Enzyme Activity of Glucose Oxidase Immobilized on Zns AND Znse Quantum Dots: In View of Glucose Sensing

S. Narkhede[1], R. Gedam[2], S. A. Acharya[3]
[1,2]*Department of Applied Physics, Vishwesharya national Institute of Technology, Nagpur, M.S. India*
[3]*Ph. D, Advanced Materials Research Laboratory, Department of Physics, RTM Nagpur University, Nagpur, M.S. India*

***Abstract*—Bioconjugation of various enzymes with semiconductor or metal Quantum dots (QDs) provides hybrids systems with new possibilities in biosensor. It also helps to enhance the enzymatic activity and stability due to the large surface area of QDs. In the present attempt, we report the conjugation of glucose oxidase (GOx) onto luminescent ZnS and ZnSe QDs using 1-ethyl-3-(3-dimethylaminopropy) carbodiimide (EDC)/N-hydroxysuccinimide (NHS) as coupling reagents for glucose biosensing based on the effective quenching of the phosphorescence (PL) spectra of PVD capped-ZnS and MPA capped-ZnSe QDs by the H2O2 generated from GOx catalyzed oxidation of glucose. A typical room temperature PL spectrum of ZnS and ZnSe are dominated by characteristics deep level emission peak at 540 and 566 nm, related with transition from conduction band to sulphur and selenium vacancies defect structure, respectively; which are found effectively quenched by glucose content. The obtained bioconjugate provided enzymatic performance with Michaelis-Menten constant (Km and Vmax) 10.20 mM/L and 0.5 for the ZnS and 6.85 mM/L and 0.125 for ZnSe, respectively. It clearly indicates that enzyme affinity for ZnSe is more as compared to ZnS QDs, however rate of enzymatic reaction is found to be more for ZnS than ZnSe.**

***Index Terms*—ZnS and ZnSe QDs, glucose detection, PL Quenching**

I. INTRODUCTION

Enzymes are multipurpose biocatalysts and find increasing applications in chemical synthesis and play prominent roles in biological systems. However, applicability of enzymes is limited due to its long-time instability and difficulty in recovery and reuse [1]. Immobilization of enzyme on certain stable substrate has been suggested to sort out the problem related to stability [2]. Immobilization of enzymes enhance it usability by (i) increasing stability, (ii) repeatability in use, (iii) easy separation from reaction mixtures and (iv) possibility of modulations of catalytic property. Nanomaterial's have been exhibited unique advantages in immobilizing enzyme and could retain its bioactivity due to the (i) desirable microenvironment, (ii) large surface-to-volume ratio, high catalytic efficiency and high surface reaction activity [3-4]. Amongst the various nanomaterial's, II-VI group semiconductors quantum dots (SC-QD) have been attracting researcher's interest owing to their unique ability to tailor optical and electrical properties, good-biocompatibility and high chemical and photochemical stability [5-8]. Immobilization of enzymes on SC-QDs by developing QDs-enzyme hybrid systems have great interest in biosensing as the biocatalytic processes induced by enzymes can be readily read out from optical behaviour of the QDs. The immobilization of enzymes on quantum dots supports through a variety of techniques that includes physical adsorption, electrostatic binding, specific recognition and covalent coupling [9-12]. As the affinity of enzyme for the substrate which directly effect on immobilization process depends upon surface properties of substrate and energy band structure of QDs.

Glucose sensing is becoming important due to their wide use in biological, chemical, clinical synthesis, food industry, environmental monitoring and so forth [13-15]. The glucose level in blood and urine is used as clinical indicators for diabetic patient. Precise measurement and regular monitoring of glucose level in blood and urine is essential in the diagnosis and management of diabetes, which is emerging as a critical health care issue, currently. It makes glucose as most commonly tested analyses. A great deal of

efforts has been focused on fabricating smart sensors for precisely monitoring the glucose level with high sensitivity, high reliability, fast response, good selectivity and low cost [16]. Most of the earlier research attempt on QDs-enzyme nanohybrids for biosensing have been focused on the electrochemical biosensors and use of the fluorescence of QDs in glucose detection [17- 22]. However, the short-lived autofluorescence and scattering light from biological matrixes limits the use. Bio conjugated phosphorescent of QDs eliminate the drawbacks of fluorescence detection, as phosphorescence allows long lifetime and avoid problem associate with scattering of light due to biological matrix.

In the present attempt, bioconjugation of GOx onto ZnS and ZnSe QDs and its influence on enzymatic activity for phosphorescent sensing of glucose are systematically studied by PL spectra. The kinetics of enzymatic activity is studied; Michaelis-Menton kinetic parameter (Km and Vmax) are estimated for both the QDs. Affinity of enzyme for QDs and its influence on enzymatic activity and glucose detection are systematically correlated for ZnS and ZnSe QDs. For this study quenching of PL sensitive S and Se defect energy states are used for monitor glucose content. According to our knowledge this is the first attempt to use pure ZnS and ZnSe QDs for glucose sensing.

II. EXPERIMENTAL SECTION

A. Materials

All chemicals were of analytical grade and of highest purity available. ZnCl2 (zinc chloride), Na2S (sodium sulphide), polyvinalpirolidone, mercaptopropanic acid, 2- mercaptoethanol, thioglycerol, Zn (NO3) (zinc nitrate), NaBH4 (sodium borohydrate), Se (selenium powder), EDA (ethylene di amine) were used as received. Glucose oxidase (GOx), EDC (1-ethyl-3-(3-diamethylaminopropyl) carbodiamide), NHS (N-hydrroxysuccinimide), PBS (phosphate-buffer saline) and glucose were purchased from Sigma Aldrich. Ultrapure water was used as a solvent.

B. Synthesis of water soluble ZnS quantum dots

Luminescent ZnS quantum dots were synthesized according to previously reported method (23). In 100 ml ultrapure water add 1.3628 g ZnCl2 and 0.08 g polyvinalpyrolidone (PVD, (C6H9NO) n) with vigorous stirring at 700C for three hours in the presence of nitrogen atmosphere. PVD is used as surfactant. The sample was kept overnight for complete dissolution to get transparent solution. An equimolar solution of Na2S was added drop wise to this solution, till it appears completely milky. The prepared sample were kept overnight for stabilization. The samples were washed several times by ultrapure water.

C. Synthesis of water soluble ZnSe quantum dots

For the synthesis of luminescent ZnSe quantum dots method reported elsewhere was used [24]. In 100 ml ultrapure water add 0.5 percent hydrazine hydrate then add 1 mM Zn (NO3)2 and 1 mM mercaptopropanic acid (C3H6O2S) with stirring continuously. PH of the solution was adjusted to above 9. In 100 ml EDA dissolved 1 mM Selenium powder and 1 mM NaBH4. The EDA solution was added drop wise with vigorous stirring. The reaction was proceeded at 1000C and refluxing for 24 hours. Both the as-synthesized samples ZnS and ZnSe are water soluble

D. Instrumentation

A Perkin Elmer UV-VIS spectrophotometer model Lambda-35 was used to measure the absorption spectra. A spectroflurometer (Jasco-Spectroflurometer, FP-8200) was used to measured photoluminescence (PL) spectra under ambient atmospheric conditions. Microstructure of the prepared sample was measured by transmission electron microscope (TEM). (TEM) was carried out using a Technai 20 G2 operated at an accelerating voltage of 200 keV using carboncoated formvar grids and the sample was prepared by dispersing the nanostructures in N, N dimethylformamide (DMF) using ultrasonication.

III. RESULTS AND DISCUSSION

A. Bioconjugation of GOx-QDs

Glucose oxidase was dissolved in phosphate buffer saline solution (PBS, 10 mm, PH 7.4) to obtain a solution (1.00 mg mL-1) that was stored at 40C. The conjugation proceeds by

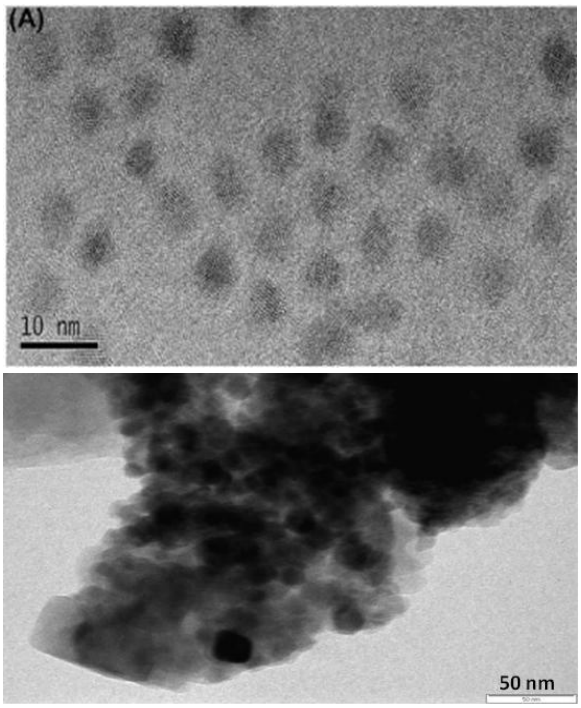
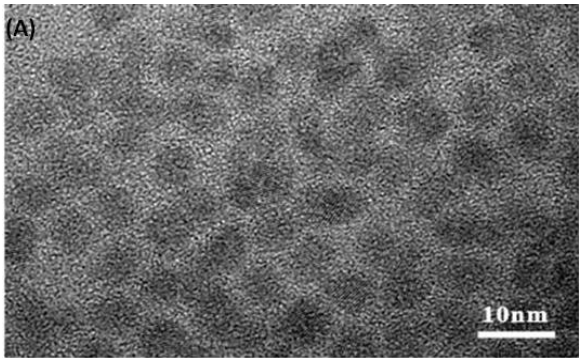


Fig 1: TEM image of (A) ZnS QDs and (B)ZnS-GOx bioconjugate

N-hydroxysuccinimide (NHS) and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) forming active esters to conjugate the carboxyl groups of quantum dots to primary amine groups of GOX. EDC (0.50 mg) and NHS (0.25 mg) were added to ZnS and ZnSe quantum dots stock solution (1.00 ml) to activate the quantum dots in PBS and it was incubated at 30 min at room temperature with continuous gentle mixing. Next, the activated quantum dots (25 µl) and GOx solution were incubated at room temperature for another 2 h with continuous gentle mixing and then stored at 40 C.

B. PL measurement



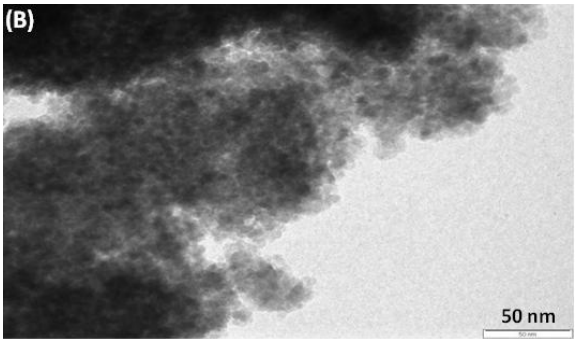


Fig 2: TEM image of (A) ZnSe and (B) ZnSe-GOx co5njugate

In 5 ml ultrapure water adds 0.50 ml phosphate buffer saline solution 2µl GOx quantum dots bioconjugate and 0 g to 3 mM of glucose were sequentially added. For dilution of solution ultrapure water was used. Then the mixture was incubated at 280C for 30 min with gentle mixing. Then photoluminescence spectra were measured at an excitation wavelength

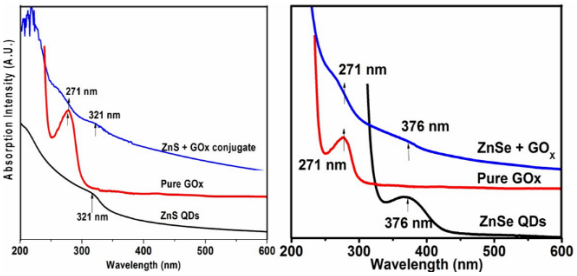


Fig 3: UV-V is spectra of QDs, GOx and QDs-GOx conjugate

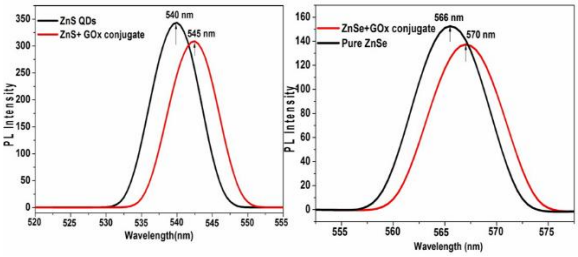


Fig 4: PL spectra QDs and QDs-GOx conjugates

340 nm for ZnS and 370 nm for ZnSe.

C. Characterization of ZnS and ZnSe quantum dots:
Fig 1a and 2a shows TEM images of as-synthesized ZnS and ZnSe QDs, respectively. It clearly illustrates nearly monodispersed QDs of both the samples having size varying in between 7 to 10 nm. TEM images of ZnS+ GOx and ZnSe+ GOx conjugate system exhibits heavy agglomeration of QDs after conjunction. It confirms successful conjugation of GOx onto ZnS and

ZnSe QDs as revealed from TEM images Fig 1b to 2b, respectively after conjugation.

UV-absorption and emission spectra of PVA capped-ZnS and MPA capped ZnSe QDs are also found noticeably changed after conjunction (see Fig 3 and 4). UV-absorption of ZnS and ZnSe QDs shows absorption edge near 321 and 376 nm respectively. Blue shift in both the absorption edge exhibits quantum confinement in both the QDs. Sharp absorption edge in pure GOx is detected around 271 nm [25]. Bioconjuction of QDs-GOx can be rationalized by UV, as both absorption edges (QDs and GOx) are detected.

A typical room temperature photoluminescence (PL) spectrum of

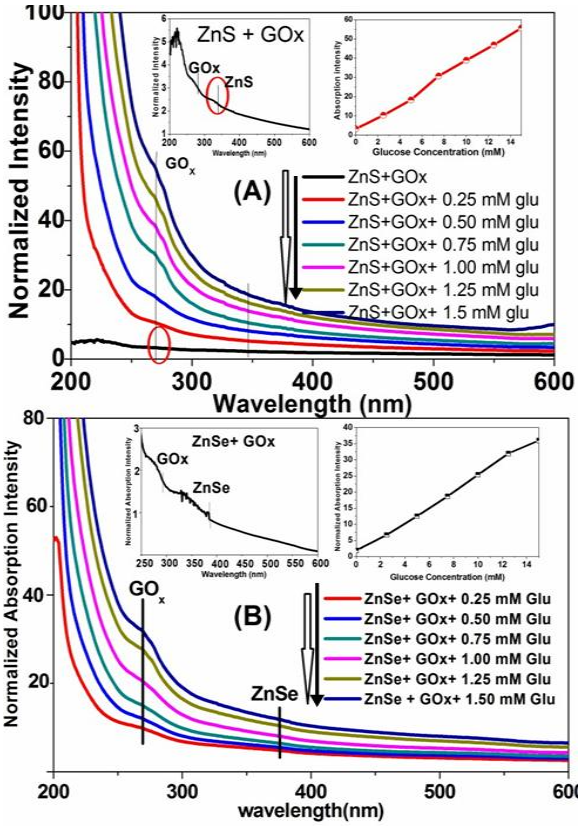


Fig 5: UV-absorption spectra of (a) ZnS-GOx-Glucose (b) ZnSe-GOx-Glucose conjugate for different percentage of Glucose

ZnS and ZnSe shows (Fig 4) that the spectrum is dominated by characteristics deep level emission peak about 540 and 566 nm respectively. The deep level emission is often attributed to stacking faults and non-stoichiometric defects such as intrinsic point defects (vacancies and interstitials). Vacancies and interstitials having different charged states (VS/Se, V+S/Se, V2S/Se+ VZn, V-Zn, Zni, Zn+i, Zn2+i, S/Sei, S/Se-i, S/Se2i) introduced different localized energy levels within the band gap and also combine into donor-acceptor pairs. S/Se vacancies and Zn interstitials introduce energy levels close to the edge of the conduction band hence acts as donor states. However, S/Se interstitials and Zn vacancies introduced energy levels closer to the edge of the valence band hence acts as acceptor levels. The observed PL emission bands around 540 for ZnS and 566 nm for ZnSe after excitation by 340 nm and 360 nm, respectively correspond to transition from conduction band to S/Se vacancies. The PL spectra of QDs-GOx conjugate shows redshift in PL emission spectra of around 5 nm in both the spectrum may attribute to increase in size of GOx conjunction ZnS and ZnSe QDs. Both UV and PL spectra confirm the GOx conjugation on ZnS and ZnSe QDs.

D. UV-VIS Spectroscopic studies of (ZnS/ZnSe) QDs + GOx + Glucose interactions

To study the excited state reaction between QDs + GOx + glucose, it is important to know the type of interaction between them in ground state. The structural changes and complex formation of donor-acceptor system for different glucose level at ground state can be investigated by using UV- Vis absorption measurement [26, 27]. In the present study, we have recorded UV-VIS absorption spectra of ZnS/ZnSe QDs + GOx bioconjugate by adding different content of glucose (0.25 - 3 mM, only up to 1.5 mM shown here) in clinically relevant regime. As shown in fig 5, addition of glucose to a fixed quantity of ZnS / ZnSe + GOx bioconjugate system led to a gradual increase in absorption, keeping the location of peak unchanged. The above results can be rationalized in terms of strong interaction between QDs + GOx and Glucose in the ground state through complex formation.

E. Glucose Sensing Mechanism of the GOD-Mn-Doped ZnS QD Bioconjugate

In general, enzyme induced biocatalytic processes can be probed by II-VI semiconductor QDs using fluorescence resonance energy transfer processes or electron-transfer quenching. In all of these processes, there is need of quencher for energy or electron transfer. Quencher is acted like a stimulator to start

enzyme activities in the QDs based assays. In literature [28-34], H2O2 has been reported to be an effective quencher for CdSe@ZnS or CdTe QDs. It is the product of many enzymatic processes such as GOD, lysine oxidase, and chlorine oxidase etc.

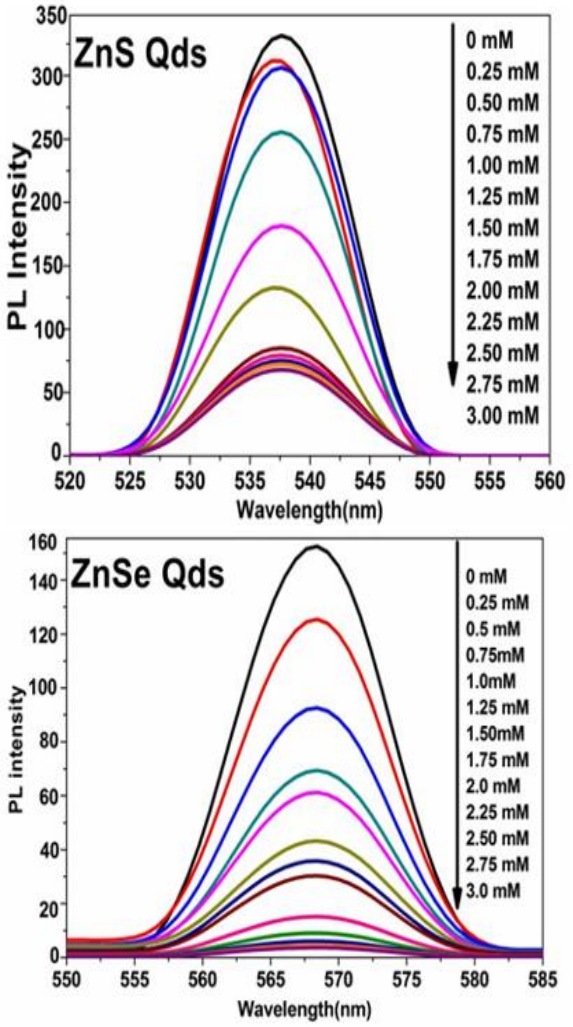


Fig 6: Effect of Glucose content on the PL spectra of GOx + ZnS/ZnSe Qdx

According to Peng Wu et al [35] coupling of QDs with these enzymes provides promising biosensing strategy without extra quenching units for QDs. In particular, the II-VI semiconductor QDs-GOD bioconjugate hybrid system has been readily employed for glucose sensing. In the present attempt, we observed that the PL spectra around 540 and 566 nm of ZnS and ZnSe QDs are effectively quench with glucose content (Fig 6a and b). The PL emission of ZnS and ZnSe QDs are correlated with transition from conduction band to sulphur and selenium vacancies defect structure, which acts as doubly ionized donor centres. It can be speculated as high surface to volume ratio in QDs, surface S2-/Se2- ions on the surface were oxidised to S/Se which existed as defect in the product. The intense emission peak at this region may be due these defects. In principle, the emission of QDs has similarities with fluorescence emission by CdSe@ZnS or CdTe QDs, so it is rationalized as the PL quenching observed in the present attempt are by H2O2 toward ZnS and ZnSe QDs through an electron-transfer mechanism, which can be understood as:[28] electrons at the conduction band of QDs can be captured by H2O2, hence radiative recombination rate of electrons and holes is slowed down, which decides the PL emission intensity, which is found to be systematically reduced with increasing glucose content in the ZnS/ZnSe QDs-GOx bioconjugated hybrid system. Therefore, the mechanism of the GOx-ZnS/ZnSe QD bioconjugate for glucose sensing can be ascribed to the H2O2 (generated from oxidation of glucose)-induced quenching of the PL spectra of ZnS/ZnSe QDs.

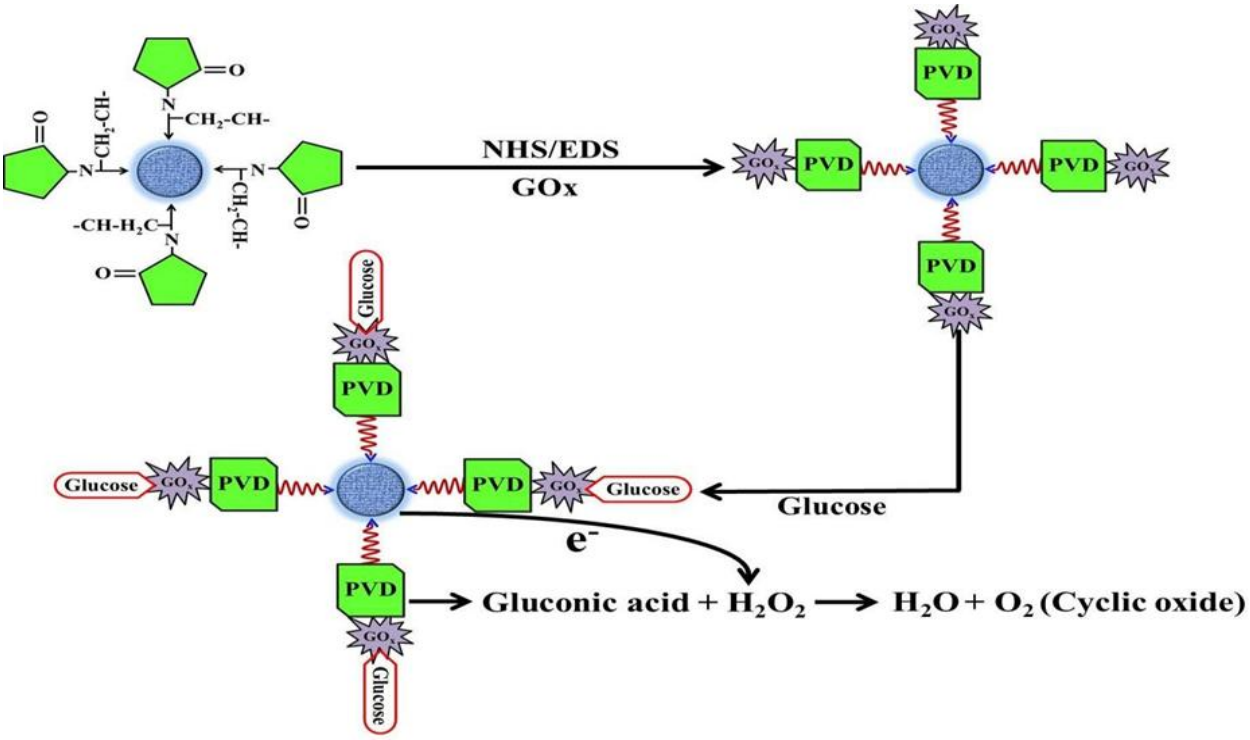


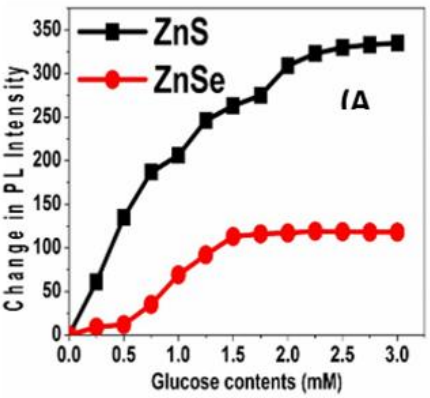
Fig 7: Glucose detection mechanism in ZnS.

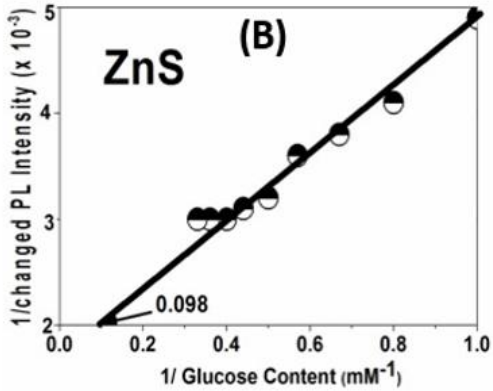
In the presence of glucose oxidase, glucose can be oxidised to produced gluconic acid and H2O2. The produced O2 can be further participated in the catalyzed reaction of Gox, forming cyclic electron transfer mechanism on glucose oxidation [35].

Glucose + O2 + H2O GOD Gluconic acid + H2O2.

The mechanism is speculated as Fig 7.

The enzymatic activity can be estimated by using method proposed in [35]. It clearly predicts that H2O2 production increases with increasing glucose content. The H2O2 is the quenching unit in the hybrid system. The extent of PL spectrum intensity quenching





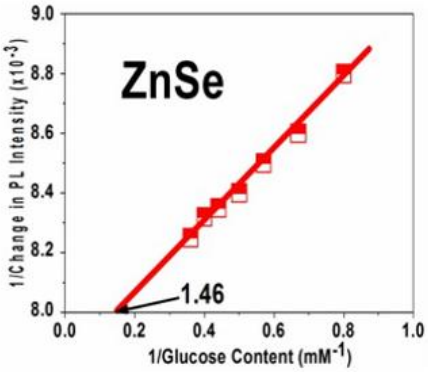


Fig 8: (A) Plots of the changed in quenched PL intensity of QDs + GOx as a function of glucose concentration

(B) Lineweaver-Burke plots can be used to quantify the enzymatic activity (Fig 8). According to the theory of kinetics of enzymatic- catalyzed reaction, Michaelis-Menton kinetic parameters (Km and V max), can be determined by the analysis of enzyme-substrate reactions. The Km parameter is used to estimate the affinity of the enzyme for the substrate in enzymatic reaction. Vmax provides the maximum rate of enzymatic reaction when the enzyme is saturated by the substrate. These parameters have been estimated using the Lineweaver-Burke plot, that is, the inverse of absorption versus the inverse of glucose concentration. The inverse of the intercepts at the x-axis and y- axis gives the value of Km and Vmax respectively. The smaller value of Km indicates the increased affinity of enzyme for substrate. The values of Km in the present enzymatic assay are found to be 10.20 mM/L and 6.85 mM/L for the ZnS and ZnSe, respectively. While, the value Vmax are obtained to be 0.5 and and 0.125 for ZnS and ZnSe, respectively. It clearly indicates that enzyme affinity for ZnSe is more as compared to ZnS QDs, however rate of enzymatic reaction in more for ZnS than ZnSe.

V. CONCLUSION

ZnS and ZnSe quantum dots were successfully synthesised by soft chemical route. TEM image confirms fine scale spherical particles having size varying in between 7 to10 nm thus ensure QDs formation. Conjugation of enzymes (GOx) on QDs are also confirmed by TEM by agglomerate QDs images. Ground state interaction QDs + GOx + Glucose are observed by UV-VIS absorption spectra; systematic gradual increase in UV- absorption peak intensity with keeping the peak location unchanged are rationalized in terms of strong interaction between QDs + GOx and Glucose in the ground state through complex formation. A typical room temperature photoluminescence (PL) spectrum of ZnS and ZnSe shows that the spectrum is dominated by characteristics deep level emission peak about 540 and 566 nm, correlated with transition from conduction band to sulphur and selenium vacancies defect structure, which acts as doubly ionized donor centres. The PL spectra of ZnS and ZnSe QDs are effectively quenched with glucose content. The observed PL quenching of ZnS and ZnSe QDs + GOx conjugate by glucose content are due to trapping of electrons at the conduction band of QDs by H2O2 which is biproduct of enzymatic reaction of GOx and Glucose, hence radiative recombination rate of electrons and holes is slowed down, which decides the PL emission intensity. The kinetics of the enzymatic-catalyzed reaction are estimated by Michaelis-Menton kinetic parameters (Km and Vmax). The values of Km and Vmax exhibits that ZnSe QDs are more supportive than ZnS QDs for GOx mobilization, however rate of enzymatic reaction in more for ZnS than ZnSe.

REFERENCES

[1] U. T. Bornscheuer, “Immobilizing Enzymes: How to Create More Suitable Biocatalysts,” Angewandte Chemie International Edition, vol. 42, pp. 3336–3337, 2003.

[2] E. Katz and I. Willner, “Integrated Nanoparticle–Biomolecule Hybrid Systems: Synthesis, Properties, and Applications,” Angewandte Chemie International Edition, vol. 43, pp. 6042–6108, 2004.

[3] E. Topoglidis, A. E. G. Cass, B. O’Regan, and J. R. Durrant, “Immobilisation and Bioelectrochemistry of Proteins on Nanoporous TiO_2 and ZnO Films,” Journal of Electroanalytical Chemistry, vol. 517, pp. 20–27, 2001.

[4] Y. H. Yang, H. F. Yang, M. H. Yang, Y. L. Liu, G. L. Shen, and R. Q. Yu, “Amperometric Glucose Biosensor Based on a Surface-Treated Nanoporous ZrO_2/Chitosan Composite Film as Immobilization Matrix,” Analytica Chimica Acta, vol. 525, pp. 213–220, 2004.

[5] R. E. Bailey, A. M. Smith, and S. Nie, “Quantum Dots in Biology and Medicine,” Physica E, vol. 25, pp. 1–12, 2004.

[6] M. V. Rigo, J. Seo, W.-J. Kim, and S. S. Jung, “Plasmon Coupling of R6G-Linked Gold Nanoparticle Assemblies for Surface-Enhanced Raman Spectroscopy,” Vibrational Spectroscopy, vol. 57, pp. 315–318, 2011.

[7] J. Wang, S. Banerji, N. Menegazzo, W. Peng, Q. Zou, and K. S. Booksh, “Glucose Detection with Surface Plasmon Resonance Spectroscopy and Molecularly Imprinted Hydrogel Coatings,” Talanta, vol. 86, pp. 133–141, 2011.

[8] G. T. Hermanson, A. K. Mallia, and P. K. Smith, Immobilized Affinity Ligand Techniques. London, U.K.: Academic Press, 1992.

[9] A. F. Collings and F. Caruso, “Biosensors: Recent Advances,” Reports on Progress in Physics, vol. 60, pp. 1397–1445, 1997.

[10] S. V. Rao, K. W. Anderson, and L. G. Bachas,

"Oriented Immobilization of Proteins," Microchimica Acta, vol. 128, pp. 127–143, 1998.
[11] W. H. Scouten, J. H. T. Luong, and R. S. Brown, "Enzyme or Protein Immobilization Techniques for Applications in Biosensor Design," Trends in Biotechnology, vol. 13, pp. 178–185, 1995.
[12] S. A. Khan, G. T. Smith, F. Seo, and A. K. Ellerbee, "Label-Free and Non-Contact Optical Biosensing of Glucose with Quantum Dots," Biosensors and Bioelectronics, vol. 64, pp. 30–35, 2015.
[13] N. G. Stan, D. Con, and C. Trial, "Diabetes Care," Diabetes Care, vol. 34, pp. S4–S10, 2011.
[14] L. Terry, S. F. White, and L. J. Tigwell, "The Application of Biosensors to Fresh Produce and the Wider Food Industry," Journal of Agricultural and Food Chemistry, vol. 53, pp. 1309–1316, 2005.
[15] D. M. Porterfield, "Measuring Metabolism and Biophysical Flux in the Tissue, Cellular and Sub-Cellular Domains: Recent Developments in Self-Referencing Amperometry for Physiological Sensing," Biosensors and Bioelectronics, vol. 22, pp. 1186–1196, 2007.
[16] S. K. Vashist, D. Zheng, K. Al-Rubeaan, J. H. T. Luong, and F. S. Shen, "Technology Behind Commercial Devices for Blood Glucose Monitoring in Diabetes Management: A Review," Analytica Chimica Acta, vol. 703, pp. 125–136, 2011.
[17] C. M. C. M. Couto, A. N. Araújo, M. C. B. S. M. Montenegro, J. Rohwedder, I. Raimundo, and C. Pasquini, "Application of Amperometric Sol–Gel Biosensor to Flow Injection Determination of Glucose," Talanta, vol. 56, pp. 997–1003, 2002.
[18] Q. Yang, P. Atanasov, and E. Wilkins, "Development of Needle-Type Glucose Sensor with High Selectivity," Sensors and Actuators B: Chemical, vol. 46, pp. 249–256, 1998.
[19] H. Ukeda, Y. Fujita, M. Ohira, and M. Sawamura, "Immobilized Enzyme-Based Microtiter Plate Assay for Glucose in Foods," Journal of Agricultural and Food Chemistry, vol. 44, pp. 3858–3863, 1996.
[20] M. Lepore, M. Portaccio, E. De Tommasi, P. De Luca, U. Bencivenga, P. Maiuri, and D. G. Mita, "Glucose Concentration Determination by Means of Fluorescence Emission Spectra of Soluble and Insoluble Glucose Oxidase: Some Useful Indications for Optical Fibre-Based Sensors," Journal of Molecular Catalysis B: Enzymatic, vol. 31, pp. 151–158, 2004.
[21] Z. Rosenzweig and R. Kopelman, "Analytical Properties and Sensor Size Effects of a Micrometer-Sized Optical Fiber Glucose Biosensor," Analytical Chemistry, vol. 68, pp. 1408–1413, 1996.
[22] X. J. Wu and M. M. F. Choi, "An Optical Glucose Biosensor Based on Entrapped Glucose Oxidase in Silicate Xerogel Hybridised with Hydroxyethyl Carboxymethyl Cellulose," Analytica Chimica Acta, vol. 514, pp. 219–226, 2004.
[23] K. Senthilkumar, T. Kalaivani, S. Kanagesan, and V. Balasubramanian, "Low Temperature Method for Synthesis of ZnS Quantum Dots and Its Luminescence Characterization Studies," Applied Surface Science, vol. 264, pp. 17–20, 2013.
[24] K. Saikia, P. Deb, and E. Kalita, "Facile Synthesis of Highly Luminescent ZnSe(S) Alloyed Quantum Dot for Biomedical Imaging," Current Applied Physics, vol. 13, pp. 925–930, 2013.
[25] J. F. Sierra, J. Galbán, and J. R. Castillo, "Determination of Glucose in Blood Based on the Intrinsic Fluorescence of Glucose Oxidase," Analytical Chemistry, vol. 69, pp. 1471–1476, 1997.
[26] Z. Truta, M. Garlovanu, S. Lerintiu, and R. Micu, "A New Method for Human Semen Glucose Concentration Evaluation," Romanian Biotechnological Letters, vol. 15, pp. 5764–5777, 2010.
[27] O. Khani, H. R. Rajabi, M. H. Yousefi, A. A. Khosravi, M. Jannesari, and M. Shamsipur, "Synthesis and Characterizations of Ultra-Small ZnS and Zn(1−x)FexS Quantum Dots in Aqueous Media and Spectroscopic Study of Their Interactions with Bovine Serum Albumin," Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, vol. 79, pp. 361–369, 2011.
[28] P. B. Kandagal, J. Seetharamappa, S. Ashoka, S. M. T. Shaikh, and D. H. Manjunatha, "Study of the Interaction Between Doxepin Hydrochloride and Bovine Serum Albumin by Spectroscopic Techniques," International Journal of Biological Macromolecules, vol. 39, pp. 234–239, 2006.
[29] L. H. Cao, J. Ye, L. L. Tong, and B. A. Tang, "New Route to the Considerable Enhancement of Glucose Oxidase Activity: The Simple Assembly of a Complex from CdTe Quantum Dots and GOx, and Its Glucose Sensing," Chemistry – A European Journal, vol. 14, pp. 9633–9640, 2008.
[30] R. Gill, L. Bahshi, R. Freeman, and I. Willner, "Optical Detection of Glucose and Acetylcholine Esterase Inhibitors by H_2O_2-Sensitive CdSe/ZnS Quantum Dots," Angewandte Chemie International Edition, vol. 47, pp. 1676–1679, 2008.

[31] L. Bahshi, R. Freeman, R. Gill, and I. Willner, "Optical Detection of Glucose by Means of Metal Nanoparticles or Semiconductor Quantum Dots," Small, vol. 5, pp. 676–680, 2009.
[32] J. P. Yuan, W. W. Guo, J. Y. Yin, and E. K. Wang, "Glutathione-Capped CdTe Quantum Dots for the Sensitive Detection of Glucose," Talanta, vol. 77, pp. 1858–1863, 2009.
[33] X. Y. Li, Y. L. Zhou, Z. Z. Zheng, X. L. Yue, Z. F. Dai, S. Q. Liu, and Z. Y. Tang, "Glucose Biosensor Based on Nanocomposite Films of CdTe Quantum Dots and Glucose Oxidase," Langmuir, vol. 25, pp. 6580–6586, 2009.
[34] J. P. Yuan, W. W. Guo, and E. K. Wang, "Quantum Dots–Bienzyme Hybrid System for the Sensitive Determination of Glucose," Biosensors and Bioelectronics, vol. 23, pp. 1567–1571, 2008.
[35] P. Wu, Y. He, H. F. Wang, and X. P. Yan, "Conjugation of Glucose Oxidase onto Mn-Doped ZnS Quantum Dots for Phosphorescent Sensing of Glucose in Biological Fluids," Analytical Chemistry, vol. 82, pp. 1427–1433, 2010.
[36] L. F. Shi, V. De Paoli, N. Rosenzweig, and Z. Rosenzweig, "Synthesis and Application of Quantum Dots FRET-Based Protease Sensors," Journal of the American Chemical Society, vol. 128, pp. 10378–10379, 2006.