Layer-by-Layer Films of Chitosan, Organophosphorus Hydrolase and Thioglycolic Acid-Capped CdSe Quantum Dots for the Detection of Paraoxon

Celeste A. Constantine,[†] Kerim M. Gattás-Asfura,[†] Sarita V. Mello,[†] Gema Crespo,[†] Vipin Rastogi,[‡] Tu-Chen Cheng,[‡] Joseph J. DeFrank,[‡] and Roger M. Leblanc*,[†]

Department of Chemistry, University of Miami, 1301 Memorial Drive, Coral Gables, Florida, 33146, and Biotechnology Team, Research and Technology Directorate, U.S. Army Edgewood Chemical Biological Center, Aberdeen Proving Ground, MD 21010-5423.

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A polyelectrolyte architecture was fabricated that was composed of chitosan and organophosphorus hydrolase polycations along with thioglycolic acid-capped CdSe quantum dots (QDs) as the polyanion. This film was imaged by epifluorescence microscopy. UV−vis and emission spectroscopies were used to monitor the growth of the bilayer film due to the enhanced optical property of QDs. Photoluminescence of the functionalized QDs improved when sandwiched between the polycations layers. The presence of organophosphorus compounds was confirmed through photoluminescence spectroscopy.

Immobilization of organic polymers on solid substrates for the development of thin films is a widely researched field due to their applications in areas such as light-emitting diodes, biomedical devices and biosensing assemblies.[1] Layer-by-layer (LbL) is one technique utilized for thin film fabrication. The layer-by-layer deposition method is based on alternating adsorption of oppositely charged macromolecules such as polymers and biomacromolecules.[2] QDs can be utilized as an emitting material in the preparation of ultrathin films[3,4] because the color emission can be tuned by size variation without altering their chemical properties.[5,6] Also, high fluorescence quantum yields can be achieved by modifying the surface of the QDs[7,8] and due to this, they are attractive for incorporating into thin films. Organophosphorus compounds such as paraoxon are used in pesticides and insecticides but they are an environmental concern because of their structural similarity to nerve agents such as sarin and soman.[9] Organophosphorus hydrolase (OPH) hydrolyzes a large variety of organophosphorus compounds by producing harmless products such as *p*-nitrophenol (PNP) and diethyl phosphate.[10]

In the present work, the versatility of the LbL system is explored where multilayers of chitosan (CS), thioglycolic acid (TGA)-capped CdSe QDs, and OPH polyelectrolytes were incorporated into the LbL architecture. OPH assumed polycationic behavior by adjusting the pH of the solution to 7.3. CS/TGA-capped CdSe QDs multilayers were used as a cushion of support, and bilayers of OPH/TGA-capped CdSe QDs were then integrated into the LbL system. The ultrathin film growth was monitored using UV−vis and photoluminescence spectroscopies. Our methodology shows that sandwiching TGA-capped CdSe QDs between polycation layers using the alternate LbL technique increases the photoluminescent properties of the QDs when compared to the ones in solution. This key characteristic allows QDs to be incorporated into a biosensing assembly system.

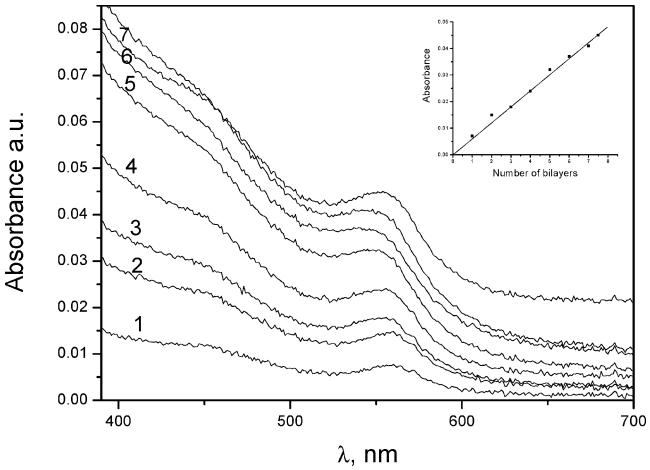

Figure 1. UV−vis spectra of growing CS/TGA-capped CdSe QDs multilayers (1−5) followed by two bilayers of OPH/TGA-capped CdSe QDs (6, 7) and a top layer of OPH (8). The inset shows a plot of λ_{max} versus the number of layers.

QDs were first prepared by following synthetic protocol[11] and then functionalized by adding thioglycolic acid (TGA) to make the modified QDs water-soluble. The size (diameter) range determined by HRTEM[12] was 3.4 nm. Scheme 1 presents an overview of the procedure used for the film assembly. This multilayer system of CS, TGA-capped CdSe QDs, and OPH from now on referred to as a *sensing assembly* was used for the detection of paraoxon solution.

The absorption spectrum of a TGA-capped CdSe QDs solution (λ_{max} 560 nm) was utilized to monitor the efficiency of the LbL bilayer growth. The results show a continuous growth of the bilayer system (Figure 1). Absorbance at λ_{max} 560 nm increased linearly with the adsorption of subsequent layers (inset). Equal amount of QDs were adsorbed after each deposition cycle.

The inset of Figure 2 shows the maximum photoluminescence intensity of each bilayer at 582 nm versus the number of bilayers. A linearity between the increase in intensity and number of bilayers was also observed. The figure also shows

* Corresponding author. Tel.: +1-305-284-2194. Fax: +1-305-284-6367. E-mail address: rml@miami.edu.
[†] University of Miami.
[‡] U.S. Army Edgewood Chemical Biological Center.

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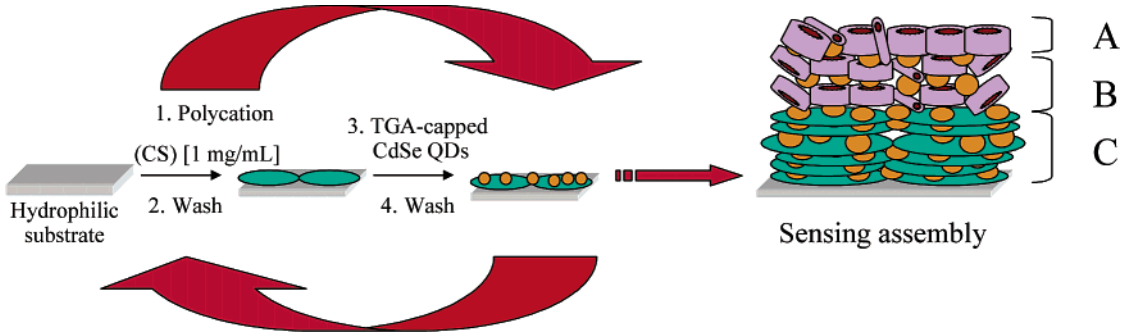
SCHEME 1: Sensing Assembly[a]



[a] (A) Top layer of OPH, (B) two bilayers OPH/TGA-capped CdSe QDs, and (C) five bilayers CS/TGA-Capped CdSe QDs.

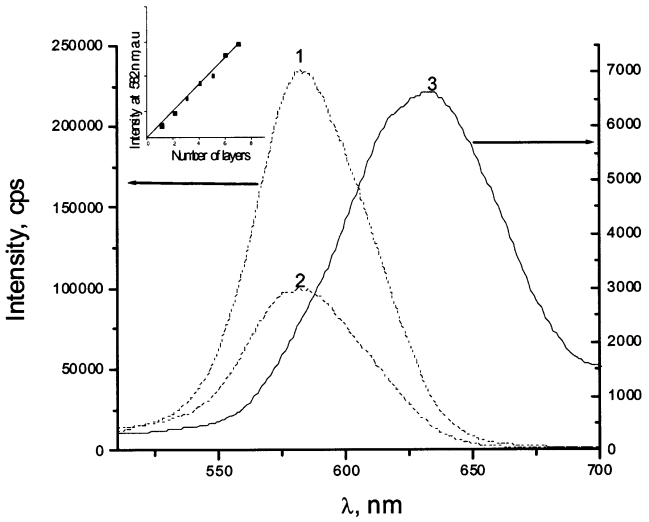


Figure 2. Photoluminescence spectra of the TGA-capped CdSe QDs in the LbL film: (1) before paraoxon exposure; (2) after paraoxon exposure. (3) Photoluminescence spectrum of TGA-capped CdSe QDs (1.6×10^{-4} M) in solution. The inset shows intensity of photoluminescence at λ_{em} versus the number of layers.

the photoluminescence spectrum of the multilayer system compared to that of the TGA-capped CdSe QDs in solution. Sandwiching QDs within the film enhances the photoluminescence intensity. This may be the result of a higher degree of surface passivation that improves the electron−hole recombination process. It is known that water molecules adsorb on the surface of QDs and passivate the surface traps.[13] Concentration changes of QDs may also influence emission intensity as well as the band spectral position as the result of interdot interactions. Transferring the QDs from solution to the LbL film environment may alter these interactions among particles. The sensing assembly was exposed to a paraoxon solution (Figure 2, curve 2). On exposure of the sensing assembly to 1×10^{-6} M paraoxon solution, there was an immediate decrease in the photoluminescence. OPH interacts with the substrate in solution, changing its conformation. This change may trigger the observed changes in photoluminescence property of the nanoparticles by influencing the degree in surface passivation. It can be postulated that the photoexcited electron or hole interacts with OPH in a donor−acceptor charge transfer manner. This cascade of reactions ultimately leads to a decrease in photoluminescence. The system works both to detect the presence of paraoxon solution and to detoxify it using OPH. As a control experiment, TGA-capped CdSe QDs in solution (1.6×10^{-4} M) were exposed to paraoxon solution (1×10^{-6} M). There was no change in the photoluminescence intensity. This means that the sensing assembly is truly responsible for the detection of the organophosphorus compound.

Epifluorescence imaging was utilized to examine the topography of the film. Images showed that the film was homogeneous with uniformed dispersal of the quantum dots. QDs emitted in the yellow region of the visible spectra as shown in Figure 1. This image was captured by epifluorescence showed a green film (Figure 3a) before paraoxon exposure. This green emission occurs because the emission from the film covered wavelengths that correspond to green in the visible spectrum. Although it is in the yellow region, it lies on the cusp of the green region, which ends at 570 nm. After the sensing assembly was exposed to an aqueous solution of paraoxon (1×10^{-6} M), the QDs lose their photoluminescence, as shown in Figure 3b.

This paper has presented results that show that TGA-capped CdSe QDs can be incorporated into the LbL film, improving the photoluminescence of the QDs. As a result of the improved optical photoluminescence, the system was used analytically to detect the presence of paraoxon solution. The fabricated LbL thin film showed an attractive alternative to using polyelectrolytes with optical properties for biosensing assembly use.

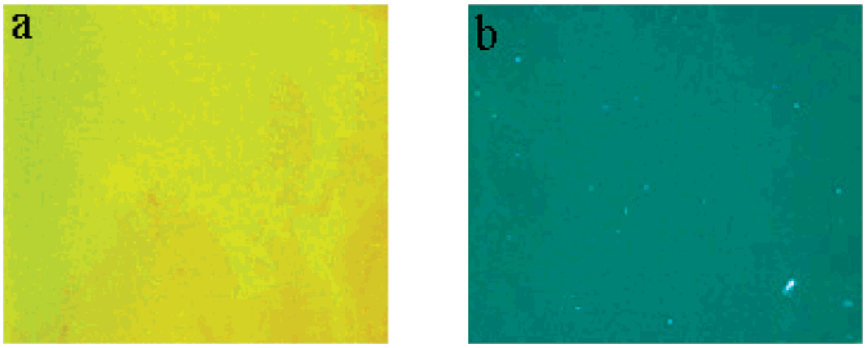


Figure 3. Epifluorescence microscope images of the TGA-capped CdSe QDs in the LbL film: (a) before paraoxon exposure; (b) after paraoxon exposure. Image size 895 μm × 713 μm.

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