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Development of nanobiosensor for urea by immobilizing jack bean meal urease on CdS QDs

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ABSTRACT

Semiconductor nanoparticles (NPs) have attracted much attention as a new class of fluorescent probe for many biological applications including biosensors for glucose, cholesterol, cysteine etc., which were based on their size dependent unique electrical and photophysical properties. One of the major challenges in fabricating nanomaterial based biosensor is conjugation of a suitable compound to quantum dots which is preferably selective to the analyte of interest. Here we report our studies on synthesis, characterization and application of jack bean meal urease immobilized on CdS quantum dots (QDs) for sensing of urea. The CdS QDs were synthesized by chemical precipitation method using Mercaptoacetic acid (MAA) for controlling size as well as for imparting functional group for conjugating urease. The urease immobilize on MAA capped CdS nanoparticle was characterized by an array of techniques, like, UV- visible, Fluorescence, XRD, FT-IR and SEM EDAX. The detection capability of urea was studied by fluorescence spectroscopy at an excitation wavelength of λ_{ex} = 430 nm and emission wavelength of λ_{em}= 546 nm. This method was capable to detect urea in the concentration range of 0.1 µM to 1 mM.

Keywords: CdS quantum dots; biosensor; urease; urea; fluorescence spectroscopy.

R. K. Dutta obtained his Ph.D. degree (1999) in Chemistry working at UGC-DAE CSR, Kolkata Centre, degree awarded by Utkal University, Bhubaneswar. He worked as a postdoctoral fellow at four international labs, i.e., University of Witwatersrand in South Africa, University of Bordeaux in France, and Technical University of Eindhoven in Netherlands and National University of Singapore in Singapore. He has eighteen years research experience. Currently he is Associate Professor in the Department of Chemistry, IIT Roorkee. His current research interests are applications of nanomaterial in drug delivery, environmental remediation, interaction with biological systems and food chemistry.

Introduction

There has been a rapid growth in the development of nanomaterial based biosensors [**1**]. Usually, the biosensors consist of sensitive biological element, namely, enzymes, nucleic acids, micro organisms, whole cells, antibodies, cell receptors or biologically derived materials, which can specifically interact with analyte of interest. In addition, biosensors also consist of a physicochemical transducer, which converts the biochemical signal into measureable signals. Some common transducers are based on electrochemical, thermometric, optical, piezoelectric or magnetic properties. For example, electrochemical biosensors which are known for high selectivity and quick response to specific substrates like enzymes, suffer from electrical barriers between enzyme's redox center and solid electrode surfaces resulting in inefficient electrical communication [**2**]. Advent of nanotechnology could be judiciously used to overcome these adversaries and led to the development of newer class biosensors using nanoparticles, (referred to as nanobiosensor) which exhibit size dependent optical properties.

A nanobiosensor is a device incorporating a biological sensing element, which probes the desired analyte of interest, is either intimately connected to or integrated within a transducer i.e., a nanoparticle. Specific molecular

recognition is a fundamental prerequisite, based on affinity between complementary structures such as enzyme-substrate, antibody-antigen and receptor- hormone, and this property in nanobiosensor is used for the production of concentration–proportional signals [**3**]. Among various types of nanomaterials, semiconducting quantum dots like CdSe, ZnO, ZnS, CdS, CdTe have shown tremendous potential as a transducer for nanobiosensor, which is associated with unique electrical, optical and catalytic properties such as broad excitation spectrum, size dependant tunability of emission, longer fluorescence lifetime than organic fluorophores and negligible photo-bleaching [**4**]. Some notable nanobiosensors were developed for detecting and quantifying glucose, maltose, urea, cysteine, adenine and ascorbic acid [**5**].

Detection and quantification of urea in biological system has drawn significant attention as it is one of the final metabolite, along with creatine and uric acid, in nitrogenous compounds of mammals and is one of the essential indexes in diagnosing liver and kidney disorders. Various methods have been reported for the determination of urea in tissue and body fluids, including ion chromatography, electrochemical methods and enzymatic methods. We report the results of our investigation to determine the feasibility of replacing the isolated enzyme urease with the less expensive biocatalytic material jack bean meal for the construction of urea biosensors [**6**]. In the present work, MAA capped CdS QDs were prepared by modifying the reported method [**7**]. And Jack bean meal urease is conjugated to CdS QDs and it is used for the determination of urea.

Experimental

Materials

Mercaptoacetic acid (MAA), Cadmium chloride ($CdCl_2 \cdot 10H_2O$), sodium sulphide, jack bean meal, urea, sodium hydroxide, toluene were procured from Himedia, India. Urea was dissolved in double distilled water to prepare stock solutions (1 mM) which was then stored at 4 ^oC. All chemicals were of analytical grade and used without further purification. All aqueous solutions were prepared with double distilled deionised water.

Synthesis of urease immobilized on CdS NPs

To 50 mM $CdCl_2$ aqueous solution, 87.2 μL of MAA dissolved in toluene was added drop wise. The above solution was stirred for 30 min. To this solution a freshly prepared solution of jack bean meal urease in toluene (2% w/v) was added. The pH of the resulting solution was raised to 8.0 using 0.1 N NaOH. Then the aqueous suspension of sodium suphide was added and stirred for 3 h. The aqueous phase was separated and acetone was added to precipitate the CdS QDs. The CdS quantum dots (QDs) were separated by centrifuging at 4000 rpm for 5 min. The supernatant was discarded and the light yellow colored precipitate so obtained was washed with deionized water and centrifuged as given above. The supernatant was discarded and the residue comprising urease immobilized on to MAA capped CdS QDs was lyophilized and stored at 4 ^oC till further used. This batch of sample will be henceforth referred to as CdS-MAA-urease QDs. In addition to this, control sample without jack bean meal urease, made of MAA capped CdS QDs (CdS-MAA QDs) was synthesized in the same process as discussed above.

Characterization

The X-ray diffraction measurements of CdS-MAA-urease QDs were performed with powder diffractometer (Bruker ARS D8 Advance) operated at 40 KV using graphite monochromatized Cu K_α radiation source with a wavelength of 1.54 Å in a range between 20° to 80° on a 2θ scale. The particle size distribution and morphology of CdS-MAA-QDs were studied by field emission scanning electron microscope coupled to energy dispersive X-ray analyzer (FESEM EDAX) using FEI-Quanta 200F, operated at 20 kV. After suitable dilution, the dispersed NPs were sprayed on a cleaned glass plate, which was dried and then coated with a thin layer of Au. The immobilization of urease on to CdS NPs was studied by recording infrared spectroscopy at room temperature in the range of 4000−400 cm^{-1} using KBr pellets, using PerkinElmer RXI FT–IR spectrometer. The absorption spectra of CdS-MAA QDs, CdS-MAA-urease NPs and CdS-MAA-urease NPs treated with different concentrations of urea were recorded using UV-visible spectrophotometer (Shimadzu, UV-1800). The emission spectra of the above batches were measured by Shimadzu–RF–5301 PC fluorescence spectrophotometer in the range of 300–800 nm. The excitation and emission slit width of 5 nm was maintained for all the measurements. All measurements were performed in triplicate. All optical measurements were performed at room temperature and under ambient conditions.

Detection of urea

The as-synthesized CdS-MAA-urease NPs were used for detecting Urea. From a 5 mM stock solution of Urea, different concentrations of urea solution, namely, 1 mM, 0.1 mM, 0.01 mM, 1 μM, 0.01 μM were prepared in double distilled water. To 2 mL of each of these different concentrations of urea solution, 3 mL of aqueous dispersion of CdS-MAA-urease NPs was added and its absorption and emission spectra were recorded. The maximum λ_{abs} was measured at 420 nm which matched with that of CdS-MAA QDs. The excitation wavelength of λ_{exc} = 430 was used for recording emission spectra for all the batches in the range of 430 - 800 nm. The excitation wavelength of 430 nm also restricted the absorption by any amino acid in the sample and hence the emission observed was only due to emission by CdS QDs.

Results and discussion

Characterization of CdS-MAA NPs

The XRD pattern of CdS-MAA QDs exhibited prominent broad peaks at 2θ values of 27^o, 44^o and 52^o which corresponded to (111), (220) and (311) planes (**Fig. 1**), and matched with the cubic structure of CdS (JCPDS – file No. 10-454). Our results were in good agreement with literature [**8**]. The broadened peaks indicated sub 10 nm particle sizes. The average crystallite size was determined to be 3.4 nm, from the full-width and half-maximum (FWHM) of the

most intense peak making by using Debye Scherrer's formula:

$$d = 0.89\lambda/\beta \cos \theta.$$

Where, λ is the wavelength of X-ray radiation, β is the FWHM in radians of the XRD peak and 2θ is the angle of diffraction (27°).

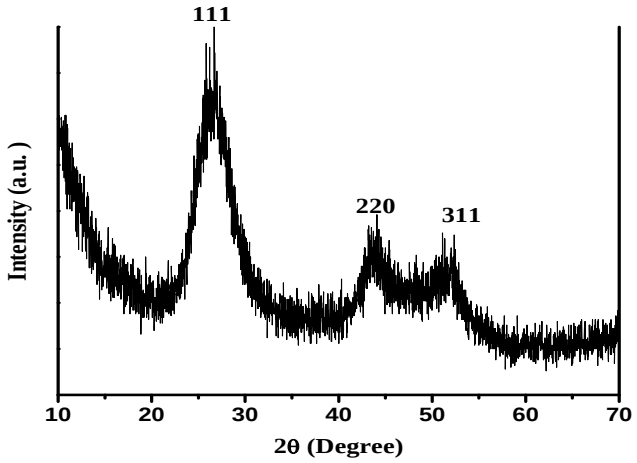

Fig. 1. X-Ray diffraction (XRD) pattern of MAA capped CdS nanoparticles exhibiting characteristic broad peaks at 2θ values of 27°, 44° and 52° corresponding to cubic CdS phase.

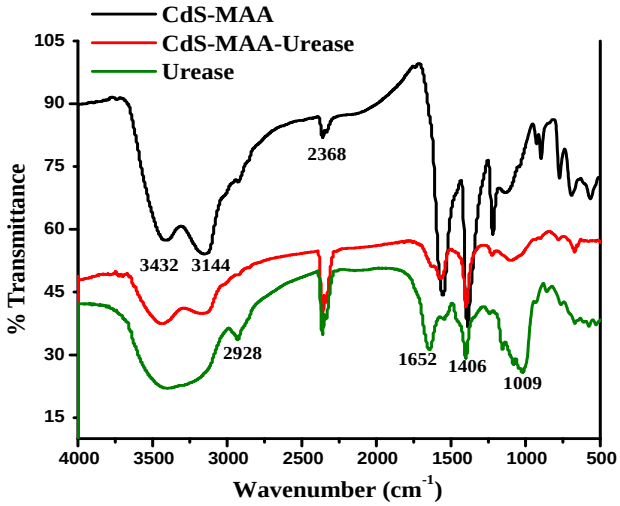

Fig. 2. FT-IR spectra of MAA capped CdS QDs, urease immobilized on MAA capped CdS QDs (CdS-MAA-urease) and urease.

The FT-IR spectra of MAA capped CdS QDs (CdS-MAA), urease immobilized on MAA capped CdS QDs (CdS-MAA-urease NPs) and urease are shown in **Fig. 2**. The most pronounced IR absorption bands occurred at 3422 cm^{-1}, 3144 cm^{-1} (νOH, COOH), 1560 cm^{-1} (ν_S COOH), 1406 cm^{-1} (bending mode of νOH, COOH) for CdS-MAA QDs. Notably, the IR absorption peak due to vibration of S-H bond at 2550-2670 cm^{-1} was absent, which indicated the formation of a covalent bond between thiols and Cd atom on the QDs surface and corroborated with the literature report on MAA capped CdS quantum dots [**9**]. In the case of CdS-MAA-urease NPs, in addition to the broad peak at 3000 – 3500 cm^{-1} due to (ν_{OH}, COOH) and 1406 cm^{-1} (bending mode of ν_{OH}, ν_{COOH}) prominent peaks were observed at 1652 ((ν_{N-H} of urease), which overlapped with the (ν_S, COOH of MAA). The peaks at 1250 cm^{-1} and 1009 cm^{-1} were due to ν_{C-O} vibration frequency. The peaks observed for CdS-MAA-urease NPs were evident in the IR spectrum of urease. These observations strongly indicated immobilization of urease on to the CdS-MAA QDs. Further, the scanning electron microscopy of CdS-MAA-urease NPs revealed the morphology of the as synthesized nanoparticles which were found to be agglomerated (**Fig. 3**).

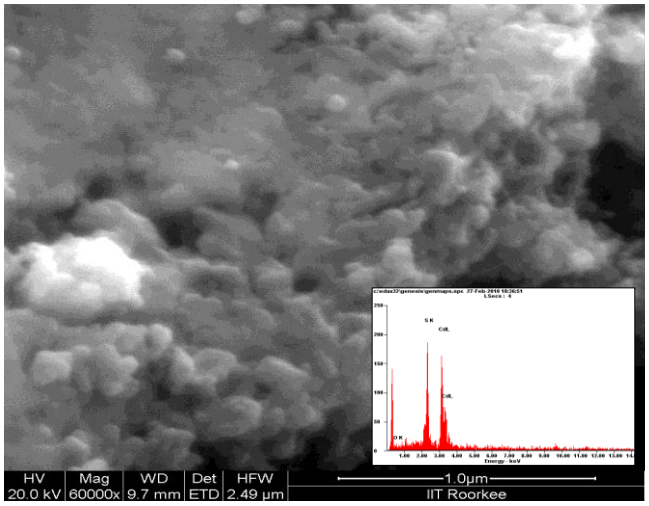

Fig. 3. Scanning electron microscopy image showing spherical shaped CdS quantum dots, inset showing Characteristic L X-ray of Cd and K_α X-ray of S.

The optical properties of MAA capped CdS QDs were characterized by UV–visible absorption spectrophotometry and fluorescence spectrophotometry. The characteristic absorption peak of the MAA capped CdS QDs was observed at λ_{abs} = 420 nm as shown in **Fig. 4**. The fluorescence emission maximum of the MAA functionalised CdS QDs is obtained at 546 nm using λ_{ex} = 430 nm.

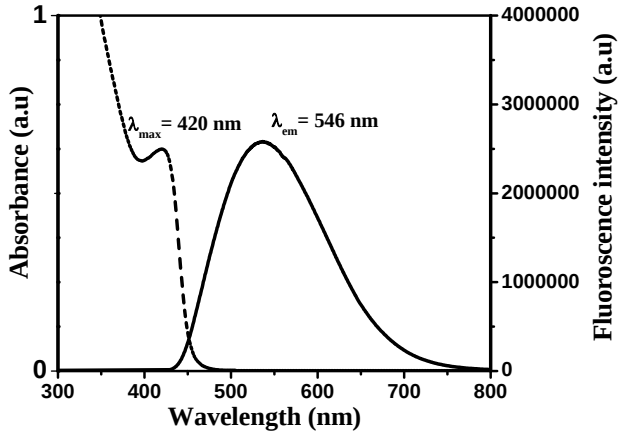

Fig. 4. Absorption (dashed) and emission (solid) spectra of MAA capped CdS nanoparticles. The characteristic absorption peak of the MAA capped CdS nanoparticles is determined at 420 nm and the corresponding maximum fluorescence emission is determined at 546 nm using λ_{ex}= 430 nm.

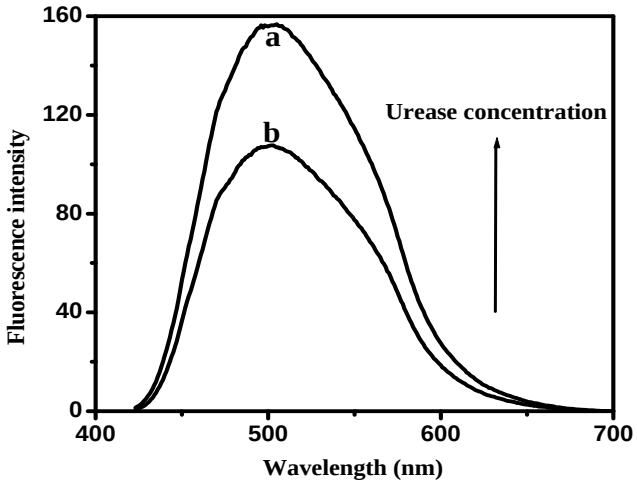

Fig. 5. Fluorescence spectra of CdS NPs (0.05 M) measured using λ_{ex}= 430 nm for (a) 0.3 M urease immobilized on MAA capped CdS QDs; (b) without urease immobilization.

Emission behavior of urease immobilized CdS NPs

The optical property of CdS-MAA-urease also showed λ_{abs} = 420 nm, which was characteristic of CdS QDs as discussed above. The fluorescence emission spectrum of CdS-MAA-urease NPs at pH 8.0 was found to be higher in intensity as compared to CdS-MAA QDs at the similar pH (**Fig. 5**).

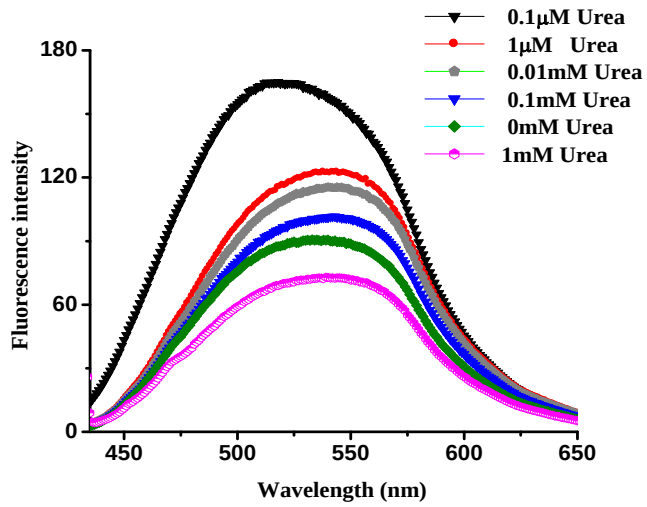

Fig. 6. Fluorescence spectra of CdS-MAA-urease treated with varying concentration of urea, measured at λ_{ex}= 430 nm.

The fluorescence emission of CdS-MAA QDs is due to radiative recombination of charge carriers in trap sites. The critical influence of QDs surface on photoluminescence can be understood in terms of the trap states, which can be caused by structural defects, such as atomic vacancies, local lattice mismatches, dangling bonds, or adsorbates at the surface [**5**]. The excited electron or hole can be trapped by these local energy minima and lead to nonradiative recombination [**10**]. However at this point, the most important factor that seemed to play a major role in governing the fluorescence enhancement could be attributed to the passivation of trap states or defects on the surfaces of the CdS QDs. The passivation of surface defects can be can be achieved by various means. In the case of urease, its isoelectric point is 5.0-5.2 and above this point, it carries a net negative charge. At pH 8.0 negative charges on the protein surface may interact electrostatically with Cd^{2+} ions on the surface of CdS NPs. The other possible mechanism for the increase in fluorescence intensity could be attributable to the electrostatic interaction occurring between the Cd^{2+} enriched CdS nanopaticles and –COOH of tyrosine/ phenyl alanine amino acids in the urease enzyme resulting in passivation of surface defects and increase in luminescence has been observed [**11**].

Detection of urea by emission spectroscopy

The concentration of CdS-MAA-urease QDs was kept fixed, which was determined from the intensities of fluorescence intensity at λ = 546 nm, corresponding to an excitation at 430 nm. The same volume of different concentration of urea solution in the range of 0.1 μM – 1 mM was added, the fluorescence intensities varied as shown in **Fig. 6**. Compared to the control samples, i.e., CdS-MAA-urease NPs, the fluorescence intensity was enhanced for the batches treated with 0.1 mM, 0.01 mM, 1 μM, 0.1 μM urea. On the other hand, the batch treated with 1 mM of urea showed 35 % quenching of fluorescence intensity with respect to the control. The quenching could be attributed to reduction of non-radiative recombination at surface vacancies [**12**]. An inverse linear correlation between fluorescence intensity against concentration of urea (R^2 = 0.947, **Fig. 7**) was obtained, which indicated the feasibility of using the as developed CdS-MAA-urease nanobiosensor for detecting urea in a quantitative manner.

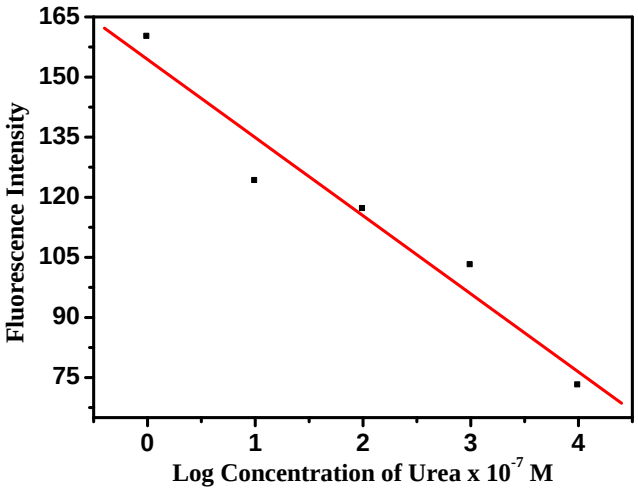

Fig. 7. Showing linear response (inverse) of fluorescence intensity of CdS-MAA-urease for increasing concentration of urea (R^2 = 0.947).

Conclusion

Herein we have presented a highly sensitive and selective method for the determination of urea using fluorescent CdS-MAA-urease QDs as a potential nanobiosensor. The concentration of urea of the level of 0.1 mM or less showed enhancement of fluorescence yield while the concentration of 1 mM of urea exhibited fluorescence quenching effect. Since the concentration of urea in human blood is of the order of 2.5 – 7.9 mM levels, so it may be surmised that the

novel developed - low cost, CdS-MAA-urease system could be further developed as a potential nanobiosensor for urea in blood.

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