

Synthesis and Spectral Studies of CdTe–Dendrimer Conjugates

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Abstract In order to couple high cellular uptake and target specificity of dendrimer molecule with excellent optical properties of semiconductor nanoparticles, the interaction of cysteine-capped CdTe quantum dots with dendrimer was investigated through spectroscopic techniques. NH_2 -terminated dendrimer molecule quenched the photoluminescence of CdTe quantum dots. The binding constants and binding capacity were calculated, and the nature of binding was found to be noncovalent. Significant decrease in luminescence intensity of CdTe quantum dots owing to noncovalent binding with dendrimer limits further utilization of these nanoassemblies. Hence, an attempt is made, for the first time, to synthesize stable, highly luminescent, covalently linked CdTe–Dendrimer conjugate in aqueous medium using glutaric dialdehyde (G) linker. Conjugate has been characterized through Fourier transform infrared spectroscopy and transmission electron microscopy. In this strategy, photoluminescence quantum efficiency of CdTe quantum dots with narrow emission bandwidths remained unaffected after formation of the conjugate.

Keywords Quantum dots · Dendrimer · Conjugate · Infrared spectroscopy · Luminescence

Introduction

Rapid advances in nanotechnology and nanoscience have spurred interests in developing a variety of nanostructured materials. In this context, semiconductor nanoparticles (also

known as quantum dots) are the most promising ones due to their high photochemical stability and size-tunable photoluminescence [1]. Quantum dots (QDs), in particular, have potential applications in optoelectronics, biosensing and biolabelling, etc. [2, 3] Recently, integration of these QDs with biological macromolecules greatly expands the impact of optical imaging, sensing and also of therapeutic strategies [4, 5]. The binding of different categories of molecules to QDs has been studied by optical methods to elucidate binding mechanism, because the surface of nanoparticles (NPs) affects the electronic states, which, in turn, influence the photoluminescence (PL) emission of QDs [6]. In our earlier reports, the binding of amino acids, DNA bases, biological relevant metal ions, enzyme and peroxydinitrite (PN), a powerful biological oxidant with semiconductor QDs was investigated, and a quantitative correlation was established therein [7–11]. However, the binding of dendrimer with QDs is of special interest as immobilization of semiconductor nanocrystal onto dendrimer has great implication in the field of material science in view of amalgamation of excellent luminescent properties of semiconductors with varied functionalities of dendrimer molecules. PAMAM Dendrimers are synthetic spherical macromolecules with a well-defined surface, comprising a core, branching sites and a large number of terminal groups [12]. The biomimetic properties and low cytotoxicity of dendrimer molecules make them potentially useful for many biological applications such as gene transfection, diagnostics, drug delivery as well as nanoscale building blocks [13, 14]. The molecules are small enough to pass into the cell membrane and can be used to deliver substances such as drugs, genetic materials or chemical markers right into the cells [15]. Thus, CdTe–Dendrimer conjugates can act as new luminescent multi-functional nanostructured materials. To achieve binding specificity and targeting

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ability, QDs can be linked to monoclonal antibodies, peptides, oligonucleotides, small inhibitor or hydrophilic segment (such as polyethylene glycol [PEG]). It is expected that the self-assembly of the dendrimer molecules onto NPs provide a route to modifying the NPs for targeted imaging of cancer cells [16]. In general, assembly process of NPs largely relies on noncovalent interactions. However, the drawback is the inherent instability of these conjugates under varied environmental parameters, such as low pH, higher temperature, ionic strength, etc. Thus, an alternative pragmatic approach to preparing QD assemblies is interfacing through covalent binding [8, 17, 18]. This method of functionalizing QDs is simple and can avoid complicated synthesis and characterization of the intermediate products of QDs, when reactions are performed on the QD surfaces. A number of reports have been published in which biological molecules have been attached onto the surface of QDs. However, optimization of conjugation process is a prerequisite for the use of QDs in biomedical applications.

In the present work, the interactions between CdTe QDs and dendrimers of different generations have been studied fluorimetrically. The binding constants K_{SV} and K_b and binding stoichiometry of the complex (n) have been determined. Significant differences on the values of binding constants for dendrimers of different generation suggest that generation has strong influence upon the interaction between CdTe QDs and dendrimer. We, further, demonstrate a simple method of the preparation of CdTe–Dendrimer conjugates through covalent binding using Glutaric dialdehyde (G) linker and describe their optical properties. Semiconductor–dendrimer conjugate, thus synthesized, represents a hybrid material in which fluorescence of semiconductors convolutes with the biomimetic properties of dendrimer, which is ideally suited for various biomedical applications such as fluorescence imaging and probing of biological systems.

Experimental

Materials

L-Cysteine hydrochlorides and cadmium nitrate tetrahydrate were purchased from Merck, Germany. The starburst dendrimers (PAMAM) of generation 2.0, 4.0, 5.0 (NH_2 terminated) and glutaraldehyde were obtained from Sigma Aldrich, Germany. Telluric acid (H_2TeO_4 , $2\text{H}_2\text{O}$) and sodium borohydrate (NaBH_4) were purchased from BDH.

Synthesis of CdTe Quantum Dots and CdTe–Dendrimer Conjugate

CdTe QDs were synthesized following our method as reported earlier [19, 20]. An aqueous solution of Cd^{2+} ion

(4.68×10^{-2} M) and L-cysteine (11.70×10^{-2} M) was prepared, and pH was adjusted to 11.2–11.8 (using Jenway 3345 ion meter). Then, NaHTe solution was added under nitrogen atmosphere. The resultant solution was refluxed at 100 °C. The as-prepared CdTe QD solutions were further diluted 10 times, and concentrations were so chosen that absorbance was kept <0.1 to avoid self-absorbance effects. The aqueous solution of 3.47×10^{-7} M PAMAM dendrimer of Generation 2, 4 or 5 was prepared, and few microlitres (10–50 μL) of these solutions were added to the different sets of 3 mL solution of CdTe QDs. In a typical synthesis of CdTe–Dendrimer conjugate, 3 mL aqueous solution of CdTe NPs (5×10^{-6} M) was mixed with 50 μL of glutaraldehyde (10% in water) and 1 mL dendrimer (7.0×10^{-6} M). The solution was allowed to react at room temperature for 24 h and dialyzed against the phosphate buffer.

Structural Characterization

UV-Vis absorption and photoluminescence (PL) spectra of nanoparticles solution were recorded on Shimadzu UV-1601PC and Perkin Elmer LS-55 luminescence spectrometer respectively. Size distribution of CdTe QDs was determined by dynamic light scattering spectrophotometer (Model DLS—nanoZS, Zetasizer, Nanoseries, Malvern Instruments). Samples were filtered several times through a 0.22- μm Millipore membrane filter prior to recording measurements. The Fourier transform infrared spectroscopy (FTIR) spectra were recorded with Perkin Elmer, Spectrum GX equipment using KBr pellet with a resolution of 2 cm^{-1} . Transmission electron microscopy (TEM) was carried out on JEOL-2010 with acceleration voltage of 200 kV. A drop of as-prepared solution of CdTe–Dendrimer conjugate was placed on a carbon-coated copper grid of 300 meshes and dried before putting it on to the TEM sample chamber.

Results and Discussion

The as-synthesized CdTe QDs were stable and highly luminescent [19]. The FWHM (full-width at half maximum) of the PL spectrum is 36 nm, which suggests narrow size distribution. The average size of CdTe QDs was found to be 3.2 nm as determined from TEM images, which is in good agreement with the size (3.0 nm) determined from the correlation of particle size and optical band gap. Further, average size and size distribution of CdTe QDs obtained from UV spectra and TEM image were also supported by DLS histogram ($d_{av} = 3.4\text{ nm}$ with narrow size distribution).

The interaction of dendrimer with these QDs was followed by fluorescence spectroscopy. The fluorescence intensity of QDs was quenched, and the plot of F_0/F against dendrimer concentration is linear (Fig. 1), which follows Stern-Volmer relation (Eq. 1),

$$\frac{F_0}{F} = 1 + K_{SV}[\text{Dendrimer}] \quad (1)$$

where F_0 and F are the fluorescence intensities QDs in the absence and in the presence of quencher, K_{SV} the Stern-Volmer constant. Additionally, binding constant (K_b) and binding capacity (n) were evaluated following the method of Tedesco et al. [21] (Eq. 2),

$$\frac{F_0 - F}{F - F_\infty} = \left(\frac{[\text{Dendrimer}]}{K_{diss}} \right)^n \quad (2)$$

where F_∞ is the intensity of QDs saturated with the quencher. The binding constant K_b is obtained by plotting $\log (F_0 - F)/(F - F_\infty)$ versus $\log [\text{Dendrimer}]$. A typical plot is shown in the inset of Fig. 1. The slope of the plot gives the number of equivalent binding sites or binding capacity (n). The value of $\log [\text{Dendrimer}]$ at $\log (F_0 - F)/(F - F_\infty) = 0$ equals to logarithm of dissociate constant (K_{diss}), and the reciprocal of K_{diss} is the binding constant (K_b). Both Stern-Volmer constants and binding constants were found to increase with increasing dendrimer generation (Table 1). With increasing dendrimer generation from 2 to 5, the quenching constant (K_{SV}) increased by factor 20. The number of surface amine groups increases linearly with increasing dendrimer generation, and this results in increased interaction of QDs with the higher generation dendrimer. Similar studies, based on the formalism

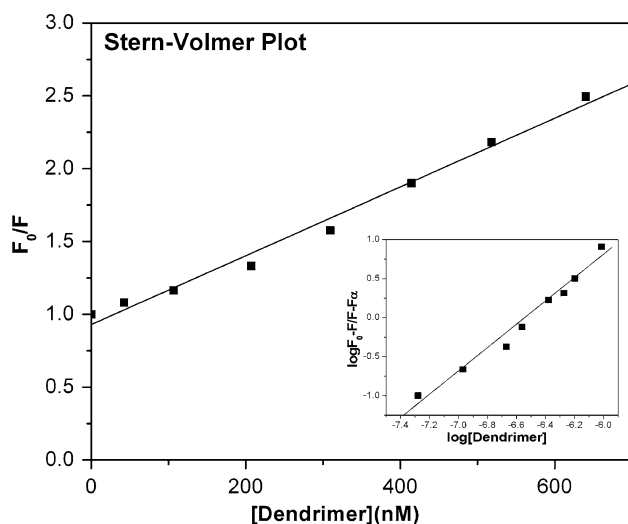


Fig. 1 Stern-Volmer plots for the PL quenching of CdTe QDs, (Here, F_0 and F are the PL intensities of the respective CdTe QDs in absence and presence of dendrimer, respectively) *Inset* double-logarithmic plot of the quenching of CdTe using Tedesco's method

Table 1 Binding constants and binding capacity of dendrimer with CdTe QDs

CdTe-dendrimer conjugates	Binding constant		Binding capacity (n)
	K_{SV} (10^7 M^{-1})	K_b (10^7 M^{-1})	
CdTe-Dendrimer G2.0	0.23	0.29	1.29
CdTe-Dendrimer G4.0	1.9	2.2	1.98
CdTe-Dendrimer G5.0	4.7	4.2	2.02

presented here, have been successfully used to evaluate the binding properties of nanoparticles to the biological macromolecules [21, 22]. To establish nature of quenching, the effects of ionic strength as well as solvent polarity (solvents of different dielectric constants) on the quenching of QDs by dendrimer have been studied. Solvents of different dielectric constants were prepared by mixing methanol ($\epsilon = 33.0$) and water ($\epsilon = 80.1$) in different proportions. If electrostatic binding or charge transfer process is involved in the interaction, the quenching constant should be adversely affected by decreasing the polarity of the solvent [23]. Hence, quenching of CdTe QDs by dendrimer has been studied by changing the composition of the solvent. With decrease in the polarity of mixed solvent, the Stern-Volmer quenching constants decreased sharply (Table 2). The observed trend indicates that charge transfer might have occurred between CdTe QDs and dendrimer at the excited state, since there was no change in ground-state absorption spectrum of CdTe QDs in the presence of dendrimer. Greater the polarity of the solvent, more stable will be the product; hence, more quenching was observed in aqueous solution. In the solvent of reduced polarity (methanol), the charge transfer process gets hampered showing lesser quenching. Thus, it can be inferred that quenching is caused by the electrostatic interaction between CdTe QDs and dendrimer.

Hence, efforts have been made to bind the dendrimer molecules with QDs *via* covalent bond. Here, the formation of CdTe-Dendrimer conjugate is followed through FTIR measurements. Figure 2 illustrates the FTIR spectra of CdTe, pure PAMAM dendrimer, and CdTe-Dendrimer conjugates. It appears that the band between 1,720 and 1,740 cm^{-1} due to carbonyl groups of the glutaraldehyde disappeared during conjugation. This suggests that QDs are

Table 2 Effect of solvent polarity on Stern-Volmer (K_{SV}) constant

Solvent	Dielectric constant	K_{SV} (Dendrimer G5.0) 10^7 M^{-1}
Water	80.1	4.7
20% Methanol	71.3	3.0
40% Methanol	61.93	2.4
90% Methanol	37.55	0.6

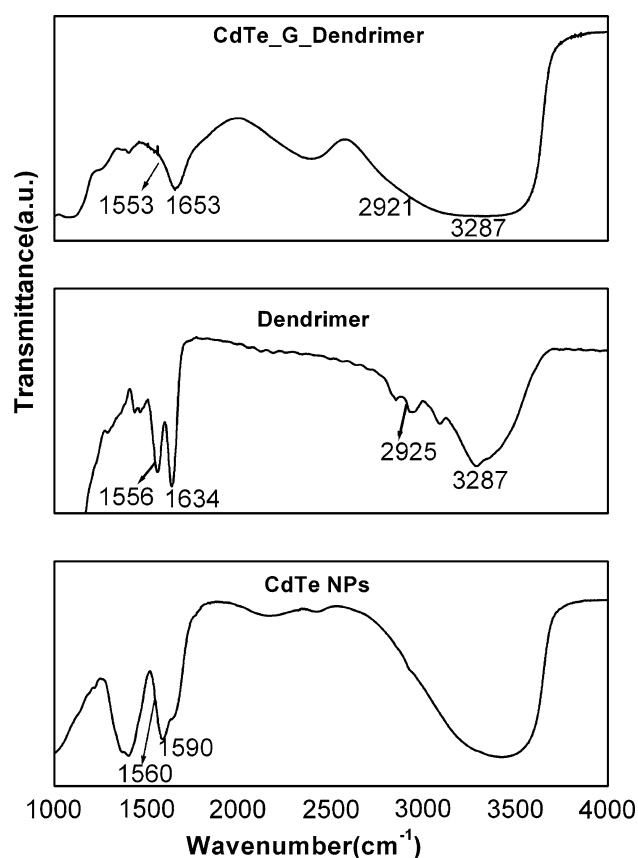
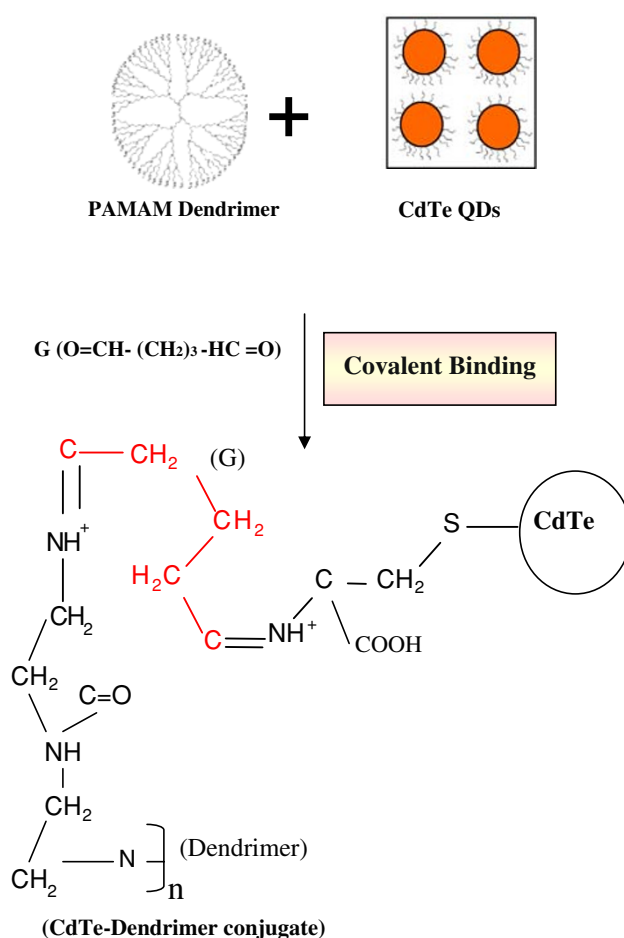


Fig. 2 FTIR spectra of CdTe, dendrimer and CdTe-dendrimer conjugate

covalently attached to the dendrimer through the Schiff base linkages formed between carbonyl groups of glutaraldehyde with surface amino (NH_2) groups of dendrimer as well as amino groups of the cysteine-capped CdTe QDs (Scheme 1). It is also observed that the band at $3,278\text{ cm}^{-1}$ corresponding to the stretching mode of surface amine group (NH_2) of dendrimer is present in the CdTe–Dendrimer conjugate. This suggests that a fraction of surface amine groups of dendrimer is used for coupling with QDs, and the rest remained unaffected during conjugation. Further, amide I and amides II bands at $1,634$ and $1,556\text{ cm}^{-1}$ are also invariant after binding of CdTe with dendrimer molecules. This observation confirms that this method of conjugation has enabled to produce covalently bonded, stable, highly fluorescent CdTe–dendrimer conjugate, keeping surface functionality of dendrimer available for biological applications, like sensing, labelling, drug delivery, etc.

Figure 3 illustrates a typical TEM image of CdTe–Dendrimer conjugate. The size of the particles was about 3.6 nm . This shows a pattern of association of few particles, which is possibly due to conjugation of QDs with dendrimers which is not observed in case of CdTe QDs alone (Inset of Fig. 3). Some particles agglomerated also due to dipolar interaction between dendrimers in aqueous medium.



Scheme 1 The synthetic route used to prepare the QDs-dendrimer conjugate

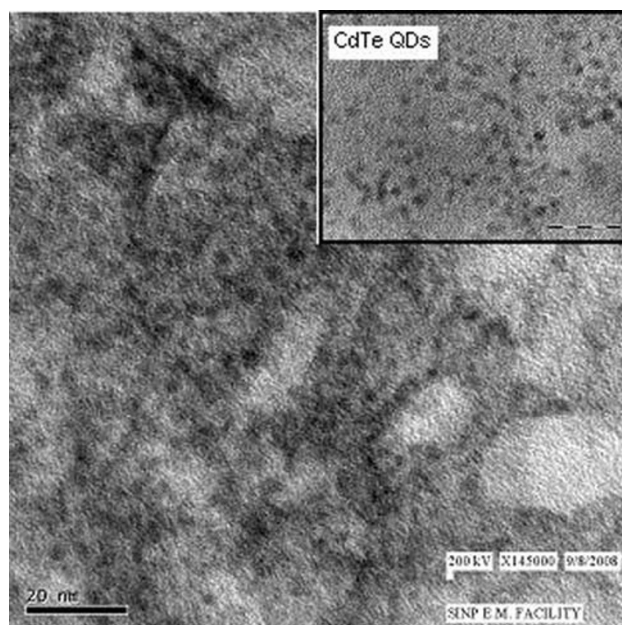


Fig. 3 TEM image of CdTe-dendrimer conjugate. Inset a typical TEM image of Cystein capped CdTe QDs

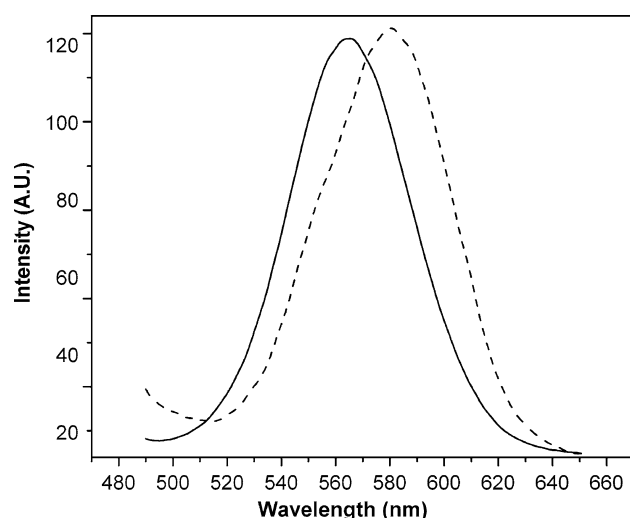


Fig. 4 The normalized luminescence spectra of CdTe QDs before (dash dot line) and after conjugation with dendrimer (solid line)

The luminescence spectrum of CdTe–Dendrimer conjugate showed a characteristic band-edge emission peak of CdTe QDs (centered at 580 nm), a 15-nm red shift from the pure QDs whose peak intensity was maximum at 565 nm (Fig. 4). The growth of NPs due to the reaction with linker molecule (G) may be responsible for spectral shift. This demonstrates that the frequency of the excitonic emission in QD–Dendrimer conjugate is lower than that of pure quantum dots. The quantum yield of QDs remained unaltered on conjugation with dendrimer. However, in comparison with organic dyes such as rhodamine [24], the QD–dendrimer system displays an emission spectrum that is nearly symmetric and of much narrower peak width, which is ideal for medical imaging diagnosis.

Conclusions

In summary, binding constants (K_{sv} and K_b) and binding capacity (n) of dendrimer to CdTe QDs have been determined fluorimetrically on the basis of noncovalent interaction. Further, dendrimer molecules are attached to the QDs through covalent binding using Glutaraldehyde. The successful assembly of QDs and dendrimer with desired functionality has significant implications in material research and demands most extensive inquiries into the luminescent electronic and chemical properties of these unique building blocks as they are incorporated into new and functional nanostructured materials. This approach may also be applicable for conjugation with other semiconductor nanoparticles. The combination of the spectroscopic characteristics of the nanocrystal with biomolecular function of dendrimer molecule can potentially make high

impact on current biomedical technologies and possibly in nanoelectronics, microphotonics and related fields.

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