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Structural and Optical Characterizations of CdSe Nanoparticles-Protein Bioconjugate

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Abstract : *In the present work a simple chemical reduction route was followed to grow CdSe (size controlled) nanoparticles (NPs) at room temperature. The grown sample was ultrasonicated in ethanol. The dispersed sample was characterized structurally and optically. The result supports the formation of nanoparticles and hence optical bandgap increased compared to that of bulk CdSe (bulk bandgap is 1.74 eV). The defect state related photoluminescence spectrum showed visible emission of CdSe NPs. We used optical spectroscopy like UV–VIS and fluorescence spectra and high resolution transmission electron microscopy (HRTEM) images to study the interaction and the formation of bioconjugate of lysozyme (a model protein) and CdSe NPs. Our result showed occurrence of spontaneous binding process between tryptophan (Trp) of the lysozyme and CdSe NPs. Formation of ground state complex with small red shift of the absorption peak of lysozyme was observed due to binding of the lysozyme with CdSe NPs. Our nanoparticles quench the fluorescence emission of tryptophan in the lysozyme. We calculated the Stern–Volmer quenching constant and the number of binding sites. HRTEM study showed the behaviour of the CdSe nanoparticles inside lysozyme molecules. HRTEM results indicated that core CdSe nanoparticles were coated by shell lysozyme. Due to interaction a fibrillar like structure was found in the CdSe-lysozyme composite system.*

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I. Introduction

Lysozyme was used as the model protein, whose dimensions (4.5 × 3.5 × 3.5 nm) were comparable in size to the 4-nm nanoparticles [1]. Chicken egg white lysozyme (molecular weight (MW) = 14.6 kDa) is a small globular protein, consisting of 129 amino acid residues with four disulfide bonds [2]. The importance of lysozyme relies on its extensive use as a model system to understand the underlying principles of protein structure, function, dynamics, and folding through theoretical and experimental studies. High natural abundance is also one of the reasons for choosing lysozyme as a model protein for studying protein-NP interaction. Another important aspect of lysozyme is its ability to carry drug. X-ray analysis revealed that, lysozyme possesses a relatively rigid structure [3-8]. It contains six tryptophan (Trp) residues. Three of them are located in the substrate binding site, two are located in the core hydrophobic region, and one is separated from all other residues. Trp62 and Trp108 are the most dominant fluorophores [9-12].

CdSe NPs are important semiconductor labelling materials for biomedical applications. However, there are still many concerned problems about how to apply them safely in biological systems. The nanoscale effects about nanoparticles and protein molecules have not been well understood. Lysozyme (Lyz) is a small monomeric globular protein. It contains protein structural elements like α -helix, β-sheet, turns and disorder. Its structure is formed by 129 tactic amino residues containing 6 tryptophanes, 3 tyrosines and 4 disulfide bonds. The interactions between proteins and non-metal ions, metal ions have been extensively investigated [13-16]. However, the detailed studies about the interaction between different semiconductor nanomaterials and proteins are very rare [17]. In the peptidoglycan of certain microorganisms lysozyme hydrolyzes the β(1→4) glucosidic linkages between N-acetylmuramic acid and N-acetylglucosamine [18]. Recent study showed that the biomolecules have potential ability in killing HIV virus, different antibacterial activity, and delivery of drug molecules [19]. In this work, the water-soluble CdSe NPs were synthesized directly in a cost effective chemical method. The optical properties like absorption and fluorescence spectroscopy have been used to investigate the interaction of CdSe NPs and Lyz. Here we try to study the binding mechanism of CdSe NPs to Lyz with respect to the binding sites. Our aims to gain insight into lysozyme- CdSe NPs interaction and binding mechanism. In this study, the effect of CdSe nanoparticles on the lysozyme (a model protein) is studied in detail. Formation of complex between the lysozyme and CdSe NPs induced a steady state reduction in the emission intensity of Trp of Lyz at different concentrations of nanoparticles. Lyz emission quenching spectra suggested that CdSe NPs act as a foreign quencher. The quenching of lysozyme showed that lysozyme has undergone slide structural

perturbations in the CdSe -Lyz composite system compared to the bare Lyz. The quenching constant was studied by analysis of the Stern-Volmer plot. Our results of this paper will help biomedical safety of the CdSe NPs in biomedical applications.

II. Experimental

A particular amount of anhydrous $CdCl_2$, selenium powder and sodium borohydride was taken for reaction. Ethylenediamine was used as a capping agent. Sodium borohydride was taken to initiate the reaction at room temperature. The stirring was continued for 3 hours at a particular speed. For TEM and TED measurements, the as-prepared CdSe nanoparticles were dispersed in ethanol by ultrasonification. A small drop of dispersed CdSe nanoparticles were taken on a thin carbon film supported on the copper grid and kept some time for drying. TEM, transmission electron diffraction (TED) and energy dispersive x-ray spectroscopy (EDX) of the as- prepared sample has been taken using JEOL-JEM-200 transmission electron microscope operating at 200 kV. Optical absorption measurements of the dispersed samples were studied in the range of 500nm-800nm using Shimadzu Pharmaspec 1700 UV-VIS spectrometer. Photoluminescence spectra of the same sample were obtained using Hitachi F-7000 FL Spectrophotometer. The as prepared CdSe NPs were dispersed in Millipore water using ultrasonication for 20 mins with the variation in concentration of CdSe NPs from 200 μM to 900μM. Lyz-CdSe NPs mixed solutions were prepared by mixing 0.1mg/mL of Lyz with CdSe NPs, ranging from 200 μM to 900μM with proper ratio.

III. Result and Discussion

3.1 Structural Characterization

The measured x-ray diffraction (XRD) data are plotted in the Figure 1. The pattern shows the peaks mostly correspond to hexagonal phase of the crystal unit cell [21]. Here, the intensities of different diffraction peaks are different signifying the formation of various planes.

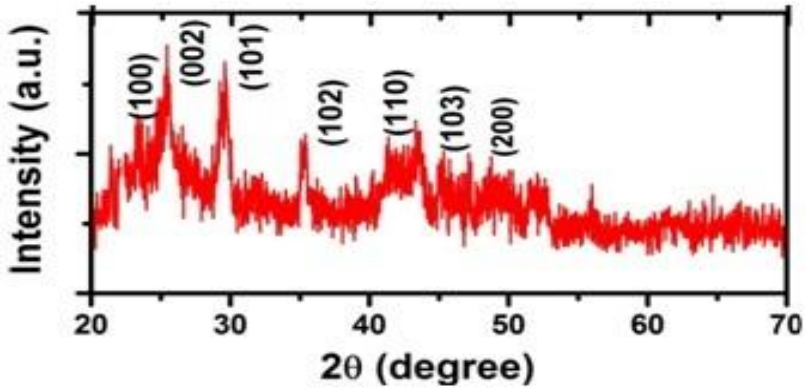


Fig. 1The XRD pattern of the as-prepared CdSe nanoparticles

Figure 2(a) shows the TEM image of the synthesized CdSe nanostructures. The average diameter of the nanostructure is approximately 5-8 nm with spherical in shape. The selected area electron diffraction (SAED) (inset of Figure 2(a)) pattern of pure CdSe-nanoparticles represented its polycrystalline nature.

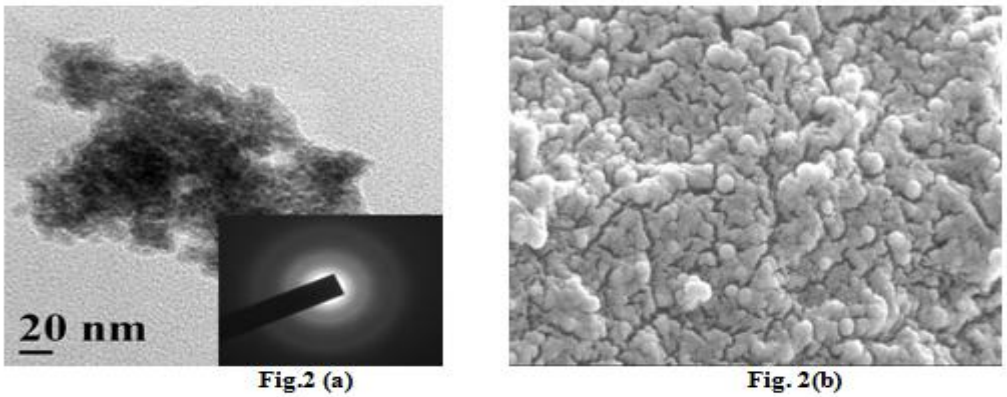


Fig.2 (a) Fig. 2(b)

Fig. 2 The TEM and FESEM image of the as-prepared CdSe NPs

Figure 2(b) exhibits the field emission scanning electron micrograph (FESEM) of the synthesized materials.

The surface morphology of CdSe is clearly obtained from the FESEM, which is spherical in shape and supports the TEM image. Figure 3 shows the process of mixing of lysozyme with the CdSe nano powder.

Figure 3d shows the behaviour of the CdSe nanoparticles inside Lysozyme molecules. TEM image of the lysozyme conjugated CdSe nanoparticle indicates that core CdSe nanoparticles are coated by shell lysozyme and is shown in Figure 4. Due to the interaction, a fibrillar like structure is found in the CdSe-lysozyme composite system [24]. Schematic of the Trp-CdSe NPs conjugation is shown in Figure 5.

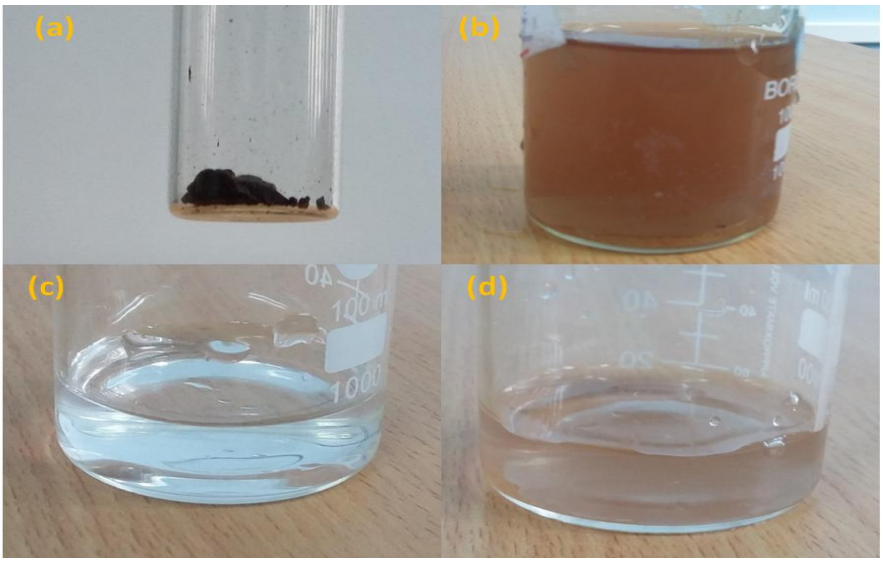


Fig. 3 Images of the (a) CdSe nanocrystals, (b) CdSe nanocrystals disperse in water, (c) Pure Lysozyme dispersed in water, (d) CdSe NPs- lysozyme in water solution.

The morphology of the CdSe NPs-Lysozyme bioconjugate was observed in a ZEISS Field emission scanning electron microscope (FESEM) operated at 5 kV. A Typical FESEM image of the bioconjugated material is shown in Figure 6. A globular and modular structure formation is represented by the picture.

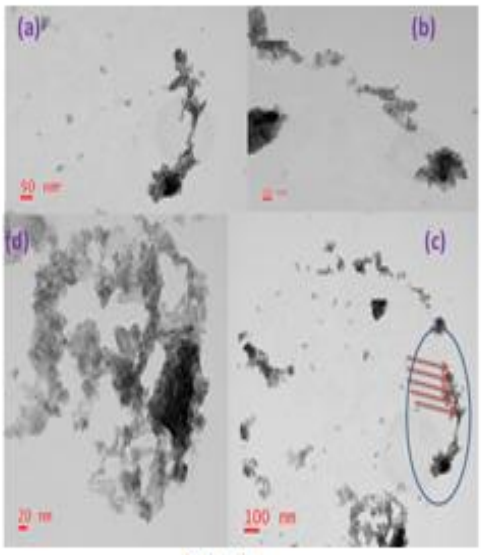


Fig. 4

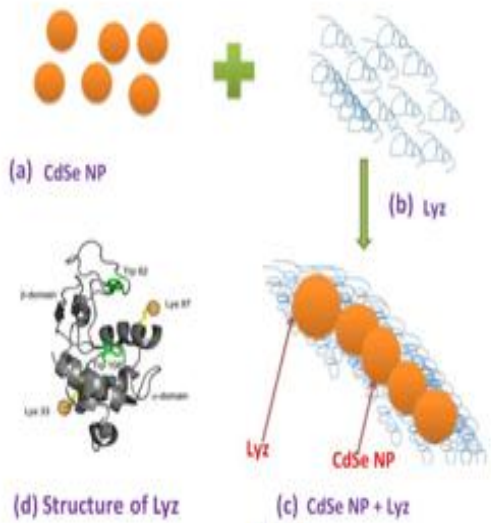


Fig. 5

Fig. 4 Bio-conjugation of CdSe nanoparticle and lysozyme are taking place gradually and its fibrillar like TEM structure is encircled in 'c' sub-section.

Fig. 5 Schematic diagram of the Trp-CdSe NPs conjugation [24].

The easy access of CdSe Nanoparticle into the cavity of the lysozyme is by diffusion process [25]. The formation of bioconjugate and well protein coated CdSe nanoparticles are clear from FESEM images (Figure 6).

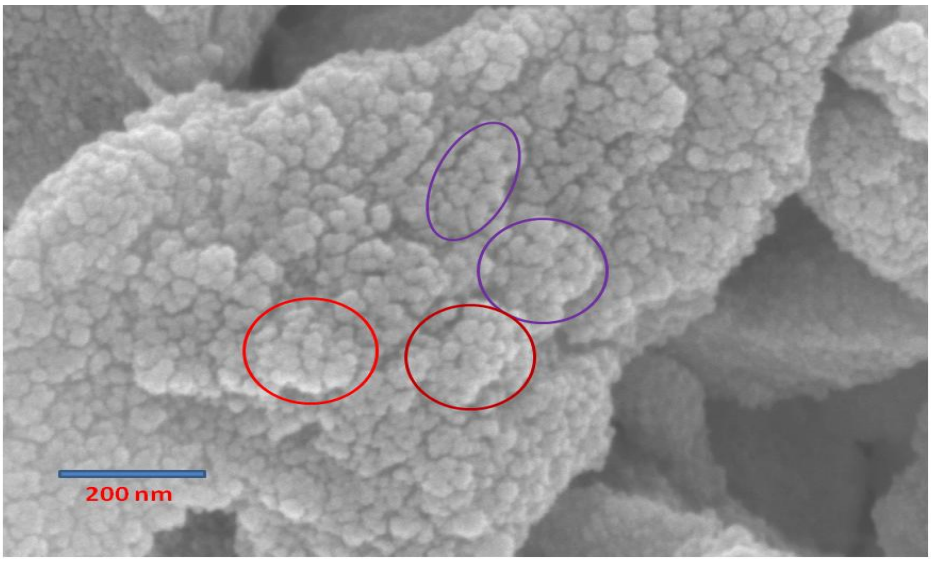


Fig. 6 FESEM images of the bioconjugated material

3.2 Optical Characterization

The Figure 7(a) shows the optical absorption spectrum of as-prepared CdSe NPs and the corresponding bandgap determination plot is represented in Figure 7 (b). Synthesized CdSe nanoparticles shows a strong absorption around 351 nm with a band gap of 2.02 eV as seen from Figures 7(a) & 7(b). The UV-VIS spectra presented in Figure 8 illustrate the effect of binding of CdSe NPs with Lyz. The tryptophan (Trp) in the Lyz exhibits absorption peak at ~278 nm (Figure 8) due to the π-π* transition of aromatic amino acid residues. In Figure 8 (b-g) the results showed that the absorbance at 278 nm increases in increases with C_{CdSe}.

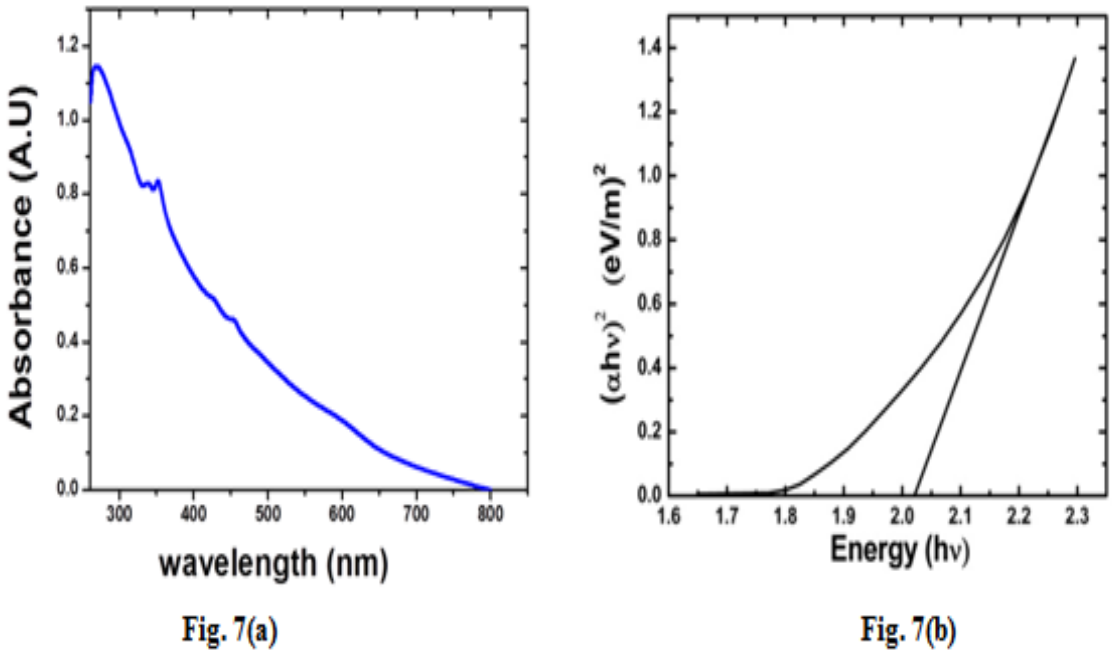


Fig. 7 (a) The optical absorption spectra of as prepared CdSe nanoparticles, (b) the corresponding bandgap determination plot.

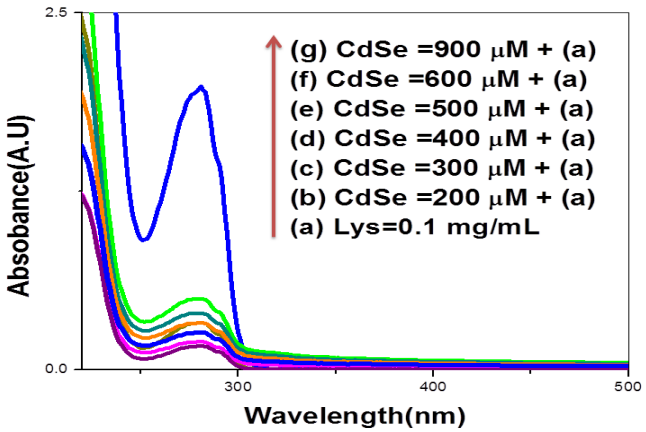


Fig. 8 : Absorption spectra of (a) pure lysozyme (0.1 mg/mL), (b) lysozyme with 200 µM CdSe NPs, (c) lysozyme with 300 µM CdSe NPs, (d) lysozyme with 400 µM CdSe NPs, (e) lysozyme with 500 µM CdSe NPs, (f) lysozyme with 600 µM CdSe NPs; (g) lysozyme with 900 µM CdSe NPs .

The increase in intensity of absorbance of Trp in the presence of CdSe may be due to binding of Trp with CdSe NPs and the formation of the ground state complex [20]. The increase in the absorbance of the tryptophan with the increase of the CdSe nanoparticle concentration is linear.

Fluorescence spectroscopy is an important tool to study about the deformation/ conformational changes of protein molecules. The emission spectrum of pure CdSe NPs is shown in Figure 9.

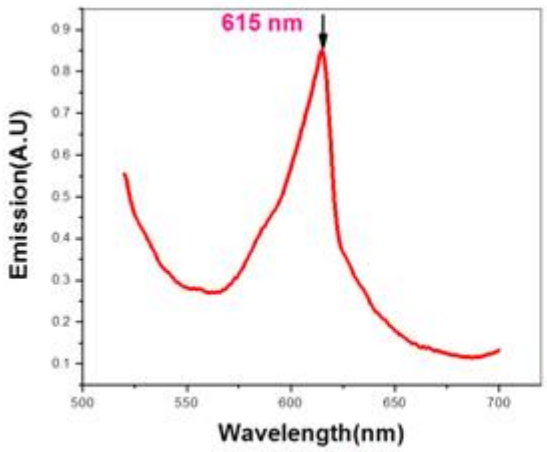


Fig.9

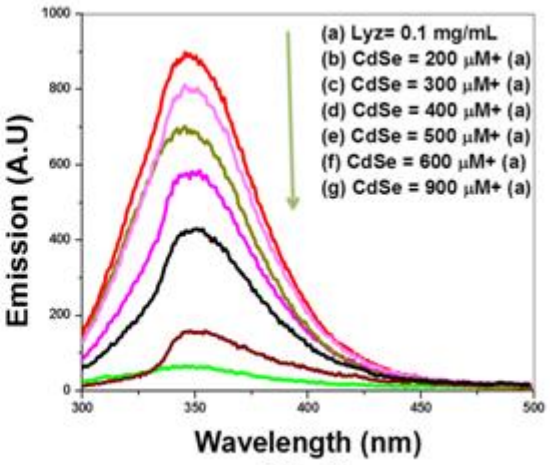


Fig. 10

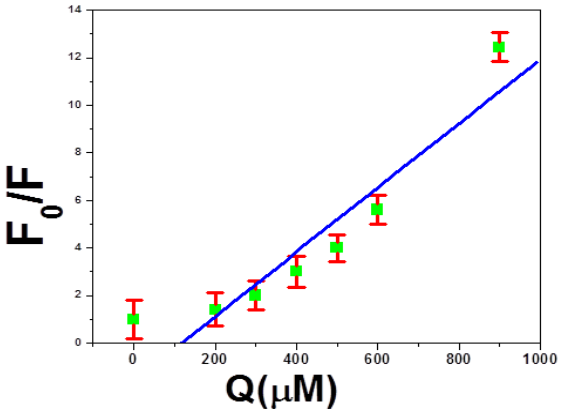
Fig. 9 The photoluminescence spectrum of the as-prepared CdSe nanoparticles

Fig. 10 Emission spectra of (a) pure lysozyme (0.1 mg/mL), (b) lysozyme (0.1 mg/mL) with 200 µM CdSe NPs, (c) lysozyme with 300 µM CdSe NPs, (d) lysozyme with 400 µM CdSe NPs, (e) lysozyme with 500 µM CdSe NPs, (f) lysozyme with 600 µM CdSe NPs; (g) Lysozyme with 900 µM CdSe NPs.

It shows a strong visible emission around 615 nm. The fluorescence quenching measurements have been analyzed to study the binding of CdSe NPs-Lyz bio-conjugates. The addition of CdSe NPs of different concentrations (C_{CdSe}) with lysozyme results a change in the maximum fluorescence emission spectrum intensity (I_{max}) of the Lyz, in Figure 10, suggesting the occurrence of quenching process [22]. The quenching occurs via the adsorption and interaction of the tryptophan residues accessible to the metallic surface of the CdSe NPs signifies the unfolding as well as denaturation of Trp in the presence of CdSe NPs. To disclose the mechanism and quenching, the Stern-Volmer equation was used [23]. The graphical plot of the Stern-Volmer equation,

$$\frac{F_0}{F} = K_{SV}[Q] + 1 \qquad \log\left[\frac{F_0}{F} - 1\right] = \log K + n\log[Q]$$

and corresponding binding sites determination plots are shown in Figure 11(a) &11(b) respectively.



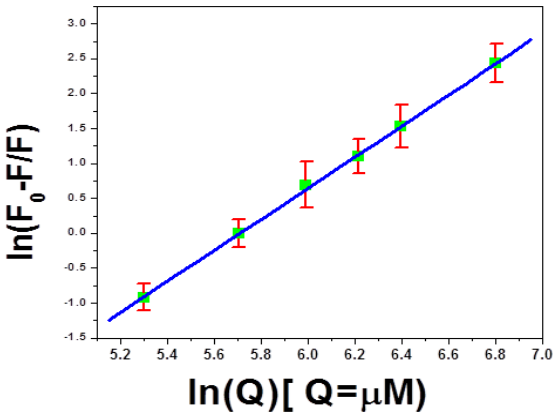


Fig. 11(a) **Fig. 11(b)**

Fig. 11a. & 11b. Variation of (A) F_0/F vs Q(μM); (11b) ln $[(F_0-F)/F]$ vs ln[Q]

The binding constant *K* along with the number of binding sites (*n*) between Lyz and CdSe NPs are 2.21 $(mM/L)^{-1}$ and 0.82 respectively. This indicates that a negative cooperative took place [23].

IV. Conclusions

In conclusion, we have synthesized CdSe nanoparticles of average size 5-8 nm using a simple wet chemical method. The XRD pattern of the as synthesized samples shows mostly the hexagonal phase. The emission quenching of the nanoparticle-lysozyme system showed a strong denaturation phenomenon of protein. The interaction between CdSe nanoparticles with Lyz showed negative cooperative reaction phenomenon. The TEM picture and FESEM pattern of the CdSe NPs-Lyz bioconjugate system indicates that the NPs were completely covered by Lyz protein molecules and formed fibrillar structure. The present investigation provides details about the binding mechanism and interaction of the physiologically important protein lysozyme with optically important semiconductor CdSe NPs. This study possesses potential applications in biomedical sciences and nano-bio interface sciences including biotechnology.

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References

[1]. Alexey A. Vertegel, Richard W. Siegel, and Jonathan S. Dordick, Silica Nanoparticle Size Influences the Structure and Enzymatic Activity of Adsorbed Lysozyme, Langmuir 2004, 20, 6800-6807.
[2]. Soumyananda Chakraborti, Tanaya Chatterjee, Prachi Joshi, Asim Poddar, Bhabatarak Bhattacharyya, Surinder P. Singh, Vinay Gupta, and Pinak Chakrabarti, Structure and Activity of Lysozyme on Binding to ZnO Nanoparticles, Langmuir 2010, 26(5),3506–3513.
[3]. Bruchez Jr M, Moronne M, Gin P, weiss S , Alivisatos A P, 1998, Science 281, 2013
[4]. Coe S, Woo W K, Bawendi M G, Bulovic V , 2002, Nature (London) 420, 800.
[5]. Colvin L, Schlamp M C, Alivisatos A P , 1994, Nature 370, 354
[6]. Cui Y, Wei Q Q, Park H K, Lieber C M, 2001, Science 293, 1289.
[7]. Hambrock J, Birkner A, Ficher R A, 2001, J.Mater.Chem.11, 3197
[8]. Jung, K.T., Shu, Y.G, Anpo, M. and Yamashita, H, 1997, Korean J.Chem.Eng.14, 213.
[9]. Lifshitz E, Dag I, Litvin I, Hodes G, Gorer S, Reisfeld R, Zelner M , Minti H, 1998, Chem. Phys. Lett.288, 188.
[10]. Mattoussi H, Mauro J M, Goldman E R, Green T M, Anderson G P, Sundar V C , Bawendi M G, 2001,Status Solidi , B224, 277.
[11]. Blake, C. C. F. Koenig, D. F. Mair, G. A. North, A. C. T. Phillips, D. C. Sarma, V. R. Nature 1965, 206, 757–761.
[12]. Imoto, T. Foster, L. S. Ruoley, J. A. Tanaka, F. Proc. Natl. Acad. Sci.U. S.A. 1972, 69, 1151–1155.
[13]. YP Wang, YL Wei, C Dong. J Photochem Photobiol A. 2006,177, 6-11.
[14]. YJ Hu, Y Liu, RM Zhao, JX Dong, SS Qu. J Photochem Photobiol A. 2006, 179, 324- 329.
[15]. J Tang, F Luan, X Chen. Bioorg Med Chem. 2006, 14(9), 3210-3217.
[16]. C Dufour, O Dangles. BBA-Gen Subjects. 2005, 1721(1), 164-173.
[17]. XC Shen, XY Liou, LP Ye, H Liang, ZY Wang. J Colloid Interf Sci. 2007, 311(2), 400- 406.
[18]. H Schwalbe, SB Grimshaw, A Spencer, M Buck, J Boyd, CM Dobson, C Redfield, LJ Smith. Protein Sci. 2001, 10, 677-688.
[19]. T Kuang, Y Liu, T Gong, P Tiantian, H Xiangfang, X Hu, Z Yu. Curr Nanosci. 2016, 12(1), 38-46.
[20]. AK Bhunia, T Kamilya, S Saha. *Chem Select*. 2016, 1(11), 2872-2882.

[21]. A. Manna, R. Bhattacharya, S. Saha, Structural, Optical and Electrical Characterization of CdS Nanoparticles, Chalcogenide Letters Vol. 6, No. 9, September 2009, p. 477 – 482.
[22]. AK Bhunia, PK Samanta, S Saha, T Kamilya. *Appl Phys Lett.* 2013, 103(14), 143701.
[23]. AK Bhunia, PK Samanta, S Saha, T Kamilya. *Appl Phys Lett.* 2014, 104(12), 123703.
[24]. AE Nel, L Madle, D Velegol, T Xia, EMV Hoek; P Somasundaran, F Klaessing, V Castranova, M Thompson. *Nat Mater*. 2009, 8, 543-557.
[25]. M Mahato, P Pal, T Kamilya, R Sarkar, A Chaudhuri, GB Talapatra. *J Phys Chem B*. 2010, 114(20), 7062-7070.

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