Synthesis of Stable Block-Copolymer-Protected NaYF₄:Yb³⁺, Er³⁺ Up-Converter Phosphor Nanoparticles

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Near-infrared-to-visible up-conversion of light by rare earth ion-doped nanophosphors (NaYF₄:Yb³⁺,Er³⁺) opens new possibilities for improved biolabeling. A major obstacle to applications of up-converting nano phosphors (UCNPs) has been obtaining samples stable in serum media for biological applications. Previous researchers have made UCNPs stable in DI water, but not serum media. In this study, hexagonal phase nanophosphors were prepared using one-step cothermolysis utilizing oleic acid (OA) and trioclyl phosine (TOP) ligands. Two routes to polymer surface modification of the UCNPs were studied: direct ligand exchange using poly(acrylic acid) (PAA) and amphiphilic copolymer encapsulation via flash nano-precipitation (FNP). FNP-coated UCNPs were produced using three block-copolymers: poly(ethylene glycol)-block-poly(caprolactone) (PEG-b-PCL), poly(ethylene glycol)-block-poly(lactic-coglycolic acid) (PEG-b-PLGA), and poly(ethylene glycol)-block-lactic acid (PEG-b-PLA). Both surface modification routes produced colloidally stable UCNP dispersions in DI water. However, for the first time, we report the successful preparation of colloidal UCNPs stable in buffers and serum media (Leibovitz L15 media with added fetal bovine serum) using FNP and PEG surface coatings. The stabilizing block-copolymer layer added ca. 15 nm to the diameter of the phosphor crystals. UCNPs assembly of amorphous PLA or PLGA is strikingly different than for crystallizable PCL. These polymer-modified UCNPs provide promising new materials for applications in bioimaging and photodynamic therapy.

Introduction

Up-converting nano phosphors (UCNPs) are lanthanide-doped nanocrystals that potentially open new doors to a next generation of bioimaging.¹² UCNPs are capable of converting two or more near-infrared (NIR) photons to one visible light photon via sequential electronic excitation and energy transfer processes.³ Unlike conventional dyes or quantum dots, UCNPs are resistant to photobleaching and have low toxicity, minimal auto-fluorescence (background noise), sharp absorption and emission lines, high quantum yields, and long life times.⁴,⁵ Most importantly, the utilization of NIR excitation allows deep penetration imaging¹ and the different emissions produced by UCNPs upon NIR excitation permit multiple biolabeling.⁶ Photodynamic therapy (PDT) will also benefit from the deep tissue penetration of NIR excitation required by the phosphors and the low cost of NIR lasers. Currently, PDT utilizes visible light excitation to activate photosensitizers to produce cytotoxic singlet oxygen.⁷ The limited tissue penetration of visible light prohibits cancer cell destruction at distances greater than a few millimeters from the light source. The use of UCNPs, colocalized with photosensitizers, will significantly enhance PDT’s efficacy. We have previously demonstrated a successful colocalization of NaYF₄-based UCNPs and photosensitizers within PEG-containing block-copolymers.⁸ These PEGylated nanoparticles are able to produce singlet oxygen upon NIR radiation. NaYF₄-based UCNPs can be produced as cubic (α) or hexagonal (β) phase crystalline structures, but the β-phase NaYF₄ is the most efficient host matrix for up-conversion.⁹,¹⁰ Different lanthanide dopants (Er³⁺/Yb³⁺ or Tm³⁺/Yb³⁺) are embedded in the NaYF₄ to tune

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up-conversion emission spectra (green or blue emitting, respectively).\(^{10}\) Lanthanide-doped \(\text{NaYF}_4\) can be prepared via cothermolysis of trifluoroacetic precursors in the presence of various coordinating ligands to control the sizes of UCNPs with high up-conversion luminescence.\(^{2,11–15}\) The selection of a proper coordinating ligand or ligand combinations dictates the particle nucleation and growth and is one of the key factors for achieving monodisperse and size-tunable colloidal UCNPs. In the cothermolysis method, oleic acid (OA),\(^{2,11,12}\) oleylamine (OM),\(^{15}\) trioctylphosphine (TOP),\(^{14}\) trioctylphosphine oxide (TOPO),\(^{13}\) and OA-TOP\(^{16}\) ligands have been studied and proven to be effective in controlling the NaYF\(_4\) synthesis.

Since the UCNPs synthesized with the above ligands are inherently hydrophobic, a surface modification is required in order to make them hydrophilic and to facilitate stability in physiological conditions. There have been several reported attempts to make the surface of NaYF\(_4\)-based UCNPs hydrophilic, but all of them suffer from significant drawbacks. Chen and co-workers\(^{17}\) prepared oleic acid-capped UCNPs and then performed an oxidation reaction using Lemieux–von Rudloff reagent. The reagent is a strong oxidizer and cleaves the \(\text{C}_9 = \text{C}_{10}\) double bond in oleic acid. This reaction shortens the hydrophobic chains by \(9 - \text{C}_8\) units, leaving carboxylic groups at the end of the hydrophobic chains, yielding azelaic acid. As verified by Capobianco and co-workers,\(^{18}\) the carboxylic groups at the ends of the ligands did not provide sufficient water dispersibility and stability. Furthermore, prolonged oxidation led to excessive brown MnO\(_2\) formation which was difficult to separate and caused the UCNPs to aggregate and show weak up-conversion luminescence.\(^{18}\) Silica coating\(^{19,20}\) and amphiphilic poly(acrylic acid) (PAA) encapsulation\(^{19,21}\) have been attempted to produce water dispersibility but these methods resulted in UCNPs that aggregated in water within a few hours\(^{19}\). Surface stabilization by ligand exchange of oleic acid ligand with PAA produced stable UCNPs in DI water\(^{22}\) but particle aggregation occurred in buffers.\(^{18}\) Lastly, UCNPs have also been successfully stabilized with poly(ethylene imine) (PEI),\(^1\) but the cytotoxicity of PEI makes such formulation problematic for in vivo studies.\(^{23}\) The reported imaging results under NIR excitation showed serious particle aggregation with cells.\(^{1,19}\) For successful bioimaging, the particle aggregation must be addressed and could be solved by using UCNPs with an appropriate surface modification to impart colloidal stability in vitro and in vivo. Thus, more efficient surface modification methods need to be developed.

The phosphors used in this study are either 70 or 140 nm NaYF\(_4\)-Yb\(_3^+\), \(\text{Er}^3^+\) crystals produced via cothermolysis using OA-TOP co-ordinating ligand\(^{16}\) to produce bright \(\beta\)-phase UCNPs with hydrophobic surfaces.

This paper presents a new method that produces UCP NPs with dense PEG layers deposited by a novel block-copolymer-directed assembly process — Flash NanoPrecipitation (FNP). The amphiphilic block copolymers are irreversibly attached to the surface and create UCP NPs that are stable in buffers and serum media, which has not been previously achieved for NaYF\(_4\) NPs. As a control, UCNPs prepared with a PAA polymer by ligand exchange following Zhang et al.\(^{22}\) are prepared. The stabilities of both types of UCNPs in water, buffers and physiological media are studied. The encapsulation using FNP involves the rapid precipitation and self-assembly of hydrophobic UCNPs and PEG-diblock copolymers. The rapid mixing is achieved using a multi-inlet vortex mixer (MIVM).\(^{24,25}\) FNP has been used previously to form nanoparticles containing hydrophobic drug compounds,\(^{26,27}\) 5 nm gold nanostructures,\(^{28}\) molecular imaging agents,\(^{29}\) and peptides.\(^{30}\) The FNP process relies on rapid micromixing to produce supersaturation and kinetically controlled aggregation of hydrophobic solutes, with the block-copolymer directing self-assembly. In FNP, the following three time scales are important: (1) mixing time \((\tau_{\text{mix}})\), (2) nucleation and growth time \((\tau_{\text{ng}})\), and (3) self-assembly time of the block-copolymer \((\tau_s)\). To achieve homogeneous mixing, \(\tau_{\text{mix}}\) must be much smaller than \(\tau_{\text{ng}}\) and \(\tau_s\), and this is achieved using high intensity multi inlet vortex mixer (MIVM).\(^{24,31}\) Careful selection of stabilizers such that \(\tau_{\text{ng}}\) of the hydrophobic solute matches \(\tau_s\) of the stabilizer permits the formation of uniform-sized polymer-protected nanoparticles. An advantage of this FNP is that the block copolymer synthesis and purification can be performed prior to UCPN formation. In contrast, other techniques that might involve covalent reaction of PEG onto UCNPs particles coated with silica, PAA,
or PEI would require subsequent purification and isolation steps. In this paper, three different biodegradable polymers are studied as polymeric stabilizers for UCNPs prepared by the FNP process: methoxy-poly(ethylene glycol)-block-poly(caprolactone) (mPEG-b-PCL), methoxy-poly(ethylene glycol)-block-poly((D,L)-lactic-co-glycolic acid) (mPEG-b-PLGA), and methoxy-poly(ethylene glycol)-block-poly((L)-lactic acid) (mPEG-b-PLA). The choice of block-copolymer dictates the size and physio-
logical stability of the resulting UCNPs, with the PLA and PLGA polymers producing the most stable UCNPs. The unexpected role of block-copolymer crystallinity on stability is a major result of this study. The stability of the coated UCNPs opens new opportunities for in vivo bioimaging and photodynamic therapy.

Experimental Section

Synthesis of OA-TOP Stabilized NaYF₄:Yb³⁺:Er³⁺ UCNPs. Oleic acid (OA) (99%), trioctylphosphine (TOP) (90%), octa-
decene (ODE) (90%), sodium trifluoroacetate (98%) (reagent grade) and ethanol were purchased from Sigma-Aldrich and used as received. M₂O₃ (M = Y, Yb, and Er) (99.99%) were provided by Sunstone Inc. (Philadelphia, PA) and M-
(CF₃COO)₃ (=M(TFA)₃) were synthesized according to our previously published procedure. The synthesis of OA-TOP coated NaYF₄:Yb(20%), Er(2%) UCNPs was performed as follows: a mixture of 2.25 mmol Na(TFA), 1.46 mmol Y(TFA)₃, 0.375 mmol Yb(TFA)₃, and 0.0375 mmol Er(TFA)₃ was dispersed in OA/TOP/ODE (2 mL/2 mL/16 mL). Under vigorous stirring in a 50 mL flask, the mixture was first heated in an oil bath at 100 °C under a vacuum for 30 min to remove water, and then nitrogen was purged into the solution periodically. Under nitrogen blanket, the solution was heated with a heating mantle to a reflux temperature of 315 °C within 10–15 min. The reaction was stopped after 1 h of heating at reflux. The cooled solution was divided into two centrifuge tubes (45 mL) and ethanol (30 mL) was added to each tube to precipitate UCNPs. The UCNPs were isolated by centrifugation and were washed with excess ethanol three times. The product was then left to dry in the open atmosphere and the yield was between 250–300 mg.

Synthesis of mPEG-Protected NaYF₄:Yb³⁺:Er³⁺ UCNPs. Milli-Q water, with a resistivity of 18.2 MΩ cm, was used in nanoparticle preparation. Tetrahydrofuran (THF) was purchased from Sigma-Aldrich and triethylene glycol (TEG) anhydrous (97%) was purchased from Fluka. PAA ligand exchange on OA-TOP protected UCNPs was performed as follows: A TEG solution (15 mL) containing PAA (2 g) was heated to 110 °C with vigorous stirring under a N₂ flow. A toluene solution of OA-
TOP-capped UCNPs (20 mg) was injected into the hot solution, which became turbid immediately. The system was heated to 210 °C and kept at this temperature for 5 h at which point the solution became clearer. After the solution cooled to room temperature, 15–20 mL of dilute hydrochloric aqueous solution was added, and a white sticky powder was obtained by ultra-
centrifugation (50 000 rpm). The powder was washed three more times with DI water. These washed powders can be dispersed in water directly.

Synthesis of Methoxy-poly(ethylene glycol-block-caprolactone) (mPEG-b-PCL), Methoxy-poly(ethylene glycol-block-
lactic acid) (mPEG-b-PLA), and Methoxy-poly(ethylene glycol-
block-(lactic-co-glycolic acid)) (mPEG-b-PLGA) block-copoly-
mers. All reagents were purchased from Sigma-Aldrich, unless otherwise noted. ε-Caprolactone (ε-CL) monomer was distilled from calcium hydride and DL-lactide monomer was purified three times by recrystallization in toluene before use. Mono-
methoxy poly(ethylene glycol) (mPEG–OH, Mn = 5000 g/mol) was purchased from Nektar Inc. (Huntsville, AL) and was pretreated by azeotropic distillation in toluene to remove water. The synthesis of mPEG-b-PCL (with a target molecular weight of 5000-7000 Da) was performed on the basis of Shibasaki and co-workers’ procedure. mPEG–OH (2 g) and ε-CL monomers (2.8 g) were dissolved in anhydrous dichloromethane (100 mL). Hydrochloric acid (2 M in diethyl ether) (0.2 mL) was added as a catalyst and the reaction proceeded at 25 °C under nitrogen for 24 h. mPEG-b-PCL block copolymers were precipitated into ice-cold hexane, filtered, and vacuum-dried. The synthesis of mPEG-b-PLA (with a target molecular weight of 5000-10 000 Da) was performed on the basis of Ko and co-workers’ procedure. mPEG–OH (2 g) and DL-lactide monomer (4 g) were dissolved in anhydrous toluene (28 mL) in a silanized round-bottom flask. Tin(II) 2-ethylhexanoate (162 mg) was added into the solution as a catalyst. The reaction proceeded at 95 °C under nitrogen for 24 h. The toluene was evaporated to dryness and the polymer was redissolved in dichloromethane (10 mL) and filtered. The solution was then precipitated into an ice-cold mixture of 320 mL of hexane and 80 mL of diethyl ether, filtered, and vacuum-dried.

All manipulations of the somewhat moisture- and air-sensitive materials used in the synthesis of mPEG-b-PLGA block-copolymers were carried out in flame- or oven-dried glassware on a high vacuum-line or in a glovebox under a nitrogen atmosphere. Monomethoxy polyethylene glycol (mPEG) initiator (0.694 g) with a molecular weight of 2000 g/mol was added to a dry Schlenk flask equipped with a magnetic stirring bar. The flask was attached to the high-vacuum line and placed in a 90 °C oil bath for 12 h. Tin(II) 2-ethylhexanoate (40.0 mg), DL-lactide (2.00 g), and glycolide (1.62 g) were added to the Schlenk flask in the glovebox, and the resulting mixture was stirred for 24 h at 150 °C.4 The bulk polymerization mixture was cooled, dissolved in THF, and transferred to a dialysis tube (Spectra/Pro 7 RC, MWCO = 1000). The polymer solution was dialyzed with methanol for 2 days, and the contents of the bag were concentrated under vacuum. The 1H NMR spectra and polydispersity indices of these polymers can be found in the Supporting Information section.

Synthesis of PEG-Protected NaYF₄:Yb³⁺:Er³⁺ UCNPs. Poly-
(ethylene glycol) (PAA) (MW 1800) was purchased from Sigma-
Aldrich and triethylene glycol (TEG) anhydrous (97%) was purchased from Fluka. PAA ligand exchange on OA-TOP capped UCNPs was performed as follows: A TEG solution (15 mL) containing PAA (2 g) was heated to 110 °C with vigorous stirring under a N₂ flow. A toluene solution of OA-
TOP-capped UCNPs (20 mg) was injected into the hot solution, which became turbid immediately. The system was heated to 210 °C and kept at this temperature for 5 h at which point the solution became clearer. After the solution cooled to room temperature, 15–20 mL of dilute hydrochloric aqueous solution was added, and a white sticky powder was obtained by ultra-
centrifugation (50 000 rpm). The powder was washed three more times with DI water. These washed powders can be dispersed in water directly.

resulting solution of nanoparticles was dialyzed against Milli-Q water to remove THF using a Spectra/Por dialysis bag with MWCO of 6000–8000 (g/mol) (Spectrum Laboratories Inc., California) at room temperature. The synthesis of mPEG-b-PLGA- or mPEG-b-PLA-protected UCNPs is the same as described above, except mPEG-b-PLGA (MW 2000–10000 Da) or mPEG-b-PLA (MW 5000–10000 Da) block-copolymers was used instead of mPEG-b-PCL.

PEG-Protected Nanoparticle Stability in Water, PBS Buffer, and Culture Media. Leibovits L-15 (L-15), fetal bovine serum (FBS), and penicillin streptomycin were purchased from American Type Culture Collection (ATCC), Manassas, Va. The culture medium contained L-15 medium (45 mL), FBS (5 mL), and penicillin streptomycin (1 mL) and stored at 4 °C.

Dialyzed nanoparticles were stored at 4 °C in three different media: water, PBS buffer (10 mM, pH 7.4), and culture medium. The nanoparticles in different media were sampled at a specific time interval and diluted 1000 fold with DI water. The nanoparticle sizes and size distributions were characterized at 25 °C via dynamic light scattering (DLS, Nano ZS, Malvern Instruments, Worcestershire, U.K.).

Characterization

Transmission electron microscopy (TEM) images of uncoated UCNPs were obtained using a LEO/Zeiss 910 TEM (Carl Zeiss, Inc., Thornwood, NY) with a point-to-point resolution of 0.2 nm. A drop of uncoated UCNPs in hexanes was deposited onto a carbon film supported by a copper grid and dried in vacuum. Powder X-ray diffractometer (XRD, Miniflex, Rigaku, Japan) was used for crystal phase identification. Uncoated UCNPs were pasted on an alumina substrate and scanned in the 2θ range of 10–70°. The photoluminescence (PL) measurements were performed on solid UCNPs at 25 °C. A 980 nm laser diode (1 W, Lasermate Group, Inc., Pomona, California), focused (12 cm focal length) to a spot size of 0.5 mm was used as the excitation source. The PL signal was collected and detected by a photomultiplier module (H6780–04, Hamamatsu Corp, location) and was amplified by a lock-in amplifier (SR510, Stanford Research System, location) with an optical chopper (SR540, Stanford Research Systems). The signal was stored and analyzed using SpectraSense software data acquisition/analyser system (Princeton Instruments, Trenton, NJ).

Block-copolymers’ structures and molecular weights were determined by high-resolution 1H NMR (Varian INOVA 400 MHz spectrophotometer) in deuterated chloroform. The block-copolymer’s polydispersity indices were measured in tetrahydrofuran by gel permeation chromatography (GPC) (Waters, Inc., Milford, MA) equipped with Phenogel columns and a differential refractive index detector, calibrated with polystyrene standards (Polysciences Inc., Warrington, PA). Scanning electron microscopy (SEM) images of PEG-coated UCNPs were obtained using FEI XL30 FEG-SEM Sirion. To obtain SEM images, we spin-coated the dialyzed nanoparticles (Chemat Technology Spin Coater KW-4A, Northbridge, CA) on a clean silicon substrate at 14 000 rpm for 2 min, vacuum-dried at 25 °C overnight, and coated with a 5 nm iridium layer using VCR IBS/TM250 ion beam sputter.

Results and Discussion

The UCNPs are hexagonal prisms with an aspect ratio of approximately 1 and an average diameter of 140 nm as observed in the TEM image (Figure 1b, or 70 nm as shown in Figure 4). XRD measurements reveal that the crystalline NaYF₄ phase is hexagonal (β) (see the Supporting Information), which is the most efficient host material for green up-conversion.¹⁰ Up-conversion was confirmed by a PL intensity measurement. Upon excitation by near-infrared light source (λₙIR = 980 nm), green (λ = 540 ± 20 nm), and red (λ = 660 ± 10 nm) emissions are observed, as shown in Figure 2.

Polymeric Surface Modification. PAA Surface Modification. Fourier transform infrared (FT-IR) spectroscopy was employed to characterize the −COOH groups present on the surface of PAA-exchanged UCNPs.
In Figure 3 (top), the weak stretching mode at about 1700 cm\(^{-1}\) suggests the presence of trace amounts of free OA on the surface of UCNPs before ligand exchange. After the ligand exchange, a new peak is formed at 1730–1740 cm\(^{-1}\), signifying the presence carboxylic acid from the PAA chains.

Another direct demonstration of the presence of PAA ligand is that PAA coated UCNPs could be easily dispersed in water, forming a very stable solution without precipitation over months. Moreover, from the TEM images (Figure 4) of the two different-sized UCNPs before and after PAA exchange, it is seen that the sizes of the crystals are retained.

Although the primary crystal sizes are unaffected by the PAA ligand exchange process (Figure 4), dynamic light scattering (DLS) measurements on the particles suspended in water show that there are significant size increases of the two PAA ligand-exchanged UCNPs (Figure 5). Two samples dispersed and sonicated in water show average hydrodynamic diameters of 140 and 210 nm (shown in Figure 5) for the original crystal sizes of 70 and 140 nm by TEM, respectively. This indicates there is some level of aggregation that has occurred during surface functionalization, probably caused by PAA chains bridging between crystal surfaces, or anhydride linkages being created between acid residues on different crystal surfaces during the high-temperature PAA ligand-exchange step.

The instability of the PAA-coated UCNPs in buffers and in cell culture media with free proteins confirms what was observed by Capobianco and co-workers.\(^{(18)}\) In their work, 15 nm NaGdF\(_4\) Ho\(^{3+}\)/Yb\(^{3+}\) were used as their up-converting nanoparticles and PAA ligand exchange was performed on the OA-stabilized nanoparticles. Their results show that the PAA nanoparticles have prolonged stability in water but instability (aggregation) in buffer and serum media. Although the PAA-coated UCNPs are negatively charged (zeta potential of \(-40.5\) mV), the aggregation in buffer indicates that the PAA layer is not adequately dense to provide stabilization when the charges are screened by salt. The greater instability with serum protein is similar to instability observed for highly negatively charged liposomes in serum which has been attributed to protein adsorption and bridging.\(^{(35–38)}\) This instability greatly limits the application of PAA direct ligand exchanging coating technology for cell culture and tissue culture studies. In addition, studies on anionic liposomes\(^{(39,40)}\) indicate that protein adsorption onto highly anionic PAA surfaces will limit in vivo circulation of PAA-coated UCNPs.

PEG Surface Modification. PEG was chosen as the stabilizing polymer because it is known to prevent protein adsorption and bridging.\(^{(35–38)}\) This instability greatly limits the application of PAA direct ligand exchange coating technology for cell culture and tissue culture studies. In addition, studies on anionic liposomes\(^{(39,40)}\) indicate that protein adsorption onto highly anionic PAA surfaces will limit in vivo circulation of PAA-coated UCNPs.

References:

adsorption, which we believe was the mechanism of destabilization of the PAA UCNP s in serum. The diblock copolymer structure is chosen so that there can be no bridging between UCNP crystals— one end of the polymer is hydrophobic and anchors on the UCNP surface and the PEG end extends out into the aqueous phase. In contrast, the homopolymer PAA, which uses the same COOH groups to provide coordination anchoring on the UCNP surface and charge stabilization in the aqueous phase, can bridge between two particle surfaces. This is a common mechanism by which polymer induces flocculation of colloids.41,42 The PCL, PLA, and PLGA hydrophobic blocks were chosen because they are also known to be biocompatible and will ultimately degrade to nontoxic monomers over time scales from days to months.43,44 The PLGA has faster hydrolysis kinetics than PLA, and the crystallizable PCL block has the slowest hydrolysis kinetics.44,45 The three polymers displayed surprisingly different behaviors in stabilizing the UCNP s. The mPEG-b-PLGA and mPEG-b-PLA polymers provide excellent stabilization on the UCNP s. Figure 6a shows a size distribution of dialyzed mPEG-b-PLGA protected phosphors in DI water. Two populations are detected in the DLS measurement: one has a peak diameter of 160 nm, whereas the other has a peak diameter of 36 nm (Figure 6a). The smaller-sized population represents the inevitable formation of empty block-copolymer micelles, which can be easily removed by centrifugation. The 160 nm nanoparticles are the polymer-protected phosphors. As shown in the TEM image in Figure 2, the diameter of unprotected phosphors is 140 nm. This indicates that the PEG (MW: 2000 Da) chain contributes to approximately 10 nm of corona radius. The SEM image (Figure 6b) and the DLS measurements confirm that each nanoparticle consists of a single phosphor without the aggregation seen with PAA.

An mPEG-b-PLA stabilized UCNP sample was dialyzed and then centrifuged (Centrifuge 5415C, Brinkmann Instruments, Westbury, NY) for 15 min at 14 000 rpm and the pellet washed three times with DI water to remove micelles. These nanoparticles were reconstituted in three different aqueous phases: DI water, PBS buffer at 10 mM, and the culture medium with proteins. DLS measurements of unfiltered samples reveal that mPEG-b-PLA-protected phosphors are stable and the diameters are identical at 190 nm under these conditions (Figure 7). The centrifugation removes the micelle phase (Figure 7). The higher molecular weight PEG (MW: 5000 Da) produced a thicker corona layer of approximately 25 nm. The smaller peaks in the size distribution for nanoparticles incubated in culture medium represent the protein present in the medium. A size distribution of culture medium alone is available in the Supporting Information. The nanoparticles remain stable in all three media for at least up to 7 days as demonstrated in Figure 8.

Surprisingly, PEG-b-PCL UCNP s were significantly larger in size and more polydisperse than UCNP s stabilized by PEG-b-PLA or PEG-b-PLGA. DLS analysis in Figure 9a showed an average size of about 300 nm but

![Figure 6](image_url) (a) DLS size distribution and (b) SEM image of 140 nm UCNP coated with mPEG-b-PLGA polymer by Flash NanoPrecipitation. The small population of 30 nm objects in the DLS distribution are block-copolymer micelles, which are also visible in the SEM as gray smudges (i.e., low electron density objects) on the silicon wafer.

![Figure 7](image_url) Size distributions of mPEG-b-PLA-protected UCNP s (from Figure 6) in water (■), PBS (●), and culture media (▲). Centrifugation has been used to remove the micelle phase. The peaks at 10 and 30 nm are proteins in the fetal bovine serum culture media (see the Supporting Information for DLS data on culture media alone).

with a distribution that extends out to 3 μm, which would be a significant problem for IV applications of these particles. The broad distribution was stable in water over months without observed precipitation. However, these PEG-b-PCL UCNPs precipitated relatively rapidly in culture media. The TEM image of the PEG-b-PCL UCNPs confirmed there was no change in primary crystal size (Supporting Information Figure S4). However, the SEM in Figure 9b shows large domains of aggregated polymer. Similar behavior and instabilities of PEG-b-PCL micelles has been observed by Eisenberg and van de Ven.46 Furthermore, Zhang and co-workers also reported that micelles containing crystalline PCL core aggregate to form cylindrical micelles.47

The proposed mechanism involves the initial diffusion limited aggregation of the stabilizing polymer onto the surface of the UC crystal during the rapid FNP process. The packing on the surface is initially controlled by the packing of the soluble PEG chains.30 As the THF cosolvent phase is removed, the low T_g PCL chains are sufficiently mobile on the surface that they rearrange and begin to crystallize. That has one of two possible effects. In one scenario, the much denser packing of the PCL crystal chains on the particle surface scavenges the PEG chains into domains and leaves regions of the hydrophobic UCNP crystal phase exposed so that aggregation occurs. In another scenario, the lamellar morphology of the PCL crystal phase would result in PCL crystal sheets with unprotected PCL edges that would allow aggregation into larger lamellar structures. This later mechanism has been observed and studied with neutron scattering for lamellar crystallizing wax systems.48 Further studies will be required to unambiguously assign

Figure 8. Normalized size distributions of mPEG-b-PLA-protected UCNPs in (a) water, (b) PBS buffer, and (c) culture media for storage period of 7 days. There is no indication of particle growth or aggregation in any of the media.

Figure 9. DLS size distribution of mPEG-b-PCL protected up-conversion phosphors shows both nanometer sized particles (average diameter, ca. 300 nm) and micrometer-sized particles (a). The SEM image confirms this observation (b). The aggregated morphology is caused by the PCL crystallization which destabilizes the nanoparticles.

a mechanism. However, the conclusion is that the crystalline PCL block fails to create UCNPs that are stable under the required physiological conditions. In contrast, the amorphous PLA-b-PEG and PLGA-b-PEG polymers form coated UCNPs with the required stability and biocompatibility that has heretofore been unobtainable.

Conclusions

Hexagonal (β)-phase NaYF₄:Yb³⁺,Er³⁺ UCNPs with uniform size distributions of 70 and 140 nm were successfully synthesized via one-step cothermolysis using oleic acid/trioctyl phosphine (OA-TOP) ligand. Upon irradiation by near-infrared (NIR, λ = 980 nm) light, the nanophosphors produce two strong emissions at 540 nm (green) and 660 nm (red). The up-conversion properties of such phosphors are useful for addressing the short tissue penetration problem encountered by ultraviolet (UV) or visible light used in conventional down-converting imaging agents. We have developed the first UCNP dispersion that is stable under physiological conditions, in contrast to earlier silica, PAA, or PEI coatings. Block copolymer stabilizing layers produced by rapid, block copolymer directed precipitation (i.e., Flash NanoPrecipitation (FNP)) using PEG steric stabilizing layers and either PLA or PLGA hydrophobic anchoring blocks were demonstrated. The 2K PEG block produced a corona layer of approximately 10 nm and the 5K PEG a layer of 25 nm. The UCNPs were stable in DI water, PBS buffer or culture media with proteins without a change in size over 7 day periods. Interestingly, UCNPs prepared with crystallizable PCL blocks resulted in a broad particle size distribution material that was stable in DI water, but unstable in buffer or culture media.

The ability of the PEG-based polymer protected nanoparticles to remain stable in physiological condition potentially allows such formulations to be used for deep-penetration bioimaging and NIR photodynamic therapy. Our initial interest has been on preparing UCNPs for delivery to solid cancer tumors by the Enhanced Permeation and Retention mechanism (EPR) where particles in the size range 70–200 nm pass through defects in the microvasculature of solid tumors and are selectively deposited. For other imaging applications, sub-50 nm UCNPs will be required that more effectively transport through healthy tissue and can be systemically cleared. There will be additional challenges to make these smaller imaging agents. UCNP brightness decreases with decreasing size which provides additional challenges in the crystal synthesis. In addition, the relatively large hydrophobic groups (10K PLA or PLGA) that we have used to irreversibly anchor the polymer on the UCNP surface are probably larger than is desirable. Smaller hydrophobes and 2K or smaller PEG chains would maximize the fraction of the UCNP that is crystal relative to the volume taken by the stabilizer. However, as the anchoring hydrophobic polymer gets smaller the polymer may partition off of the UCNP surface in vivo and limit stability. To irreversibly anchor smaller hydrophobes on the surface, we may be required to cross-link the polymers to the surface. We are currently investigating surface cross-linking chemistries. The phosphors’ capability to utilize low energy, by NIR light in place of UV–visible light to produce sharp red and green emissions opens new opportunities for a next generation of photodynamic therapy. We are also addressing the efficacy of photosensitizer coated UCNP particles in vitro biological assays. Our ability to functionalize the PEG chains and incorporate targeting ligands is also being pursued to enhance the delivery of cytotoxic singlet oxygen to cancer cells and avoid damage to healthy tissue.

Supporting Information Available: ¹H NMR spectra of PEG-b-PLGA, PEG-b-PLA, and PEG-b-PCL; XRD of OA-TOP-capped NaYF₄:Yb³⁺, Er³⁺; and DLS size distribution of serum media alone (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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