PRAMANA
— journal of
physics

Vol. 75, No. 6
December 2010
pp. 1215–1220

A confocal laser scanning microscopic study on thermoresponsive binary microgel dispersions incorporated with CdTe quantum dots

J BRIJITTA[1], B V R TATA[1,*], K SARAVANAN[1], B K PANIGRAHI[1] and T KALIYAPPAN[2]

[1]Materials Science Group, Indira Gandhi Centre for Atomic Research, Kalpakkam 603 102, India

[2]Department of Chemistry, Pondicherry Engineering College, Pondicherry 605 014, India

*Corresponding author. E-mail: tata@igcar.gov.in

Abstract. Monodisperse poly(*N*-isopropylacrylamide) (PNIPAM) particles loaded with cadmium telluride (CdTe) quantum dots (QDs) of two different sizes (4.7 nm and 5.6 nm) were synthesized in aqueous medium by bonding the capping agent on the quantum dots to the amide groups of PNIPAM and incubating the samples at 45°C. A huge increase in the photoluminescence (PL) intensity (green and red regions) is observed for the PNIPAM–CdTe QDs composites compared to the parent CdTe QDs. We report here for the first time the imaging of binary dispersion of green and red luminescent PNIPAM–CdTe QDs composites using a fluorescence confocal laser scanning microscope. These composites have potential applications both in material science and biology.

Keywords. Confocal laser scanning microscope; poly(*N*-isopropylacrylamide); microgel dispersion; cadmium telluride quantum dots; photoluminescence.

PACS Nos 78.55.-m; 78.67.Hc; 81.07.Ta

1. Introduction

Fluorescent microspheres have attracted widespread interest because of their application as markers for biological detection. To achieve fluorescence, monodisperse microspheres are embedded with organic dyes. The fluorescent properties of such microspheres are not long-lasting as these dyes get bleached off when exposed to light. Semiconductor quantum dots (QDs) serve as an alternative to the common dyes routinely used for biological detection. QDs have size tunable emission colour, narrow emission profile and are free of photobleaching. Hence, the incorporation of QDs into the microspheres provide new hybrid materials with potential applications in biological labelling and for investigating the real space structure of binary colloidal alloys and other soft matter systems. Hydrogel microspheres are widely used for drug delivery, because of their aqueous inner environment and biocompatibility. Thus, QD-incorporated hydrogels are promising candidates for biomarking

and clinical detection. One such potential hydrogel is poly(N-isopropylacrylamide) (PNIPAM), which can be synthesized in macro and nano/microforms. PNIPAM undergoes a temperature (T) induced volume phase transition (VPT) at 34°C, below which the PNIPAM particles are highly water swollen, whereas above VPT, the particles collapse and de-swells. These gel particles are also sensitive to the environmental pH. Taking into account the T- and pH-sensitive behaviour of the PNIPAM particles, several ways of loading the QDs into them have been developed in recent years. To get uniform luminescence, the QDs are to be directly incorporated into the gel particles. Li *et al* [1] generated fluorescent microspheres by confining CdTe QDs into poly(N-isopropylacrylamide)–acrylic acid (PNIPAM–AAc) by varying the environmental pH. They observed the self-assembly of CdTe/PNIPAM–AAc into dendritic and fractal structures. But, the CdTe/PNIPAM–AAc aggregated to form a porous film, resulting in the phase separation of the CdTe and PNIPAM–AAc particles separately. Gong *et al* [2] fabricated CdTe-incorporated PNIPAM particles by hydrogen bonding between the capping agent and the PNIPAM network by incubating the CdTe–PNIPAM mixture. But, after incubation, they observed that the PNIPAM particles got deformed and the VPT of the PNIPAM particles got altered. For application in biolabelling and in materials science the monodispersity of the particles and inherent properties of the polymer should be retained. To achieve this we have synthesized aqueous suspension of monodisperse PNIPAM microgel particles and water soluble green and red luminescent CdTe quantum dots. The CdTe QDs are incorporated into PNIPAM microgel particles via hydrogen bonding by incubating the composite suspension at 45°C for 48 h with an interval of 2 h. This resulted in the successful bonding the CdTe QDs in PNIPAM particles without deforming the particle shape and altering the polymer characteristics, viz., VPT.

2. Experimental methods

2.1 *Synthesis of CdTe quantum dots*

We have used a mixed approach to synthesize CdTe QDs by clubbing synthesis procedures of Gong *et al* and Li *et al*. Conventional methods use H_2Te gas as the source of Te. But, H_2Te is highly toxic and has corrosive nature. To avoid this we have used sodium hydrogen telluride (NaHTe) which is produced by reducing tellurium powder (Te) with sodium borohydride ($NaBH_4$) (1 : 2 molar ratio) by keeping in an ice bath. The reduction proceeds for 8 h resulting in NaHTe (supernatant) and crystals of sodium tetraborate [1]. In a three-neck flask, 3.34 mM of cadmium perchlorate hexahydrate ($Cd(ClO_4)_2{\cdot}6H_2O$), 6.41 mM of thioglycerol (TGOL), and 1.60 mM of thioglycolic acid (TGA) are dissolved in 500 ml of Milli-Q water filtered through a 0.1 μm filter under stirring. The pH of the solution is adjusted to 11.2 by drop-wise addition of 1 M NaOH and de-aerated with argon gas for 1 h [2]. To this solution 2 ml of NaHTe solution is added maintaining the argon atmosphere. After 20 min the solution is refluxed at 100°C in an air condenser. CdTe QDs are generated after refluxing the solution for sufficient time. The size of the QDs is controlled by the reflux time. Half of the sample is collected after 1 h of refluxing when green luminescence is observed in the flask on excitation using an

8 W UV light source of 380 nm wavelength. The remaining half is found to show red luminescence after 48 h of refluxing.

2.2 *Synthesis of PNIPAM microgel suspension*

Aqueous suspension of PNIPAM microgel particles is synthesized by free radical precipitation polymerization [3,4]. In our earlier work we have synthesized PNIPAM particles of 273 and 520 nm by varying the surfactant concentration. In this work we have synthesized larger size PNIPAM particles by increasing the initiator concentration and using no surfactant during the synthesis. 139 mM of *N*-isopropylacrylamide (purchased from Acros, Belgium), 3.93 mM of *N*,*N*′-methylene bisacrylamide (purchased from Fluka, Germany) is dissolved in 250 ml of argon purged water. The reaction mixture is kept at 70°C for 1 h and then 5.97 mM of potassium persulphate (KPS) is added with vigorous stirring. KPS was purchased from Rankem, India. The polymerization is carried out at 70°C for 6 h under a stream of argon. Synthesized microgel suspensions are purified and concentrated by ultrafiltration technique. The molecular weight cut-off for the cellulose filtration membrane is 10,000 g/mol. The purified suspensions are kept in contact with a mixed bed of ion exchange resins (Ag501–X8, Bio-Rad Laboratories, Hercules, CA) to remove ionic impurities.

2.3 *Incorporating CdTe in PNIPAM microgel*

Green and red luminescent PNIPAM–CdTe QDs composites are prepared at room temperature by mixing 1 ml of PNIPAM microgel particles suspension with 4 ml of CdTe QDs at pH 7 with stirring. The PNIPAM–CdTe QDs composite mixture is incubated at 45°C for 48 h and then centrifuged at 15,000 rpm for 30 min. The unloaded CdTe QDs (supernatant) is decanted, followed by redispersing the centrifugate in water. After repeated washings and centrifugation, the supernatant do not show any luminescence and luminescence is observed only in the centrifugate. This ensures the loading of the QDs into PNIPAM particles. The thiol groups (capping) in QDs are responsible for the hydrogen bond formation between the amide groups of PNIPAM and QDs.

2.4 *Characterization techniques*

Particle size measurements of CdTe QDs and PNIPAM microgels and temperature dependence of PNIPAM microgel particle size are performed using dynamic light scattering (DLS) set-up (Malvern, UK, 4700 model) consisting of a goniometer and multi-tau photon correlator and a diode pumped solid state laser (Elforlight Ltd, UK) operating at a wavelength of 532 nm. Room temperature photoluminescence (PL) measurements are carried out using the 325 nm line from a He–Cd laser as the excitation source with backscattering geometry. The laser beam power on the sample is 25 mW and is maintained for all the samples throughout the

measurements. The backscattered light from the sample is dispersed by a triple grating spectrometer, Jobin Yvon-Horiba T64000 system. Gratings with the grating density of 1800 grooves/mm are used to disperse the scattered signals. The liquid nitrogen cooled CCD is used to record the spectrum. Confocal fluorescence imaging of binary suspension of PNIPAM–CdTe QDs composites are carried out using a Leica (Germany) TCS-SP2-RS confocal microscope having a scan speed of 7.4 frames/s. The PNIPAM–CdTe QDs composites are excited using a 488 nm Ar ion laser. Two channel fluorescence windows are used for imaging the green and red luminescence separately.

3. Results and discussion

The diameter and size polydispersity of the CdTe QDs in aqueous medium determined from DLS are found to be 4.7 ± 0.4 and 5.6 ± 0.3 nm respectively. The hydrodynamic diameter of the PNIPAM microgel particles at 25°C is 730 ± 5 nm. After incubating the PNIPAM particles with CdTe QDs, an increase in the PNIPAM particle size is observed. For the PNIPAM particles loaded with 4.7 and 5.6 nm CdTe QDs, the particle sizes at 25°C are 825 and 781 nm respectively. Figure 1a shows the temperature dependence of particle size for the unloaded and QDs-loaded PNIPAM particles. In all the cases VPT remained the same at ∼33°C. This indicates that the incorporation of CdTe QDs in PNIPAM particles has not altered the polymer characteristics, viz., VPT.

Figure 1a shows the PL spectra for the parent CdTe QDs and that for the PNIPAM–CdTe QDs composite particles. The PL emission for the 4.7 and 5.6 nm luminescent CdTe QDs are at 529 (green) and 591 nm (red) respectively. Hence the particles are hereafter referred to as green and red luminescent respectively. Interestingly, we observed a huge increase in the PL intensity for the PNIPAM microgel particles incorporated with CdTe QDs along with a red-shift of 7 nm and 2 nm for the green and red luminescent particles. A five- and three-fold increase in intensity is observed for the green and red luminescent PNIPAM particles compared to that of the parent CdTe QDs. The incorporation of QDs in PNIPAM particles enhances the PL emission because of the combined effect of the increased concentration of QDs in individual PNIPAM particles and the surface passivation of the CdTe QDs by the PNIPAM particles. Surface passivation diminishes the contribution of the non-radiative channel and the probability of radiative electron–hole recombination increases.

To see whether the CdTe QDs are uniformly incorporated in the PNIPAM particles we have recorded the confocal laser scanning microscope (CLSM) images. Though we could image green luminescent particles we were unable to image red luminescent particles. It can be clearly seen from figure 1b that the PL intensity of the red particles is almost one third of the PL intensity of green particles. Hence we increased the amount of red luminescent CdTe QDs in PNIPAM particles followed by incubation. With this we could record the red particles with the same settings used for imaging green particles. A 1 : 1 mixture of PNIPAM–CdTe green and PNIPAM–CdTe red QDs suspension is used for recording the images. Figure 2 shows the true colour confocal fluorescence images captured using CLSM

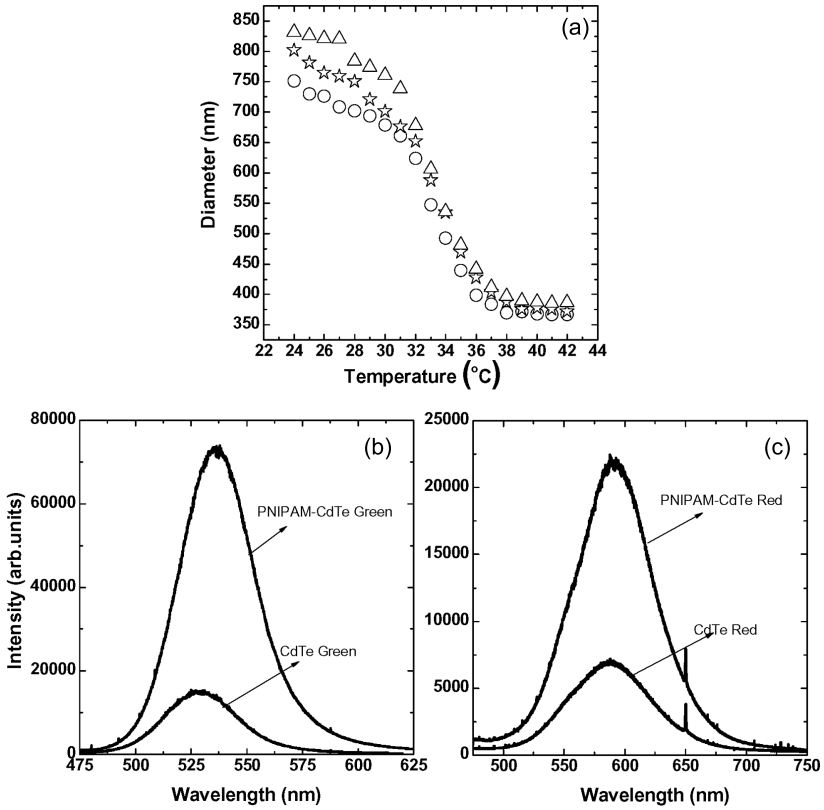

Figure 1. (**a**) Diameter as a function of temperature for PNIPAM particles (circles) and those loaded with 4.7 nm (stars) and 5.6 nm (triangles) QDs respectively, (**b**) PL spectra for the parent CdTe QDs and (**c**) for PNIPAM–CdTe QDs composites at 25°C.

for the binary suspension of green and red luminescent PNIPAM particles in two fluorescent channels. It can be seen from the figure with incubation of CdTe in PNIPAM, that it is possible to image clearly the green and red particles separately and also simultaneously in the mixture using a single-wavelength excitation of 488 nm. The images are recorded 30 μm deep inside the sample. For the first time we are successful in imaging binary suspension of PNIPAM–CdTe QDs composites.

4. Conclusions

We have synthesized aqueous suspension of monodisperse PNIPAM microgels and successfully incorporated CdTe quantum dots of 4.7 and 5.6 nm into them via, hydrogen bonding by utilizing the thiol capping in QDs and amide groups of PNIPAM. A drastic increase in the PL intensity is observed. This enhancement is attributed to the combined effect of increase in concentration of QDs in the PNIPAM particles

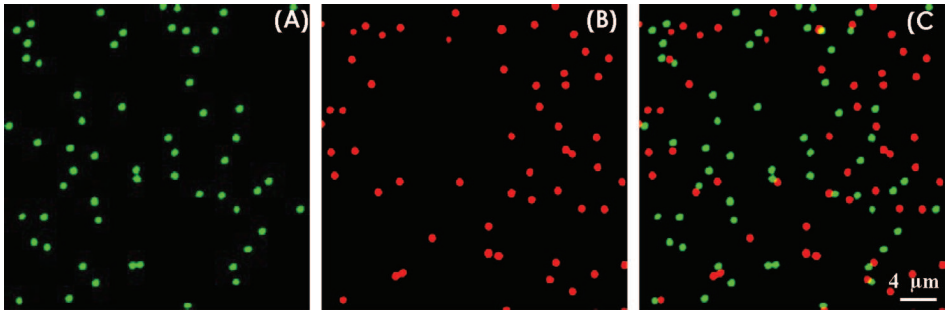

Figure 2. True colour confocal fluorescence micrograph of binary dispersion of PNIPAM–CdTe QDs composite captured using CLSM. (**A**) and (**B**) are the fluorescence images captured in two fluorescence channels and (**C**) is the overlay of A and B.

and enhancement of the electron–hole recombination because of surface passivation of QDs by PNIPAM. For the first time we are successful in imaging binary suspension of PNIPAM–CdTe QDs composites using confocal laser scanning microscope. These suspensions can be utilized for making binary colloidal alloys.

Acknowledgements

Authors acknowledges Dr Baldev Raj, Dr C S Sundar and Dr A K Arora, Materials Science Group, IGCAR for support and encouragement. First author acknowledges UGC-DAE-CSR, Kolkata for financial support.

References

[1] J Li, B Liu and J Li, *Langmuir* **22**, 528 (2006)
[2] Y Gong, M Gao, D Wang and H Mohwald, *Chem. Mater.* **17**, 2648 (2005)
[3] J Brijitta, B V R Tata and T Kaliyappan, *J. Nanosci. Nanotechnol.* **9**, 5323 (2009)
[4] J Brijitta, B V R Tata, R G Joshi and T Kaliyappan, *J. Chem. Phys.* **131**, 074904 (2009)