Size dependent interaction of biofunctionalized CdS nanoparticles with tyrosine at different pH

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Enhancement of fluorescence of CdS nanoparticles by tyrosine at pH 10 in contrast to Stern–Volmer quenching at pH 7 was observed and both the effects were found to depend on the size of the nanoparticles.

There has been considerable interest in semiconductor nanoparticles or colloidal quantum dots due to the novel electronic and optical properties arising out of quantum confinement effects.[1] The drive for expanding our understanding of semiconductor nanoparticles has been spearheaded by potential applications of these materials in various optoelectronic devices.[2] Lately, surface modification of fluorescent semiconductor nanoparticles by biomolecules like peptides and nucleic acids has added a new dimension to nanoparticle research with respect to their biological applications.[3] Utilizing these nanoparticle bioconjugates for accurate and sensitive determination of water soluble analytes such as toxins, small molecule explosives, ionic species, and various biomolecules like nucleic acids and proteins is one of the most cherished scientific goals with wide ramifications in disease diagnosis, drug development and defence applications. The effectiveness of biofunctionalized semiconductor nanoparticle probes in determination of proteins and nucleic acids as compared to conventional techniques using organic fluorophores has been quite vividly demonstrated by earlier workers.[4,5] In contrast, little attention has been paid to understanding the role of constituents of these macromolecules in interactions with the semiconductor nanoparticles

To illustrate the importance, in the present work, we take a microscopic view of the interaction of fluorescent and biocompatible CdS nanoparticles with tyrosine, a key amino acid in numerous protein and enzymes for a variety of biological functions. Contrasting behaviours of the tyrosine–CdS NPs system was observed at pH 7 and 10. Tyrosine quenched the fluorescence of CdS nanoparticles at pH 7 whereas it caused enhancement of luminescence intensity at pH 10. Interestingly, the degree of quenching and enhancement of CdS luminescence at the two pH was found to vary with the size of the nanoparticles. To the best of our knowledge, this is the first such study on the size dependent interaction of CdS nanoparticles with amino acids. The enhancement effect at pH 10 was specific for tyrosine only. Based on the differential behaviour exhibited by tyrosine at basic pH, the possibilities of developing a sensitive determination technique for tyrosine or proteins with exposed tyrosine moieties in biological systems can be explored.

CdS nanoparticles, biofunctionalized by surface capping with an amino acid, cysteine, were synthesized by a colloidal chemistry route as reported earlier by us.[6] The characteristic optical absorption and emission profiles of the CdS nanoparticles selected for these purposes are displayed in Fig. 1. The average sizes of the CdS nanoparticles as determined from the absorption onset are 2.8 nm (CdS-I), 3.3 nm (CdS-II) and 4.0 nm (CdS-III). The interaction of tyrosine with these CdS nanoparticles was investigated at pH 7 and pH 10 by fluorescence spectroscopy.

The luminescence behaviour of CdS nanoparticles in presence of tyrosine is shown in Fig. 2(a). The fluorescence intensity of the

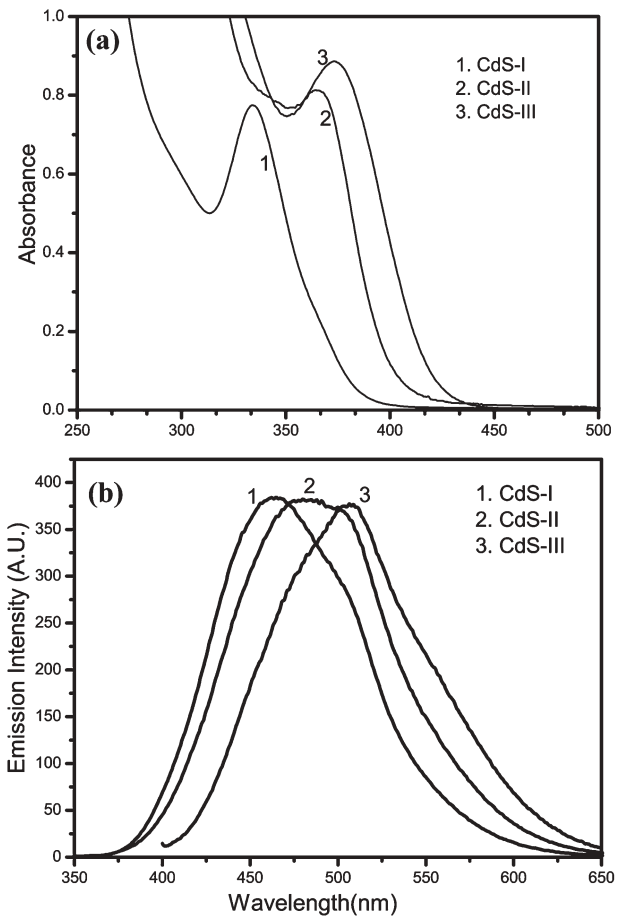

Fig. 1 Optical properties of CdS nanoparticles, (a) UV–visible absorption and (b) photoluminescence spectra.

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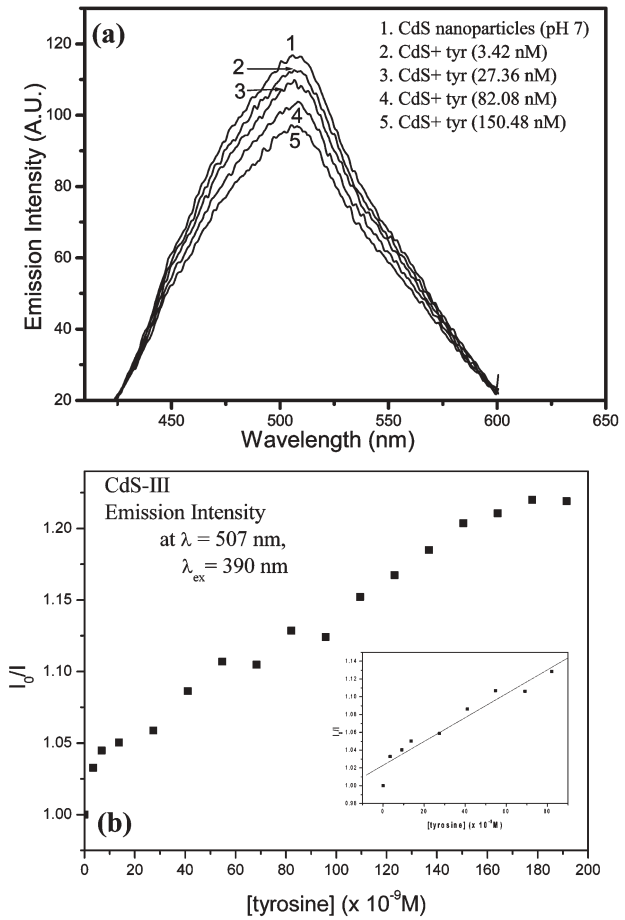

Fig. 2 Effect on luminescence of CdS nanoparticles (CdS-III) with varying tyrosine concentration at pH 7. (a) Photoluminescence spectra, (b) a typical plot of I_0/I against tyrosine concentration, inset: Stern–Volmer plot in the linear range.

particles was found to be quenched by even nanomolar concentrations of tyrosine at pH 7. The plot of I_0/I against tyrosine concentration is linear up to certain extent beyond which it curves towards the *x*-axis. A typical plot is shown in Fig. 2(b). In the inset, the linear range (Table 1) is shown where it follows the Stern–Volmer equation:

$$I_0/I = 1 + K_{S-V}\,[\text{tyrosine}]$$

It was quite fascinating to observe size dependence in the quenching pattern of nanoparticle fluorescence by tyrosine. It was observed from these quenching phenomena that Stern–Volmer constants were decreasing with increasing particle size. A similar observation was also made by Matsumoto *et al.*[7] in quenching of CdS fluorescence by TiO_2 colloids. To establish the nature of quenching, fluorescence lifetime measurements were carried out on a single photon counting set up. No change in the fluorescence lifetime of CdS nanoparticles was observed on addition of tyrosine suggesting the quenching to be static. The $-NH_2$ group in the amino acid could be responsible for quenching the fluorescence as reported earlier in the case of quenching of CdS fluorescence by aniline.[8] At pH 7, the carboxyl group would be fully ionized and the protonated amino group could scavenge electrons from the electron–hole pairs formed as a result of excitation of CdS nanoparticles. As the size of the nanoparticles decreases, the energy of the conduction band shifts to higher energy due to the quantum confinement effect. Redox potentials of the conduction band become more negative[7,9] thereby enhancing the reducing power with a decrease in particle size. Due to higher surface to volume ratio in smaller nanoparticles, most of the constituent atoms reside on the surface of the particles[10] and can have more efficient transfer of electrons to the suitable species adsorbed on the surface, reducing the chances of radiative recombination of e^-–h^+ pairs. So, enhanced reducing power and higher ratio of surface to core atoms could account for the observed increase in the value of Stern–Volmer constants with decreasing particle size. To check the specificity of this interaction for tyrosine, two other amino acids: glycine, the simplest one, and tryptophan, an aromatic amino acid, were also tried. They also exhibited a similar quenching effect on

Table 1 Variation in quenching behaviour with particle size

Sample	Particle size/nm	Linear range (10^{-9} M)
CdS-I	2.8	0–16
CdS-II	3.3	0–30
CdS-III	4.0	0–82

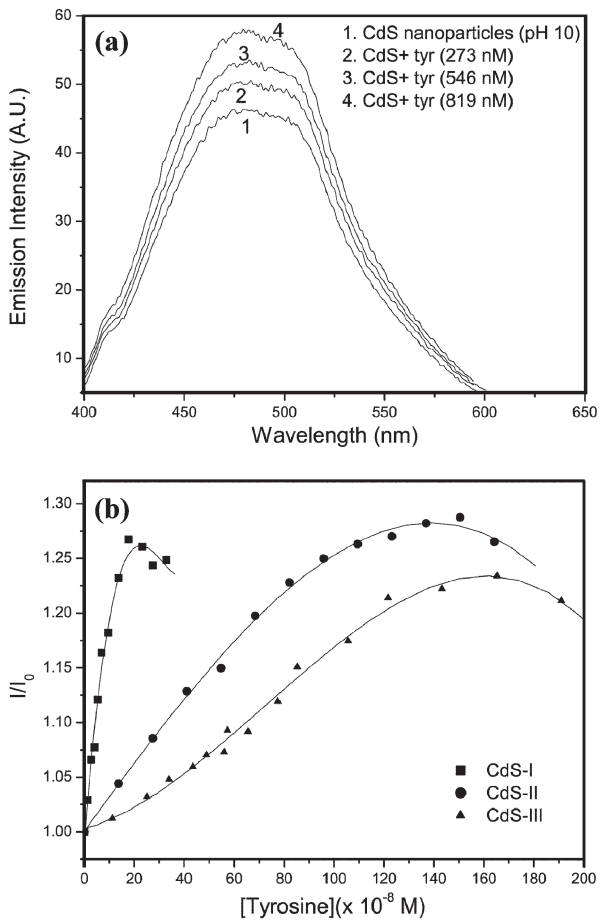

Fig. 3 Effect on luminescence of CdS nanoparticles at pH 10 with varying tyrosine concentration. (a) Photoluminescence spectra (CdS-II), (b) a plot of I/I_0 against tyrosine concentration for nanoparticles of different sizes.

CdS luminescence at pH 7 which supports the view that quenching is due to $-NH_2$ group in the amino acids.

In contrast to the effects of tyrosine at pH 7, the luminescence of CdS nanoparticles at pH 10 was found to be enhanced with gradual addition of tyrosine, even at low concentrations as displayed in Fig. 3(a). The other two amino acids namely, glycine and tryptophan, could only quench the fluorescence of the particles even at this pH which makes this enhancement effect on CdS fluorescence quite specific for tyrosine. To reveal the nature of the interaction of CdS nanoparticles with tyrosine at this pH, the effect of ionic strength on the enhancement of luminescence in the presence of tyrosine was studied. If the interaction of nanoparticles and tyrosine is mainly due to electrostatic forces, the ionic strength should have some effect on the interaction.[11] The fluorescence intensity remained unchanged with a gradual increase in KCl concentration, which indicates that the nature of interaction is not electrostatic; rather, it is covalent. Considering that the pK_a for the dissociation of the phenolic –OH in tyrosine is 10.1,[12] tyrosine is expected to be more dissociated into the tyrosinate form and this enhancement could be attributed to binding of the phenolate group of tyrosine to the surface of the CdS nanoparticles. This may lead to more passivation of the trap states on the nanoparticle surface thereby increasing the fluorescence intensity of the nanoparticles. The maximum enhancement achieved is almost the same for nanoparticles of different sizes, but the amount of tyrosine required is different as displayed in Fig. 3(b). For the same change in the concentration of tyrosine, the enhancement is greater for the smaller nanoparticles. Smaller nanoparticles have a greater ratio of surface to core atoms and therefore most of the electronic defects originate on the surface.[13] A greater number of trap states become accessible to the tyrosinate ligand as the size of the particle decreases. With increase in particle size, accessibility of trap states to the added ligand is affected drastically and we get less enhancement in fluorescence.

In summary, this is the first report showing the size dependent interaction of CdS nanoparticles with tyrosine. pH dependent differential interactions of tyrosine with CdS nanoparticles, in contrast to other amino acids, suggest the possibility of using it as probe for tyrosine in a mixture of amino acids, *e.g.*, in determination of tyrosine in dissolved free amino acids (DFAA) present in fresh water. Most significantly, the enhancement of fluorescence of CdS nanoparticles at pH 10 is specific for tyrosine and could be attributed to the tyrosinate anion. The linear range of the enhancement plot can be used for determination of tyrosine. The observed sharp rise in luminescence for smaller particles with variation in tyrosine concentration suggests better sensitivity of detection can be achieved with particles of smaller size.

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