Published in final edited form as:

ACS Appl Mater Interfaces. 2016 April 06; 8(13): 8608–8619. doi:10.1021/acsami.6b00727.

Block-Copolymer-Encapsulated CaWO₄ Nanoparticles: Synthesis, Formulation, and Characterization

Jaewon Lee¹, Nicholas J. Rancilio², Jean M. Poulson², and You-Yeon Won^{1,3,*}

¹School of Chemical Engineering, Purdue University, West Lafayette, IN 47907, USA

²Department of Veterinary Clinical Sciences, Purdue University, West Lafayette, IN 47907, USA

³Purdue University Center for Cancer Research, West Lafayette, IN 47907, USA

Abstract

We envision that CaWO₄ (CWO) nanocrystals have the potential for use in biomedical imaging and therapy because of the unique ways this material interacts with high energy radiation (Figure 1). These applications, however, require development of nanoparticle (NP) formulations that are suitable for in vivo applications; primarily, the formulated nanoparticles should be sufficiently small, chemically and biologically inert, and stable against aggregation under physiological conditions. The present study demonstrates one such method of formulation, in which CWO nanoparticles are encapsulated in bio-inert block copolymer (BCP) micelles. For this demonstration, we prepared three different CWO nanocrystal samples having different sizes (3, 10 and 70 nm in diameter) and shapes (elongated vs. truncated rhombic). Depending on the specific synthesis method used, the as-synthesized CWO NPs contain different surfactant materials (citric acid, cetyl trimethylammonium bromide, or a mixture of oleic acid and oleylamine) in the coating layers. Regardless of the type of surfactant, the original surfactant coating can be replaced with a new enclosure formed by BCP materials using a solvent exchange method. Two types of BCPs have been tested: poly(ethylene glycol-block-n-butyl acrylate) (PEG-PnBA), and poly(ethylene glycol-block-D,L-lactic acid) (PEG-PLA). Both BCPs are able to produce fully PEGylated CWO NPs that are stable against aggregation under physiological salt conditions for very long periods of time (for at least three months). The optical and radio-luminescence properties of both BCP-encapsulated and surfactant-coated CWO NPs were extensively characterized. The study confirms that the BCP coating structure does not influence the luminescence properties of CWO NPs.

TOC Graphic

*To whom correspondence should be addressed. yywon@ecn.purdue.edu.

Supporting Information: Photographs demonstrating the photo and radio luminescence of CWO NPs (Figure S1); ¹H NMR spectra and GPC traces for the BCPs used in this study (Figures S2 and S3, respectively); Optical micrographs of the B-CWO samples used in this study (Figure S4); TGA profiles of the BCPs used in this study (Figure S5); ¹H NMR spectra of the surfactants used in the syntheses of CWO NPs (Figure S6); Fluorescence and phosphorescence spectra of the surfactants and BCPs used in this study (Figure S7); UV absorption spectra of uncoated, surfactant-coated and BCP-coated CWO NPs (Figure S8); UV-visible absorption spectra of the BCPs and surfactants used in this study (Figure S9); OSL emission spectra of BCP-coated CWO NPs (Figures S10, S11, S12 and S13).

Keywords

Block copolymer; encapsulation; calcium tungstate; nanoparticle; stabilization; fluorescence; phosphorescence; radioluminescence

3. Introduction

CaWO₄ (CWO) crystals have been extensively studied for their unique luminescence properties.^{1–4} CWO produces a wide emission spectrum under excitation at wavelengths < 250 nm because of the tetrahedral coordination of WO₄ and the octahedral coordination of CaO₈;¹ the photographs shown in Figure S1 demonstrate the emission of blue light by CWO NPs under 250 nm UV or 6 MV X-ray excitation. Molecular orbital calculations suggest that WO₄^{2–} in the WO₄ tetrahedral state has a valence band composed of the oxygen 2p orbitals (O(2p_x + 2p_y + 2p_z)) and a conduction band composed of the tungsten 5d orbitals (W 5d_{xy} + 5d_{yz} + 5d_{xz}) (Figure 1).^{5–6} An electron transition from the W 5d_{xy} to O 2p molecular orbitals of WO₄ causes an emission of blue light at a wavelength of 420 nm.^{1, 5, 7–10} A green emission at 480 – 490 nm has been attributed to the oxygen-vacancy defect centers in WO₃ or the electron transition from the W 5d_{xy} to O 2p molecular orbitals of the WO₄^{2–} complex ion.^{1, 5, 7, 10} Red emission has also been observed from quenched CaWO₄ with longer-wavelength excitation and has been ascribed to electron transitions in higher tungstate complexes.^{2, 5, 9–10} The singlet state of CWO has a relatively long decay time constant (8 μs), which makes this material suitable for use in integrated X-Ray detection systems as a scintillation material.^{3, 7, 11} The optically and thermally stimulated luminescence properties of radiated CWO have also been studied in relation to potential applications in dosimetry.^{3, 12–14} Bulk CWO crystals are currently used in various applications including X-ray intensifying screens,¹⁵ light emitting diodes,⁸ scintillators,^{3, 11} and dosimetry.^{11–12}

CWO is chemically stable, does not liberate WO₄^{2–} ions and is insoluble in aqueous solution. Its MSDS classifies it as a nonhazardous substance possessing no carcinogenic properties and requires no precautionary statements. Studies investigating dental filling materials possessing radiopacity have tested unencapsulated CaWO₄ for biocompatibility and found no associated toxicity against periodontal and osteogenic cell lines.¹⁶ Additionally,

encapsulated CaWO_4 has been shown to possess no detectable cytotoxicity against HeLa cells.¹⁷ Currently, there are no published data on the toxicity of CWO in its nanoparticulate form. CWO NPs have poor colloidal stability in aqueous solution.¹⁸ Standard synthesis procedures for preparation of CWO NPs involve uses of such surfactant materials as sodium citrate^{19–21}, cetyl trimethylammonium bromide (CTAB),^{22–23} or poly(ethylene glycol) (PEG)²⁴ as a reaction-controlling and stabilizing agent. However, the stabilization capacities of these surfactants are very limited. Citrate is an anionic ligand.^{19, 25} CTAB forms a cationic coating.^{22, 26–27} PEG does not physisorb onto CWO, and the PEG-functionalization (“PEGylation”) of CWO NPs requires chemical modification of PEG.²⁴ One study reported the use of a non-ionic surfactant TritonX-100 for production of CWO NPs within the cyclohexane phase of a microemulsion system.²²

PEGylation of nanoparticles provides a steric barrier to the adsorption of opsonization proteins, resulting in reduced toxicity and improved pharmacokinetic properties.^{28–29} Chemical conjugation of pre-made PEG chains to the nanoparticle surface is a common method for producing PEGylated NPs.^{30–31} However, this method does not produce dense PEG grafted layers because of the steric hindrance caused by initially grafted PEG chains.³² Incomplete PEGylation of NPs results in poor colloidal stability. Alternatively, PEG-based amphiphilic block copolymers (BCPs) can be used to encapsulate NPs within PEG-coated polymer micelle structures via a solvent exchange process.^{30, 33–34} This method has been demonstrated to produce highly stable BCP/NP assemblies that maintain their stability and integrity after exposure to physiological environments.^{30, 35–36}

The present study demonstrates that stable BCP-encapsulated CWO NPs can also be produced by using this same solvent exchange technique. For this study, four different CWO samples were used: a commercially available bulk CWO powder sample (of 2 – 3 μm diameter; named hereafter as “B-CWO”), and three monodisperse CWO NP samples having different sizes (70, 10 and 3 nm in diameter) synthesized in our laboratory (called hereafter as “L-CWO”, “M-CWO”, and “S-CWO”, respectively). The as-purchased B-CWO material has no coating on the surface. The CWO NP samples were synthesized using three different surfactants: citric acid (L-CWO), CTAB (M-CWO), and a mixture of oleic acid and oleylamine (S-CWO). Shapes were also different among the different NP samples: elongated rhombic (L-CWO), truncated rhombic/round (M-CWO), and irregular (S-CWO). Regardless of the type of surfactant, the original surfactant coating could be successfully replaced with BCP materials using the solvent exchange method developed in our laboratory.³⁰ Two types of BCPs have been tested: poly(ethylene glycol-block-n-butyl acrylate) (PEG-PnBA), and poly(ethylene glycol-block-D,L-lactic acid) (PEG-PLA). Both polymers were found to be able to produce fully PEGylated CWO NP products that are essentially devoid of surfactant and are stable against aggregation under physiological salt concentrations for very long periods of time (for at least three months). Regardless of the size of the bare CWO nanocrystal material used, the mean hydrodynamic diameters of the resulting PEG-PLA BCP-encapsulated CWO NPs were determined to be always greater than those of the PEG-PnBA-encapsulated CWO NPs; in the PEG-PnBA/CWO assemblies single or a small number of CWO NPs were encapsulated within each BCP micelle, whereas in the PEG-PLA/CWO assemblies the NPs were, in general, encapsulated as clusters within the hydrophobic core domains of the BCP micelles. The optical and radio-luminescence

properties of both BCP-encapsulated and surfactant-coated CWO NPs were extensively characterized. The polymer encapsulation did not alter the luminescence activity of CWO. Nor did the polymer type, i.e., whether CWO NPs were encapsulated as single NPs or clusters.

4. Experimental

Syntheses of CWO NPs

B-CWO was purchased from Sigma-Aldrich and used as received. The L-CWO, M-CWO and S-CWO NP samples were synthesized using different procedures.

The L-CWO NPs were synthesized by a hydro-thermal method.²⁴ First, 0.006 mol of citrate acid (99.5%, Sigma-Aldrich) was dissolved in 20 ml of Milli-Q purified water. Next, 0.003 mol of Na_2WO_4 (99%, Acros Organics) and 0.006 mol of CaCl_2 (99%, Mallinckrodt Chemicals) were added to the solution. Then, an appropriate amount of NaOH (98.7%, Macron Chemicals) was added to the solution in order to adjust the pH to about 9. At pH < 4, no particles form. After the solution was then transferred into a Teflon-lined stainless steel autoclave, the temperature was increased to 160 °C, and the reaction was run for 24 hours. After the 24 hour incubation, the autoclave was gradually cooled to room temperature. The product was washed and centrifuged with Milli-Q water three times.

The M-CWO NPs were synthesized by a micro-emulsion method.²² First, 20 ml of cyclohexane was mixed with 2 ml of 1-hexanol. CTAB (2 mmol) (99%, Sigma) was added to this solvent mixture, and then the solution was heated to 70 °C or until the solution became transparent (Solution 1). Meanwhile, 0.4 mmol of Na_2WO_4 (99%, Acros Organics) was dissolved in 0.6 ml of Milli-Q water (Solution 2). Next, 0.4 mmol of CaCl_2 was dissolved in a mixture of 0.564 ml of Milli-Q water and 0.036 ml of 0.1 M HCl solution (Solution 3). Solutions 2 and 3 were immediately injected to Solution 1, and the resulting mixture was vigorously stirred. After about a minute, the mixture was transferred into a Teflon-lined stainless steel autoclave, and the autoclave was heated to 160 °C and maintained at that temperature for 24 hours. Afterwards, the autoclave was gradually cooled to room temperature. The product was collected by centrifugation and washed twice with ethanol and chloroform to remove residual cyclohexane and excess CTAB and 1-hexanol.

The S-CWO NPs were synthesized by a solvo-thermal method.^{8, 37} 0.001 mmol of Na_2WO_4 and 0.001 mmol of $\text{Ca}(\text{NO}_3)_2$ (99%, Sigma-Aldrich) were dissolved in 30 ml of ethylene glycol containing oleic acid (0.005 mmol) (99%, Sigma-Aldrich) and oleylamine (0.005 mmol) (70%, Aldrich) in a 50 ml round bottom flask. The mixture was rapidly heated to 160 °C in an oil bath and maintained at that temperature for 12 hours. During the reaction, the reactor was purged with nitrogen. Afterwards, the oil bath was removed, and the reactor was gradually cooled to room temperature. The product was purified by washing and centrifugation with excess ethanol.

Syntheses of BCPs

The PEG-PnBA BCP was synthesized by Reversible Addition-Fragmentation Chain-Transfer (RAFT) polymerization of nBA using monomethoxy PEG (PEG-ME, M_n 5,000 g/

mol, PDI 1.17, Polysciences, Inc.) as the starting material. 4-Cyano-4-(phenylcarbonothioylthio)pentanoic acid(CPCP, 97%, Aldrich)-end functionalized PEG (PEG-CPCP) was prepared by reacting PEG-ME with CPCP under the catalytic influence of 4-dimethylaminopyridine (DMAP, 99%, Aldrich) and *N,N'*-dicyclohexylcarbodiimide (DCC, 99%, Sigma-Aldrich).^{38–39} CPCP and DMAP were dissolved in dichloromethane (DCM, 99.8%, Sigma-Aldrich) at below 0 °C in a round bottom flask (Solution A). DCC was dissolved in DCM at below 0 °C in a different flask (Solution B). Solution B was added into Solution A at 0 °C temperature. The reaction mixture was sealed under nitrogen and was allowed to react at room temperature with agitation for 24 hours. Afterward, the reaction product was filtered and precipitated into excess ether to purify. This process was repeated two additional times to remove CPCP. The resulting precipitate was dried under vacuum and stored at low temperature.

For the RAFT polymerization a commercial grade of nBA monomer was passed through an Al₂O₃/MgSO₄ column to remove free radical inhibitors. PEG-CPCP was dissolved in THF. An appropriate amount of the purified nBA was added to this PEG-CPCP solution. The polymerization reaction was initiated by adding 4,4'-azobis(4-cyanovaleric acid) (ACVA) initiator to the above solution at room temperature. The molar ratio of ACVA to PEG-CPCP was 0.1:1. The reaction mixture was sealed in a round bottom flask under nitrogen and placed in a 80 °C oil bath. The reaction was allowed to proceed with stirring for 48 hours. To remove unreacted nBA the polymerization product was filtered and precipitated into excess ether. This process was repeated two additional times. The final precipitate was dried under vacuum and stored at low temperature.

The PEG-PLA diblock copolymer was synthesized by 1,8-diazabicyclo[5.4.0] undec-7-ene(DBU, 98%, Aldrich)-catalyzed ring-opening polymerization of lactide (LA, a racemic mixture). 0.45 g of PEG-ME was dissolved in DCM (22 ml) dried with molecular sieves. After a day LA (0.35 g) was added into the PEG-ME solution. The polymerization was initiated by adding 2 ml of a DBU solution (3.35 mmol of DBU dissolved in 30 ml of DCM) to the LA/PEG-ME mixture at room temperature. The polymerization reaction was run for 1 hour at room temperature. Afterward the reaction was terminated by adding 10 mg of benzoic acid (99.5%, Sigma-Aldrich). The polymerization mixture was added drop-wise to 1000 ml petroleum ether for precipitation. After the PEG-PLA product settled to the bottom, the supernatant was decanted. The polymer was dried in a vacuum oven.

Encapsulation of CWO NPs with BCPs

PEG-PnBA or PEG-PLA-encapsulated B-CWO and L-CWO samples were prepared as follows. 100.0 mg of PEG-PnBA or PEG-PLA was dissolved in 3.9 g of dimethylformamide (DMF, 99.9%, Sigma-Aldrich). 1.0 mg of B-CWO or L-CWO was dispersed in 2.1 g of Milli-Q water. The two solutions were mixed rapidly, mechanically stirred (15000 rpm), and ultrasonicated for 30 minutes. The mixture was then dialyzed using a dialysis bag (molecular weight cutoff 50 kDa) for 3 days against a total of 1.0 liter of Milli-Q water (replaced with fresh Milli-Q water three times) to remove DMF.³⁰

PEG-PnBA or PEG-PLA-encapsulated M-CWO and S-CWO samples were prepared slightly differently than the above. 1.0 mg of M-CWO or S-CWO (purified by centrifugation) was

dispersed in 1.0 g of DMF. 100.0 mg of PEG-PnBA or PEG-PLA was added to 2.9 g of the above nanoparticle suspension. This mixture was stirred using a high speed overhead mechanical stirrer (at 15000 rpm) with simultaneous sonication. 2.1 ml of Milli-Q water was added to the DMF solution. The resulting mixture was emulsified with a mechanical stirrer and then ultrasonicated in a sonication bath for 30 minutes. This emulsion was placed in a dialysis bag (molecular weight cutoff 50 kDa) and dialyzed for 3 days against a total of 1.0 liter of Milli-Q water (regularly replaced with fresh Milli-Q water) to remove DMF.³⁰

Characterizations of pristine CWO NPs

For **XRD** measurements, a small droplet of the as-synthesized CWO NP sample in solution (i.e., purified L-CWO NPs re-dispersed in water, purified M-CWO NPs re-dispersed in cyclohexane, or purified S-CWO NPs re-dispersed in chloroform) was placed on a glass microscope slide, and dried in a vacuum oven at room temperature overnight. This glass slide containing dried NP specimens was mounted on an X-ray diffractometer. Diffraction patterns were obtained over an angular (2θ) range from 10° to 90° with a scan rate of $1^\circ/\text{min}$. **TEM** specimens were prepared by placing a 10- μl drop of a CWO NP suspension (prepared as described in the previous subsection) on a Formvar/carbon-coated copper grid (Ted Pella) and drying the solution. TEM imaging was performed at 200 kV with an FEI Tecnai 20 transmission electron microscope. The resulting images were processed with Gatan's Digital Micrograph software (to correct for dark current and gain variations).

Characterizations of BCP-encapsulated CWO NPs

The sizes of BCP-coated CWO NPs dispersed in water were measured by **DLS** (ZetaPALS, Brookhaven Instruments). All measurements were performed at a fixed scattering angle of 90° using aqueous samples containing 0.025 mg/ml quantities of CWO NPs. The mean hydrodynamic diameters of BCP-encapsulated CWO NPs were estimated by the cumulant analysis. For **TEM** investigation of BCP-encapsulated CWO NPs, samples were prepared as follows. A Formvar/carbon-coated TEM grid was first plasma cleaned for 30 s in a Gatan's Solarus Plasma Cleaning System. Then, a 10- μl drop of an aqueous suspension containing about 0.25 mg/ml BCP-coated CWO NPs was placed on the plasma-cleaned TEM grid and dried under atmospheric conditions. Before the suspension was completely dried, 10 μl of 2% uranyl acetate solution in water was instilled on the sample-loaded grid. Once in place, excess solution was soaked up using filter paper, and then the sample was let completely dry at atmospheric conditions. These negatively stained samples were imaged using a 200 kV FEI Tecnai 20 transmission electron microscope.

Acquired images were processed with Gatan Digital Micrograph. **TGA** on dried BCP-coated CWO NP samples (initial weight: 30 mg) was performed in helium by heating the sample from room temperature to 700°C at a heating rate of $4^\circ\text{C}/\text{min}$ (SDT-Q600 TGA/DSC, TA Instruments). Chloroform-soluble extracts from BCP-coated CWO NPs were analyzed by ^1H **NMR** spectroscopy. Samples were prepared as follows. Water suspensions containing BCP-encapsulated CWO NPs were centrifuged at 5000 rpm for 30 minutes. The precipitate was isolated by decanting the supernatant liquid and dried in a vacuum oven at room temperature for 2 hours. The resulting solid mass was re-dispersed in deuterated chloroform

(Cambridge Isotope Laboratories). The chloroform-soluble components were characterized by NMR using a Bruker ARX300 spectrometer operating at 300 MHz.

Photo/radio luminescence characterizations

The fluorescence and phosphorescence properties of CWO NPs were characterized using a Cary Eclipse fluorescence spectrophotometer (Varian). Detailed measurement parameters are presented in the main text. For measurements of the optically stimulated luminescence (OSL) properties, CWO NP samples were irradiated with 6 MV X-rays using a Varian EX Linear Accelerator at a designated total X-ray dose (5 or 20 Gy) with a dose rate of 0.4 Gy/min.

5. Results & Discussion

Detailed structural characteristics (lattice structure and spacing, grain size and shape, and crystallinity) of CWO nanocrystals were characterized using the X-ray diffraction (XRD) (SmartLab, Rigaku) and high-resolution TEM (Tecnai 20, FEI) techniques. Figure 2 shows that the XRD patterns of all CWO samples agree with a simulated XRD pattern (PDF #07-0210), confirming that the NP syntheses were successful. The values of the full width at half maximum (FWHM) for the peaks at $2\theta = 28.775^\circ$ are estimated to be 0.21° (B-CWO), 0.76° (L-CWO), 0.49° (M-CWO), and 1.26° (S-CWO). It is known that the FWHM quantity is inversely related to the size of the crystalline domains; this relationship is known as the Scherrer equation.^{40–42}

The representative TEM image shown in Figure 3a demonstrates that L-CWO NPs have a rhombus shape, and their diagonal length is about 70 ± 25 nm. As shown in Figures 3d and 3g, M-CWO and S-CWO NPs were, on the other hand, observed to be close to cube and sphere-shaped, respectively; their diameters were estimated to be approximately 10 ± 2 and 3 ± 1 nm, respectively. It is interesting to note that this trend in NP size does not correlate directly with the trend in the size of the crystalline domains expected from the XRD FWHM data based on the Scherrer equation. It can be deduced that L-CWO NPs are composed of smaller crystal domains (“grains”) than M-CWO NPs. We also note that even though all NP synthesis reactions were run at an identical temperature (160°C) for an identical period of time (12 hours), the crystalline properties were found to vary among the three different NP samples depending on surfactant or solvent type used. The high-magnification images of the samples (Figures 3b, 3e and 3h) were further analyzed by Fast Fourier Transform (FFT). The spots in the FFTs were assigned to specific planes of reflection for the CWO crystal (Figures 3c, 3f and 3i). As shown in the figures, measured angles between two identified planes of reflection agree well with predictions, confirming the CWO crystal structure of the NPs.

CWO samples were encapsulated with PEG-PnBA or PEG-PLA using a solvent exchange method.³⁰ The number-average degrees of polymerization of the PnBA and PLA blocks of these PEG-PnBA and PEG-PLA BCPs were determined by ^1H NMR spectroscopy (ARX300, Bruker) to be 45 and 46, respectively (Figure S2 of the Supporting Information (SI)); thus in the SI these polymers are also denoted as PEG₁₁₃-PnBA₄₅ and PEG₁₁₃-PLA₄₆.

The polydispersity indices of these polymers were determined by GPC (Waters Breeze HPLC System) to be 1.38 (PEG-PnBA) and 1.16 (PEG-PLA) (Figure S3).

The hydrodynamic diameters of PEG-PnBA-encapsulated L-CWO, M-CWO and S-CWO NPs in Milli-Q water were measured by DLS (ZetaPALS, Brookhaven Instruments) to be approximately 70, 60 and 40 nm, respectively (Figures 4a, 4b and 4c). The hydrodynamic diameters of PEG-PLA-encapsulated L-CWO, M-CWO and S-CWO NPs were 190, 175 and 165 nm, respectively (Figures 4d, 4e and 4f). These BCP-encapsulated CWO NPs were confirmed to be stable against aggregation in physiological salt conditions; their hydrodynamic sizes did not change after addition of 150 mM NaCl over, at least, a 30-day period similarly to what was observed with Au nanorods.³⁰ PEG-PnBA and PEG-PLA-encapsulated B-CWO samples were also prepared and used to measure the size of the B-CWO particles by optical microscopy; the BCP coating delays the aggregation of B-CWO in water and thus enables imaging of individual B-CWO particles by microscopy. From the images shown in Figure S4, the sizes of the B-CWO particles were estimated to be in the range of about 2 – 3 μm .

Detailed morphologies of BCP-encapsulated CWO NPs were examined using TEM. In Figure 5 we present representative images of this study. The samples were negatively stained with uranyl acetate; in the negatively stained images shown in Figure 5, the light (i.e., unstained) regions correspond to the hydrophobic domains formed by the PnBA or PLA blocks, and the dark (electron dense) objects represent CWO NPs. As shown in Figures 5a through 5c, when CWO NPs were encapsulated with PEG-PnBA, typically less than a few NPs were incorporated within each BCP assembly. However, when encapsulated with PEG-PLA, CWO NPs formed clusters during the self-assembly processes (Figures 5d through 5f). These trends are in good agreement with the size data obtained by DLS testing.

TGA measurements were performed to determine the compositions of the BCP-encapsulated CWO NPs. As shown in Figure 6a, in PEG-PnBA-encapsulated L-CWO, M-CWO and S-CWO samples, the major weight loss occurred in two steps during heating. The first began as early as a little over 100 $^{\circ}\text{C}$ and continued to about 380 $^{\circ}\text{C}$ (which likely corresponds to the degradation of the PnBA block, the major component of the block copolymer), and the second ended at about 450 $^{\circ}\text{C}$ (corresponding to the degradation of the minor PEG component); this degradation pattern exactly matched what was observed with the pristine PEG-PnBA material (Figure S5a). The non-degradable solid content represents CWO. Different samples exhibited different levels of weight loss; i.e., a 38% weight loss was observed for PEG-PnBA-coated L-CWO NPs, a 47% weight loss for PEG-PnBA-coated M-CWO NPs, and a 51% weight loss for PEG-PnBA-coated S-CWO NPs. Figure 6b also displays results obtained from PEG-PLA-encapsulated L-CWO, M-CWO and S-CWO NPs. Again, the thermal degradation processes were seen to occur in two steps; the initial phase of weight loss continued up to about 320 $^{\circ}\text{C}$, and the second to about 380 $^{\circ}\text{C}$ (similarly to the TGA profile of pristine PEG-PLA (Figure S6b) except that in the presence of CWO NPs the PLA blocks degraded at significantly higher temperatures). PEG-PLA-coated L-CWO, M-CWO and S-CWO samples exhibited 42, 45 and 47% total weight losses, respectively, upon heating to 700 $^{\circ}\text{C}$. We note that in both PEG-PnBA-coated S-CWO and PEG-PLA-coated S-CWO samples (green curves Figures 6a and 6b), there was a slight weight loss at

approximately 150 °C, which indicates that there remained small amounts of residual oleic acid and oleylamine (4% in PEG-PnBA-coated S-CWO NPs, and 3% in PEG-PLA-coated S-CWO NPs); these surfactants were not completely removed during solvent exchange because of their strong hydrophobicity and hence high affinity for solids. Conversely, the TGA data confirm that all other BCP-encapsulated CWO NP formulations were indeed free of residual surfactants (sodium citrate, CTAB or 1-hexanol). As will be discussed below, these results were also confirmed by NMR analysis of chloroform extracts from BCP-coated CWO NPs.

As summarized in Table 1, from the TGA data, we were able to determine the values of the effective surface area of CWO NPs covered by a BCP molecule (α). In these calculations, we assumed for simplicity that regardless of the shape of the BCP/CWO NP assembly, each primary NP is fully coated with a BCP monolayer, because we do not have any information as to how the BCP molecules are arranged in the interstitial space within the cluster domain; therefore, the resulting α values represent the maximum likelihood estimates of PEG surface coverage. The values of the dimensionless PEG surface coverage, $\alpha/\pi R_{g,PEG}^2$ (where $R_{g,PEG}$ is the radius of gyration of the PEG block in the self-avoiding random-walk configuration), were also estimated and listed in the table. The $\alpha/\pi R_{g,PEG}^2$ values were found to lie in the range of 0.01 – 0.18, which suggests that the PEG corona chains are truly in the “brush” configuration (i.e., $\alpha/\pi R_{g,PEG}^2 < 1/\pi$).^{43–44} These results provide an explanation of why the BCP-encapsulated CWO NPs are so stable against aggregation (Figure 4).

In order to confirm whether the original surfactants used in the syntheses of CWO NP samples (i.e., sodium citrate, CTAB, 1-hexanol, oleic acid, and oleylamine) have been removed to the highest degree possible during the solvent exchange processes, chloroform-soluble extracts from the BCP-coated CWO NP products were analyzed by ¹H NMR spectroscopy; these surfactant compounds are inappropriate particularly for biomedical applications because of their cytotoxicity. As shown in Figure 7, NMR spectra showed no sign of residual surfactant (sodium citrate, CTAB, or 1-hexanol) in BCP-coated L-CWO and M-CWO samples; see Figure S6 of the SI for ¹H NMR spectra for citrate, CTAB and 1-hexanol. However, the NMR spectra of BCP-coated S-CWO NPs (Figures 7c and 7f) showed an additional peak at 5.4 ppm, which can be assigned to the ethylene protons of oleic acid and oleyl amine (Figures S6d and S6e). This data is consistent with the TGA results data (Figure 6), and suggests that the original oleic acid/oleylamine coating was not completely replaced with a new enclosure formed by PEG-PnBA/PEG-PLA BCPs. The possible influence of oleic acid/oleylamine on NP's toxicity in cells remains to be evaluated. It should also be noted that there is no significant difference in the detailed features of the NMR spectra (i.e., the relative areas under the peaks corresponding to the two component blocks) between the pristine BCPs and the chloroform extracts from the BCP-coated CWO NPs, which suggests that both the PEG-PnBA and PEG-PLA materials used in the present study contain a negligible amount of unreacted PEG homopolymer precursors; if the BCPs used had contained PEG homopolymer impurities, those homopolymer compounds should have been removed during the process of extraction of BCPs from BCP-coated NPs, resulting in changes in the relative areas of various peaks.

The luminescence spectra of the B-CWO, L-CWO, M-CWO and S-CWO samples in their non-BCP-coated forms were recorded with 200 nm excitation (Cary Eclipse, Varian) (Figure 8); this wavelength is shorter than that corresponding to the reported band gap energy of bulk CWO ($5.27 \text{ eV} = 235 \text{ nm}$)¹ and longer than that corresponding to the work function of CWO ($12 \text{ eV} = 103 \text{ nm}$).^{1, 5} All measurements were performed at an identical CWO concentration of 0.1 mg/ml. The B-CWO and L-CWO samples were dispersed in water. The M-CWO and S-CWO samples were dispersed in chloroform. As shown in Figure S7, organic components in the samples (i.e., BCPs, residual surfactants, and solvents) do not produce any significant luminescence signals. For comparison, the luminescence spectra of PEG-PnBA or PEG-PLA-encapsulated CWO samples (at a CWO concentration of 0.1 mg/ml in Milli-Q water) were also measured with 200 nm excitation. The emission intensities were measured in the 350 – 600 nm region in the fluorescence mode under zero delay-time/decay-time conditions. As can be seen from Figure 8a, for a given total amount of CWO material, smaller particles, in general, produce higher emissions because they have larger surface areas. However, among the four samples tested M-CWO (instead of S-CWO having the smallest size) showed the highest fluorescence because of the relatively poor crystallinity of the S-CWO sample (Figure 2). Despite the differences in intensity, the shapes of the spectra were found to be identical among all samples tested. Also as shown in Figures 8b and 8c, the PEG-PnBA and PEG-PLA coating structures did not influence the main features of the fluorescence spectra nor the intensity trends among the different CWO samples.

The fluorescence band gap energies of the different CWO samples were evaluated by measuring their emission intensities at the peak emission wavelength of 420 nm under varying excitation wavelengths in the range of 200 – 280 nm, as demonstrated in the literature^{1, 45} (Figures 8d, 8e and 8f). Of note, UV absorbance measurement could not be used for the determination of the band gap energy because, as shown in Figures S8 and S9, BCP and surfactant materials used in this study strongly absorb UV light. However, none of these materials produces a significant emission that can interfere with the CWO fluorescence at 420 nm (Figure S7). From the data shown in Figure 8d, for instance, the band gap energies of the non-BCP-coated CWO materials were estimated to be 4.62 eV (B-CWO), 5.44 eV (L-CWO), 5.46 eV (M-CWO) and 5.46 eV (S-CWO). Also from Figures 8e and 8f, the following band gap energy values were obtained: 4.58 eV (PEG-PnBA-coated B-CWO), 5.45 eV (PEG-PnBA-coated L-CWO), 5.47 eV (PEG-PnBA-coated M-CWO), and 5.48 eV (PEG-PnBA-coated S-CWO); 4.58 eV (PEG-PLA-coated B-CWO), 5.44 eV (PEG-PLA-coated L-CWO), 5.46 eV (PEG-PLA-coated M-CWO), and 5.50 eV (PEG-PLA-coated S-CWO) (Table 2). These results clearly indicate that the nanoscale size causes a slight (but significant) blue shift of the emission spectra due to the quantum confinement effect.^{45–46} Also, there might exist differences in molecular orbitals between the bulk and nanoparticle forms of CWO; it has been reported that the band gap energy of CWO decreases to 4.11 eV when W is dislocated away from O by 0.3 Å.² Figures 8d, 8e and 8f also demonstrate that non-BCP-coated and BCP-coated CWO samples give similar excitation spectra, indicating that the BCP encapsulation process does not influence the electronic structure of the CWO material.

The phosphorescence spectra of the CWO samples were also measured with 200 nm excitation using the phosphorescence mode with a delay time of 0.2 ms and a gate time of 5 ms. All measurements were performed at an identical CWO concentration of 0.1 mg/ml. All spectra showed two broad peaks at around 480 nm (due to charge transfer transitions within the WO_4^{2-} molecular orbitals) and 680 nm (likely due to electron transitions in higher tungstate complexes)^{1, 5} (Figures 9a, 9b and 9c). The sample-dependent trends in phosphorescence intensity were similar to those seen in the fluorescence measurements; M-CWO shows the highest phosphorescence, whereas B-CWO shows the lowest (Figures 9a, 9b and 9c). PEG-PnBA and PEG-PLA did not affect the phosphorescence spectra of CWO.

The phosphorescence emission intensities at the peak emission wavelength of 480 nm were measured as a function of excitation wavelength from 200 to 330 nm. The results are presented in Figures 9d, 9e and 9f; note in these figures the data are presented in normalized form. From the data shown in Figure 9a, for instance, the phosphorescence band gap energies of the non-BCP-coated CWO materials were estimated to be 4.39 eV (B-CWO), 4.38 eV (L-CWO), 4.39 eV (M-CWO) and 4.38 eV (S-CWO). Also from Figures 9e and 9f, the following band gap energy values were obtained: 4.40 eV (PEG-PnBA-coated B-CWO), 4.41 eV (PEG-PnBA-coated L-CWO), 4.39 eV (PEG-PnBA-coated M-CWO), and 4.40 eV (PEG-PnBA-coated S-CWO); 4.40 eV (PEG-PLA-coated B-CWO), 4.43 eV (PEG-PLA-coated L-CWO), 4.40 eV (PEG-PLA-coated M-CWO), and 4.40 eV (PEG-PLA-coated S-CWO) (Table. 2). These values are significantly different from the fluorescence band gap energies (4.62 – 5.46 eV); the fluorescence and phosphorescence processes involve different electron pathways (Figure 1). In both non-BCP-coated and BCP-coated CWO samples the nanoscale size was found to have no influence on the phosphorescence band gap energies (Figures 9d, 9e and 9f), which is in contrast to what was observed in the analysis of the fluorescence data (Figures 8d, 8e and 8f). Overall, the BCP coating did not significantly influence the phosphorescence properties of CWO.

The optically stimulated luminescence (OSL) properties of BCP-encapsulated CWO NPs were characterized following irradiation with 6 MV X-rays (Varian EX Linear Accelerator). Measurements were performed at two different CWO concentrations: 1.0 and 0.1 mg/ml CWO in Milli-Q water. Samples were irradiated at two different total X-ray doses (20 and 5 Gy) with the same dose rate of 0.4 Gy/min. The OSL spectra of the X-ray irradiated samples were measured with 700 nm excitation in the fluorescence mode under zero delay-time/decay-time conditions (Cary Eclipse, Varian) (Figures 10, S10 through S13). The emission intensities were recorded in the 250 – 650 nm wavelength range. As schematically explained in Figure 1, electrons trapped in defects within CWO crystal lattices are re-excited by 700 nm light, and the release of these re-excited electrons into the near-valence-band states can thus produce emissions at wavelengths shorter than the 700-nm excitation wavelength.¹³ We suspect that oxygen vacancies in the crystal structure of CWO and/or dangling bonds at the material's surface serve as the electron-trapping sites, which appears to be supported by the observation that B-CWO displays almost no OSL characteristics; see Figures 10, S10 through S13. Also as shown in these figures, CWO NPs clearly exhibited OSL signals, which typically decayed within 2 – 3 hours.

6. Conclusions

CWO NPs of different sizes and shapes were prepared by the reaction of sodium tungstate dihydrate with calcium salt using different combinations of solvent and surfactant. These CWO samples exhibited differing degrees of crystallinity. These CWO NPs were encapsulated in biocompatible BCPs such as PEG-PnBA or PEG-PLA. BCP-encapsulated NPs were stable against aggregation in physiological salt conditions for very long periods of time (for at least three months). The luminescence properties of both pristine and BCP-encapsulated CWO NPs were extensively characterized. For an identical mass of CWO material, NPs showed much stronger luminescence (fluorescence/phosphorescence/OSL following X-ray irradiation) than bulk powder because NPs have larger surface areas. CWO NPs were measured to have higher fluorescence band gap energies than bulk CWO. The BCP coating structure did not influence the luminescence properties of CWO NPs. BCP-encapsulated CWO NPs are promising materials for biomedical applications because of their colloidal stability, biocompatibility, and unique optical and radio-luminescence properties. The present study provides a strong basis for further studies of BCP-encapsulated CWO NPs for specific applications.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported by the “NIH New R01 Program” of the Purdue University Office of Executive Vice President for Research and Partnerships and the US National Science Foundation (CBET-1264336). The authors gratefully acknowledge the support from the Purdue University Center for Cancer Research (NIH Grant P30 CA023168).

References

1. Cavalcante LS, Longo VM, Sczancoski JC, Almeida MAP, Batista AA, Varela JA, Orlandi MO, Longo E, Li MS. Electronic Structure, Growth Mechanism and Photoluminescence of CaWO₄ Crystals. *CrystEngComm*. 2012; 14:853–868.
2. Orhan E, Anicete-Santos M, Maurera MAMA, Pontes FM, Souza AG, Andrs J, Beltrn A, Varela JA, Pizani PS, Taft CA, Longo E. Towards an Insight on the Photoluminescence of Disordered CaWO₄ from a Joint Experimental and Theoretical Analysis. *J Solid State Chem*. 2005; 178:1284–1291.
3. Nikl M. Scintillation Detectors for X-Rays. *Meas Sci Technol*. 2006; 17:R37–R54.
4. Manjn FJ, Errandonea D, Lpez-Solano J, Rodrguez-Hernndez P, Muoz A. Negative Pressures in CaWO₄ Nanocrystals. *J Appl Phys*. 2009; 105:094321.
5. Lou Z, Cocivera M. Cathodoluminescence of CaWO₄ and SrWO₄ Thin Films Prepared by Spray Pyrolysis. *Mater Res Bull*. 2002; 1573–1582.
6. Zhang Y, Holzwarth NAW, Williams RT. Electronic Band Structures of the Scheelite Materials CaMoO₄, CaWO₄, PbMoO₄, and PbWO₄. *Phys Rev B*. 1998; 57:12738–12750.
7. Moszy ski M, Balcerzyk M, Czarnacki W, Nassalski A, Szcz niak T, Kraus H, Mikhailik VB, Solskii IM. Characterization of CaWO₄ Scintillator at Room and Liquid Nitrogen Temperatures. *Nucl Instrum Methods Phys Res Sect A*. 2005; 553:578–591.
8. Basu S, Sanyasi Naidu B, Viswanadh B, Sudarsan V, Jha SN, Bhattacharyya D, Vatsa RK. Nature of WO₄ Tetrahedra in Blue Light Emitting CaWO₄ Probed through the EXAFS Technique. *RSC Adv*. 2014; 4:15606–15612.

9. Zhao J, Wu W, Sun J, Guo S. Triplet Photosensitizers: From Molecular Design to Applications. *Chem Soc Rev.* 2013; 42:5323–5351. [PubMed: 23450221]
10. Endicott JF, Chen Y-J. Charge Transfer-Excited State Emission Spectra of Mono- and Bi-Metallic Coordination Complexes: Band Shapes, Reorganizational Energies and Lifetimes. *Coord Chem Rev.* 2007; 251:328–350.
11. Zdesenko YG, Avignone FT Iii, Brudanin VB, Danevich FA, Nagorny SS, Solsky IM, Tretyak VI. Scintillation Properties and Radioactive Contamination of CaWO₄ Crystal Scintillators. *Nucl Instrum Methods Phys Res Sect A.* 2005; 538:657–667.
12. Shmlevich A, Weiss D, Chen R, Kristianpoller N. Phototransferred Thermoluminescence of CaWO₄ Crystals. *Radiat Prot Dosim.* 1999; 84:131–133.
13. Caruba R, Iacconi P, Cottrant JF, Calas G. Thermoluminescence, Fluorescence and Electron Paramagnetic Resonance Properties of Synthetic Hydrothermal Scheelites. *Phys Chem Miner.* 1983; 9:223–228.
14. McKeever SWS. Optically Stimulated Luminescence Dosimetry. *Nucl Instrum Methods Phys Res Sect B.* 2001; 184:29–54.
15. Blasse, G., Grabmaier, BC. *Luminescent Materials.* Springer-Verlag; New York, NY: 1994.
16. Cornelio ALG, Salles LP, da Paz MC, Cirelli JA, Guerreiro-Tanomaru JM, Tanomaru M. Cytotoxicity of Portland Cement with Different Radiopacifying Agents: A Cell Death Study. *J Endod.* 2011; 37:203–210. [PubMed: 21238803]
17. Zhai X, Yu M, Cheng Z, Hou Z, Ma P, Yang D, Kang X, Dai Y, Wang D, Lin J. Rattle-Type Hollow CaWO₄:Tb³⁺@SiO₂ Nanocapsules as Carriers for Drug Delivery. *Dalton Trans.* 2011; 40:12818–12825. [PubMed: 21879092]
18. Jia PY, Liu XM, Li GZ, Yu M, Fang J, Lin J. Sol–Gel Synthesis and Characterization of SiO₂@CaWO₄, SiO₂@CaWO₄:Eu³⁺/Tb³⁺ Core–Shell Structured Spherical Particles. *Nanotechnology.* 2006; 17:734–742.
19. Su Y, Li G, Xue Y, Li L. Tunable Physical Properties of CaWO₄ Nanocrystals via Particle Size Control. *J Phys Chem C.* 2007; 111:6684–6689.
20. Ryu JH, Yoon J-W, Lim CS, Oh W-C, Shim KB. Microwave-assisted synthesis of nanocrystalline MWO₄ (M: Ca, Ni) via water-based citrate complex precursor. *Ceramics International.* 2005; 31:883–888.
21. Su Y, Li L, Li G. Synthesis and Optimum Luminescence of CaWO₄-Based Red Phosphors with Codoping of Eu³⁺ and Na⁺ Chemistry of Materials. 2008; 20:6060–6067.
22. Mai M, Feldmann C. Microemulsion-Based Synthesis and Luminescence of Nanoparticulate CaWO₄, ZnWO₄, CaWO₄:Tb, and CaWO₄:Eu. *J Mater Sci.* 2011; 47:1427–1435.
23. Guo Y, Zhang G, Gan H, Zhang Y. Micro/Nano-Structured CaWO₄/Bi₂WO₆ Composite: Synthesis, Characterization and Photocatalytic Properties for Degradation of Organic Contaminants. *Dalton Trans.* 2012; 41:12697–12703. [PubMed: 22964585]
24. Chen S-J, Li J, Chen X-T, Hong J-M, Xue Z, You X-Z. Solvothermal Synthesis and Characterization of Crystalline CaWO₄ Nanoparticles. *J Cryst Growth.* 2003; 253:361–365.
25. Huynh KA, Chen KL. Aggregation Kinetics of Citrate and Polyvinylpyrrolidone Coated Silver Nanoparticles in Monovalent and Divalent Electrolyte Solutions. *Environmental science & technology.* 2011; 45:5564–5571. [PubMed: 21630686]
26. Huff TB, Hansen MN, Zhao Y, Cheng J-X, Wei A. Controlling the Cellular Uptake of Gold Nanorods. *Langmuir.* 2007; 23:1596–1599. [PubMed: 17279633]
27. Javadian S, Ruhi V, Heydari A, Asadzadeh Shahr A, Yousefi A, Akbari J. Self-Assembled CTAB Nanostructures in Aqueous/Ionic Liquid Systems: Effects of Hydrogen Bonding. *Ind Eng Chem Res.* 2013; 52:4517–4526.
28. Park J, Fong PM, Lu J, Russell KS, Booth CJ, Saltzman WM, Fahmy TM. PEGylated PLGA Nanoparticles for the Improved Delivery of Doxorubicin. *Nanomedicine.* 2009; 5:410–418. [PubMed: 19341815]
29. Owens DE 3rd, Peppas NA. Opsonization, Biodistribution, and Pharmacokinetics of Polymeric Nanoparticles. *International Journal of Pharmaceutics.* 2006; 307:93–102. [PubMed: 16303268]

30. Kim DH, Wei A, Won YY. Preparation of Super-Stable Gold Nanorods via Encapsulation into Block Copolymer Micelles. *ACS applied materials & interfaces*. 2012; 4:1872–1877. [PubMed: 22471403]
31. Lee J, Yang J, Ko H, Oh S, Kang J, Son J, Lee K, Lee SW, Yoon HG, Suh JS, Huh YM, Haam S. Multifunctional Magnetic Gold Nanocomposites: Human Epithelial Cancer Detection via Magnetic Resonance Imaging and Localized Synchronous Therapy. *Adv Funct Mater*. 2008; 18:258–264.
32. Grabinski C, Schaeublin N, Wijaya A, D'Couto H, Baxamusa SH, Hamad-Schifferli K, Hussain SM. Effect of Gold Nanorod Surface Chemistry on Cellular Response. *ACS Nano*. 2011; 5:2870–2879. [PubMed: 21405102]
33. Gao GH, Lee JW, Nguyen MK, Im GH, Yang J, Heo H, Jeon P, Park TG, Lee JH, Lee DS. pH-Responsive Polymeric Micelle Based on PEG-Poly(beta-amino ester/amido amine) as Intelligent Vehicle for Magnetic Resonance Imaging in Detection of Cerebral Ischemic Area. *Journal of controlled release : official journal of the Controlled Release Society*. 2011; 155:11–17. [PubMed: 20854855]
34. Kim DH, Won Y-Y. In Situ Polymerized Carbon Nanotube/Polyimide Nanocomposites: Effect of Reaction Stoichiometry on the Glass Transition Properties of the Nanocomposites. *Macromol React Eng*. 2012; 6:45–56.
35. Lv Y, Ding G, Zhai J, Guo Y, Nie G, Xu L. A Superparamagnetic Fe₃O₄-Loaded Polymeric Nanocarrier for Targeted Delivery of Evodiamine with Enhanced Antitumor Efficacy. *Colloids and surfaces B, Biointerfaces*. 2013; 110:411–418. [PubMed: 23759382]
36. Won Y-Y, Davis HT, Bates FS. Molecular Exchange in PEO–PB Micelles in Water. *Macromolecules*. 2003; 36:953–955.
37. Sharma KG, Singh NR. Synthesis of CaWO₄:Eu³⁺ Phosphor Powders via Ethylene Glycol Route and Its Optical Properties. *J Rare Earths*. 2012; 30:310–314.
38. Demirci S, Celebioglu A, Aytac Z, Uyar T. pH-Responsive Nanofibers with Controlled Drug Release Properties. *Polym Chem*. 2014; 5:2050–2056.
39. Lee H, Lee J, Park J, Kwon T, Hong S, Won Y-Y. CO₂-Producing Polymer Micelles. *Polym Degrad Stab*. 2015; 120:149–157.
40. Borchert H, Shevchenko EV, Robert A, Mekis I, Kornowski A, bel GG, Weller H. Determination of Nanocrystal Sizes_ A Comparison of TEM, SAXS, and XRD Studies of Highly Monodisperse CoPt₃ Particles. *Langmuir*. 2005; 21:1931–1936. [PubMed: 15723491]
41. Patterson AL. The Scherrer Formula for X-Ray Particle Size Determination. *Phys Rev*. 1939; 56:978–982.
42. Monshi A, Foroughi MR, Monshi MR. Modified Scherrer Equation to Estimate More Accurately Nano-Crystallite Size Using XRD. *World J Nano Sci Eng*. 2012; 02:154–160.
43. Lee H, Kim DH, Park HW, Mahynski NA, Kim K, Meron M, Lin BH, Won YY. Reduced Water Density in a Poly(ethylene oxide) Brush. *J Phys Chem Lett*. 2012; 3:1589–1595. [PubMed: 26285713]
44. Lee H, Kim DH, Witte KN, Ohn K, Choi J, Akgun B, Satija S, Won YY. Water Is a Poor Solvent for Densely Grafted Poly(ethylene oxide) Chains: A Conclusion Drawn from a Self-Consistent Field Theory-Based Analysis of Neutron Reflectivity and Surface Pressure-Area Isotherm Data. *J Phys Chem B*. 2012; 116:7367–7378. [PubMed: 22616550]
45. Lin H, Huang C, Li W, Ni C, Shah S, Tseng Y. Size Dependency of Nanocrystalline TiO₂ on Its Optical Property and Photocatalytic Reactivity Exemplified by 2-Chlorophenol. *Appl Catal B*. 2006; 68:1–11.
46. Alivisatos AP. Semiconductor Clusters, Nanocrystals, and Quantum Dots. *Science*. 1996; 271:933–937.

Figure 1. Energy band diagram explaining the molecular electron transitions associated with the photo and radio-luminescence processes of CWO.

Figure 2. XRD patterns of B-CWO (black), L-CWO (red), M-CWO (blue) and S-CWO (green) in comparison with the standard diffraction pattern of bulk CWO (PDF #07-0210).

Figure 3. Representative low-magnification TEM images of (a) L-CWO, (d) M-CWO and (g) S-CWO. High-resolution TEM images of (b) L-CWO, (e) M-CWO and (h) S-CWO. Shown in (c), (f) and (i) are 2D Fourier transforms of the marked regions of (b), (e) and (h), respectively.

Figure 4.

Mean hydrodynamic diameters of (a) PEG-PnBA-coated L-CWO, (b) PEG-PnBA-coated M-CWO, (c) PEG-PnBA-coated S-CWO, (d) PEG-PLA-coated L-CWO, (e) PEG-PLA-coated M-CWO and (f) PEG-PLA-coated S-CWO in Milli-Q water (red) and 150 mM NaCl (blue). All measurements were performed at an identical CWO concentration of 0.025 mg/ml. As of the time of final revision of this paper (i.e., at 100 days), the hydrodynamic diameter of the PEG-PLA-coated M-CWO NPs in 150 mM NaCl has been confirmed to be still unchanged (≈ 180 nm) (data not included).

Figure 5. Representative TEM images of (a) PEG-PnBA-coated L-CWO, (b) PEG-PnBA-coated M-CWO, (c) PEG-PnBA-coated L-CWO, (d) PEG-PLA-coated L-CWO, (e) PEG-PLA-coated M-CWO, and (f) PEG-PLA-coated S-CWO. All samples were negatively stained with 2% uranyl acetate.

Figure 6. TGA weight loss profiles of (a) PEG-PnBA-coated L-CWO (red), PEG-PnBA-coated M-CWO (blue), PEG-PnBA-coated L-CWO (green), and (b) PEG-PLA-coated L-CWO (red), PEG-PLA-coated M-CWO (blue), (f) PEG-PLA-coatedS-CWO (green). These measurements were performed under continuous heating at a rate is 4 °C/min in helium environment.

Figure 7. ^1H NMR spectra of (deuterated) chloroform-soluble extracts from (a) PEG-PnBA-coated L-CWO, (b) PEG-PnBA-coated M-CWO, (c) PEG-PnBA-coated S-CWO, (d) PEG-PLA-coated L-CWO, (e) PEG-PLA-coated M-CWO and (f) PEG-PLA-coated S-CWO samples. Also shown on top for comparison are ^1H NMR spectra of the pristine PEG-PnBA and PEG-PLA BCPs. ^1H NMR spectra for citrate, CTAB, 1-hexanol, oleic acid and oleylamine are presented in Figure S6 of the SI.

Figure 8.

Fluorescence emission spectra of (a) non-BCP-coated, (b) PEG-PnBA-coated and (c) PEG-PLA-coated CWO samples under 200 nm excitation. Fluorescence intensities of (d) non-BCP-coated, (e) PEG-PnBA-coated and (f) PEG-PLA-coated CWO samples measured at a fixed emission wavelength of 420 nm as a function of excitation wavelength. Also shown are the linear fits to the data used to calculate the fluorescence band gap energies (i.e., the x-intercepts). All measurements were performed at an identical CWO concentration of 0.1 mg/ml.

Figure 9.

Phosphorescence emission spectra of (a) non-BCP-coated, (b) PEG-PnBA-coated and (c) PEG-PLA-coated CWO samples under 200 nm excitation (with a total decay time of 4 ms, a delay time of 0.2 ms, a gate time of 5 ms, and an averaging time of 100 ms).

Phosphorescence intensities of (d) non-BCP-coated, (e) PEG-PnBA-coated and (f) PEG-PLA-coated CWO samples measured at a fixed emission wavelength of 420 nm as a function of excitation wavelength. Also shown are the linear fits to the data used to the data used to calculate the fluorescence band gap energies (i.e., the x-intercepts). All measurements were performed at an identical CWO concentration of 0.1 mg/ml.

Figure 10.

OSL intensities of (a) PEG-PnBA-coated B-CWO, (b) PEG-PnBA-coated L-CWO, (c) PEG-PnBA-coated M-CWO, (d) PEG-PnBA-coated S-CWO, (e) PEG-PLA-coated B-CWO, (f) PEG-PLA-coated L-CWO, (g) PEG-PLA-coated M-CWO and (h) PEG-PLA-coated S-CWO measured at a fixed emission wavelength of 550 nm under 700 nm excitation as functions of time following 6 MV X-ray irradiation (at a total dose of 20 Gy and a dose rate of 0.4 Gy/min). All measurements were performed at an identical CWO concentration of 1.0 mg/ml in Milli-Q water.

Table 1

The values of the surface area of CWO NPs covered by a PEG brush chain (α) determined from the TGA data (Figure 6) for the various BCP-encapsulated CWO NP samples. $R_{g,PEG}$ denotes the radius of gyration of the PEG block ($M_n \approx 5.0$ kDa) in the self-avoiding random-walk configuration (≈ 26 Å).³⁰

BCP-Coated NP Sample	PEG Surface Coverage, α (nm ² per Chain)	$\alpha/\pi R_{g,PEG}^2$
PEG-PnBA/L-CWO	0.421	0.020
PEG-PnBA/M-CWO	1.333	0.063
PEG-PnBA/S-CWO	3.730	0.176
PEG-PLA/L-CWO	0.260	0.012
PEG-PLA/M-CWO	1.107	0.052
PEG-PLA/S-CWO	3.147	0.148

Table 2

Summary of the fluorescence and phosphorescence band gap energies determined from the excitation spectra shown in Figures 8 and 9.

NP Sample	Fluorescence Band Gap (eV)				Phosphorescence Band Gap (eV)			
	Non-BCP-Coated	PEG-PnBA-Coated	PEG-PLA-Coated	Non-BCP-Coated	PEG-PnBA-Coated	PEG-PLA-Coated	Non-BCP-Coated	PEG-PnBA-Coated
B-CWO	4.62	4.58	4.58	4.39	4.40	4.40	4.40	4.40
L-CWO	5.44	5.45	5.44	4.38	4.41	4.43	4.43	4.43
M-CWO	5.46	5.47	5.46	4.39	4.39	4.40	4.40	4.40
S-CWO	5.46	5.48	5.50	4.38	4.40	4.40	4.40	4.40