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Effect of CdO Nanoparticles on Bovine Serum Albumin: A Spectrofluorometric Study

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The conformational behaviour of bovine serum albumin (BSA) on conjugation with nanoparticles, especially metallic oxide nanoparticles receives great attention in the recent years due to their key impact in biomedical field. Cadmium oxide nanoparticles prepared by the simple low cost precipitation method were characterized by FTIR, XRD and SEM. Cadmium oxide nanoparticles are of antibacterial and anticancer properties. Therefore, the interaction between BSA and CdO nanoparticles and the effect of CdO nanoparticles on the conformation of BSA has been analyzed by various spectroscopic techniques. The BSA-CdO nanoparticle conjugate made significant changes in BSA fluorescence emission parameters and thus confirmed the conformational changes.

Keywords: Bovine serum albumin, CdO nanoparticles, Fluorescence, Conformational changes.

INTRODUCTION

The nano-sized materials of dimension < 100 nm are used in various scientific, industrial and medical fields [1]. Metal oxide nanoparticles have found many applications in a variety of areas including use in biosensors and medicine [2]. Cadmium oxide nanoparticles is an inorganic compound and is a category of an important practical n-type semiconductor metal oxide. There are several synthesis methods such as hydrothermal method, template assisted method, solvothermal methods, mechano-chemical method, thermal decomposition and sono-chemical method for the preparation of CdO nanoparticles. In this study, CdO nanoparticles have been prepared by the simple low cost precipitation method. Because of their unusual optical, chemical, photo electrochemical and electrical properties, CdO nanoparticles are widely applied in various scientific and industrial fields as well as for medical imaging and targeting of pharmaceutical agents to sites of disease [2-4]. Nano CdO has been reported for antibacterial properties [5,6]. Cadmium oxide nanoparticles can be useful in the treatment of infectious diseases caused by *E. coli* [7,8]. Now-a-days, cadmium/CdO is used in multi-vitamin pills, dietary supplements [9,10] and in various cosmetics and anti-solar creams. Hence, CdO can be considered as a chemical compatible with the body [11,12].

The heavy metals such as cadmium, copper and cobalt can eliminate cancer cells in low concentrations. The various forms of CdO nanoparticles are widely interested in the field of medicinal and pharmaceutical researches, especially the researches about its applications on cancer treatment. Cadmium oxide nanoparticles are not dangerous for human and mammalian cells and having anticancer properties [13]. Its effect on cancer cells is through DNA and protein damage and also destruction of the cell wall, which makes them very useful for eliminating cancer cells [14,15]. For the first time the applications of CdO nanoparticles on eliminating cancer cells, the effect of CdO nanoparticles on DNA of human cancer cells and the interaction of CdO nanoparticles with DNA of cancer cells were studied [13]. Cadmium oxide nanoparticles are used in various fields such as delivery of drug to the tumor cells, pulling out the cancer of live cells, attacking to cancer cells, improving the sensitivity of cancer cells to imaging [16-18], to identify the precise location of the tumor before surgery, to detect risk margin around the tumor and to track responses to treatment after surgery [19-22]. As CdO nanoparticles stick to cancer cells, they can easily detect and observed by MRI [23]. Thus, CdO nanoparticles are used for the prevention of cancer and its treatment and CdO nanoparticles based therapy has emerged as a new branch of nano-based treatments [13,24].

Bovine serum albumin, the most abundant proteins in blood plasma manage transportation of drugs and nutrition through human body and are also responsible for drug deposition and efficacy [25]. Among the three intrinsic fluorophores of BSA, tryptophan (trp), tyrosine (tyr) and phenylalanine (phy), the major contribution of intrinsic fluorescence is due to tryptophan residues with only a minor contribution by the numerous tyrosine depending on the excitation wavelength

selected. Bovine serum albumin is an ideal protein for intrinsic fluorescence measurements due to the presence of two intrinsic tryptophan residues: trp-134 in the first domain located on the surface of molecule, which can be quenched and trp-212 in the second domain, located within a hydrophobic binding pocket [26]. Since tryptophan is highly sensitive to its local environment it can be used to observe changes in the fluorescence emission spectra due to protein conformational changes, binding to substrates *etc*. [27].

Nanoparticles can easily accumulate on biomolecules as they have dimensions comparable to the biological molecule. They provide high loading capacity for drugs and can be used for biomedical applications like targeted drug delivery, bio sensing, bio imaging *etc*. [28,29]. The direct conjugation of semiconductor nanoparticles with proteins was studied [30]. Proteins may undergo structural rearrangements called conformational changes during adsorption on nanoparticles. It was reported that in most of the cases biomolecules especially proteins undergo some structural changes at the boundary surface of nanoparticles [31]. Among the several optical techniques a large amount of information is acquired about the structural fluctuations and the microenvironment surrounding the fluorescent labels of proteins from the measurements and analysis of fluorescence spectra. Therefore fluorescence spectroscopy plays a crucial role in the study of interactions between nanoparticles and proteins. The interaction of proteins and other macromolecules with nanoparticles has been shown to result in marked changes to the physical properties of the nanoparticles as well as the structure and function of the proteins [32]. Conformational changes were observed by the interaction of BSA with colloidal capped CdS nanoparticles [33,34], quantum dots [35], ZnO nanoparticles [36] and TiO_2 nanoparticles [37].

Bovine serum albumin readily undergoes conformational changes, hence BSA provides a good model for investigating the effect of nanoparticles on protein conformational change. Cadmium oxide nanoparticles have many advantageous such as remarkable catalytic activity, simple preparation procedure, long-term stability, low cost [38], the lowest toxicity comparing with nanoparticles of other toxic semiconductors [39,40] and especially having anticancer properties [13]. Thus it is of prime importance to study the interaction mechanism between BSA and CdO nanoparticles. To the best of our knowledge there is no prior report about the fluorescence measurements of BSA-CdO nanoparticle interaction. Therefore, the present work is focused to study the interaction between BSA protein and CdO nanoparticles by using various spectroscopic techniques.

EXPERIMENTAL

Bovine serum albumin was purchased from Sigma-Aldrich, USA. Cadmium acetate monohydrate and ammonium hydroxide were purchased from Merck, India. The samples were used as received without any further purification.

Steady state fluorescence measurements: The fluorescence measurements were carried out with JASCO FP-8600 spectrofluorometer. The excitation wavelength was 290 nm. The excitation slit width 2.5 nm, emission slit width 2 nm and scan rate 500 nm/min were maintained constant for all measurements. The quartz cuvette (4 cm × 1 cm × 1 cm) with path length of 1 cm was used.

Time resolved fluorescence 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).

Preparation of CdO nanoparticles: The cadmium acetate and ammonium hydroxide were used as starting materials for preparing CdO nanoparticles by the simple low cost precipitation method. The predetermined amount of cadmium acetate was dissolved in deionized water in a 250 mL conical flask with constant stirring. Then, ammonium hydroxide was added slowly into the already prepared aqueous solution of cadmium acetate under constant stirring until the pH of the solution reached 8. The obtained white precipitate was allowed to settle down for 2-3 h, which indicated the formation of cadmium hydroxide. This was washed filtered and washed with distilled water several times until the reacted products were washed away. Thus obtained precipitate was dried at 100 °C for 24 h and then grinded well to make it as a fine powder. The resultant fine powder was calcinated at 500 °C for 2 h in muffle furnace. The obtained reddish brown powder was confirmed the formation of CdO nanoparticles.

Stock preparation of BSA and CdO nanoparticles: Bovine serum albumin stock solution was prepared by dissolving 100 mg of BSA in 10 mL double distilled water and it was used for further studies. Similarly stock solution of CdO nanoparticles was prepared by dissolving 13 mg of CdO nanoparticles in 10 mL double distilled water and then subjected to ultrasonic vibration for 20 min. This dispersion was used further for the interaction studies.

Interaction of BSA with CdO nanoparticles: Bovine serum albumin was interacted with various concentrations of CdO nanoparticles. This mixture was homogenized and kept for 0.5 h for incubation. All measurements were recorded after 0.5 h of incubation required for the interaction between CdO nanoparticles and BSA. 3 mL of the sample was subjected to analysis at the excitation wavelength of 290 nm and emission spectra in the range 310-470 nm. All measurements were performed at room temperature.

RESULTS AND DISCUSSION

FTIR study: The FTIR spectrum of CdO nanoparticles is shown in Fig. 1. The O-H stretching and vibrations peaks are observed at 3245 cm^{-1} and 1650 cm^{-1}, which are found to be the absorption of water during pellet preparation process. The Cd-O metal oxide stretching is seen at 650 cm^{-1}. It is confirmed the formation of CdO nanoparticles.

XRD pattern for CdO nanoparticles: The structure of CdO nanoparticles has also been confirmed by XRD technique. Fig. 2 shows the typical XRD pattern of pure CdO. It shows the diffraction peaks of (1 1 1), (2 0 0), (2 2 0), (3 1 1) and (2 2 2) which are characteristics of the pure CdO with the cubic phase. All the existing peaks in the pattern are in good agreement with the JCPDS data (File No. 05-0640). There are no unwanted peaks in the pattern, which confirm the purity of CdO. The size of the CdO nanoparticles is calculated as 42 nm using well known Scherrer's formula.

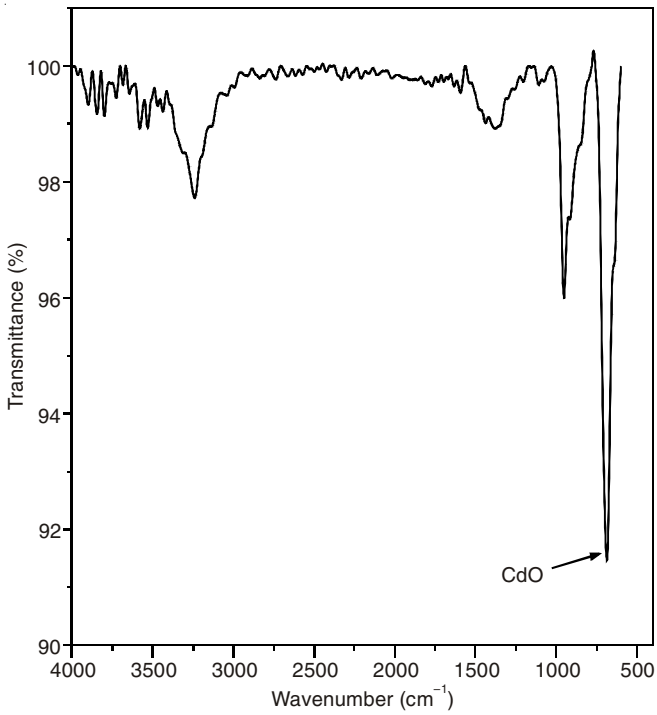

Fig. 1. FTIR spectrum of cadmium oxide nanoparticles

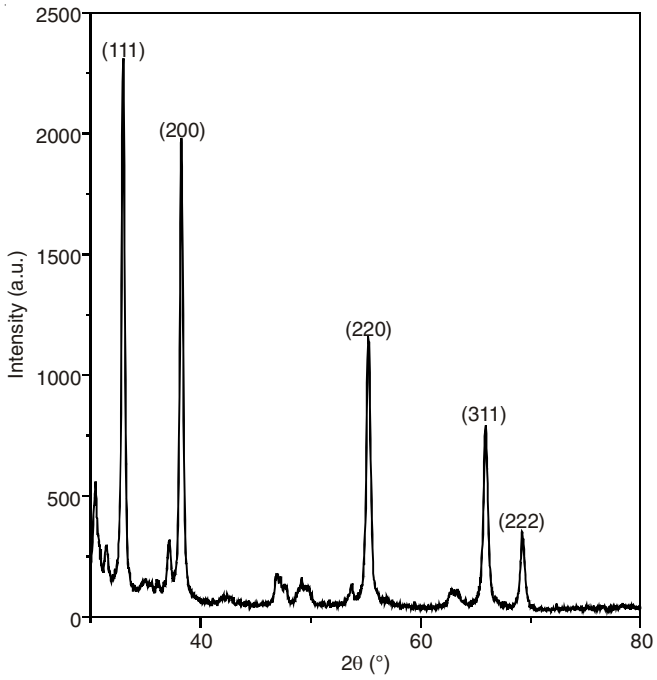

Fig. 2. XRD pattern of pure CdO

Scanning electron microscopy measurement: The morphology of the prepared CdO nanoparticles is analyzed using scanning electron microscope. Fig. 3 shows the SEM micrograph of CdO nanoparticles. It is observed that CdO nanoparticles are more or less in spherical morphology.

Steady state fluorescence analysis: The intrinsic fluorescence spectra for native BSA and BSA-CdO nanoparticle complex were analyzed at the excitation wavelength of 290 nm to evaluate the conformational changes around tryptophan residues (Fig. 4). When excited at 290-295 nm the emission of proteins is generally dominated by the tryptophan fluorescence

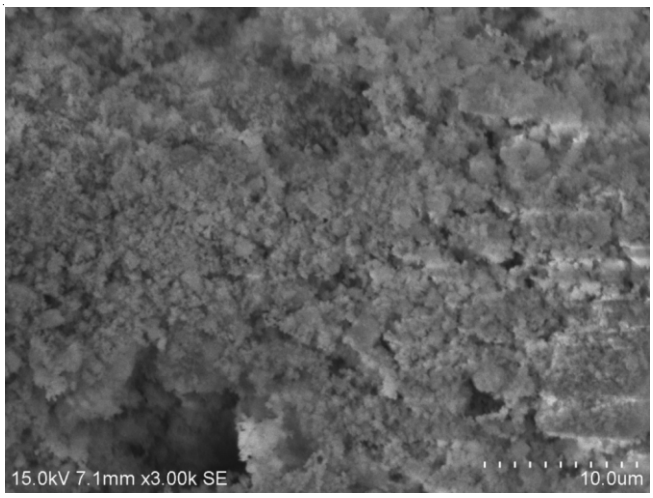

Fig. 3. SEM micrograph of CdO nanoparticles

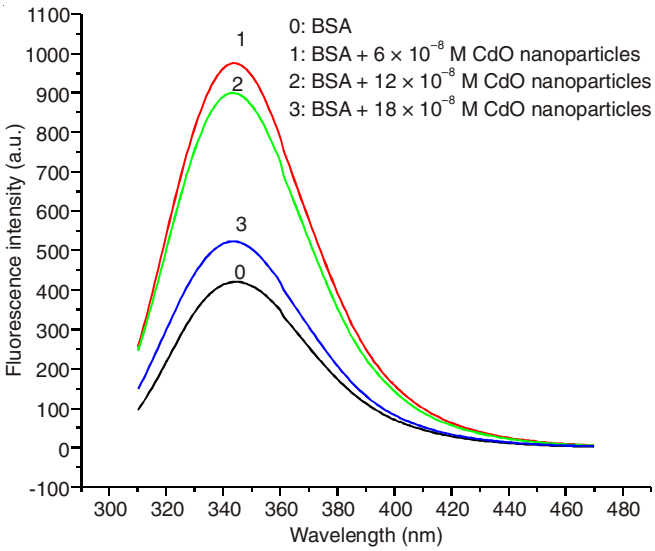

Fig. 4. Fluorescence spectra of BSA at different concentrations of CdO nanoparticles (0, 6, 12 and 18 × 10^{-8} M)

[41]. The figure clearly shows that BSA has a strong emission band at 345 nm when excited with 290 nm wavelength. Several previous works have also reported emission band at the same wavelength for BSA [37]. Fluorescence spectra of CdO nanoparticles attached with BSA were found to be different than that of the native BSA (Fig. 4). After the addition of CdO nanoparticles the maximum emission wavelength of BSA showed a blue shift of 2 nm indicates a change of the micro-environment polarity around the tryptophan residues and they were exposed to a hydrophobic environment. The blue shift in the spectral maximum suggests a decrease in the polarity or an increase in the hydrophobicity of the microenvironment surrounding the fluorophore site [42]. The fluorescence intensity of BSA increased for all the three concentrations of CdO nanoparticles with maximum increase observed for the lowest concentration. While increasing the concentration of CdO nanoparticles a gradual decrease in fluorescence intensity was observed. Thus it is concluded that the fluorescence intensity increase of BSA is due to lower concentrations of CdO nanoparticles and at higher concentrations of CdO nanoparticles fluorescence of native BSA can be quenched.

The fluorescence intensity increase at low concentrations of CdO nanoparticles may be due to conformational changes leading to a more folded protein aroused from BSA-CdO nanoparticles interaction. The change in fluorescence intensity is due to change in local environment around the tryptophan residues and also due to quenching [43]. This suggests that CdO nanoparticles change the native state of BSA and altering the microenvironment around the tryptophan residues. This result is consistent with the studies in which it was observed that when BSA interacted with gold nanoparticles an increase in fluorescence intensity occurred at lower concentrations of gold nanoparticles [44]. Similarly an increase in fluorescence intensity occurred at lower concentrations of Al_2O_3 nanoparticles when interacted with BSA [45]. Mercaptoacetic acid-coated cadmium sulfide nanoparticles conjugated with bovine serum albumin exhibited stronger fluorescence intensities than the non-conjugated cadmium sulfide nanoparticles [34]. Conjugation of BSA with quantum dots resulted in enhancement of fluorescence intensity, which might be due to the stabilizing effect of BSA on quantum dots or due to energy transfer from tryptophan moieties of albumin to quantum dots [35].

A quenching in BSA fluorescence with a shift in maximum of the emission peak (red/blue/no shift) was observed with increasing concentrations of $AgTiO_2$ nanoparticles [46], ZnO nanoparticles [47] and colloidal capped CdS nanoparticles [33]. But in all these works either the nanoparticle concentration was more [46] or BSA concentration was less [47] or lower BSA concentration with higher nanoparticle concentration [33] was used for the interaction studies compared to that of the present study. Thus it is concluded that in the present study fluorescence quenching of native BSA was not observed because of the use of either lower concentrations of CdO nanoparticles or higher concentrations of BSA. The interaction of Cd(II) complex with bovine serum albumin and human serum albumin was studied by fluorescence spectroscopy and while increasing the concentrations of the cadmium complex the fluorescence intensities of the proteins are regularly decreased [48].The interaction between human hemoglobin and CdS quantum dots was studied by spectroscopic methods [49].

Time resolved fluorescence analysis: The lifetime measurements are the most authoritative method of distinguishing between dynamic and static quenching because the steady state fluorescence quenching involves the total process (static and/or dynamic) whereas in the time resolved fluorescence quenching only the dynamic quenching is measured [27]. The fluorescence effect of BSA in the absence and presence of CdO nanoparticles were measured. The exponential decay curves of BSA and BSA with different concentrations of CdO nanoparticles are shown in Fig. 5. The fluorescence decays of BSA were fitted with two exponentials T_1 = 7.0 ns and T_2 = 3.56 ns. This result is consistent with the studies in which it was shown that the lifetimes of tryptophan fluorescence are rather short and often multi exponential [50]. The two lifetimes indicated that BSA contained two tryptophan residues that fluoresced in two different environments [37]. The significant changes between the two lifetimes indicated that one of the tryptophan residues in the protein may be relatively exposed

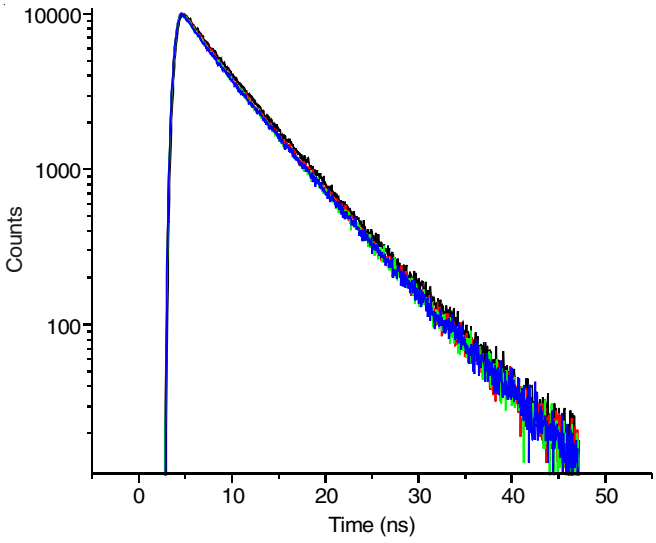

Fig. 5. Time resolved fluorescence decay of BSA at different concentrations of CdO nanoparticles (0, 6, 12 and 18 × 10^{-8} M)

whereas the other tryptophan residue appears to be deeply buried inside the protein [26].

The lifetime of both tryptophan residues in BSA changed with increasing CdO nanoparticle concentrations and the changes were more prominent in lifetime T_2 compared to that of lifetime T_1. The fluorescence lifetime of BSA decreased for lifetime T_1 from 7.0 ns to 6.39 ns, 6.46 ns and 6.30ns whereas for lifetime T_2 from 3.56 ns to 2.74 ns, 2.84 ns and 2.36 ns upon interaction with different concentrations of CdO nanoparticles respectively (Fig. 5 and Table-1). For a static quenching the complex formation will not disturb the fluorescence lifetime of BSA, however the fluorescence lifetime will be cut down due to the collision between the excited protein fluorophore and nanoparticles in a dynamic quenching procedure [27]. Therefore, in this case the changes in fluorescence lifetimes of tryptophan residues of BSA indicated dynamic quenching process in the BSA-CdO nanoparticle complex. Time resolved fluorescence measurements showed that consistent dynamic quenching occurred by the interaction of BSA with TiO_2 nanoparticles [37] and silver nanoparticles [51] while static quenching mechanism was confirmed when BSA interacted with colloidal capped CdS nanoparticles [33], colloidal $AgTiO_2$ nanoparticles [46] and gold nanoparticles [44].

Monitoring conformational changes of CdO nanoparticle-bound BSA: Conformational changes in proteins may disturb the microenvironment around the tryptophan residues and thus influence the fluorescence emission. Therefore the tryptophan fluorescence is widely used to monitor conformational changes in proteins [52]. The interaction of BSA with different concentrations of CdO nanoparticles resulted in significant changes in the fluorescence intensity of BSA with a blue shift in the fluorescence emission maximum. When the proteins bind to other substances or the conformation of protein is changed the corresponding fluorescence intensity and/or the wavelength of the maximum peak of the protein will generate some alterations [53]. Therefore this behaviour means that the conformation around the tryptophan residues of the BSA-CdO

TABLE-1
EMISSION MAXIMUM, CORRESPONDING FLUORESCENCE INTENSITY AND LIFETIMES OF BSA AND BSA WITH DIFFERENT CONCENTRATIONS OF CdO NANOPARTICLES

Sample	Emission maximum (nm)	Fluorescence intensity (AU)	Lifetime T_1 (ns)	Lifetime T_2 (ns)
BSA	345	420.32	7.00	3.56
BSA + 6×10^{-8} M CdO nanoparticles	344	975.08	6.39	2.74
BSA + 12×10^{-8} M CdO nanoparticles	343	899.98	6.46	2.84
BSA + 18×10^{-8} M CdO nanoparticles	343	522.77	6.30	2.36

nanoparticle complex changes as compared to that of the native BSA. The lifetime of the tryptophan residues in BSA changed with increasing CdO nanoparticle concentrations and the changes were more prominent in the lifetime T_2 compared to that of lifetime T_1. Only the dynamic quenching process reduces the fluorescence lifetimes compared to that of static quenching process [27]. Therefore, the significant decrease in the fluorescence lifetime indicated consistent dynamic quenching process in the BSA-CdO nanoparticle complex. Depending on the hydrophobicity of the surrounding of a tryptophan residue the center of its emission band can vary over several tens of nanometers as well as its quantum yield and lifetime changes. Therefore steady state spectroscopic studies even with proteins containing several tryptophan residues can provide insight into conformational changes upon substrate binding or other catalytic processes. Hence, in the present study the blue shift in the fluorescence emission maximum, significant changes in fluorescence intensity as well lifetimes (Figs. 4 & 5 and Table-1) implies the probable conformational changes induced by CdO nanoparticles on BSA. Spectroscopic investigations showed conformational changes when BSA interacted with colloidal CdS nanoparticles [33], ZnO nanoparticles [36], TiO_2 nanoparticles [37] and colloidal $AgTiO_2$ nanoparticles [46].

Conclusion

In the present study, CdO nanoparticles were prepared by the simple low cost precipitation method and the interaction between CdO nanoparticles and BSA has been studied by various spectroscopic techniques. The blue shifts of fluorescence emission peak and the changes in fluorescence intensity of CdO nanoparticles-BSA conjugated system indicated that the microenvironment close to tryptophan residues of BSA is perturbed and the hydrophobicity of both residues increased. The analysis of lifetime measurements indicated that the lifetime of the tryptophan residues in BSA changed with increasing CdO nanoparticle concentrations confirmed that the dynamic quenching was dominant in this reaction process. The significant changes made by the BSA-CdO nanoparticle conjugate in BSA fluorescence emission parameters indicated that CdO nanoparticles induced conformational changes in the structure of BSA.

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