads with a hydroxyl surface (diameter 150 ± 30 nm, density 1.96 g/cm^3) were purchased from Polysciences, Inc., in an aqueous suspension. Highly polished single-crystal silicon wafers of a $\langle 100 \rangle$ orientation (Semiconductor Processing Co.) were used as substrates for model experiments and atomic force microscopy (AFM) imaging. The wafers were first cleaned in an ultrasonic bath for 30 min, placed in a hot (80 °C) "piranha" solution (3:1 conc. H_2SO_4:30% H_2O_2) for 1 h, and then rinsed several times with high-purity water. Glycidyl methacrylate, inhibitor removing column, and 2,2′-azobisisobutyronitrile were obtained from Aldrich Chemical Co., Inc. Rhodamine B was purchased from Alfa Aesar and used as received. Carboxy-terminated poly(2-vinyl pyridine) ($M_n = 40600$ g/mol, PDI 1.08) and carboxy terminated poly(ethylene glycol) ($M_n = 20500$ g/mol, PDI 1.08) were obtained from Polymer Source, Inc. Oligo(ethylene glycol) methyl ether methacrylate (OEGMA, typical $M_n = 300$ g/mol) was purchased from Aldrich. Copper(I) bromide (CuBr), copper(II) bromide ($CuBr_2$), 2,2′-bipyridine, and α-bromo- *iso*-butyric acid were used as received from Aldrich.

Characterization. Atomic force microscopy (AFM), thermogravimetric analysis (TGA), fluorescence spectroscopy (FS), fluorescent microscope, gel permeation chromatography (GPC), elemental analysis (EA), ^{1}H NMR, dynamic light scattering (DLS) analysis, and scanning electron microscopy (SEM) were employed in this study.

^{1}H NMR was performed on a Bruker Avance 300 spectrometer. EA of C, N, and H was performed using a PerkinElmer 2400 Series II CHNS/O elemental analyzer at 925 °C combustion temperature. The molecular weight and molecular weight distribution were determined at 30 °C by a Waters Breeze GPC system equipped with a 1525 binary HPLC pump, Styragel HR4E and HR5E columns heated to 33 °C, and a Waters 2414 refractive index detector. A mobile phase of $CHCl_3$ at a flow rate of 1 mL/min was used. Polystyrene samples of known molecular weights with narrow molecular weight distributions were used as standards for the molecular weight calculation. TGA data were obtained from a Hi-Res TGA 2950 thermogravimetric analyzer, TA Instruments. (The method for estimation of the thickness of polymer layers by TGA is provided in the Supporting Information.) A TA Instruments DSC 2920 differential scanning calorimeter was used to establish the glass-transition temperatures of PGMA. UV−vis absorption spectra were measured on a computer-controlled Shimadzu UV-3101 PC UV–vis−NIR scanning spectrophotometer. Emission spectra were recorded on a Jobin Yvon Fluorolog 3–222Tau spectrofluorometer. A fluorescent microscope, Olympus MVX, was also utilized in this study.

Ellipsometry was performed with a COMPEL automatic ellipsometer (InOmTech, Inc.) at an angle of incidence of 70°. Reproducibility of the ellipsometry measurements was better than ±10%. The refractive index used to calculate the thickness of PGMA was 1.525.[30] The particle diameter was determined by AFM in the taping mode, on a Dimension 3100 (Digital Instruments, Inc.) microscope. Silicon tips with spring constants of 50 N/m and radii of 10–20 nm were used. Imaging was done at scan rates in the range 0.75–1 Hz. For AFM imaging, nanoparticles were deposited (from a suspension in THF) on the surface of a silicon wafer by dip-coating. The hydrodynamic particle diameter was estimated with a DLS instrument (Brookhaven Instruments Corp., Austin, TX) operating in the ZetaPlus particle sizing extension mode. SEM studies were performed using a FESEM-Hitachi S4800.

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Synthesis of PGMA-RhB. PGMA ($M_n = 382000$ g/mol, PDI 2.5) was synthesized by the free-radical polymerization of glycidyl methacrylate in methyl ethyl ketone (MEK, Mallinckrodt Chemicals) at 45 °C. The obtained polymer was purified by multiple precipitations from the MEK solution using diethyl ether (VWR International). Azobisisobutyronitrile was used as an initiator. The polymer was then fluorescence-labeled with Rhodamine B (RhB) in the MEK solution (17:1 RhB:PGMA molar ratio). The reaction was allowed to proceed for 24 h at 70 °C. The polymer was then purified by multiple precipitations from the MEK solution using diethyl ether. The GPC analysis of the labeled PGMA-RhB showed a M_n of 402000 g/mol and PDI of 2.2.

Deposition of PGMA-RhB on Flat Model Surface. To determine the conditions for the modification of the nanoparticles with PGMA-RhB, we employed a linear temperature gradient heated stage. A uniform PGMA-RhB layer with a thickness of 150 nm was deposited onto a silicon wafer by dip-coating from a MEK solution. A copper bar was heated at one end by an embedded heating device; the other end was cooled by water. The temperatures of the hot and cold ends of the stage were 110 and 40 °C, respectively. The wafers covered with the PGMA-RhB layer were brought into close contact with the copper bar. After a certain period of annealing in the apparatus, the modified wafers were taken out and rinsed thoroughly with MEK to remove the unattached polymer.

Fabrication of Nanoparticles Covered with PGMA-RhB Shell. Functionalization of the silica nanoparticles was performed using 5.2 wt % (initial concentration) of the silica particles in water (as-received). Bare silica particles were dispersed in tetrahydrofuran (THF) by adding the aqueous suspension of the nanoparticles dropwise into the THF under constant ultrasonication. Water was completely removed by several centrifuge precipitations and the redispersing of the silica nanoparticle in the THF. Thus, a stable colloidal suspension of the particles in THF (about 1 wt %) was obtained and used for the further modification. Ten milliliters of the THF suspension containing the bare particles was injected dropwise into 24 mL of 3 wt % PGMA-RhB solution in THF under ultrasonication. The mixture was sonicated for 1 h, and the solvent was then rotary-evaporated under a nitrogen steam. The residual was annealed in a vacuum/nitrogen condition in an oil bath preheated to 60 °C for 30 min The particles were then redispersed in THF and purified. The purification process was typically repeated four times to remove any ungrafted polymer from the nanoparticle (process employs a centrifugation step for the isolation of the nanoparticles and ultrasound bath for redispersing the particles in THF).

Anchoring of the Fluorescent Nanoparticles To Polyester Fibers. Poly(ethylene terephthalate) (PET) polyester fabric (Dacron type heat set 122 g/m^2 fabric) obtained from Test Fabrics (style 777H) was used as a fibrous substrate. The fabric was initially rinsed in several solvents (water, acetone, toluene, ethanol, and final rinsing with water) to remove any finishes and other contaminants. The cleaned textile material was then subjected to a generally accepted hydrolysis procedure to increase the reactivity of a polyester fabric.[31] Namely, a 40% sodium hydroxide (NaOH) treatment for 2 min was carried out to hydrolyze the PET on the fiber surface and form additional carboxyl and hydroxyl groups on the fiber boundary. The fabric was then thoroughly rinsed in deionized water for about 1 h to remove all residuals and then dried in an oven at 80 °C until constant weight was acheived. The fabric was immersed under constant stirring in a suspension (0.1 – 0.5 wt % in THF) of the epoxydized silica nanoparticles at 50 °C for 3 h. After the treatment, the PET fabric was thoroughly rinsed in THF under constant ultrasonication, to remove unattached particles from the fibers.

Grafting of Carboxy-Terminated Polymers To the Fluorescent Nanoparticles. A THF suspension of the particles covered with PGMA-RhB (~1 wt %, 5 mL) was added into a 4% THF solution of P2VP-COOH or PEG-COOH (0.2 g) under ultrasonication. The samples were kept at room temperature for 1 h under ultrasound. The solvent was then rotary-evaporated under a stream of nitrogen. The samples containing particles dispersed in the polymer matrix were annealed in a vacuum at 110 °C for 30 min (P2VP) and at 70 °C for 24 h (PEG). The particulates were then rinsed with fresh THF four times (to remove any ungrafted polymers), employing a centrifuge for the separation.

Attachment of α-Bromo-iso-butyric Acid and Polymerization Initiated from the Nanoparticles. α-Bromo- *iso*-butyric acid (BBA) was attached to the nanoparticles modified with PGMA (nonfluorescent version) by the dispersion of the particles in a 10× excess (by weight) of a 3% solution of BBA in acetone in an ultrasonic bath. The solvent was removed by rotary evaporation and the residue was reacted at 60 °C for 1 h. After dilution with acetone, particles were recovered by centrifuging and purified from an excess of unreacted acid via multiple centrifuging and redispersing in ethanol.

Atom-transfer radical polymerization of OEGMA was carried out in oxygen-free conditions. For polymerization, 0.2 g of the nanoparticles, 10 mL of OEGMA, 5 mL of water, and 5 mL of ethanol were used. The amount of ethanol introduced together with particles was taken into account. The molar ratio of the components for ATRP was 300:1:0.5:3.1 OEGMA:CuBr:CuBr$_2$:biPy. First, a solution of monomer, water, and activating−deactivating copper complexes was prepared. A water solution of OEGMA was added to CuBr, CuBr$_2$, and biPy under a N$_2$ atmosphere. The mixture was degassed and stirred at room temperature until a homogeneous solution was obtained (approximately 2 h). The deoxygenated ethanol suspension of particles was then added. The reaction mixture was treated in an ultrasonic bath for a few minutes and allowed to polymerize for 4 h without agitation at 30 °C. Particles were centrifuged off (after 3-time dilution with ethanol) and purified by multiple centrifuging and redispersion in ethanol.

Results and Discussion

Synthesis of Rhodamine B-Labeled Poly(glycidyl methacrylate). To obtain fluorescent reactive nanoparticles, we used poly(glycidyl methacrylate) (PGMA) modified with a fluorescent dye for modification of the particle surface. We have selected the polymer with epoxy functionality, because the epoxy groups are quite universal. They can react with different functional groups (carboxy, hydroxy, amino, thiol, and anhydride) that are often present or can be created on the surface/boundary of various materials/objects. The epoxy groups of the polymer chemically anchor the PGMA to a surface.[32] The glycidyl methacrylate units located in the "loops" and "tails" sections of the attached macromolecules are not connected to a substrate boundary. These free groups serve as reactive sites for the subsequent attachment of molecules with functional groups, which exhibit an affinity for the epoxy-modified surface. Because epoxy groups are highly active in various chemical reactions, the approach

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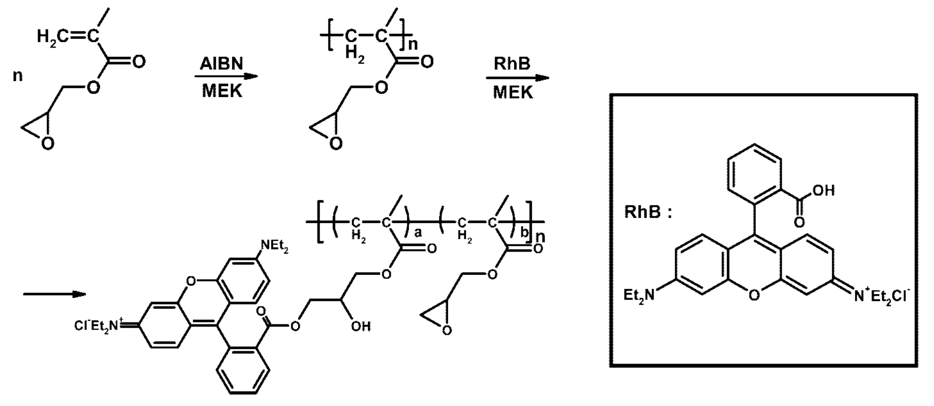
Scheme 1. Synthesis of Rhodamine B-Labeled Poly(glycidyl methacrylate)



becomes virtually universal toward both surface and functionalized (macro)molecules. We studied the attachment of PGMA to various surfaces and found that the polymer layer containing epoxy could be employed for grafting to inorganic (silicon, silica, glass, titanium, alumina, gold, silver) and polymeric (PET, cotton, nylon, polyethylene, polypropylene, polyvinylidenedifluoride (PVDF), silicon resin) surfaces.[22–24]

PGMA was synthesized by a free-radical polymerization in solution and fluorescently labeled with Rhodamine B, RhB, via the reaction between the epoxy groups of the polymer and carboxyl groups of the dye (Scheme 1). The obtained PGMA-RhB macromolecules are readily soluble in common organic solvents, such as THF, acetone, and MEK. A GPC analysis of the labeled PGMA-RhB (M_n = 402000 g/mol, PDI 2.2) did not show significant change in molecular weight and molecular weight distribution when compared with the starting polymer (M_n = 382000 g/mol, PDI 2.5). However, after labeling, the GPC profile contained an additional peak of a higher-molecular-weight fraction with the area of approximately 10% of the major peak. This result indicated that a certain cross-linking of the PGMA during the labeling process occurred.

The UV−vis absorption spectra of the PGMA-RhB and unlabeled PGMA in THF (1 wt %) are presented in Figure 1. The PGMA-RhB solution in THF exhibits a strong absorption peak at 560 nm, which is not present in the spectrum of the PGMA solution. The peak at 560 nm is due to the $\pi \rightarrow \pi^*$ transition of Rhodamine B segments. Fluorescence spectra were obtained as well for the PGMA-RhB solution and thin film (4.5 nm) dip-coated from the THF solution on a silicon wafer (Figure 2). Strong fluorescence emission (the excitation wavelength of 522 nm) was observed for the solution and film at 582 nm, which is characteristic of RhB. The emission was not observed for the solution and film obtained from the unlabeled polymer. The spectroscopic results indicated that RhB was indeed introduced into the PGMA macromolecules. The significant fluorescence signal obtained from the ultrathin layer of the polymer deposited on the silica surface, in fact, indicated that PGMA-RhB could be used to generate fluorescent nanoparticles via their surface modification with the polymer.

A ^{1}H NMR comparative study was conducted for the PGMA and PGMA-RhB macromolecules. Qualitatively, we observed a signal from the aromatic rings present in RhB. These peaks were not found for the unlabeled PGMA. In general, the spectra recorded were virtually identical for the polymers, indicating that the incorporated amount of RhB was on the border of the method sensitivity. Therefore, a very low number of the dye molecules were incorporated in the PGMA macromolecules, and epoxy groups were still present in the fluorescent polymer. The epoxy groups were, thus, still available for the anchoring to the particles and further chemical modifications. Elemental analysis showed that the labeled PGMA contained about nine RhB molecules per polymer chain (~0.3% of epoxy groups reacted with carboxylic groups of RhB, or 1 dye molecule per 311 monomer units). TGA measurements of PGMA-RhB were performed in nitrogen at a heating rate of 20 °C/min. There was no appreciable weight loss below 250 °C. The significant decomposition of the polymer took place at 275 °C. The thermal stability of the labeled polymer was practically the same as the stability of the unlabeled PGMA.

Covering of Silica Nanoparticles with an Ultrathin Layer of PGMA-RhB. The synthesized PGMA-RhB was used to prepare a fluorescent reactive anchoring polymer layer on the surface of nanoparticles. The first step toward the synthesis of the epoxydized fluorescent particles was to optimize the deposition parameters of PGMA-RhB. There are two potential methods to deposit the fluorescent reactive shell on the surface of the silica particles: adsorption from the solution and anchoring from polymer melt. In our work on the flat substrates, we favored the deposition from the melt, because dense cross-linked layers with exceptional stability were formed with this method.[33] Namely, the polymer was deposited from a solution by dip-coating and, after the solvent was quickly evaporated, the layer was annealed at an elevated temperature (close or above glass-transition temperature for PGMA, T_g = 59–61 °C [DSC]). During the annealing, self-cross-linking of the PGMA layer and stabilizing of the reactive coating occurred. It was demonstrated that a significant number of the epoxy groups survived the annealing procedure and were available for further reactions. Conversely, the PGMA layers obtained by adsorption from the solution demonstrated decreased stability.[33] Therefore, an annealing stage, where the nanoparticles incorporated into the bulk of the PGMA-RhB were kept at

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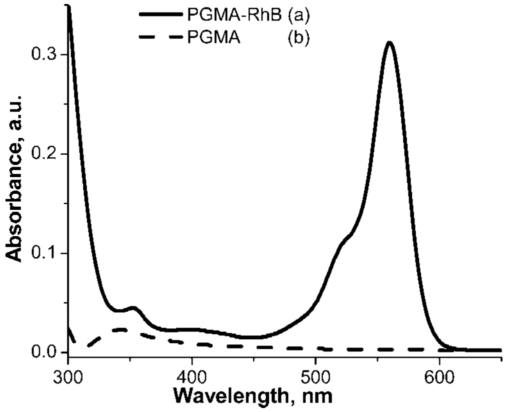

Figure 1. UV−vis spectra of the PGMA and PGMA-RhB solution in THF (1 wt %).

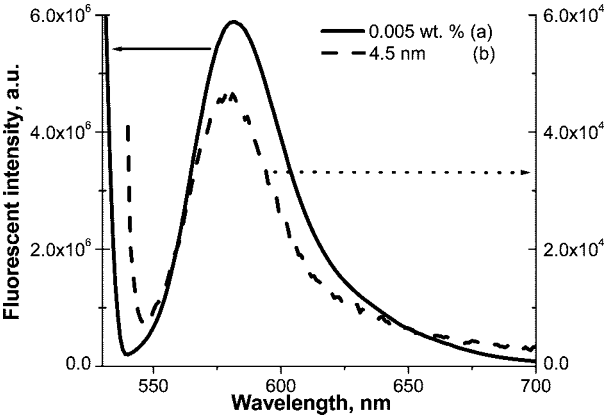

Figure 2. Fluorescent spectra of (a) PGMA-RhB solution in THF and (b) PGMA-RhB film deposited on the surface of a Si wafer by dip coating.

an elevated temperature, was included in our protocol for the surface modification.

In our preceding work, it was established (for flat substrates) that the thickness of the PGMA layer permanently attached to a surface from melt/bulk is dependent upon the temperature and time of the annealing process.[34] To determine the conditions for the modification of the nanoparticles with PGMA-RhB, a linear temperature gradient heated stage was employed. It was found that the thickness of the PGMA-RhB film attached to the surface of a model substrate (silicon wafer) strongly depends on the annealing time and temperature (Figure 3). The results obtained are in agreement with our earlier work, which also showed virtually linear growth of the thickness of the anchored polymer layer with temperature.[34] Therefore, the introduction of the fluorescent label did not significantly alter the physicochemical properties of the PGMA macromolecules. For the shorter time (30 min), the variation of the anchoring layer thickness along the substrate was between 3 and 10 nm. When the time was increased, a continuous gradient from 5 to 13 nm was created. Thus, by variation of temperature and time, an epoxydized fluorescent shell with a thickness between 3 and 13 nm can be readily fabricated.

The modification of the silica nanoparticles included several consecutive procedures. First of all the particles were removed from the water environment and redispersed in THF. Diameter and level of aggregation of the silica particles were determined from AFM images after their deposition on the surface of silicon wafers (Figure 4a). The average vertical distance (diameter) of original particles was about 150 nm (average deviation 12.5). Determination of average diameter was performed by measuring the cross-section of at least 60 single particles. AFM imaging indicates that no significant aggregation occurred (beside the lateral aggregation caused by capillary forces during drying) when the particles were transferred in THF from the water medium. The AFM results were confirmed with DLS measurements (Figure 5). The average hydrodynamic size of the particles in THF was about 118 nm.

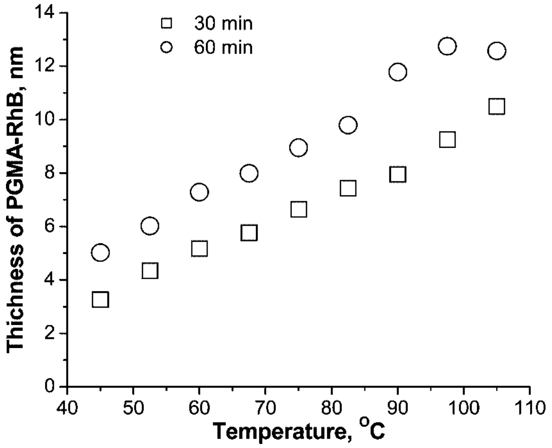

Figure 3. Thickness of the PGMA-RhB anchoring layer vs temperature for different times of annealing. Substrate was Si wafer.

Next, the colloidal suspension was injected dropwise into a 3 wt % PGMA-RhB solution in THF under ultrasonication. The mixture was sonicated for 1 h, and the solvent was then rotary-evaporated under a nitrogen stream. The residual nanoparticles/PGMA-RhB mixture was annealed in a vacuum. The following annealing conditions (giving the coating of about 5 nm for the flat substrate) were chosen for the attachment of PGMA-RhB to the nanoparticles: 30 min at 60 °C. The particles modified with PGMA-RhB were then dispersed in THF and purified via four centrifugal precipitation and redispersion in a fresh solvent. The UV/vis and fluorescence spectra for the colloidal suspension modified with PGMA-RhB are presented in Figure 6. The modified particles exhibit a strong absorption peak at 560 nm, which is not present in the spectrum of the unmodified colloidal suspension. A strong fluorescence emission (the excitation wavelength of 522 nm) was observed for the nanoparticles dispersed in THF as well. The spectroscopic results suggested that the PGMA-RhB layer was deposited permanently on the surface of the particles via covalent bonding between the surface silanol groups and the epoxy of the PGMA-RhB.

From the AFM imaging (Figure 4b), it was found that aggregation was minimal for the nanoparticles modified with PGMA-RhB, beside the lateral aggregation caused by capillary forces during drying. The average diameter of the particles, obtained from the cross-sectional AFM measurements, was 157 nm (average deviation 11.0). Accordingly, the thickness of the polymer layer was estimated to be

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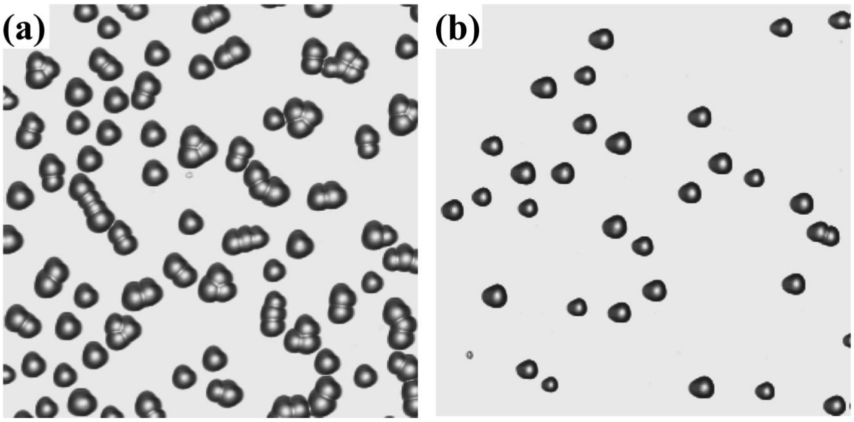

Figure 4. AFM images of (a) bare silica particles and (b) PGMA-RhB-covered particles deposited onto silicon wafer by dip coating. Image size: 5 × 5 μm^2.

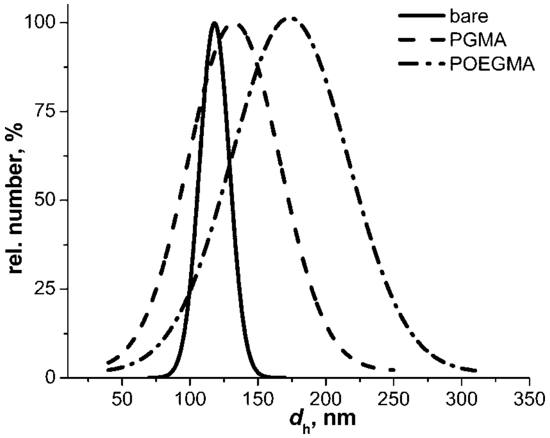

Figure 5. Hydrodynamic particle diameter d_h distribution of bare, PGMA-covered, and poly(OEGMA) brush nanoparticles dispersed in ethanol.

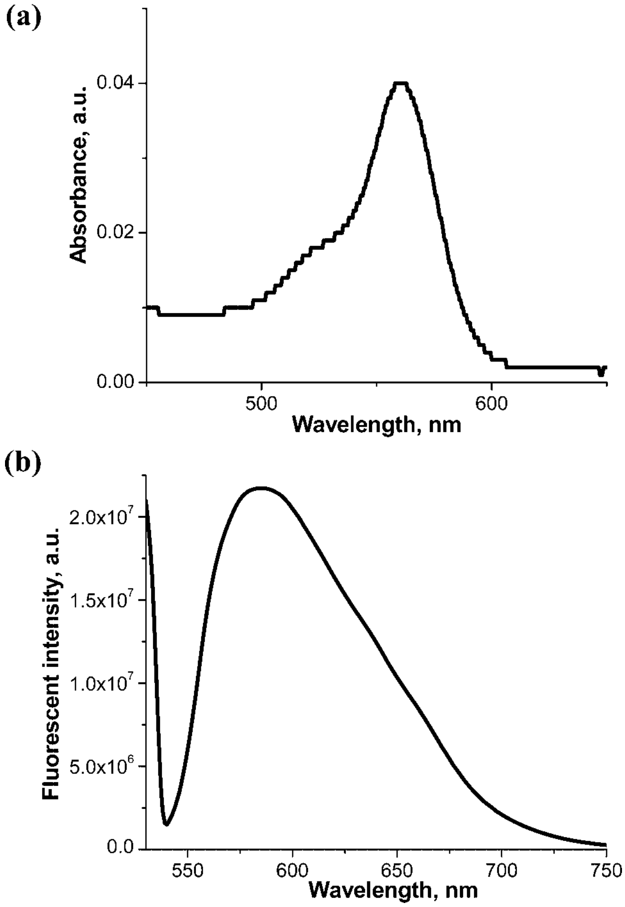

Figure 6. (a) UV−vis and (b) fluorescence spectra of PGMA-RhB-covered nanoparticles (0.1 wt % in THF).

~3–4 nm. The increase in particle size is close to the values expected from the model experiment conducted on the silicon wafer. DLS measurements were carried out for the particle modified (at the same conditions) with nonfluorescent PGMA to avoid complications with RhB UV−vis adsorption during the DSL experiment. Figure 5 shows the result of the measurements. The hydrodynamic radius for the particles in THF increased from 118 to 132 nm with somewhat increased polydispersity. Provided that the PGMA layer is capable of swelling in THF, the DSL results are in accord with the AFM thickness measurements. The thickness of the PGMA-RhB layer was additionally estimated by TGA and elemental analysis EA. TGA indicated that the thickness of the PGMA-RhB layer was 3.5 nm. EA shows the presence of C (3.260%) and H (1.455%) in the PGMA-RhB-covered nanoparticle sample. Such a result corresponds to ~3 nm of the polymer layer. Therefore, TGA and EA data were in agreement with the AFM measurements.

The surface coverage Γ, the PGMA-RhB chain density Σ, and the surface concentration of the epoxy groups present on the boundary of the functionalized silica nanoparticle (epoxy groups/nm^2 = ΣN, where N is the degree of polymerization) were also calculated for the 4 nm thick layer as described elsewhere.[35] The values of Γ and Σ are estimated to be 4.32 mg/m^{2}and 0.006 chain/nm^2, respectively. The surface concentration of epoxy groups is about 18 epoxy groups/nm^2. The concentration value represents the upper bound for the surface concentration of the epoxy functionalities in the PGMA-RhB layer.[36] The fraction of these groups is spent for the PGMA attachment to the silica particles. Additionally, the epoxy groups in the loops and tails may be lost because of PGMA self-cross-linking. Therefore, it is estimated (according to ref 36) that the PGMA-RhB layer deposited has a surface concentration of

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(35) Iyer, K. S.; Luzinov, I. *Macromolecules* **2004**, *37*, 9538.

(36) Zdyrko, B.; Varshney, S. K.; Luzinov, I. *Langmuir* **2004**, *20*, 6727.

active epoxy groups not less than 5 groups/nm^2. Therefore, by deposition of minute amounts of PGMA-RhB on the particle surface, a significant number of reactive groups were anchored to the nanoparticles.

During our fluorescence measurements, it was noticed that the fluorescent intensity of nanoparticles modified with PGMA-RhB is greater than the intensity of the polymer solution containing the equivalent concentration of the polymer. For example, the 0.1 wt % suspension of particles covered with ~3 nm of PGMA-RhB layer corresponds to 0.005 wt % polymer solution (close amount of polymer). However, the fluorescence signal from the colloidal system is more than five times higher (Figures 2 and 6b). Two possible reasons for the observed phenomena were suggested. First, PGMA-RhB anchored to the surface has a higher quantum yield than the polymer in solution. Second, during adsorption of the polymer on the surface (taking place during 1 h ultrasonication of the particles in PGMA-RhB solution prior the annealing) polymer fractionation occurs. Specifically, in view of the fact that there is a certain distribution of the dye molecules among the polymer chains, the macromolecules containing more dye molecules may preferentially adsorb on the particles. This preferential adsorption can be driven by the presence of nitrogen (capable of forming hydrogen bonding with silanol groups on the particle surface) in the dye molecule.

To differentiate between the two scenarios, we performed the following experiment. The PGMA-RhB layer was deposited on the silicon wafer in two different ways: by dip-coating the silicon wafer with a 0.16 wt % polymer solution (sample 1) and adsorption for 12 h in a 1 wt % solution (sample 2). The ellipsometric thicknesses of the layers obtained were 4.5 and 4.4 nm, respectively, for the dip-coated and adsorbed layers. In fact, it was found that the intensity of the fluorescence signal for sample 1 was much smaller than the intensity for sample 2. Therefore, we suggested that the polymer chains possessing a larger amount of RhB attached adsorb preferentially on the particle surface.

Reactivity of the Epoxydized Nanoparticles. The epoxy groups in the fluorescent polymer shell surrounding the silica particles can be used for subsequent reactions with various functional molecules/materials containing chemical groups active in reactions with epoxy functionalities. To confirm the reactivity, we modified the surface of the polyester fibers with the nanoparticles. The permanent attachment of the particles was realized via a reaction between epoxy groups on the nanoparticle surface and carboxy/hydroxy groups on the fiber surface. The fabric was immersed under constant stirring in a THF suspension of the epoxydized silica nanoparticles at 50 °C for 3 h. After the treatment, the PET fabric was thoroughly rinsed in THF under constant ultrasonication to remove unattached particles from the fibers. SEM studies were performed to observe the modified PET fabric. Figure 7a shows the SEM micrographs of the PET fabric after nanoparticle attachment. In general, the functionalized nanoparticles form a monolayer and are homogeneously distributed with very little aggregation. A strong fluorescence signal was observed from the fabric, confirming the deposition of the reactive nanoparticles (Figure 7b). Observation of the textile material under a fluorescent microscope confirmed that all fibers were evenly covered with the nanoparticles.

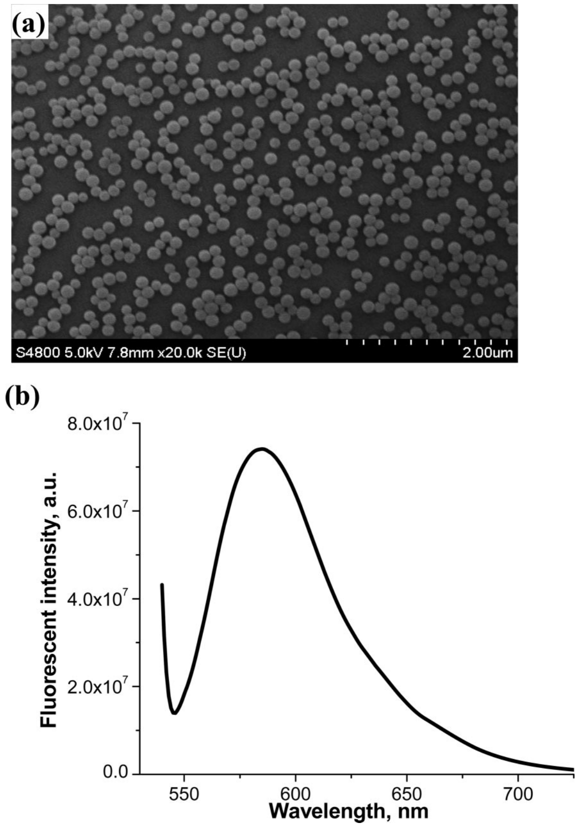

Figure 7. (a) SEM micrograph, (b) fluorescence spectrum of the PET fabric modified with PGMA-RhB nanoparticles.

The ability to functionalize the particles with anchored polymer chains was demonstrated by grafting polymers to the particle surface utilizing a “grafting to” approach, where end-functionalized polymer molecules react with complementary functional groups located on the surface to form tethered chains.[26] Namely, carboxy-terminated polymers poly(2-vinylpyridine) (P2VP) and poly(ethylene glycol) (PEG) were grafted to the particle surface via a reaction between carboxy and epoxy functionalities. Previously, we demonstrated grafting PVP and PEG brushes to a flat silicon surface activated with a PGMA-reactive layer.[25,30] Anchoring of P2VP macromolecules was used to prepare pH-responding particles.[37] Grafting of PEG chains yielded fluorescence-biocompatible nanoparticles.[38]

For the grafting, the dispersion of PGMA-RhB particles was added into a THF solution of P2VP-COOH or PEG-COOH under ultrasonication. The samples were kept at room temperature for 1 h in an ultrasonic bath. Then the solvent was rotary-evaporated. The samples containing particles dispersed in the polymer matrix were annealed in a vacuum at 110 °C for 30 min (P2VP) and at 70 °C for 24 h (PEG). The temperatures chosen for the grafting were above glass-transition temperatures for P2VP (98 °C) and close to melting

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(37) Li, D.; He, Q.; Cui, Y.; Li, J. *Chem. Mater.* **2007**, *19*, 412.

(38) Tshikhudo, T. R.; Wang, Z.; Brust, M. *Mater. Sci. Technol.* **2004**, *20*, 980.

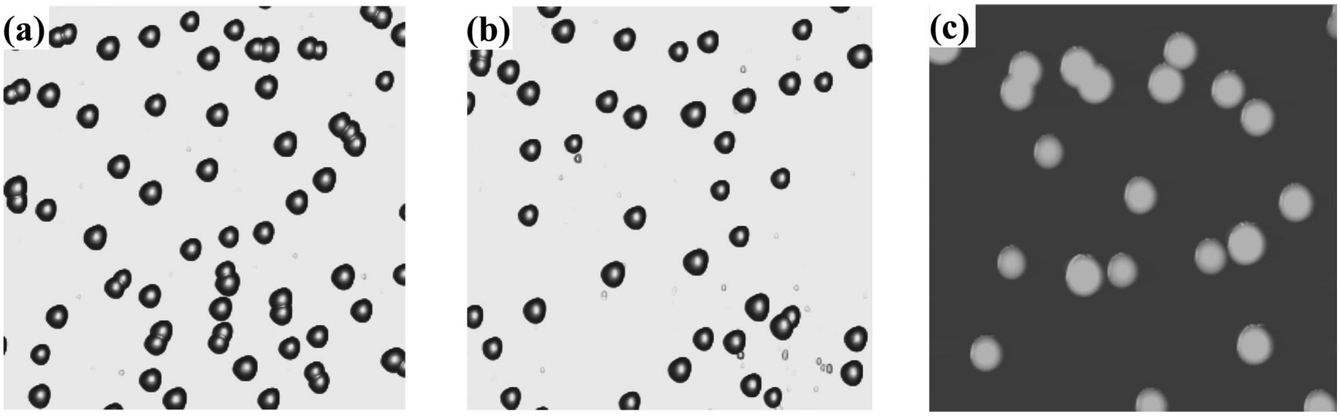
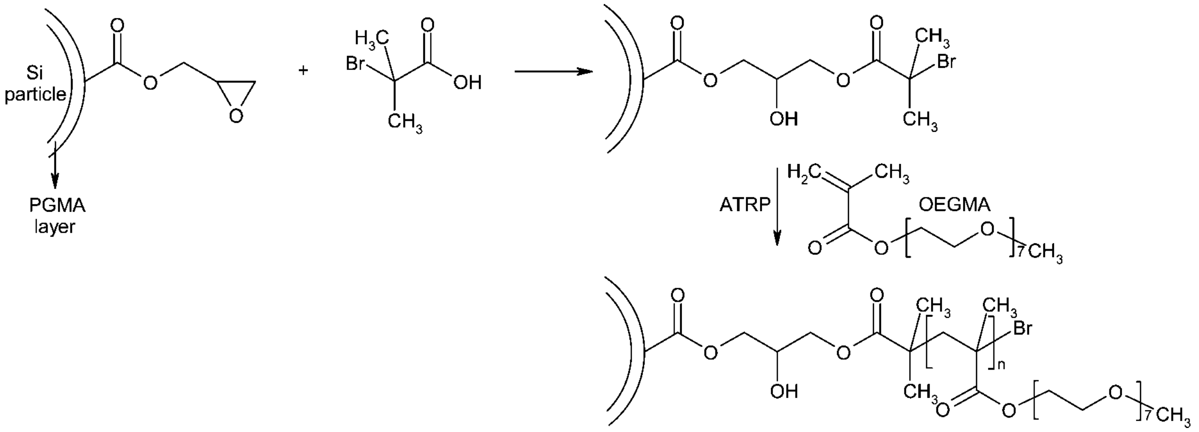


Figure 8. AFM images of (a) P2VP, (b) PEG, and (c) poly(OEGMA) particles deposited onto silicon wafer by dip coating. Image size: (a, b) 5 × 5 μm^2, (c) 3 × 3 μm^2.

Scheme 2. Synthesis of Poly(OEGMA) Brush on the Nanoparticles



temperature for PEG (62–76 °C).[39] Next, the particulates were precipitated by centrifuging and redispersed in fresh THF four times (to remove any ungrafted polymers). Finally, the stable fluorescent colloidal dispersion of the nanoparticles modified with polymer brushes was obtained.

AFM imaging (Figure 8) demonstrated that aggregation was insignificant for the nanoparticles modified with grafted P2VP and PEG chains. From the AFM images (Figure 8a), the average diameter of P2VP particles was 171 nm (average deviation 13) and, accordingly, 10 nm of the thickness of polymer layer (4 nm PGMA-RhB + 6 nm P2VP) was found. For PEG particles (Figure 8b), the average diameter was 164 nm (average deviation 16) and accordingly 7 nm of the thickness of polymer layer (4 nm PGMA-RhB + 3 nm PEG) was detected. Fluorescence spectroscopy revealed that further modification of the nanoparticles with the polymer brushes did not result in a significant decrease of the photoluminescence intensity.

To demonstrate modification of the nanoparticles by low-molecular-weight functional molecules, we reacted the nanoparticles, covered with PGMA (nonfluorescence version), with α-bromo-*iso*-butyric acid (BBA). The reaction was conducted at 60 °C for 1 h. After the reaction, particles were recovered by centrifuging and purified from excess of unreacted acid via multiple centrifuging and redispersing in ethanol. The reaction between the epoxy groups and the carboxyl functionality of the BBA produces an α-bromo-*iso*-butyric ester derivative of the PGMA. Such α-bromoesters are known as effective initiators for atom-transfer radical polymerization (ATRP) of styrene, acrylic, and some other vinyl monomers.[40] Consequently, the silica particles covered with the ATRP macromolecular initiator, covalently anchored to the surface, were obtained.

To verify the efficiency of the preparation of the particle initiators, we synthesized a poly(OEGMA) brush on the particle surface using a "grafting from" approach (Scheme 2). (In the "grafting from" technique, the polymerization is initiated from the substrate surface by attached initiating groups.[26]) The polymerization was performed by the addition of the proper amount of monomer and the CuBr/$CuBr_2$/bipyridine catalytic system, leading to the particles covered with the poly(OEGMA) brush.

The nanoparticles covered with the brush were analyzed with AFM and DLS. AFM imaging demonstrated that no significant aggregation was caused by the polymerization (Figure 8c). In general, individual particles were observed. It was impossible to accurately determine the thickness of the grafted layer from the AFM images, because the glass-

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(39) Bicerano, J. *Prediction of Polymer Properties*; Marcel Dekker: New York, 2002.

(40) Matyjashewski, K. In *Controlled Radical Polymerization*;ACS Symposium Series No. 685; American Chemical Society: Washington, D.C.; Chapter 16, p 258.

transition temperature of poly(OEGMA) is significantly below room temperature ($T_g = -11$ °C, estimated according to ref 39). The hydrodynamic size (Figure 5) of the particles modified with poly(OEGMA) (number average diameter 174 nm) increased upon polymerization, if compared to the particles modified with PGMA (number average diameter 132 nm). The obtained results indicated a successful grafting of the poly(OEGMA)-grafted layer on the surface of the silica nanoparticles.

Conclusions

We developed a novel method for the synthesis of fluorescent core–shell nanoparticles with a high surface concentration of reactive epoxide functional group. Namely, the particles were obtained through the modification of their surface with an epoxy-containing fluorescent polymer. Strong fluorescence emission was observed for the colloidal suspension of the nanoparticles. The reactive nanoparticles are a convenient and straightforward platform to generate particles with various molecules attached to their exterior. Reactivity of the particles was confirmed via modification of the surface of polyester fibers with the nanoparticles and synthesis of polymer grafted layers on their surface using "grafting to" and "grafting from" approaches. This newly designed technique will be applicable toward particles made from a wide variety of organic and inorganic materials, as long as the surface of material contains functional groups reactive with epoxy moiety.

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Supporting Information Available: Estimation of the thickness of PGMA-RhB layer on nanoparticles from TGA and EA data, fluorescence spectra of PGMA-RhB film deposited on the silicon wafer by adsorption and dip-coating, and fluorescence spectra of PGMA-RhB-coated and P2VP brush nanoparticles (PDF). This information is available free of charge via the Internet at http://pubs.acs.org.

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