Use of CdSe/Zns core/shell Quantum Dots For Detection of Protoporphyrin IX and Polyaromatic Hydrocarbon Compounds

Jong Il Rhee

Abstract: In this work, some CdSe/ZnS core/shell quantum dots (QDs) were synthesized and entrapped into a mixture of 3-glycidoxypropyltrimethoxysilane (GPTMS) and methyltrimethoxysilane (MTMS). The electron energy transfer from the QD-entrapped GA (mixture of APTMS and GPTMS) membrane to protoporphyrin IX (PpIX) or to polycyclic aromatic hydrocarbon compounds (PAHs) led to the quantification of PpIX in the linear detection range of 1-50 μM, of anthracene (ANT) and phenanthrene (PHE) in the linear detection range of 0.01-0.1 μM, and of pyrene (PYR) in the range of 0.005-0.05 μM.

Key-Words: Anthracene, Electron energy transfer, Phenanthrene, Protoporphyrin IX, Pyrene, CdSe/ZnS QDs ,

I. INTRODUCTION

Semiconductor nanoparticle quantum dots (QDs) show many attractive properties for applications in the field of light-based technology, including as lasing media [1], electronic devices [2], and sensors [3], due to their high quantum yield and photo-stability [4]. Fluorescent semiconductors conjugated with polymers can be used as energy donors when they are conjugated to a corresponding acceptor. QDs can act as an energy donor by being sized-tuned to give better spectral overlap with the acceptor. Protoporphyrin IX (PpIX) is produced from the conversion of 5-aminolevulinic acid (ALA) in the synthesis of Haem and used as a photosensitizer. Anthracene (ANT), phenanthrene (PHE) and pyrene (PYR) are polycyclic aromatic hydrocarbon compounds (PAHs). According to the U.S. EPA (environmental protection agency), they are included in the list of 16 PAHs considered as priority pollutants.

In this work, the CdSe/ZnS QDs were synthesized and entrapped in a sol-gel matrix (GA) of APTMS and GPTMS for fabricating the QD membranes by a simple and general procedure on bottom of wells of the 24 well microtiter plate. The QD-entrapped sol-gel membranes were evaluated by measuring their energy absorption and sensitivity to various concentrations of PpIX. Concentrations of PAHs like PYR, ANT and PHE at trace levels were detected based on the amplification of fluorescent emission of QDs through the mechanism of energy transfer between PAHs and QDs.

II. EXPERIMENTAL

II.a MATERIALS

Cadmium acetate, selenium, zinc acetate, hexamethyldisilathiane ($(TMS)_2S$), trioctylphosphine oxide (TOPO), trioctylphosphine (TOP), mercaptopropionic acid (MPA), 4-dimethylamino pyridine (DMAP), 1-hexadecylamine, stearic acid, 3-glycidoxypropyltrimethoxysilane (GPTMS), 3-aminopropyltrimethoxysilane (APTMS), tetraethoxysilane (TEOS), methyltrimethoxysilane (MTMS), protoporphyrin IX (PpIX), anthracene (ANT), phenanthrene (PHE), and 1-pyrenemethylamine hydrochloride (PYR) were purchased from Sigma-Aldrich Chemical Co. (USA) N,N-dimethylformamide (DMF), methanol, ethanol, and chloroform were obtained from Honeywell International Inc., Burdick & Jackson (USA). Other chemicals such as sodium phosphate, sodium chloride, etc., used for the preparation of phosphate buffer saline (PBS) were of analytical grade and used without further purification.

The author is with the School of Chemical Engineering,
Chonnam National University
YongBong Ro 77, 61186 GwangJu
REPUBLIC OF KOREA
jirhee@jnu.ac.kr

II.b SYNTHESIS OF HYDROPHILIC CdSe/ZnS CORE/SHELL QDs

The synthesis of the CdSe-ZnS core-shell QDs was based on the theories and little modified. CdSe nanoparticles were synthesized using a modified version of the method of Qu and Peng [5] and Gaunt et al [6], as described in our previous work [7]. For the synthesis of the hydrophilic CdSe/ZnS QDs, the CdSe/ZnS QDs were coated with mercaptopropionic acid (MPA). The synthesis of the MPA-coated QDs was performed according to the protocol described in the literature with a slight modification [8]. The absorption and emission spectra of the hydrophilic CdSe/ZnS QDs were determined by a Multiskan Spectrum Spectrophotometer (Thermo electron Co., Finland) and Fluorescence Spectrophotometer (Model: F-4500, Hitachi Co., Japan), respectively.

II.c PREPARATION OF THE QD-ENTRAPPED SOL-GEL MEMBRANES

The MPA-coated QDs in DMF (2 mL) were precipitated by adding 1 mL of DMAP solution (25 mg DMAP/1.0 mL DMF). After centrifugation, the collected QDs were mixed with 300 μL of sol-gel GA and then kept at room temperature for several hours to ensure their complete dissolution and conjugation. 6 μL of QDs dissolved in the sol-gel were spread on the bottom of a well of a 96 well microtiter plate (NUNC Co., Denmark) and dried at room temperature for 1 hour before heating them at a temperature of 65 ^{o}C for 24 hours. In this work, four kinds of QD-entrapped sol-gel membranes were prepared by adding hydrophilic QDs with different emission wavelengths of 535 nm, 545 nm, 555 nm and 575 nm (i.e. QD535, QD545, QD555 and QD575, respectively) to the sol-gel GA.

II.d MEASUREMENTS

II.d.1 Measurement of PpIX

100 μL of PpIX in the concentration range of 1-50 μM were added to each well in which the QD-entrapped sol-gel membrane was deposited and its absorption spectrum was measured using Multiskan a Spectrum spectrophotometer (Thermo electron Co., Finland). The repeatability of the QD-entrapped sol-gel membranes was also tested with 1-50 μM PpIX after washing each well several times with distilled water.

II.d.2 Measurements of PAHs (ANT, PHE and PYR)

Anthracene (ANT) was dissolved in acetonitrile to obtain a 1.0 μM stock solution. From this stock solution, various ANT samples were prepared in the concentration range of 0.01 μM to 0.1 μM. Pyrene (PYR) was dissolved in ethanol to form a 0.5 μM stock solution from which various samples in the concentration range of 0.005 μM to 0.05 μM were prepared by dilution with distilled water. A 1.0 μM stock solution of phenanthrene (PHE) was also prepared in ethanol and various samples in the concentration range of 0.01 μM to 0.1 μM were made by diluting it with distilled water. 1 ml of different concentrations of ANT or PHE or PYR was added to one well of a 24 well microtiter plate on which a QD-entrapped membrane was deposited.

III. RESULTS AND DISCUSSION

III.A PROPERTIES OF CDSE/ZNS QD AND PPIX

The CdSe/ZnS core-shell QDs synthesized herein had a particle size of about 5 nm and high fluorescence intensity (quantum yield of 60 %). The quantum yield of the QDs after covering their surface with carboxyl groups (MPA) was decreased to about 50 % of its initial value. After ligand exchange, the resulting water-soluble CdSe/ZnS QDs can be used for one month before their precipitation. Fig. 1 shows the absorption and emission spectra of the water-soluble CdSe/ZnS QDs with an FWHM (full width at half maximum of the emission spectrum) of 35 nm.

A difference in the absorption spectra of PpIX in DMF and 10 mM PBS is observed in Fig. 1. One spectral band of PpIX in DMF was blue shifted to 375nm and the other spectral bands were slightly red-shifted to longer wavelengths. However, the spectral bands of PpIX in PBS had higher energy absorption values than those in DMF, because PpIX in aqueous solution is preferable to a hydrophilic medium in biological applications. Therefore, PpIX solutions were prepared in 10 mM PBS for all experiments in this work.

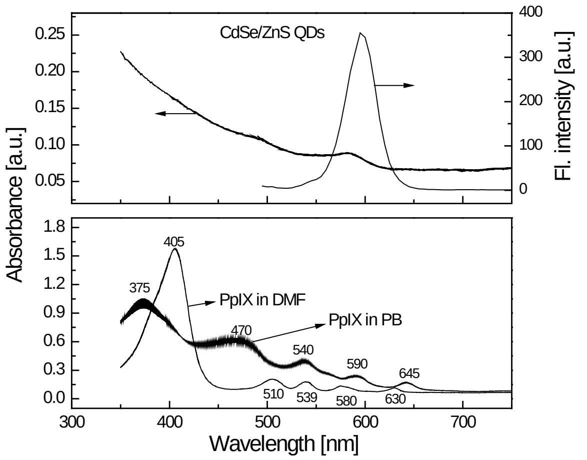

Fig.1 Absorption and fluorescence emission spectra of water-soluble CdSe/ZnS QDs and of PpIX in DMF and 10 mM PBS in a quartz cuvette

Four kinds of QDs with different emission wavelengths (535 nm, 545 nm, 555 nm, 575 nm) were also synthesized. Fig. 2 shows the emission and absorption spectra of the water-soluble CdSe/ZnS QDs with an FWHM (full width at half maximum of the emission spectrum) of 30 nm being observed for both the green and orange QDs. The photos of the emission fluorescence of the water-soluble QDs (QD535, QD545, QD555 and QD575) under UV light and their TEM images (about 2.5-3.0 nm for QD535, QD545 and QD555, and 5 nm for QD575) were also investigated.

As in theory [9], fluorescence resonance energy transfer (FRET) is a nonradiative process whereby an excited state donor transfers energy to a proximal ground state acceptor through long range dipole-dipole interactions. The acceptor must absorb energy at emission wavelength(s) of the donor. The rate of energy transfer is highly dependent on many factors, such as the extent of spectral overlap, the relative orientation of the transition dipoles and most importantly, the distance between the donor and acceptor molecules. Herein, with broad absorption range (~ 300-580 nm, the four QDs in this study can absorb the emission energy from other compounds in this range. Some PAHs such as anthracene (ANT), phenanthrene (PHE) and pyrene (PYR) emit fluorescence in the range of 380-480 nm (Fig. 3). Their emission spectra are overlapped with the absorption spectra of the QDs that could lead to an energy transfer from the PAHs to the QDs when they are being excited.

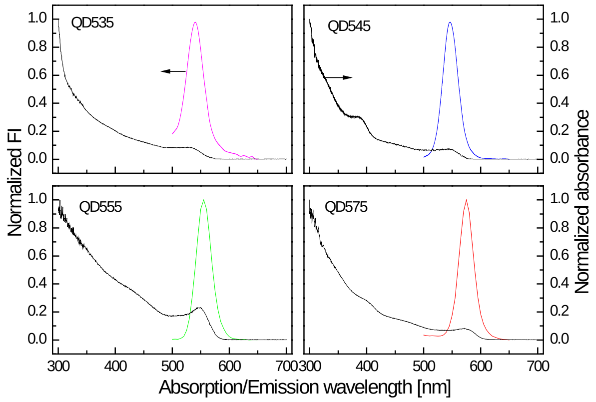

Fig. 2. Emission and absorption wavelengths of hydrophilic CdSe/ZnS QDs (QD535, QD545, QD555, QD575).

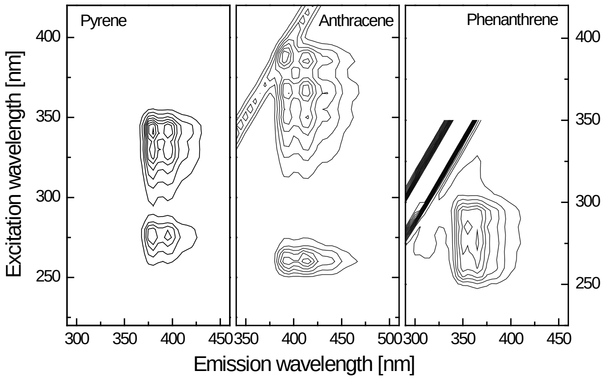

Fig. 3. 2D fluorescence spectra of 0.005 µM pyrene (PYR), 0.01 µM anthracene (ANT) and 0.01 µM phenanthrene (PHE)

III.b QUANTIFICATION OF PPIX THROUGH THE CDSE/ZNS QD-ENTRAPPED GA MEMBRANE

The CdSe/ZnS QD-entrapped membranes in particular and the CdSe/ZnS QDs probes in general were used in our previous works [16,17] for the detection of some analytes. In this work, the absorption spectrum of PpIX increased significantly in the presence of the QD probes, as mentioned above, and therefore, this technique was applied to the quantification of PpIX using the QD-entrapped membrane. In Figs. 4a and 4b, the QD-entrapped membrane responded to different concentrations of PpIX (1-50 µM) with high repeatability and sensitivity. The highest sensitivity was obtained at an absorption wavelength of 475 nm (Slope=0.012, R^2=0.9929), as shown in Fig. 4c. In comparison with the control samples without the QD-entrapped membrane, i.e. only PpIX solutions with QD probes (Fig. 4d), the sensitivity of the QD-entrapped membrane to PpIX solution was increased by 25 % - 44 % in the spectral bands. The repeatability of the QD-entrapped membrane was very high, since the slope values (SI) of the linear detection range did not change very much for any of the absorption peaks (Fig. 4b). Compared with the sensitivity and repeatability of the QDs probes, the QD-entrapped sol-gel membrane showed higher sensitivity and reuse with high productivity for the quantification of PpIX samples.

III.C. RESPONSE OF THE QD-ENTRAPPED MEMBRANES FOR PAHS

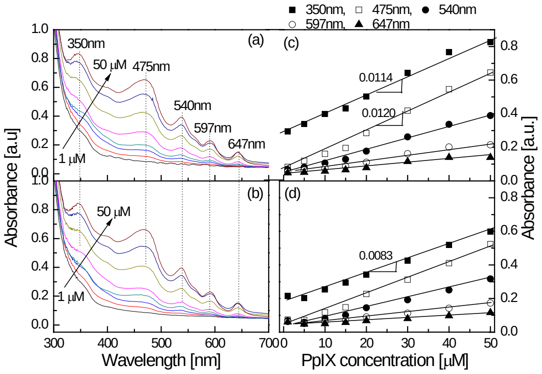

Fig. 4 Response and repeatability of the CdSe/ZnS QD-entrapped sol-gel membrane in the PpIX concentration range of 1-50 μM; (a) the first and (b) the second measurement. (c) Linear calibration curves for PpIX solution with the QD-entrapped membrane at various excitation wavelengths; (d) absorption values of QD probes for 1-50 μM PpIX solution as control samples

Fig. 5 shows the response characteristics of the four QD-entrapped membranes for different concentrations of ANT, PHE and PYR. All of the membranes were very sensitive to ANT, PHE and PYR with linear detection ranges of 0.005-0.05 μM for PYR and 0.01-0.1 μM for ANT and PHE. Their response time was also very short in all cases, viz. 8 seconds after inserting the microtiter plate into the measurement chamber of the microtiter plate reader. When comparing three PAHs, the sensitivity of the QD-entrapped membrane for PYR was higher than those for ANT or PHE, since its slope value for PYR was about twice as high as that for ANT or PHE (data not shown). This might be due to the influence of the solvent (acetonitrile for ANT and ethanol for PHE) on the QD-entrapped membrane, even though the volume of the solvent was less than 10 % of the total sample volume. The compatibility of the PAHs originating from the less polar stock solution with the aqueous solution is another reason for this difference. When using 100 % acetonitrile as the solvent for the dilution of the ANT sample, the sensitivity of the QD-entrapped membrane was decreased for ANT (data not shown) and the membrane was cracked after being used three or four times.

When comparing the four QD-entrapped membranes in terms of their sensitivity to PYR (ANT or PHE), no significant differences were observed. Thus, the indirect activation of the QDs occurred by the FRET mechanism from the PAHs to QDs, since the fluorescence intensity of the QDs increased with increasing concentration of PYR, ANT and PHE. A supposition is also attributed for the amplification of the fluorescence intensity of QDs. That is, in a fluorescent semiconductive conjugated polymer, absorption of photon generates excitons. The migration of excitons is known to follow the downhill energy gradient and to be trapped at low-energy sites. In the same time, analytes are introduced to the conjugated polymer and generate trapping sites. These trapping sites can serve as energy sinks that effectively harvest all of the excitons generated in the polymer and this type of energy harvesting constitutes an amplification mechanism of signal [12,13]. As in this case, sol-gel matrix GA could contribute an important role in the interaction of QDs and PAHs.

The high responses of QD535, QD545, QD555 and QD575 for the detection of ANT, PHE and PYR showed that a variety in emission wavelengths of QDs could be applied for fabricating QD-entrapped membranes. These QD-entrapped membranes can be used as an independent sensor or as a reference for the detection of PAHs. In a test of the QD-entrapped membranes for the analysis of PAHs in real water samples, such as tap water and mineral water, their capability to recover from fixed concentrations of ANT, PHE and PYR was very high (data not shown). It ranged from 97.4 % to 106.9 % for all of the QD-entrapped membranes. This means that some interfering elements, such as ions, usually present in these kinds of water did not affect the measurement of the PAHs significantly.

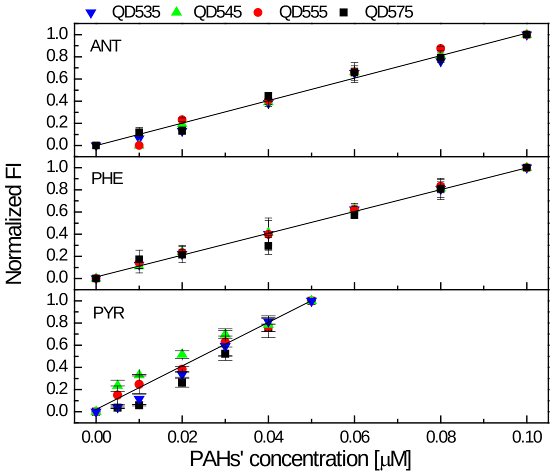

Fig. 5. Change in fluorescence intensities of four QD-entrapped membranes with increasing concentrations of ANT, PHE and PYR.

VI CONCLUSION

The electron energy transfer mechanism from the CdSe/ZnS QDs and sol-gel GA matrix to PpIX could be used to quantify the amount of PpIX by using the QD-entrapped GA membrane. The linear detection range for PpIX was of 1-50 µM. The high response of the CdSe/ZnS QD-entrapped GA membrane makes it promising for certain applications in the sensor area. Four kinds of hydrophilic QDs (QD535, QD545, QD555, QD575) were successfully used to fabricate the QD-entrapped sol-gel membranes. The linear detection ranges of the four types of QD-entrapped membranes were 0.005-0.05 µM for PYR and 0.01 - 0.1 µM for ANT and PHE. The easy and simple preparation of these QD-entrapped membranes should enable them to be applied in environment analysis.

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