6 Spectroscopic Studies on the Interaction of Colloidal Uncapped and capped CdX Nanoparticles and Quantum dots with Albumins

6.1. Introduction

Nanoparticle probes compared with organic dyes acting as biosensors in chemical and biochemical fields have been researched recently and their applications are becoming more extensive. These probes have been applied to the ultrasensitive detection of proteins, DNA sequencing and clinical diagnostics etc [1]. Compared to conventional dyes, confinement of electronic states of quantum dots makes them quite attractive, showing some unique optical properties such as high quantum yield, symmetrical emission spectra, broad-band excitation, photostability and readily tunable spectra [2-6].

The interaction of proteins with inorganic surfaces involving mainly silica particles and to a lesser extent hematite [7], hydroxiapatite [8] and titanium dioxide [9,10] were reported. From our group people reported the binding of TiO$_2$ colloid with serum albumin [11,12]. Major interactions involved in protein adsorption can be classified as electrostatic, hydrophobic and hydrogen-bonding, etc. [13]. One of the goals in the study of protein adsorption is the follow-up of the process dynamics through in situ techniques that allow the analysis of the possible conformational changes that may take place during adsorption [14,15]. This aspect has been studied actively for decades because of its importance in the wide range of biomedical applications, such as artificial tissues and organs [16], drug delivery system [17], biosensor [18], solid-phase immunoassay [19], immunomagnetic cell separation [20] and immobilized enzyme or catalyst [21] and so on.

Quenching measurement of albumin fluorescence is an important method to investigate the interactions of drugs with serum albumins. It can reveal the accessibility of quenchers to albumin’s fluorophore groups, helps to understand
the binding mechanisms of albumins with drugs and provide clues to the essential of binding phenomenon [22].

In this work, bovine serum albumin (BSA), human serum albumin (HSA) and lysozyme are selected as our protein models because of their medicinal importance, low cost, commercial availability, and unusual ligand-binding properties [23]. BSA has two tryptophan residues that possess intrinsic fluorescence, Trp-212 is located within a hydrophobic binding pocket of the protein and Trp-134 is located on the surface of the molecule [24-26]. HSA is a protein with the molecular weight of 66,500 containing 585 amino acid residues. There is only a single tryptophan (Trp) residue within HSA at the position of 214 in domain II, which makes it very convenient to study the protein intrinsic fluorescence [27-29]. Lysozyme is a 14.6 kDa single chain protein and is formed by 129 amino acid residues, including α-helix, β-sheet, turns and disorder. It contains six tryptophan (Trp) residues, 4 disulfide bonds and 3 tyrosines in its structure [30-32]. Three of the tryptophan residues are located at the substrate binding sites, two in the hydrophobic matrix box, while one is separated from the others [33]. Among the amino acid residues Trp 62 and Trp 108 are the most dominant fluorophores, both being located at the substrate binding sites [34].

Ions such as Cd$^{2+}$, S$^{2-}$, Se$^{2-}$ and Te$^{2-}$ are the component parts of QDs which are harmful to human body. By researching the interaction of CdX nanoparticles with BSA, the effect of CdX to protein in real cells can be stimulated. The interaction of the CdS nanocrystals with its environment or capping agents plays a crucial role in determining its luminescent properties. Organic and inorganic capping agents such as polymers, amines, tri-n-octyl phosphine oxide, thiols and silica are used during the wet chemical synthesis for capping the surface of particles to prevent non-radiative recombination at surface sites and also control of growth kinetics to prevent the aggregation via steric
hindrance [35,36]. It is also believed that the capping agent on the surface of particles plays an important role on the transfer of photogenerated electrons and holes [37].

Among the available techniques, fluorimetry is extensively used and is considered to be superior to the other techniques (equilibrium and dynamic dialysis, ultra filtration, gel filtration) because, to a first approximation, they do not disturb the binding equilibrium upon separation [38]. In this chapter, synthesis of uncapped and capped CdX nanoparticles and QDs and the effect of these prepared capped CdX nanoparticles on intrinsic tryptophan fluorescence quenching of albumins has been studied and to characterize the type of chemical association taking place.

6.2. Experimental section
6.2.1. Materials

Cadmium chloride hemipentahydrate (CdCl2· 2.5H2O), cadmium acetate dihydrate (Cd(OAc)2· 2H2O), thioacetamide and sodiumhexametaphosphate were purchased from Qualigens and they were used as such. Starch, glacial acetic acid, 2-methoxy ethanol and acetone were purchased from Loba Chemicals. Thioglycolic acid (TGA, 98%), mercapto propionic acid (MPA), Selenium powder (99.997%), Sodium borohydride (95%) and thioacetamide (99.0%) were purchased from Sigma-Aldrich. Disodium hydrogen phosphate and potassium dihydrogen phosphate (phosphate buffer) were purchased from Merck, Germany. Bovine serum albumin, Human serum albumin and Lysozyme were purchased from Himedia, India. Stock solutions were prepared in phosphate buffer of pH 7.0 and stored at 0–4 °C. The stock concentration of albumins was determined spectrophotometrically using their molar extinction coefficient (ε280) [39]. All measurements were performed at ambient temperature (25 °C).
6.2.2. Preparation procedures
6.2.2.1. Preparation of colloidal CdS nanoparticles

The preparation of uncapped and starch capped CdS followed by the modified methods were adopted from literature [37,40]. 10 ml of 0.1 M cadmium chloride, 10 ml of 0.1 M thioacetamide and 10 ml of 0.1 M sodium hexametaphosphate were mixed with constant stirring. The pH of the mixture was adjusted to 10.4 by 0.1 M NaOH, under N₂ atmosphere. The mixture was kept at room temperature for 35 min for the growth of colloidal CdS nanoparticles. The resulting yellow colloid was diluted to a required concentration and was stored at 4 °C.

6.2.2.2. Preparation of Starch capped CdS nanoparticles (SCdS)

0.1 g of starch was dissolved in 5 ml of hot water to get a clear solution. Then 0.1 M (0.0266 g) cadmium acetate dihydrate dissolved in 20 ml of glacial acetic acid was added into the starch solution and stirred for 5 min at room temperature. After this 0.1 M (0.0075 g) thioacetamide in 20 ml 2-methoxy ethanol was added to the above mixture and stirred in an oil bath at 85-90 °C for 1 h. The resulting lemon yellow coloured solid products were centrifuged, washed and finally dispersed in water for optical study. In starch solution, the hydroxyl groups acted as stabilizer agent of the synthesized CdS nanoparticles.

6.2.2.3. Preparation of Sodium hydro selenide (NaHSe)

NaHSe was prepared according to the previously reported procedure with some modifications [41]. Briefly, a mixture of 0.0197 g of selenium (Se) powder and 0.0284 g of sodium borohydride (molar ratio of Se to NaBH₄ is 1:3) was first loaded into a 50 ml single necked flask fitted with a septum connected to the vacuum line and then deaerated by alternating between a vacuum pump and nitrogen flow. Then 1 ml of N₂ saturated pure water was added into the flask
under magnetic stirring and nitrogen flow at room temperature. After the black Se powder fully disappeared and white sodium tetraborate precipitate is appeared at the bottom of the flask (about 45 min), the resulting NaHSe in clear supernatant was separated and injected by syringe into 24 ml of N₂ saturated pure water to produce a 0.01 M of NaHSe solution for use as Se precursor in the following TGA-CdSe QDs synthesis.

6.2.2.4. Preparation of water soluble TGA-CdSe and MPA-CdTe QDs

The synthesis of thiol-capped CdSe QDs was performed by using CdCl₂. 2.5 H₂O and NaHSe as precursors according to previously reported methods with some modifications [42,43]. Briefly, in a three necked flask with a condenser attached, Cd precursor solutions were prepared by mixing a solution of CdCl₂. 2.5 H₂O (0.2279 g, 0.0125 M) in the presence of TGA (0.2763 g, 0.03 M) as stabilizing agent and were then adjusted to pH 8.5-9.0 by using 1 M NaOH. The resulting solution was deaerated with N₂ gas for 30 min. Under vigorous stirring, the oxygen free NaHSe solution as above prepared was injected. In our experiments the typical molar ratio of Cd²⁺: NaHSe: TGA was 1: 0.2: 2.4. The resulting yellow colloidal solution indicated the formation of TGA-CdSe QDs which was further refluxed under nitrogen flow at 100° C for 8 h. **Scheme 1** represents the preparation of TGA capped CdSe QDs.

![Scheme 1: Preparation of colloidal TGA capped CdSe QDs](image-url)
The resulting products were precipitated by acetone and superfluous of TGA and Cd\(^{2+}\) that did not participate in the reaction was removed with centrifugation at 4000 rpm for 5 min. The resultant precipitate was re-dispersed in water and re-precipitated by acetone for more than two times, then kept at 4 °C in dark for further use. The re-dispersed colloidal solution can be kept for three months as stable without any obvious aggregation.

Preparation of MPA-CdTe QDs is similar to the method explained in Chapter V, only difference is the mercapto propionic acid was used as a capping agent instead of thio glycolic acid.

### 6.2.3. Instrumentation

X-ray powder diffraction patterns were recorded on a Bruker AXS B8 Discover model using CuK\(\alpha\) radiation \((\lambda = 0.154 \text{ nm})\) and a graphite monochromator in the diffracted beam. CdS and SCdS sample was in the form of powder. A scan rate of 0.05° min\(^{-1}\) was applied to record a pattern in the 2\(\theta\) range of, \(2\theta = 20-80°\). SEM picture of the prepared colloidal CdS has been taken by using HITACHI S 3400 instrument. TEM picture of the prepared colloidal TGA-CdSe and MPA-CdTe QDs has been taken by using TECNAI G2 model.

Absorption spectra were recorded using Cary 300 UV-Visible spectrophotometer. The samples were carefully purged using pure nitrogen gas for 10 min. Quartz cells (4 x 1 x 1 cm) with high vacuum Teflon stopcocks were used for purging. The fluorescence quenching measurements were carried out with JASCO FP-6500 spectrofluorometer. The excitation and emission slit width (each 5 nm) and scan rate (500 nm/min) were maintained constant for all the measurements. Fluorescence lifetime measurements were carried out in a picosecond time correlated single photon counting (TCSPC) spectrometer. The excitation source is the tunable Ti-sapphire laser (Tsunami, Spectra Physics,
USA). The fluorescence decay was analyzed by using the software provided by IBH (DAS-6).

6.2.4. Methods

A 3 ml solution containing appropriate concentration of albumins (1 x 10^{-5} M) were titrated by successive additions of 3 μl stock solution of colloidal uncapped and capped CdX nanoparticles separately. Titrations were manually done by using micro-pipette for the addition of colloidal CdS and starch capped CdS. UV-Visible spectra of all the solutions were recorded in the range of 200-800 nm.

Fluorescence spectra were then measured by using Quartz cells. In synchronous fluorescence spectra also the same concentration of albumins and CdX nanoparticles and quantum dots were used and the spectra were measured at two different Δλ values such as 15 and 60 nm.

6.3. Characterization of CdX nanoparticles and QDs

6.3.1. Determination of particle size of colloidal CdS Nanoparticles

The prepared colloidal CdS nanoparticles were analyzed by UV-Visible and fluorescence spectroscopy to identify the presence of nanoparticles. The diameter of the prepared colloidal CdS has been determined from the relationship between band gap shift (ΔE_g) and radius (R) of quantum size particles using equation (1),

$$\Delta E_g = \frac{\pi^2 h^2}{2 \mu R^2} - \frac{1.8 e^2}{\varepsilon r} + \text{Polarization terms}$$

where h is Planck’s constant, R is radius of the particle, e is the electronic charge and ε is the relative permittivity of the semiconductor. A value of 0.153 m_e was used for the reduced effective mass of the exciton (1/μ = 1/m_e + 1/m_h) of CdS, m_e
and \( m_h \) are the effective masses of \( e^- \) (electron) and \( h^+ \) (hole), respectively, the columbic and polarization terms in the equation (1) are neglected. The calculated size of the prepared colloidal CdS is 3.35 nm.

### 6.3.2. XRD characterization of CdS and SCdS nanoparticles

For XRD measurement the resulting lemon yellow coloured solid products were centrifuged, washed and dried in a vacuum oven at 40 °C. **Figure 1** shows the XRD pattern of uncapped CdS and SCdS nanoparticles. The patterns are considerably broadened due to very small size of the CdS. The XRD pattern exhibits prominent broad peaks at 2\( \theta \) values of 27°, 44° and 52° which are identified for cubic CdS phase, the 2\( \theta \) values are similar to the reported [44]. The 2\( \theta \) values at which major peaks appear have been found to be almost the same for SCdS sample when compared to pure CdS, except the intensities of the peaks. This may be due to the fact that capping does not affect the crystal structure of SCdS nanoparticles. The average size of the sample determined is in the range of 6.02 nm from full-width at half-maximum (FWHM) of the most intense peak making by using Scherrer’s equation (2),

\[
d = \frac{0.9\lambda}{\beta \cos \theta}
\]

where \( \lambda \) is the wavelength of X-ray radiation, \( \beta \) is the FWHM in radians of the XRD peak (2.585) and \( \theta \) is the angle of diffraction (27°). The particle size calculated from XRD method (6.02 nm) is larger than the value obtained from absorption method (≈3.35 nm). This may be due to aggregation of colloidal CdS upon aging. The absorption and emission spectra of SCdS nanoparticles is shown in **Figure 2**.
**Figure 1:** X-Ray diffraction (XRD) spectrum of uncapped CdS and SCdS nanoparticles.

**Figure 2:** Absorption (A) and emission (B) spectra of SCdS nanoparticles.
6.3.3. Spectral characterization of TGA-CdSe and MPA-CdTe QDs

The particle size of the prepared CdSe and CdTe QDs were determined from the first absorption maximum (Figure 3a\&b) according to equations (3) and (4) respectively [45]:

\[
D = (1.6122 \times 10^{-9}) \lambda^4 - (2.6575 \times 10^{-6}) \lambda^3 + (1.6242 \times 10^{-3}) \lambda^2 - (0.4277) \lambda + 41.57 \quad \rightarrow (3)
\]

\[
D = (9.8127 \times 10^{-7}) \lambda^3 - (1.7147 \times 10^{-3}) \lambda^2 + (1.0064) \lambda - 194.84 \quad \rightarrow (4)
\]

where D (nm) is the particle size of QDs and \( \lambda \) (nm) is the wavelength of the first excitonic absorption peak of the corresponding QDs. The results showed that the particle diameter of the CdSe and CdTe QDs were around 2.18 and 3.12 nm, corresponding with the first absorption maximum of 482 nm and 540 nm respectively. The particle size can be further confirmed by TEM measurement which is shown in Figure 4a\&b. From TEM results we identified that the fine and uniform particle size is around \( \sim 3 \) nm. It was observed that the particle size determined from both the calculation as well as TEM measurements were matched well.

It is difficult to determine the concentration of prepared QDs by gravimetric method due to the difficulty in identifying the exact number of ligands on the surface which varies under different conditions. Measurements solely based on gravimetric methods using ligands coated nanocrystals are accurate only when the interactions between nanocrystals and ligands are sufficiently strong to withstand necessary purification procedures. Therefore, the absorption spectrum method in many cases is the most practical and convenient way to determine the particle concentration, so the concentration of prepared QDs were calculated from the absorption spectrum method using Lambert–Beer’s law (equation 5) [46]:
A = \epsilon CL \rightarrow (5)

In equation (5), A is the absorbance at the peak position of the first excitonic absorption peak for CdSe, C is the molar concentration of the QDs of the same sample. l is the path length of the radiation beam used for recording the absorption spectrum, and \epsilon is the molar extinction coefficient (for CdSe QDs, \epsilon = 5857 (D)^{2.65} in which D is the particle size (2.18 nm) of CdSe QDs and for CdTe QDs, \epsilon = 10,043 (D)^{2.12} in which D is the particle size (3.12 nm) of CdTe QDs [46]). Based on equation (5) concentration of both the CdSe and CdTe QDs are $1 \times 10^{-5}$ M.

**Figure 3a:** Absorption (A) and emission (B) spectra of TGA-CdSe QDs.
Figure 3b: Absorption (A) and emission (B) spectra of MPA-CdTe QDs.

Figure 4a: TEM picture of 8 h refluxed TGA-CdSe QDs.
6.4. Results and Discussion
6.4.1. Absorption characteristics of BSA with CdS and SCdS nanoparticles

To study the excited state reactions between BSA and SCdS, it is important to know the type of interaction between them in the ground state. The absorption spectra of BSA in the absence and presence of starch capped CdS is shown in Figure 5a. Absorption of BSA is characterized by a strong band at 278 nm. Addition of starch capped CdS led to gradual increase in BSA absorption with a blue shift (shorter wavelength) of 2 nm. These observations indicate that there is a structural change (microenvironment) in BSA which has occurred upon interaction with the surface of SCdS. The above results can be rationalized in terms of strong interaction between SCdS and BSA in the ground state through complex formation. Similar type of interaction between BSA with capped CdTe QDs and TiO$_2$ has been previously reported [6,11].

Figure 4b: TEM picture of 8 h refluxed MPA-CdTe QDs.
**Figure 5a:** Absorption spectrum of BSA (1 x 10^{-6} M) in the absence and presence of SCdS nanoparticles (0–6 x 10^{-4} M). The inset is the straight line dependence of 1/A_{obs}–A_0 on the reciprocal concentration of SCdS nanoparticles.

The equilibrium for the formation of complex between BSA and SCdS is defined by equation (6) where K_{app} is the apparent association constant,

\[
\text{BSA} + \text{capped CdS} \overset{K_{app}}{\Longrightarrow} \text{BSA.....capped CdS} \quad \rightarrow (6)
\]

\[
K_{app} = \frac{[\text{BSA.....capped CdS}]}{[\text{BSA}].[\text{capped CdS}]} \quad \rightarrow (7)
\]

The K_{app} value was calculated by the method reported by Benesi and Hildebrand [47] using the following equation (8):

\[
A_{obs} = (1–\alpha)\ C_0\varepsilon_{\text{BSA}}l + \alpha C_0\varepsilon_{\text{c}}l \quad \rightarrow (8)
\]

where A_{obs} is the absorbance of the BSA solution containing different concentrations of starch capped CdS at 278 nm, \(\alpha\) is the degree of association between BSA and capped CdS, \(\varepsilon_{\text{BSA}}\) and \(\varepsilon_{\text{c}}\) are the molar extinction coefficients at
the defined wavelengths for BSA and the formed complex respectively, $C_0$ is the initial concentration of BSA and ‘l’ is the optical path length, which has been taken as unity. Equation (8) can be expressed by equation (9), where the $A_0$ and $A_c$ are the absorbance of BSA and the complex at 278 nm, with the concentration of $C_0$:

$$A_{obs} = (1-\alpha)A_0 + \alpha A_c \quad \rightarrow (9)$$

At relatively high concentrations of capped CdS, $\alpha$ can be equated to $(K_{app}[\text{capped CdS}]/(1 + K_{app}[\text{capped CdS}])$. In this case, equation (9) can be expressed as equation (10):

$$\frac{1}{A_{obs}-A_0} = \frac{1}{A_c-A_0} + \frac{1}{K_{app}(A_c-A_0)[\text{capped CdS}]} \quad \rightarrow (10)$$

Therefore, if the enhancement of absorbance at 278 nm was due to absorption of complex, one would expect a linear relationship between $1/(A_{obs} - A_0)$ and the reciprocal concentration of capped CdS with a slope equal to $1/K_{app}(A_c - A_0)$ and an intercept equal to $1/(A_c - A_0)$ (shown in the inset of Figure 5a). The calculated value of $K_{app}$ from the straight line of such plot is about $2.54 \times 10^2 \text{ M}^{-1}$.

In order to determine which group of the SCdS is responsible for interaction with BSA molecules, we have done the comparative experiments with uncapped colloidal CdS. In uncapped CdS–BSA system while increasing the amount of CdS there is only a slight change in optical density without any shift in wavelength in the absorption spectrum of BSA (Figure 5b), indicating that the interaction between uncapped CdS and BSA is very weak, simply BSA cannot be adsorbed on the surface of uncapped CdS. Therefore we conclude that the BSA molecule strongly interacts with the capping agent on the surface of the colloidal CdS nanoparticles and is involved in the ground state complex formation as shown in Scheme 2.
**Figure 5b:** Absorption spectrum of BSA (1 x 10^{-6} M) in the absence and presence of uncapped CdS nanoparticles (0–6 x 10^{-4} M).

**Scheme 2:** Interaction between BSA with starch capped and uncapped colloidal CdS nanoparticles.

6.4.2. Fluorescence quenching of BSA by SCdS nanoparticles

6.4.2.1. Steady-state measurement

The interaction of BSA with SCdS was studied by spectrofluorometer at room temperature. An aqueous solution of BSA (1 x 10^{-6} M) was titrated with increasing concentration of (0–5 x 10^{-4} M) SCdS solution as shown in Figure 6. The SCdS also has luminescent property but its excitation (415 nm) and emission
(522 nm) wavelengths are much far away from the BSA absorption and emission (280 and 345 nm respectively), so it may not interrupt the interaction study. While increasing the concentration of SCdS the emission intensity of BSA was found to decrease progressively with the blue shift of around 6 nm. The quenching of BSA fluorescence by capped CdS can be described by Stern-Volmer equation (11).

\[
\frac{F_0}{F} = 1 + K_{SV}[Q] \quad \rightarrow (11)
\]

where \(F_0\) and \(F\) are the fluorescence intensities of BSA in the absence and presence of capped CdS, respectively. \(K_{SV}\) is Stern-Volmer constant and \([Q]\) is the concentration of respective quencher, capped CdS. The ratios \(F_0/F\) were calculated and plotted against quencher concentration according to equation (11) (shown in the inset of Figure 6). The quenching constant calculated from the slope of the plot is in the order of \(0.1955 \times 10^4\) M\(^{-1}\). The Stern-Volmer constant \((K_{SV})\) is related to quenching rate constant by \(k_q = K_{SV}/\tau\). From the quenching constant we have calculated the value of quenching rate constant by using the lifetime \((\tau)\) of BSA (6 ns, from time resolved measurement) which is \(3.25 \times 10^{11}\) M\(^{-1}\) s\(^{-1}\).

In general, maximum collisional quenching constant \((k_q)\) of various kinds of quenchers to biopolymers is \(2.0 \times 10^{10}\) M\(^{-1}\) s\(^{-1}\) [48]. But for BSA–SCdS system higher quenching rate constant \((3.25 \times 10^{11}\) M\(^{-1}\) s\(^{-1}\)) was obtained. This proves that the quenching is static in nature, it depends on the formation of complex between SCdS and BSA (Scheme 3&4). Further the type of interaction between BSA and capped CdS was also confirmed by time resolved spectroscopy.

\[
BSA + SCdS \underset{h\nu}{\rightleftarrows} BSA*......SCdS \quad \rightarrow (12)
\]

\[
BSA + SCdS \underset{h\nu}{\leftrightarrow} BSA*...SCdS \quad \rightarrow (13)
\]

**Scheme 3:** Mechanism of complex formation
Figure 6: Steady state fluorescence quenching of BSA (1 x 10^{-6} M, \lambda_{exi}; 295 nm and \lambda_{emi}; 347 nm) by starch capped CdS in the concentration range of 0–5 x 10^{-4} M in water. The insert is the Stern-Volmer plot between F_0/F vs [Q].

6.4.2.2. Time resolved measurement

Fluorescence lifetime measurement is useful for understanding the type of interaction between the colloidal semiconductor-sensitizer systems. In general, the measurement of fluorescence lifetime is the most definitive method to distinguish static and dynamic quenching [49].

In the present work we have studied the effect of uncapped CdS and SCdS nanoparticles on the fluorescence lifetime of BSA. Figure 7a shows the fluorescence decay of BSA in the absence and presence of SCdS. Initially BSA in the absence of SCdS showed monoexponential decay with the lifetime of 6 ns, and then first addition of SCdS changed the decay curve from mono to biexponential with two lifetimes such as 5.27 and 2.02 ns, respectively. But further increasing the concentration of starch capped CdS the fluorescence lifetime of BSA remains unaltered. So there is no change in the fluorescence
lifetime of BSA in the presence of highest concentration of capped CdS which indicated that the quenching follows static mechanism.

**Figure 7b** shows the fluorescence decay of BSA in the absence and presence of uncapped CdS nanoparticles which indicates there is no change in fluorescence lifetime of BSA in the presence of uncapped CdS representing the absence of interaction between the two. In **Figure 7b** though the decay traces of BSA in both the absence and presence of uncapped CdS were actually plotted however the lifetime of BSA remained the same in both conditions, hence the merging of kinetic traces were observed (the plot looks like a single decay curve).

**Figure 7a:** Fluorescence decay of BSA (1 x 10^{-6} M) in the absence and presence of SCdS nanoparticles in the concentration range of 0–5 x 10^{-4} M. The black colored decay is the prompt used for calibration.
Figure 7b: Fluorescence decay of BSA (1 x 10\(^{-6}\) M) in the absence and presence of uncapped CdS nanoparticles in the concentration range of 0–5 x 10\(^{-4}\) M. The black colored decay is the prompt used for calibration.

6.4.3. Binding constant and number of binding sites

For static quenching, we can deduce the binding constant (K) resulting from the formation of ground state complex between fluorophore and the quencher. The intrinsic tryptophan fluorescence quenching of BSA by capped CdS also follows complex formation, so we can deduce the binding constant (K) which is calculated by using the following method.

If it is assumed that there are similar and independent binding sites in the BSA, the relationship between the fluorescence intensity and the quencher medium can be deduced from the following equation (14) [50]:

\[ nQ + B \rightarrow Q_n\ldots B \rightarrow (14) \]

where B is the fluorophore, Q is the quencher, Q\(_n\)…B is the postulated complex between fluorophore and n molecules of the quencher. The constant K is given by
If the overall amount of biomolecules (bound or unbound with the quencher) is $B_0$, then $[B_0] = [Q_n...B] + [B]$, where $[B]$ is the concentration of unbound biomolecules, then the relationship between fluorescence intensity and the unbound biomolecule as $[B]/[B_0] = F/F_0$ that is:

$$\log \left[ \frac{F_0-F}{F} \right] = \log K + n \log [Q]$$  \hspace{1cm} \rightarrow (16)$$

where $K$ is the binding constant of BSA with starch capped CdS, which can be determined from the plot of $\log [(F_0-F)/F]$ versus $\log [Q]$ curve as shown in Figure 8 and thus we obtained binding constant ($K$) which is $6.6 \times 10^2$ M$^{-1}$ and number of binding sites ($n$) is 0.8631 from intercept and slope of the plot respectively. The good agreement between these values of binding constant highlighted the validity of assumption proposed for the association between BSA and starch capped CdS. The number of binding sites which is close to 1 indicates, there is only one type of interaction between BSA and SCdS.

**Figure 8:** The plot of $\log (F_0 - F)/F$ versus $\log [Q]$ for BSA with SCdS.
6.4.4. Characteristics of synchronous fluorescence spectra

Influences of capped CdS on the conformational changes of BSA were assessed by synchronous fluorescence method. Synchronous fluorescence measurements provide information about the molecular microenvironment in the vicinity of fluorophore functional groups. Synchronous fluorescence spectra were obtained by simultaneous scanning of excitation and emission monochromators. According to Miller [51], the difference between excitation and emission wavelength ($\Delta \lambda$) is 15 nm, synchronous fluorescence offers the characteristics of tyrosine residues, while when $\Delta \lambda$ is 60 nm, it provides the characteristic information of tryptophan residues. Synchronous fluorescence spectra of BSA upon addition of SCdS gained at 60 nm is shown in Figure 9a.

The fluorescence intensity of both tryptophan and tyrosine were decreased but the emission wavelength of tryptophan (340 nm) is blue shifted (2 nm) with increasing concentration of capped CdS. Comparing the emission wavelength of tyrosine, no significant change was observed (Figure 9b). It indicated that the interaction of capped CdS with BSA does not affect the conformation of tyrosine micro-region. Firstly, combined with tyrosine capped CdS gradually interacts with tryptophan and brings changes to BSA and results in blue-shift of fluorescence wavelength. It is likely due to that the hydrophobic amino acid structure surrounding tryptophan residues in BSA tends to collapse slightly and thus tryptophan residues are exposed more to the aqueous phase. Similar observation has been reported [52].
Scheme 4: Mode of interaction between SCDs and BSA molecules.

Figure 9a: Synchronous spectra of BSA (1 x 10^{-6} M) in the absence and presence of SCDs nanoparticles (0–5 x 10^{-4} M) in the wavelength difference of Δλ = 60 nm.
Figure 9b: Synchronous spectra of BSA (1 x 10^{-6} M) in the absence and presence of SCdS nanoparticles (0–5 x 10^{-4} M) in the wavelength difference of Δλ = 15 nm.

6.4.5. Interaction between Lysozyme and colloidal TGA-CdSe QDs
6.4.5.1. Fluorescence characteristics

Figure 10 shows the effect of increasing concentration of colloidal TGA-CdSe QDs on the fluorescence emission spectrum of lysozyme. We have excited the lysozyme at 295 nm (Excitation at 295 nm should result in tryptophan fluorescence only, since tyrosine does not absorb in this region and also to avoid the excitation of CdSe QDs which cannot be excited at this given wavelength). The emission intensity of lysozyme decreases in the presence of CdSe QDs which shows the quenching of lysozyme has occurred.

According to equation (11) we have obtained curvature plot [inset of Figure 10] for F₀/F vs [TGA-CdSe]. The result from the fluorescence study indicated that the quenching may follow static mechanism through ground state
complex formation which has been further confirmed by the fluorescence lifetime measurements.

![Fluorescence spectral change](image)

**Figure 10:** Fluorescence Quenching of Lysozyme (1 x 10^{-6} M; \(\lambda_{\text{exi}} = 295\) nm; \(\lambda_{\text{emi}} = 345\) nm) by CdSe QDs at various concentrations (0–5 x 10^{-6} M). Inset is the plot of F0/F Vs [TGA-CdSe QDs].

**Figure 11** shows the fluorescence decay curve of lysozyme in the absence and presence of TGA-CdSe QDs (0–5 x 10^{-6} M). Lysozyme exhibited single exponential decay not only in dilute solutions but also in the presence of CdSe QDs. While increasing the concentration of colloidal TGA-CdSe QDs there is no change in the fluorescence lifetime of lysozyme (it remains constant as 2.5 ns). This observation confirmed that the quenching of lysozyme by CdSe QDs followed static mechanism. It also supported the adsorption of lysozyme on the surface of QDs and the formation of ground state complex.

For static quenching, we can deduce the binding constant (K) because static quenching arises from the formation of complex between fluorophore and the quencher. Hence the binding constant (K) was calculated by using
equation (16). The binding constant and binding sites were determined (Table 1) from the intercept of log \( [(F_0 - F)/F] \) versus log \( [Q] \) as shown in Figure 12. The value of “n” approximately equal to 1 indicates the existence of just a single binding site in lysozyme for CdSe QDs.

The synchronous fluorescence spectra of the tryptophan, a residue of lysozyme at various concentrations of CdSe QDs is shown in Figure 12a. It can be seen that an obvious red shift (around 4 nm) was observed with increasing concentration of CdSe QDs, (In contrast, no shift was observed for tyrosine residue, Figure 12b) which indicated that the interaction of CdSe QDs with lysozyme does not affect the conformation of tyrosine micro-region, but it only affects the tryptophan micro-region. It is also shown that the polarity and the hydrophilicity around the tryptophan residues were increased [53]. Similar type of conformational changes has already been reported [54,55].

![Figure 11: Fluorescence decay of Lysozyme (1 x 10^-6 M; \( \lambda_{exi} = 295 \) nm; \( \lambda_{emi} = 345 \) nm) in the absence (red) and presence (green) of CdSe QDs (0–5 x 10^-6 M). The black colored decay is the prompt used for calibration.](image-url)
**Figure 12a:** The synchronous fluorescence spectra of lysozyme ($\Delta \lambda = 60$ nm) in the presence of CdSe QDs (0–5 x 10^{-6} M).

**Figure 12b:** The synchronous fluorescence spectra of lysozyme ($\Delta \lambda = 15$ nm) in the presence of CdSe QDs (0–5 x 10^{-6} M).
6.4.5.2. Effect of metal ions on the binding constants between lysozyme and TGA-CdSe QDs

There are many metal ions existing in the blood system and participating in many biochemical processes. Some plasma proteins usually act as sequestration agent for metal ions and have a variety of metal sites with different specificities [56,57]. It is necessary to investigate the binding reaction between ligand and protein in the presence of metal ions.

In the present system the effect of metal ions such as Co$^{2+}$, Zn$^{2+}$, Mn$^{2+}$, Ni$^{2+}$ and Cu$^{2+}$ on the binding constants was investigated at room temperature by recording the fluorescence intensity of lysozyme in the wavelength range of 300–500 nm upon excitation at 295 nm, and the concentrations of lysozyme and metal ions were fixed as 1 μM. The results are shown in Table 1. The lower values of binding constant obtained in presence of metal ions are attributable to the competition between metal ions and TGA-CdSe QDs.

Hence, metal ions may combine with carboxylic or amino groups of the amino acids, which indicated the same active sites for CdSe QDs. Thus, there may be a competitive effect between the metal ions and CdSe QDs, and the active sites occupied by CdSe QDs reduced, which resulted in the decrease of binding constants. This indicates the requirement of less dose of drug for desired therapeutic effect. In this way, the trace metal ions influence the binding of drug to protein and in turn the dose limits. Similar type of competitive experiment has already been reported [58].
Table 1: Influence of metal ions on the binding constants (K), binding sites (n) and regression coefficient (R²) values for the interaction between lysozyme and CdSe QDs as well as metal ions.

<table>
<thead>
<tr>
<th>S.No.</th>
<th>System</th>
<th>K</th>
<th>n</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>lysozyme + CdSe</td>
<td>3.38 ± 0.01 x 10^8 M⁻¹</td>
<td>1.57</td>
<td>0.9991</td>
</tr>
<tr>
<td>2</td>
<td>lysozyme + Cu²⁺ + CdSe</td>
<td>1.28 ± 0.02 x 10^8 M⁻¹</td>
<td>1.48</td>
<td>0.9964</td>
</tr>
<tr>
<td>3</td>
<td>lysozyme + Mn²⁺ + CdSe</td>
<td>1.22 ± 0.01 x 10^8 M⁻¹</td>
<td>1.48</td>
<td>0.9991</td>
</tr>
<tr>
<td>4</td>
<td>lysozyme + Zn²⁺ + CdSe</td>
<td>6.91 ± 0.02 x 10^7 M⁻¹</td>
<td>1.44</td>
<td>0.9978</td>
</tr>
<tr>
<td>5</td>
<td>lysozyme + Co²⁺ + CdSe</td>
<td>4.26 ± 0.04 x 10^7 M⁻¹</td>
<td>1.41</td>
<td>0.9747</td>
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<tr>
<td>6</td>
<td>lysozyme + Ni²⁺ + CdSe</td>
<td>4.04 ± 0.02 x 10^7 M⁻¹</td>
<td>1.39</td>
<td>0.9975</td>
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</tbody>
</table>

6.4.6. Interaction of BSA and HSA with colloidal MPA-CdTe QDs

Figure 13a&b shows the fluorescence spectrum of BSA and HSA in the presence of various concentrations of MPA-CdTe QDs in the range of 280-480 nm in phosphate buffer medium of pH 7.0. From this figure we observed that while increasing the concentration of QDs the fluorescence intensity of albumins is gradually decreased. The value of Stern-Volmer constant (K_{SV}) and quenching rate constant (k_q) has been calculated and the values are shown in Table 2.

Table 2: Lifetime of BSA, HSA and Stern-Volmer constant and quenching rate constant for albumins with MPA-CdTe QDs.

<table>
<thead>
<tr>
<th>S. No</th>
<th>System</th>
<th>τ</th>
<th>K_{SV} x 10^6 M⁻¹</th>
<th>k_q x 10^{14} M⁻¹ s⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>BSA + QDs</td>
<td>6 ns</td>
<td>3.46</td>
<td>5.76</td>
</tr>
<tr>
<td>2</td>
<td>HSA + QDs</td>
<td>10⁻⁸s</td>
<td>2.03</td>
<td>2.03</td>
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</tbody>
</table>
Figure 13a: Fluorescence Quenching of BSA (1 x 10^{-6} M; \( \lambda_{\text{exi}} = 278 \text{ nm} \)) in the presence of various concentrations of MPA-CdTe QDs, \([\text{MPA-CdTe QDs}] = 0, 1, 2, 3, 4 \& 5 \times 10^{-7} \text{ M}\). Inset is the Stern-Volmer plot for the quenching of BSA by MPA-CdTe QDs.

Figure 13b: Fluorescence Quenching of HSA (1 x 10^{-6} M; \( \lambda_{\text{exi}} = 278 \text{ nm} \)) in the presence of various concentrations of MPA-CdTe QDs, \([\text{MPA-CdTe QDs}] = 0, 1, 2, 3, 4 \& 5 \times 10^{-7} \text{ M}\). Inset is the Stern-Volmer plot for the quenching of HSA by MPA-CdTe QDs.
Table 3: Binding constants (K_b), number of binding sites (n) and linear regression coefficient (R^2) for BSA and HSA systems with MPA-CdTe QDs.

<table>
<thead>
<tr>
<th>S. No</th>
<th>System</th>
<th>n</th>
<th>K x 10^6 M^-1</th>
<th>R^2</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>BSA + QDs</td>
<td>1.03</td>
<td>5.41</td>
<td>0.9972</td>
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<td>2</td>
<td>HSA + QDs</td>
<td>1.04</td>
<td>4.18</td>
<td>0.9996</td>
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</table>

In general, maximum collisional quenching rate constant (k_q) for various kinds of quenchers to biopolymers is 2.0 x 10^{10} M^{-1}s^{-1} [49]. But in the present case higher quenching rate constants were obtained (Table 2). This result indicates that the quenching mechanism of the reaction between albumins and CdTe QDs belongs to static quenching. So, static quenching arises from the formation of complex between albumins and the quencher. Hence we have determined the binding constant (K) and number of binding sites (n) by using equation (16) and the values are shown in Table 3. The value of “n” approximately equal to 1 indicates the existence of just a single average binding site in albumins for QDs (Figure 14).

The synchronous fluorescence spectra of BSA and HSA at various concentrations of MPA-CdTe QDs are shown in Figures 15a,b&16a,b respectively. From the figures, it can be seen that in the presence of QDs emission wavelength of the tyrosine residues does not have a significant shift. In contrast red shift (around 4 nm) was observed for tryptophan residues. This indicated the interaction of QDs with albumins does not affect the conformation of tyrosine micro-region, but it affects the conformation of tryptophan micro-region. It also indicated that the polarity and the hydrophilicity around the tryptophan residues were increased [53]. Similar types of conformational changes have already been reported [55].
Figure 14: Plot between log (F₀−F)/F vs log [MPA-CdTe QDs] for the determination of binding constant and number of binding sites.

Figure 15a: The synchronous fluorescence spectra of BSA (Δλ = 60 nm) in the presence of MPA-CdTe QDs (0−5 x 10⁻⁷ M). Inset is the normalized spectrum which clearly shows the red shift around (5 nm).
**Figure 15b:** The synchronous fluorescence spectra of BSA ($\Delta\lambda = 15$ nm) in the presence of MPA-CdTe QDs (0–5 x $10^{-7}$ M).

**Figure 16a:** The synchronous fluorescence spectra of HSA ($\Delta\lambda = 60$ nm) in the presence of MPA-CdTe QDs (0–5 x $10^{-7}$ M). Inset is the normalized spectrum which clearly shows the red shift around (5 nm).
Figure 16b: The synchronous fluorescence spectra of HSA ($\Delta \lambda = 15$ nm) in the presence of MPA-CdTe QDs ($0-5 \times 10^{-7}$ M).

6.5. Conclusions

Colloidal uncapped and starch capped CdS nanoparticles, TGA-CdSe QDs and MPA-CdTe QDs were prepared and their interaction with BSA, HSA and lysozyme has been studied by using UV–Visible, steady state, time resolved and synchronous fluorescence spectroscopic measurements. The albumins adsorbed on the surface of colloidal CdX through the capping agents. The apparent association constant has been calculated from absorption studies. The result presented clearly indicates that the nanoparticles and QDs quench the fluorescence of albumins through complex formation. The quenching rate constant, binding constant, and number of binding sites were calculated according to the relevant fluorescence data. From the synchronous fluorescence spectra, it is established that the conformational changes of albumins occurred especially in the tryptophan micro-region. This type of interaction studies having applications in the field of biological imaging, cancer therapy and drug delivery etc.
6.6. References


Spectroscopic Studies...


**Table 4:** Absorption studies of BSA by colloidal SCdS nanoparticles in water.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>[SCdS] (x 10^{-4} M)</th>
<th>Absorbance</th>
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<th>1/[SCdS] (x 10^{-4} M)</th>
<th>1/A_{obs} - A_0</th>
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Table 5: Fluorescence quenching of BSA by SCdS nanoparticles in water.

<table>
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<th>Intensity</th>
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<th>F_0-F</th>
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<th>Log [SCdS]</th>
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Table 6: Fluorescence quenching of Lysozyme by TGA-CdSe QDs in water.

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<th>[TGA-CdSe QDs] (x 10^-6 M)</th>
<th>Intensity</th>
<th>F₀/F</th>
<th>F₀-F</th>
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Table 7: Fluorescence quenching of BSA by MPA-CdTe QDs in water.

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<th>[MPA-CdTe QDs] (x 10^{-7} M)</th>
<th>Intensity</th>
<th>F₀/F</th>
<th>F₀-F</th>
<th>(F₀-F)/F</th>
<th>Log [MPA-CdTe QDs]</th>
<th>Log (F₀-F)/F</th>
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Table 8: Fluorescence quenching of HSA by MPA-CdTe QDs in water.

<table>
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<tr>
<th>S. No.</th>
<th>[MPA-CdTe QDs] (x 10^{-7} M)</th>
<th>Intensity</th>
<th>F_0/F</th>
<th>F_0-F</th>
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