Syntheses of Biologically Non-Toxic ZnS:Mn Nanocrystals by Surface Capping with O-(2-aminoethyl)polyethylene Glycol and O-(2-carboxyethyl)polyethylene Glycol Molecules

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Water-dispersible ZnS:Mn nanocrystals were synthesized by capping the surface of the nanocrystal with O-(2-Aminoethyl)polyethylene glycol (PEG-NH₂, Mw = 10,000 g/mol) and O-(2-Carboxyethyl)polyethylene glycol (PEG-COOH, Mw = 10,000 g/mol) molecules. The modified PEG capped ZnS:Mn nanocrystal powders were thoroughly characterized by XRD, HR-TEM, EDXS, ICP-AES and FT-IR spectroscopy. The optical properties were also measured by UV/Vis and photoluminescence (PL) spectroscopies. The PL spectra showed broad emission peaks at 600 nm with similar PL efficiencies of 7.68% (ZnS:Mn-PEG-NH₂) and 9.18% (ZnS:Mn-PEG-COOH) respectively. The measured average particle sizes for the modified PEG capped ZnS:Mn nanocrystals by HR-TEM images were 5.6 nm (ZnS:Mn-PEG-NH₂) and 6.4 nm (ZnS:Mn-PEG-COOH), which were also supported by Debye-Scherrer calculations. In addition, biological toxicity effects of the nanocrystals over the growth of wild type E. coli were investigated. They showed no biological toxicity to E. coli until very high concentration dosage of 1 mg/mL of the both nanocrystal samples.

Key Words: ZnS:Mn nanocrystal, Polyethylene glycol derivatives capping, Water-dispersible nanocrystal, Biological toxicity

Introduction

Nanosized semiconductor materials have gained significant attention over the past decade.¹ ² These materials provide a wide range of applications, such as non-linear optics or photo-electronic devices, and some advanced biomedical imaging areas, due to their unique physical, chemical, and optical properties.³ ⁴ The optical properties of these materials can vary greatly with the size of the corresponding nanocrystals, because the band gap energies are quite different, even between materials of identical chemical composition.⁵ ⁶

ZnS nanocrystals (ZnS:Mn) doped with manganese ions have attracted great interest on account of their high quantum efficiency and thermal stability, which are critically essential properties for commercial applications in electro-luminescence devices.⁸ There have been various synthetic techniques developed for such nanocrystals, including gas, solid, and aqueous solution reactions via the thermal decomposition of organometallic precursors. However, these methods often require high temperatures and pressures, and even the use of bio-hazardous chemicals.⁹

Syntheses of water-dispersible nanocrystals were developed for fluorescent labeling agents used in advanced bio-imaging techniques.¹⁰ ¹¹ Such techniques are expected to replace complicated or hazardous radioactive detection methods, and can be used to perform very sensitive biological assays. Inorganic semiconductor nanocrystals are much more efficient, sensitive, and stable than the organic dyes used for same purpose.¹² Unfortunately, the most highly luminescent semiconductor nanocrystals have hydrophobic nature, making them incompatible with biological systems. Although there are several reports of solubilized hydrophobic inorganic semiconductor nanocrystals in water produced by modifying their surfaces,¹³ ¹⁴ it still remains a great challenge to make water-dispersible and bio-compatible semiconductor nanocrystals without damaging the high quantum efficiency and stability in aqueous media. Several water-dispersible ZnS:Mn nanocrystals have been synthesized in this laboratory.¹⁵ ¹⁶ ¹⁷ They were made by passivating the hydrophobic ZnS:Mn nanocrystal surfaces with polar amino acids and mercaptoacetate molecules to impart water solubility to the originally hydrophobic inorganic nanocrystals. More recently, syntheses and characterizations of water-dispersible ZnS:Mn nanocrystals have been published, in which the surfaces of the nanocrystals are capped by polyethylene oxide (PEO) molecules of varying molecular weight.¹⁸ ¹⁹ It was revealed that the best optical properties and stability were obtained from the ZnS:Mn capped with PEO, whose molecular weight is 10,000 g/mol. This has inspired the use of PEG-NH₂ and PEG-COOH with almost the same molecular weight of 10,000 g/mol as capping ligands in the present study. The primary goal of this research is synthesizing water-dispersible and non-toxic nanocrystals that are suitable for further bio-imaging study, or environmentally friendly materials for advanced photo-electronic devices,
since nanocrystals made of zinc ions are expected to be much less toxic than those of a cadmium base, such as CdSe and CdS nanocrystals.

**Experimental Section**

**Instrumentation.** UV/Vis absorption spectra were recorded on a Perkin Elmer Lambda 25 spectrophotometer equipped with a deuterium/tungsten lamp. The FT-IR spectra were recorded on a Perkin Elmer spectrophotometer equipped with an attenuated total reflection (ATR) unit. The photoluminescence (PL) spectra were obtained via an EDXS collector equipped in the HR-TEM, with an Si-Li detector in X-ray Spectroscopy (XRD). The nanocrystals were determined by EDXS (Energy Dispersive X-ray Spectroscopy) spectra obtained via an EDXS collector equipped in the HR-TEM, with an Si-Li detector in X-ray Spectroscopy. The elemental compositions of the nanocrystals were determined by EDXS (Energy Dispersive X-ray Spectroscopy) spectra obtained via an EDXS collector equipped in the HR-TEM, with an Si-Li detector in X-ray Spectroscopy.

**Syntheses of PEG-NH$_2$ and PEG-COOH Capped ZnS:Mn Nanocrystals.** The preparation procedures are similar to those previously reported for unmodified PEG-capped ZnS:Mn nanocrystal synthesis in aqueous solution. A solution of ZnSO$_4$·5H$_2$O (1.44 g, 5 mmol) in 50 mL of water was slowly added to a 50-mL aqueous mixture containing 1.0 mmol of modified PEG molecules at ambient temperature. In another flask, MnSO$_4$·H$_2$O (0.02 g, 0.1 mmol) and Na$_2$S (0.40 g, 5 mmol) were dissolved in 20 mL of DI water. The mixture was then transferred to a flask containing the ZnSO$_4$/modified-PEG mixture with vigorous stirring. The resulting solution was refluxed for 20 h. Slow cooling to ambient temperature and the addition of ethanol solution resulted in a yellow-white precipitate at the bottom of the flask.

**Results and Discussions**

**Toxicity Tests in Bacteria.** The antibacterial effects of the ZnS:Mn-PEG-NH$_2$ and ZnS:Mn-PEG-COOH nanocrystals were tested according to the previous method suggested by Li et al., which is to evaluate toxicity effects of silver nanoparticles on the growth of *E. coli*, with suitable modifications. *E. coli* K-12 strain was grown in 5 mL of nutrient broth (beef extract 3 g/L, peptone 5 g/L, NaCl 10 g/L) with shaking at 37 °C for 16 h. The nanocrystal solids (ZnS:Mn-PEG-NH$_2$ and ZnS:Mn-PEG-COOH) were dissolved in 5 mL of the nutrient broth at 37 °C for a final concentration of 1.0 mg/mL. After making each nutrient broth containing different amounts of nanocrystal samples, *E. coli* K-12 that had been grown overnight (16 h) was inoculated into the nutrient broth. To plot the growth curve, the turbidity of the culture was examined every 30 min by measuring the optical density at 600 nm using a SpectraMax M2e microplate reader (Molecular Devices). The test was performed in triplicate.

**Statistical Analysis.** The data were expressed as means ± SD. The results were analyzed with GraphPad Prism statistics software (GraphPad Software, Inc., San Diego, CA, USA). The Student’s t-test was used to evaluate the statistical differences between the groups. A P value less than 0.05 was considered statistically significant.

**Results and Discussions**

Figures 1(a) and (b) show the HR-TEM images obtained...
from the ZnS:Mn-modified PEG nanocrystals. Unfortunately, the HR-TEM images do not show the individual discrete particles so clearly, which is probably due to the existence of some aggregates of the nanocrystal particles, which were possibly caused by hydrogen bonding interactions between the surface capping molecules. However, we were able to find some fringe images of the particles by maximum enlargement of the HR-TEM images, and we also tried to measure as many particle sizes as we could to obtain average particle sizes from those pictures. Figure 1(c) presents size distribution histograms of ZnS:Mn-PEG-NH$_2$ and ZnS:Mn-PEG-COOH nanocrystals. Both nanocrystals fairly fit into Gaussian size distribution, in which the center was located at 5.6 nm for ZnS:Mn-PEG-NH$_2$ with a standard deviation of 0.8 nm, and at 6.4 nm for ZnS:Mn-PEG-COOH with a standard deviation of 1.2 nm. Distinct lattice planes of approximately 2.7 Å separations appeared in the fringe images, which suggest that all the solid samples comprised single crystals rather than just poly-crystalline aggregates. In addition, to support our measurements, we also performed Debye-Scherrer calculations for both modified PEG-capped ZnS:Mn nanocrystals using the obtained XRD peaks. From the measured full width at half maxima (FWHM) of the selected XRD peaks, we calculated the average particle sizes for ZnS:Mn-PEG-NH$_2$ and ZnS:Mn-PEG-COOH nanocrystals, which were 6.1 nm and 6.9 nm, respectively. It is possible that the present aggregates could cause extra broadening of PL emission peaks and XRD peaks, as shown in the corresponding diagrams.

We obtained the energy dispersive X-ray spectra (EDXS) shown in Figure 2 to confirm the elemental compositions of the ZnS:Mn-modified PEG nanocrystals. The atomic ratios of Zn to S were almost 1:1, and the average doping concentration of manganese(II) ions in the nanocrystals were 0.16 (ZnS:Mn-PEG-NH$_2$) and 0.31 atomic % (ZnS:Mn-PEG-COOH), respectively. The concentrations of the manganese(II) ions in the corresponding nanocrystals were more precisely determined by inductively coupled plasma-atomic emission spectrometry (ICP-AES). The averages of the elemental proportion of Mn ions against the ZnS parent crystals were respectively measured as 0.42% (ZnS:Mn-PEG-NH$_2$) and 0.32% (ZnS:Mn-PEG-COOH). The intended manganese(II) ion-doping concentrations in the ZnS:Mn crystals were in the range of 1-2%, which is the optimum dopant amount previously reported for PL efficiency in other ligand-capped water-dispersible ZnS:Mn nanocrystals.

Figure 3 shows the UV-visible absorption spectra of ZnS:Mn-PEG-NH$_2$ and ZnS:Mn-PEG-COOH nanocrystals. In both spectra, fairly broad absorption peaks appeared from 290 to 350 nm, in which the maxima are located at 305 nm (ZnS:Mn-PEG-NH$_2$) and 320 nm (ZnS:Mn-PEG-COOH). The values are quite close to those previously reported for various PEO-capped ZnS:Mn nanocrystals with an average of 315 nm. The photoluminescence (PL) spectroscopy data of the nanocrystals were presented in Figure 4. The emission peaks obtained for both the nanocrystals were almost identical, with the peak maxima located at around 600 nm. These emission spectra were obtained by fixing the excitation wavelengths of the light source at the UV-Visible absorption wavelengths for a corresponding nanocrystal. In these spectra, all the nanocrystals showed quite large Stokes shifts between the absorption and emission wavelengths of ca. 280 to 295 nm. The large Stokes shifts are usually the result of metal or counter anion vacancies in the crystal lattice, or

![Figure 2. EDXS diagrams of (a) ZnS:Mn-PEG-NH$_2$ and (b) ZnS:Mn-PEG-COOH.](image_url)

![Figure 3. UV-vis absorption spectra of ZnS:Mn-PEG-NH$_2$ (black) and ZnS:Mn-PEG-COOH (red).](image_url)
surface defects resulting from incomplete capping by the surfactant ligand.\(^{23}\) The orange light emitted at 600 nm is attributed to the \(5T_{2g} - 6A_{1g}\) transition of the Mn\(^{2+}\) ions. During the luminescence pathway, if surface defect states are located close to the conduction band, the direct energy transfer from the ZnS host to the Mn\(^{2+}\) dopant ions is significantly interrupted, which can cause a weakening of the orange emission as well as enlarging the Stokes shift.\(^{24}\) In some cases, the previously mentioned surface defects on the ZnS:Mn nanocrystal and zinc ion vacancies are known to cause an extra blue emission at around 450 nm, but this phenomenon was not observed in the PL spectra of our ZnS:Mn-modified PEG nanocrystals.\(^{25}\) In addition, we measured and calculated the relative PL efficiencies for both nanocrystals. The calculated relative PL efficiencies of the nanocrystals were 7.68\% (ZnS:Mn-PEG-NH\(_2\)) and 9.18\% (ZnS:Mn-PEG-COOH). These efficiencies were measured and calculated using a method reported by Williams \textit{et al.},\(^{26}\) which involves calculating the relative quantum yields through a comparison with a standard material: a 0.1 M aqueous solution of Tyrosine whose reported excitation wavelength and absolute quantum yield are 275 nm and 14\%, respectively.\(^{27}\) The obtained PL efficiencies were similar or somewhat better than those in the literature for CdSe (8.9\%), CdS (6.0\%), and ZnS:Mn-Aminoacids (4.0-7.0\%) nanocrystals.\(^{16}\) 

Figure 5 shows the wide-angle X-ray diffraction patterns of a powdered sample of modified PEG-capped ZnS:Mn nanocrystals, and bulk ZnS solid in a hexagonal Wurtzite crystalline phase as a reference. In the diagram, most peaks obtained by the modified PEG-capped ZnS:Mn nanocrystals are broad, which is one of the common features for low-dimensional nano-sized semiconducting materials.\(^{28}\) Even so, there were distinct peaks in the diagram, corresponding to the (008), (110), and (118) planes, indicating that both ZnS:Mn-PEG-NH\(_2\) and ZnS:Mn-PEG-COOH nanocrystals are in Wurtzite phases and belong to the space group of \textit{P6\_3mc}.\(^{29}\) 

The modified polyethylene glycol surface-capping ligands were characterized by FT-IR spectroscopy to confirm if the capping molecules are actually coordinated onto the surface of the ZnS:Mn nanocrystals. Figure 6 presents the FT-IR spectra of the modified PEG molecules coordinated with ZnS:Mn nanocrystals, which are overlapped with the spectra of the corresponding uncoordinated free ligands for direct comparison. In the spectra, broad peaks that appeared at
around 2980 cm\(^{-1}\) were assigned as N-H (ZnS:Mn-PEG-NH\(_2\)) and O-H (ZnS:Mn-PEG-COOH) stretching peaks.\(^{30}\) Those peaks were quite shifted from those of free PEG-NH\(_2\) (3495 cm\(^{-1}\)) and PEG-COOH (3508 cm\(^{-1}\)), clearly indicating that these terminal functional groups are strongly coordinated onto the surface of the nanocrystals. In addition, the down shifts of the C-O stretching peaks for nanocrystal-coordinated PEG-NH\(_2\) and PEG-COOH molecules, which appeared at 1105 cm\(^{-1}\) and shifted from that of uncoordinated ligands at 1140 cm\(^{-1}\), indicate that some of the oxygen atoms in the PEG chains are also strongly coordinated to the heavy zinc ions on the surface of the ZnS:Mn nanocrystals.

Finally, these modified PEG-capped ZnS:Mn nanocrystals were evaluated for their potential toxicities on the growth of \(E.\ coli\), which are typical bacteria associated with people. For these experiments, the optical density (OD) 600 method was employed, which measures the degree of turbidity of the nutrient broth by filling with growing bacteria.\(^{31}\) The more turbid solution scatters more scanned light, so that the intensities of the passing light decreases with the bacterial growth. Usually, spectroscopic bacterial growth monitoring is performed at a working range of 550-600 nm, because the nutrient broth has a very low OD value at this wavelength region.\(^{32}\) Since the ZnS:Mn nanocrystals do not emit any light in response to the scanning of the 600-nm light source, they do not interrupt the OD measurements at all. Therefore, this method is valid for estimating the biological toxicity of the modified PEG-capped ZnS:Mn nanocrystals. As shown in Figure 7, surprisingly, no toxicities were seen at concentrations of 1.0 mg/mL in all of the nanocrystal samples, which correspond to PEG-NH\(_2\)[ZnS:Mn] (4.54 × 10\(^{-8}\) M) and PEG-COOH[ZnS:Mn] (4.42 × 10\(^{-8}\) M). The concentrations of the nanocrystals in aqueous solution, represented as [ZnS:Mn], were determined by elemental analysis by ICP-AES measurements, as described in the literature.\(^{33}\) The obtained Zn and Mn concentrations were combined and converted into the nanocrystal particle concentration, assuming that the nanocrystal particle holds the same density as its bulk material. This result can be compared to that shown from some previously reported aminoacids and mercaptoacetic acid-capped ZnS:Mn nanocrystals.\(^{34}\) In those studies, the prepared ZnS:Mn-Ser, ZnS:Mn-Val, ZnS:Mn-Thr, ZnS:Mn-Met and ZnS:Mn-MAA nanocrystals showed strong growth inhibition effects at a solution concentration of 100 µg/mL, which corresponds to the average of [ZnS:Mn] = 1.24 × 10\(^{-9}\) M in aqueous solution, showing much higher biological toxicity than the ZnS:Mn-PEG-NH\(_2\) and ZnS:Mn-PEG-COOH nanocrystal samples, even with biologically friendly amino-acid capping molecules. This observation is more closely related to the capping abilities on the ZnS:Mn nanocrystal surfaces. The PEG-NH\(_2\) and PEG-COOH molecules contain long hydrocarbon chains and oxygen atoms, which can strongly bind to zinc and manganese metal ions on the surface of the ZnS:Mn nanocrystals, which is supported by the FT-IR spectroscopic analyses. In addition, there are extra terminal anchoring groups in the PEG-NH\(_2\) and PEG-COOH molecules, which can more tightly immobilize the PEG chains onto the nanocrystal surface, so that they can prevent the dissociation of zinc ions from the nanocrystal surface, which is also known as a major cause of biological toxicity in most nanosized inorganic semiconductor materials.\(^{36}\)
Another important factor that can affect the toxicity effect of the nanocrystals in aqueous solution is aggregation between nanoparticles to reduce actual surface area of the nanocrystals exposing toward biological substrates. It has been reported that several water-soluble polymers capped silver nanoparticles showed significant degree of aggregation between nanocrystals in aqueous solution state.\(^\text{37}\) We expect that similar aggregation will exist for the ZnS:Mn-PEG-NH\(_2\) and ZnS:Mn-PEG-COOH nanocrystals in aqueous solution; in addition, it is probably impossible to entirely separate each nanoparticle even in very low concentration condition of the nanocrystal colloid due to the strong hydrogen bonding interaction between the modified PEG molecules. Therefore, one can suspect that the obtained non-toxic test results from the ZnS:Mn-PEG-NH\(_2\) and ZnS:Mn-PEG-COOH nanocrystals are simply induced by the reduced surface area, which was caused by aggregation of the nanoparticles, rather than by the nature of the surface capping ligand themselves. To demonstrate the surface capping ligand dependency on the toxicity effect of the ZnS:Mn nanocrystal, we performed same toxicity tests for previously reported aminoacids capped ZnS:Mn nanocrystals,\(^\text{34,38}\) which showed very similar physical and chemical properties in terms of absorption/emission spectroscopic properties and particle size distribution in solid and solution states. In addition, the aminoacids capped ZnS:Mn nanocrystals are also expected to show similar aggregation phenomena due to strong hydrogen bonding interaction between polar aminoacid molecules in aqueous solution. Figure 8 presents comparative toxicity effects of ZnS:Mn-PEG-NH\(_2\), ZnS:Mn-PEG-COOH, ZnS:Mn-His\(^\text{34}\) and ZnS:Mn-Met\(^\text{37}\) nanocrystals on the growth of \(E.\) coli K-12 strain. The concentration of [ZnS:Mn] were equally fixed at \(4.0 \times 10^{-8}\) M in aqueous solution for all the nanocrystal samples, which was the highest ZnS:Mn nanocrystal concentration in this study. The diagram clearly shows different toxicity effects between aminoacids and modified PEG capped ZnS:Mn nanocrystals at the same concentration condition. Therefore, we can conclude that the non-toxic natures of the modified PEG capped ZnS:Mn nanocrystals were mainly originated from the surface capping abilities of the capping ligands.

In addition, previously in this lab, we also have reported comparative study of toxicity effects of the two different aminoacids; Cysteine and Histidine, capped ZnS:Mn nanocrystals on different biological substrates such as \(E.\) coli, human’s lung (IMR90) and kidney (HEK293) cells.\(^\text{34}\) In that study, the two different aminoacids capped ZnS:Mn nanocrystals showed quite different toxicity effects for corresponding bacteria and human cells; however, interestingly, we found that the tendency of the toxicity effects over \(E.\) coli and human cells by the two nanocrystals are quite similar, i.e. the nanocrystal showed stronger toxicity over \(E.\) coli also showed stronger toxicity over the human cells. Therefore, from that results, we can speculate that the non-toxic nature of the ZnS:Mn-PEG-NH\(_2\), ZnS:Mn-PEG-COOH nanocrystals over \(E.\) coli would be similarly retained for other biological cells.

## Conclusion

In summary, water-dispersible ZnS:Mn nanocrystals capped with PEG-NH\(_2\) and PEG-COOH molecules were successfully synthesized, and their biological effects on enteric bacteria were investigated. The surprisingly low toxicity of the modified PEG-conjugated ZnS:Mn nanocrystals makes them suitable for biological and environmentally friendly electronic material applications. Recently, water-dispersible semiconductor quantum dots (QDs) have received much attention as a novel type of fluorophore for biomedical imaging. Since capping water-dispersible ZnS:Mn semiconductor nanocrystals with PEG-NH\(_2\) and PEG-COOH can provide a novel platform to which many biomolecules such as DNA, RNA, and proteins can be attached, the near lack of toxicity of the nanocrystals is a significant finding. In addition, the water-dispersible ZnS:Mn nanocrystals capped with PEG-NH\(_2\) and PEG-COOH should also be widely applicable in environmentally friendly electronic device applications, such as QD-LEDs, since they are more suitable for non-toxic large-scale production processes than hydrophobic inorganic semiconductor nanocrystals.

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## References
