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PEGylated Nanoceria as Radical Scavenger with Tunable Redox Chemistry

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Cerium oxide nanoparticles (CNPs) have been demonstrated to protect biological tissues against radiation induced damage and scavenging of superoxide anions, prevent laser induced retinal damage, reduce spinal injury, possess pH dependent antioxidant properties, prevent cardiovascular myopathy, and as a tool for immunoassays and other inflammatory diseases.[1a−j] It is speculated that nanoceria is a regenerative radical scavenger with the ability to regenerate the active Ce^{3+} oxidation state for radical scavenging which separates it from other nanomaterials based antioxidant systems such as hydroxylated and water-soluble C-60 and SWCNTs.[1k,l] Thus far there are no reports on controlling the regeneration of the Ce^{3+} oxidation state which is the most important parameter in the application of CNPs as a reliable, regenerative radical scavenger. There is an imminent need to increase the residence time of CNPs in the body and to control the regeneration of the Ce^{3+} oxidation state. PEG has been reported to increase the residence time of NPs and proteins inside cells and provide biocompatibility.[2] PEGylated counterparts of the Superoxide Dismutase (SOD) enzymes have shown improved performance over non-PEGylated enzymes.[2] Herein, we report our efforts to synthesize CNPs directly in PEG (600 MW) solution and determine the effect of increasing [PEG] (PEG vol % as 5, 10, 20, 40, 60, and 80) on the SOD mimetic properties exhibited by nanoceria. We also report how the active Ce^{3+}

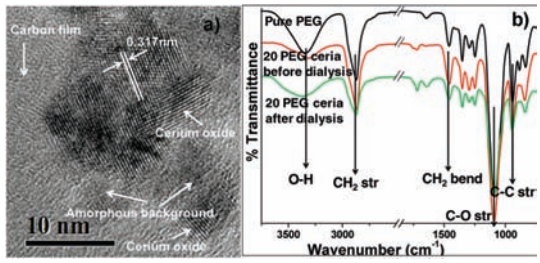

Figure 1. (a) HRTEM image of 3−5 nm nanoceria crystals over amorphous polymer layer; (b) FTIR spectra of PEG coated NPs confirming presence of PEG on nanoceria.

oxidation state can be regenerated and demonstrate the role of PEG on the redox chemistry of CNPs catalyzed by H_2O_2. Several complexes of PEGs with lanthanides have been reported and characterized.[3] To evaluate the effect of [PEG] on the complexation of cerium, UV−vis spectra of the precursor salt of cerium (cerium nitrate hexahydrate) in different solutions of PEG were obtained (SI-1). All PEG solutions

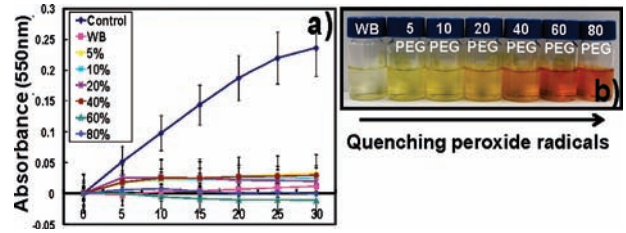

Figure 2. CNPs in varying [PEG]: (a) superoxide dismutase (SOD) mimicking activity; (b) Visible changes upon H_2O_2 induced oxidation of CNPs.

show higher absorption relative to the water based solution of cerium nitrate, but the observed nonspecific trend could not be ascribed to a systematic decrease in the solvent polarity or dielectric constant. This observation indicates the complexation of cerium ions with PEG. In contrast to this Uekawa et al.[4a,b] reported a red shift upon addition of cerium nitrate in PEG and ascribed the red shift to the complexation of PEG with cerium ions. The CNPs were synthesized as described in the experimental details (SI-2). A high resolution transmission electron micrograph (Figure 1a) demonstrates that PEG is present as an amorphous layer on CNPs confirmed by an amorphous background around the crystalline CNPs. To confirm further, CNPs synthesized in PEG were dialyzed using a 3500 MWCO cellulose membrane and the FTIR spectrum was collected from the dried powder. Figure 1b confirms the presence of PEG on the nanoceria particles from FTIR of 20% PEG CNPs.

Biocompatibility and SOD Mimetic Activity of CNPs in PEG. Cell viability analysis was performed for CNPs in PEG solution using a standard MTT assay. PEG in MWs of 400 to 1500 has been reported as benign in an acute oral dose; however, there are isolated reports of toxicity with increases in MW and concentration.[4c] It was observed (SI-3) that the cell viability of CNPs in PEG in a concentration as high as 100 μM (for 72 h) was unaffected, and the cells could thrive in the presence of PEG-CNPs. Our experiments reemphasize the benign nature of PEG by demonstrating excellent biocompatibility.

Significant progress has been made to mitigate the effect of ROS and has met with limited success in the form of CuZn SOD and their PEGylated counterparts.[2] It was shown recently that increasing the coating thickness of polymer (dextran) could reduce CNP activity.[2i] In a similar study a decrease in peroxidase activity of magnetite NPs was observed when the particles were coated with high MW dextran or PEG.[5a] To reduce the possibility of hindrance in the activity of CNPs at higher [PEG] (which may increase coating thickness), CNPs were synthesized specifically in low MW (600 MW) PEG and did not show significant differences in particle or agglomerate size (SI-4). As expected, the low MW (Figure 2a) PEG

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did not decrease the SOD mimicking activity of CNPs evaluated using a classic SOD mimetic assay, competition with ferricytochrome C for reduction by superoxide radicals as a measure of radical concentration. While the activity of 60PEG based CNPs was maximum, the SOD mimicking activity did not vary much with [PEG], suggesting that the SOD mimetic activity is independent of [PEG] but may depend upon the coating thickness achieved. Pure PEG controls showed no SOD mimicking activity (SI-5).

Hydrogen Peroxide Mediated Redox Cycling. It is important to determine the changes in redox properties of CNPs in PEG solutions to evaluate the role of PEG coating on the redox properties of nanoceria. At higher [Ce^{3+}], nanoceria forms a colorless colloidal solution (at 5 mM) and the redox reaction can be monitored by changes in characteristic UV absorbance. Nanoceria has been shown to protect H_2O_2 induced cell damage. Thus to compare the redox chemistry of CNPs in different PEG solutions, we monitored the change in oxidation state of CNPs as a function of time upon addition of equal amounts of H_2O_2 to the CNP samples. This redox procedure can briefly be summarized as follows: (a) Addition of H_2O_2 oxidizes cerium in nanoceria from the 3+ to 4+ oxidation state. (b) The acidic medium (pH 2.5−3.5) around the NPs favors the 3+ oxidation state, and thus upon aging the nanoceria undergoes surface reduction and regenerates its active 3+ oxidation state (with reduction of the 4+ state). (c) Further addition of H_2O_2 to CNPs can repeat this cycle. Figure 2b shows the visible changes in color of the solution upon addition of equal amounts of H_2O_2 to the CNPs prepared in various PEG solutions. Note that the color from the CT intensifies and shows a red shift upon increasing [PEG] from 0 to 80 vol %. UV−visible spectra depict a primary peak at 298 nm corresponding to an increase in Ce^{4+} species as shown in SI-6b (inset shows relative increase in absorbance with increase in [PEG]). A secondary peak (appearing as a shoulder) was seen between 300 and 400 nm corresponding to the CT spectra between the oxidized cerium 4+ and the etheral oxygen ($-CH_2O$) from PEG. Figure 3a depicts the bathochromic shift with respect to [PEG]. It is clear that CNPs in 40, 60, and 80PEG solutions are red-shifted as compared (maximum in absorption beyond 340 nm) to 5, 10, and 20PEG CNP solutions.

It is known that the absorption of dyes and other absorbing species changes with solvent polarity and dielectric constant.[5b] In general the spectra are red-shifted with increased solvent polarity while the spectra in Figure 3a are red-shifted with decreased solvent polarity corresponding to the negative solvatochromism phenomenon. The dielectric constant of PEG varies as 81, 77, 74, 67, 53, 39 and 25 for pure water, 5, 10, 20, 40, 60 and 80 PEG, respectively. The polarity of the solution is also expected to decrease in similar manner. The red shift upon varying [PEG] suggests that two different CT mechanisms occur from oxygen to cerium: the first being an internal CT from oxygen to cerium in CeO_2 (centered at 298 nm) and the second from the ethereal ($-CH_2O$) oxygen of PEG to cerium (centered around 320−400 nm). A nonspecific trend in absorbance with concentration (or polarity/dielectric constant) of PEG suggests that the spectrum is not entirely solvent dependent but PEG associates to a different degree with CNPs. In addition to exhibiting different CT, the regeneration of the 3+ oxidation state of nanoceria in varying [PEG] was monitored over time. Figure 3b depicts the UV−vis spectra of CNPs in varying [PEG] after 1 week of aging which shows the disappearance of the Ce^{4+} oxidation state. Note that the regeneration of the Ce^{3+} state of nanoceria is also a function of [PEG] and is possibly induced by the stability of the CT complex. Investigations on the specific mechanism are underway, and the possibilities have been described in SI-7. The UV−vis spectra clearly depict that CNPs can be tuned to regenerate the 3+ oxidation state faster or slower depending upon the requirement. A faster regeneration of the 3+ oxidation state can reduce the time lag for nanoceria to be active repeatedly for radical scavenging. The behavior of 20PEG solution upon aging for 28 days is shown in SI-8. It can be observed that the oxidation state of CNPs changes dynamically with time and by the 21st day the 3+ oxidation state (absorption max, 252 nm) was completely regenerated. This confirms the hypothesis that coating of CNPs with PEG did not interfere with their redox property. Further, synthesizing CNPs in PEG has additional advantages in tuning the regeneration kinetics. The regeneration of the 3+ oxidation state of nanoceria was a function of [PEG]. CNPs synthesized in 5−40PEG showed a fast reversal of oxidation state and will be used in future investigations. CNPs synthesized in PEG offer a promising alternative to PEG-SOD as ROS scavengers.

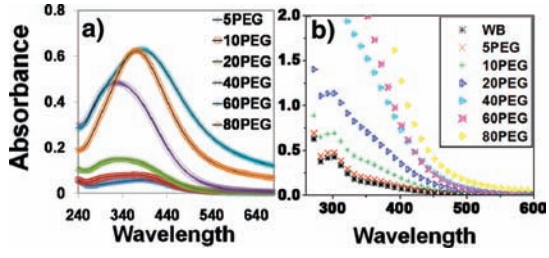

Figure 3. (a) Bathochromic shift of PEG-CNPS upon reaction with H_2O_2. PEG-CNPs showed behavior peculiar to negative solvatochromism as the spectral values are red-shifted with decrease in polarity of the solvent. (b) UV−vis spectra of CNPs in PEG solutions depicting reduction in amount of Ce^{4+} in different PEG solutions after 1 week.

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Supporting Information Available: Experimental details, UV−vis plots, TEM images, SOD activity and cell viability plots. This material is available free of charge via the Internet at http://pubs.acs.org.

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