Incorporating Fluorescent CdTe Nanocrystals into a Hydrogel via Hydrogen Bonding: Toward Fluorescent Microspheres with Temperature-Responsive Properties

Yanjun Gong,[†] Mingyuan Gao,*[,†] Dayang Wang,*[,‡] and Helmuth Möhwald[‡]

Key Laboratory of Colloid, Interface Science and Chemical Thermodynamics, Institute of Chemistry, Chinese Academy of Sciences, Beijing 100080, China, and Max Planck Institute of Colloids and Interfaces, D-14424 Potsdam, Germany

Received November 25, 2004. Revised Manuscript Received March 1, 2005

Fluorescent microspheres were constructed by incorporating CdTe nanocrastals (NCs), costabilized by both thioglycerol and thioglycolic acid, into poly(*N*-isopropylacrylamide) (PNIPAM) microspheres through hydrogen bonding between the ligands capped on CdTe NCs and the PNIPAM chains. The loading capacity of PNIPAM was found to be dependent on the incubation temperature. Under optimized conditions the average spatial distance between CdTe NCs loaded into hydrogel spheres is still greater than that required for Förster energy transfer between CdTe NCs; therefore, very little change in the photoluminescence of CdTe NCs was observed after they were loaded into PNIPAM microphores. Multiplex optical encoding was realized by loading differently sized NCs into single gel spheres. The emission color of the resultant fluorescent spheres was mainly determined by the ratio of differently sized NCs incorporated. Although CdTe NCs in the hydrogel increased the cross-linking degree of the PNIPAM network, the volume of the resultant composite spheres remained tunable against temperature. Therefore, Förster energy transfer between differently sized NCs loaded can be initiated by increasing the environmental temperature, creating a temperature-responsive emission.

Introduction

Due to their unique photoluminescence behavior, involving size-tunable emission color, a narrow and symmetric emission profile, high emission stability against bleaching, and a broad excitation range, cadmium chalcogenide nanocrystals (NCs) provide the most attractive fluorescence labels in comparison with routine dyes or metal complexes.[1−9] Microbeads are the most commonly used carriers of fluorescence labels for fluorescence detection. The integration of fluorescent NCs in microbeads should therefore provide a new generation of fluorescence markers for biological assays; especially the incorporation of differently sized NCs in one bead is expected to realize multiplex optical encoding for high throughput and fast detection.[10,11]

There exist a number of methods to produce NC-loaded microbeads for creating fluorescent markers. In situ synthesis of fluorescent NCs from their precursors embedded in microspheres provides the simplest way for creating NC-loaded beads, but the NCs obtained in spheres are polycrystalline and their size distributions are rather broad, thus not allowing the luminescence behavior of the resulting beads to be controlled.[12,13] To define the luminescence property of the resultant microbeads, one should directly incorporate the preformed NCs into microbeads. Dispersing silane-capped CdTe NCs into sodium silicate aqueous solutions, followed by hydrolysis of sodium silicates, Kotov and co-workers have encapsulated CdTe NCs within submicrometer-sized silica beads.[14] Through an inverse microemulsion technique, Murase et al. obtained luminescent CdTe−silica microspheres. However, most NCs are unexpectedly coated on the surface of silica spheres.[15] Recently, Bawendi et al. also

* To whom correspondence should be addressed. E-mail: gaomy@iccas.ac.cn (M.G.); dayang.wang@mpikg-golm.mpg.de (D.W.) Fax: +86 10 8261 3214 (M.G.); +49 331 567 9202 (D.W.).

[†] Chinese Academy of Sciences.

[‡] Max Planck Institute of Colloids and Interfaces.

(1) Murray, C. B.; Norris, D. J.; Bawendi, M. G. *J. Am. Chem. Soc.* **1993**, *115*, 8706−8715.

(2) Chan, W. C. W.; Maxwell, D. J.; Gao, X. H.; Bailey, R. E.; Han, M. Y.; Nie, S. M. *Curr. Opin. Biotechnol.* **2002**, *13*, 40−46.

(3) Guo, W. Z.; Wang, Y. A.; Peng, X. G. *Chem. Mater.* **2003**, *15*, 3125−3133.

(4) (a) Gao, M. Y.; Kerstein, S.; Möhwald, H.; Rogach, A.; Kornoeski, A.; Eychmuller, A.; Weller, H. *J. Phys. Chem.* **1998**, *102*, 8360−8363. (b) Gaponik, N.; Talapin, D. V.; Rogach, A. L.; Hoppe, K.; Shevchenko, E. V.; Kornowski, A.; Eychmüller, A.; Weller, H. *J. Phys. Chem. B.* **2002**, *106*(29), 7177−7185.

(5) Pathak, S.; Choi, S.-K.; Arnheim, N.; Thompson, M. E. *J. Am. Chem. Soc.* **2001**, *123* (17), 4103−4104.

(6) Baümle, M.; Stamou, D.; Segura, J. M.; Hovius, R.; Vogel, H. *Langmuir* **2004**, *20*, 3828−3831.

(7) (a) Bruchez, M., Jr.; Moronne, M.; Gin, P.; Weiss, S.; Alivisatos, A. P. *Science* **1998**, *281*, 2013−2015. (b) Chan, W. C. W.; Nie, S. M. *Science* **1998**, *281*, 2016−2018.

(8) Gao, X. H.; Cui, Y. Y.; Levenson, R. M.; Chung, L. W. K.; Nie, S. M. *Nat. Biotechnol.* **2004**, *22*, 969−976.

(9) Larson, D. R.; Zipfel, W. R.; Williams, R. M.; Clark, S. W.; Bruchez, M. P.; Wise, F. W.; Webb, W. W. *Science* **2003**, *300*, 1434−1436.

(10) Han, M. X.; Gao, J. Z.; Su, S.; Nie, S. M. *Nat. Biotechnol.* **2001**, *19*, 631−635.

(11) Gao, X. H.; Nie, S. M. *Anal. Chem.* **2004**, *76*, 2406−2410.

(12) (a) Chang, S. Y.; Liu, L.; Sanford, A. A. *J. Am. Chem. Soc.* **1994**, *116*, 6739−6744. (b) Moffitt, M.; Vali, H.; Eisenberg, A. *Chem. Mater.* **1998**, *10*, 1021−1028.

(13) Wang, J.; Montville, D.; Gonsalves, K. E. *J. Appl. Polym. Sci.* **1999**, *72*, 1851−1868.

(14) Rogach, A. L.; Nagesha, D.; Ostrander, J. W.; Giersig, M.; Kotov, N. A. *Chem. Mater.* **2000**, *12*, 2676−2685.

(15) Selvan, S. T.; Li, C. L.; Ando M.; Murase, N. *Chem. Lett.* **2004**, *33* (4), 434−436.

10.1021/cm047932c CCC: $30.25
Published on Web 04/23/2005

reported a robust procedure for incorporating CdSe/ZnS core/shell NCs into a silica or titania shell grown on preformed silica microspheres.[16] On the basis of electrostatic attraction, Chen and co-workers successfully obtained CdSe@ZnS NC-loaded glyconanospheres formed by the negatively charged CdSe@ZnS NCs, negatively charged CM-dextran, and positively charged polylysine.[17] As such processes require the modification of NC surfaces, the emission efficiency and the colloidal stability of NCs are usually reduced. Furthermore, to avoid the aggregation of NCs and define the NC amount as well as the ratio among different NCs within spheres might be difficult.

On the basis of the swelling behavior of the polymer beads, the preformed organic- or water-soluble NCs have recently been successfully embedded within the preformed polymer beads with little changed luminescence and defined NC loading amounts and ratios between differently sized NCs. By mixing polystyrene microbeads and organic-soluble NCs in their common solvents, Nie and co-workers have successfully incorporated differently sized CdSe/ZnS core/shell NCs into polystyrene microbeads, creating multiplex optically encoded microbeads.[10,11] The emission intensity and color can be finely tuned by the loading amount and the ratio of differently sized NCs within the beads. Following a similar method, Nabiev obtained highly fluorescent polymeric beads incorporated with CdSe/ZnS NCs.[18] On the basis of the multivalent hydrophobic interaction between the ligands capped on NCs and the templating organic molecules embedded within the inner wall of mesoporous silica microspheres, Nie et al. also succeeded in incorporating NCs into silica beads.[19]

Due to their aqueous inner environment, biological compatibility, and feasibility for conjugating with biological molecules, hydrogel microspheres have been widely employed as delivery vehicles for drugs, proteins, and genes.[20−23] The incorporation of water-soluble NCs into hydrogel spheres should therefore provide promising fluorescent markers for biological and clinical detection. Recently, by using the pH-sensitive swelling behavior of *N*-isopropylacrylamide and 4-vinylpyridine copolymer spheres, water-soluble CdTe NCs have been absorbed in their swollen state at lower pH and trapped in their collapsed networks at higher pH.[24] The loaded NCs can be maintained in hydrogel spheres in a broad pH range. Their pH-triggered release profiles indicate that the entrapment of NCs in the hydrogel spheres is dominated by the physical entanglement of the collapsed network and electrostatic interactions between the loaded NCs and the gel network. By incorporating differently sized NCs in one hydrogel sphere, we have successfully realized multiplex emission encoding.[24]

It is known that a poly(*N*-isopropylacrylamide) (PNIPAM) hydrogel will undergo a volume phase transition across the low critical solution temperature (LCST). Below the LCST, it is hydrophilic and swells in water, while, above the LCST, it becomes hydrophobic and expels water, collapsing into a smaller volume. Therefore, apart from the pH-sensitive swelling behavior, the inherent temperature-sensitive swelling properties of PNIPAM may offer another measure for uptaking the CdTe NCs at an environmental temperature higher than the LCST. However, we failed in firmly trapping the CdTe NCs capped with thioglycolic acid in the hydrogel spheres. It was found that the NCs incorporated were released again from the swollen gel network once the environmental temperature was brought below the LCST. To solve this problem, herein we prepared CdTe NCs, capped with both thioglycerol and thioglycolic acid; utilizing hydrogen bonding between thioglycerol on the CdTe NCs and amide groups on the PNIPAM chains, we successfully constructed CdTe NC-loaded PNIPAM spheres stable under ambient conditions.

Experimental Section

Materials. Thioglycerol (TGOL; 99%, Fluka), thioglycolic acid (TGA; 97+%, Aldrich), *N*-isopropylacrylamide (NIPAM; 99%, Acros), $K_2S_2O_8$ (Aldrich), *N*,*N*′-methylenebis(acrylamide) (MBA; Aldrich), Al_2Te_3 (99.5%, Aldrich), and $Cd(ClO_4)_2{\cdot}6H_2O$ (Aldrich) were used without further purification.

Synthesis of CdTe NCs Capped with TGOL and TGA. CdTe NCs, capped with TGOL and TGA, were produced on the basis of our previous study.[4a] Typically, in a three-necked flask, 1.40 g of $Cd(ClO4)_2{\cdot}6H_2O$ (3.34 mmol), 0.56 mL of TGOL (6.41 mmol), and 0.11 mL of TGA (1.60 mmol) were dissolved in 180 mL of water under stirring. The pH of the mixture solutions was adjusted to 11.2 by 1 M NaOH. After 30 min of deaeration by using N_2, H_2Te gas (*note: H_2Te gas is highly flammable and toxic by inhalation!*), generated from the reaction of 0.39 g of Al_2Te_3 (0.90 mmol) with 30 mL of 0.5 M H_2SO_4 under N_2, was passed through the mixture solution along with a slow N_2 flow for 20 min. After reflux for a certain period, CdTe NCs were generated in the solution. The particle size was controlled by the reflux time. The sizes of CdTe NCs used in the current study are estimated to be 2.5, 3.0, and 3.5 nm.

Synthesis of PNIPAM Microspheres. PNIPAM hydrogel spheres were prepared by surfactant-free radical polymerization.[25] A 1.50 g sample of NIPAM was dissolved in 150 mL of water at room temperature and the solution obtained was purged with N_2 under stirring for 30 min. A 0.068 g sample of $K_2S_2O_8$ was dissolved in 30 mL of water and the resultant solution was poured into the O_2-free NIPAM solutions. The polymerization was performed for 4 h at 68−70 °C under N_2. After the solution was cooled to room temperature, hydrogel spheres were obtained according to the self-cross-linking effect of PNIPAM chains and purified by three repetitions of centrifugation at 18000 rpm and redispersion in water. To modify the cross-linking degree of PNIPAM spheres, we added different amounts of MBA, 0.012,

(16) Chan, Y.; Zimmer, J. P.; Stroh, M.; Steckel, J. S.; Jain, R. K.; Bawendi, M. G. *Adv. Mater.* **2004**, *16*, 2092−2097.
(17) Chen, Y. F.; Ji, T. H.; Rosenzweig, Z. *Nano Lett.* **2003**, *3*, 581−584.
(18) Stsiapura, V.; Sukhanova, A.; Artemyev, M.; Pluot, M.; Cohen, J. H. M.; Baranov, A. V.; Oleinikov, V.; Nabiev, I. *Anal. Biochem.* **2004**, *334*, 257−265.
(19) Gao, X. H.; Nie, S. M. *J. Phys. Chem. B* **2003**, *107*, 11575−11578.
(20) Huang, G.; Gao, J.; Hu, Z. B.; John, J. V. St.; Ponder, B. C.; Moro, D. *J. Controlled Release* **2004**, *94*, 303−311.
(21) (a) Ding, Z. L.; Chen, G. H.; Hoffman, A. S. *Bioconjugate Chem.* **1996**, *7*, 121−125. (b) Chen, G.; Hoffman, A. S. *Bioconjugate Chem.* **1993**, *4*, 509−514.
(22) Oupicky, D.; Reschel, T.; Konak, C.; Oupicka, L. *Macromolecules* **2003**, *36*(18), 6863−6872.
(23) Dube D.; Francis M.; Leroux, J. C.; Francüoise, M. W. *Bioconjugate Chem.* **2002**, *13*, 685−692.
(24) Kuang, M.; Wang, D. Y.; Bao, H. B.; Gao, M. Y.; Möhwald, H.; Jiang, M. *Adv. Mater.* **2005**, *17*(3), 267−270.
(25) (a) Gao, J.; Frisken, B. J. *Langmuir* **2003**, *19*, 5217−5222. (b) Gao, J.; Frisken, B. J. *Langmuir* **2003**, *19*, 5212−5216.

0.0225, 0.045, and 0.15 g, into the monomer solutions, corresponding to weight percentages of MBA of 0.8, 1.5, 3, and 10 wt %, respectively.

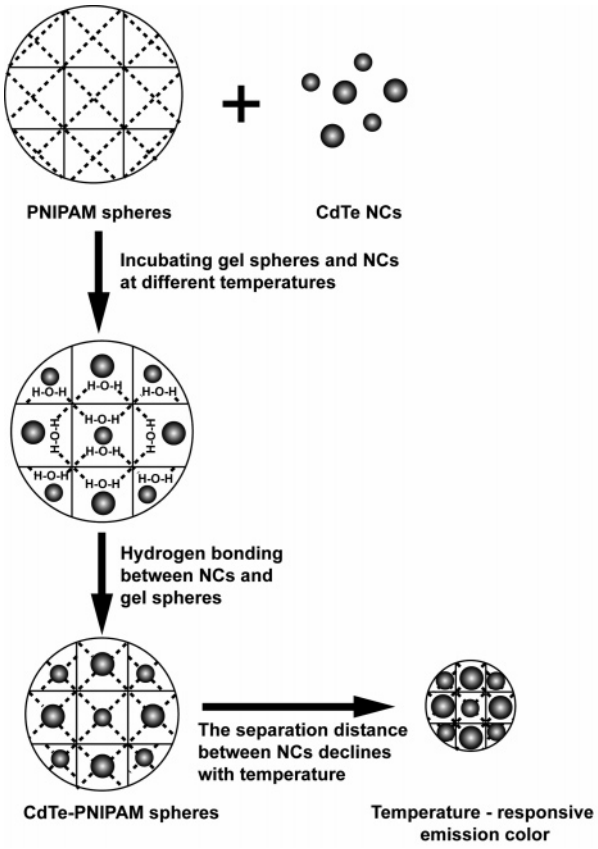
Encapsulation of NCs in PNIPAM. At room temperature, a 10 mL dispersion of PNIPAM spheres (the solid content is about 0.98 wt %) was mixed with 3 mL of an aqueous solution of CdTe NCs at pH 7 under stirring. After incubation at different temperatures, 25, 28, 30, 33, 35, 38, 40, and 45 °C, for 1−2 h, the mixed solutions were centrifuged at 18000 rpm for 20 min at 25 °C. By decanting the supernatant, followed by repeating the cycle of redispersion with water and centrifugation, the unloaded NCs were removed and PNIPAM spheres loaded with CdTe NCs were obtained, denoted as CdTe−PNIPAM.

Characterization. UV−vis absorption spectra were recorded at room temperature with a Cary 50 UV−vis spectrophotometer. Fluorescence spectra were obtained with a Cary Eclipse fluorescence spectrophotometer equipped with a single-cell Peltier accessory to control the sample temperature. True-color fluorescence images were obtained with an inverted Olympus microscope (IX-70). Broad-band excitation in the near-UV range (330−385 nm) was provided by a 100 W mercury lamp. An oil-immersion objective was used (magnification of 100×), and the total wide-field excitation power was ~5 mW. Transmission electron microscopy (TEM) images were obtained by a JEM-100CX II microscope operating at 100 kV. Hydrodynamic light scatting (DLS) measurements were performed on an ALV DLS/SLS-5022F spectrometer equipped with an ALV-5000 digital time correlator and a helium−neon laser ($\lambda_0 = 632.8$ nm).

Results and Discussion

For the synthesis of PNIPAM microspheres, as the polymerization temperature used is higher than the LCST of PNIPAM, the PNIPAM chains generated in the reaction solutions may be self-cross-linked to form gel spheres.[25,26] Scheme 1 depicts the procedure of loading CdTe NCs into PNIPAM spheres. According to previous studies[24] and current results, although TGA-capped CdTe NCs can be entrapped into the PNIPAM spheres at a temperature higher than their LCST, all loaded NCs were released out of the gel spheres again when the environmental temperature was brought below the LCST. This is because the pore size of the gel network is larger than the size of the NCs used at room temperature and there is no strong enough interaction between the NCs and the gel network. Hence, no fluorescent spheres were obtained by directly loading TGA-capped CdTe NCs into PNIPAM. In contrast, when CdTe NCs capped by TGOL were incubated with PNIPAM spheres, after centrifugation, the NCs used were observed exclusively in the sediments of the gel spheres rather than in the supernatant; therefore, stable fluorescent spheres were created. By incubating the resultant fluorescent spheres with urea solutions, the emission of the CdTe-loaded NCs was observed only in the supernatants rather than in the gel sediments after centrifugation, suggesting that most NCs loaded were released out of the PNIPAM spheres. As urea is a well-known hydrogen bond breaker,[27] this suggests that there exists hydrogen bonding between the hydroxyl groups of TGOL capped on NCs and the amide groups from the PNIPAM gel networks, which plays a crucial role in trapping the TGOL-capped CdTe in the PNIPAM spheres.

Scheme 1. Schematic Illustration of the Procedures for Loading CdTe NCs into PNIPAM Spheres via Hydrogen Bonding between the NCs and the Gel Network[a]



[a] The dotted lines represent the hydrogen bonds between amide groups within the gel spheres, amide groups and water, and amide groups and hydroxyl groups of TGOL capped on the NCs. Incubating PNIPAM spheres and NCs at high temperature enables the entrapment of NCs (top), but the open-pore structure formed at low temperature also facilitates the release of trapped NCs (middle). Capping the NCs with a hydrogen-bonding agent (TGOL) fixes the loaded NCs inside the mesh (bottom left). At a sufficiently high concentration of differently sized NCs, energy transfer takes place, and due to its strong distance dependence, the emission spectrum becomes temperature-dependent (bottom, right).

In comparison with TGA-capped ones, however, TGOL-capped CdTe NCs present a much lower fluorescence quantum yield. Therefore, both TGA and TGOL were used to costabilize the CdTe NCs. The optimized feed ratio of TGOL/TGA was found to be 4:1 for achieving the best fluorescent spheres.

Similarly to our previous report,[24] we determined the amount of TGOL/TGA-capped CdTe NCs embedded in the PNIPAM by absorption spectroscopy. Figure 1a reveals that the loading amount of TGOL/TGA-capped CdTe NCs within the PNIPAM spheres increases as a function of the incubation temperature. The loading amount of NCs remains rather low, about 0.4×10^4 NCs (2.5 nm) per gel sphere, in an incubation temperature range of 25−30 °C. When the incubation temperature is higher than 30 °C, the loading amount of NCs starts to increase in a rather linear fashion with increasing incubation temperature. The maximal amount of CdTe NCs embedded in PNIPAM spheres is achieved when the incubation temperature reaches ~45 °C, corre-

(26) Mcphee, W.; Tam, K. C.; Pelton, R. *J. Colloid Interface Sci.* **1993**, *156*, 24−30.
(27) Iimain, F.; Tanaka, T.; Kokufuta, E. *Nature* **1991**, *349*, 400−401.

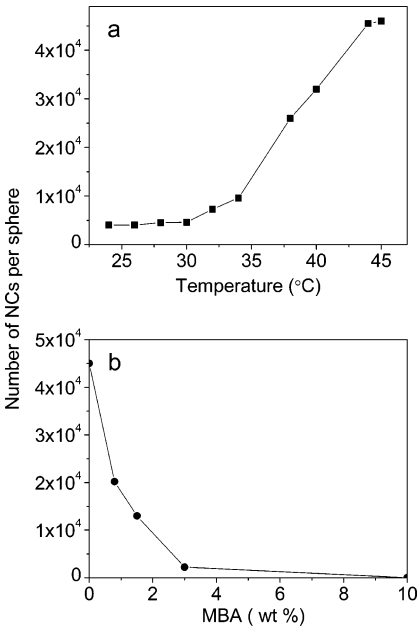


Figure 1. (a) Loading amount of 2.5 nm CdTe NCs per gel sphere versus the temperature for incubating NCs and PNIPAM spheres. (b) Loading amount of 2.5 nm CdTe NCs per gel sphere versus the feed concentration of MBA used in the preparation of PNIPAM spheres. The incorporation of NCs into PNIPAM spheres was conducted by incubating them at 45 °C.

sponding to 4.5×10^4 NCs (2.5 nm) per gel sphere. The maximal amount of 3.5 nm CdTe NCs loaded under similar conditions was 2.3×10^4 NCs per gel sphere.

Apart from the self-cross-linking ones, PNIPAM spheres synthesized in the presence of different amounts of cross-linking agent, MBA, were also used in the preparation of fluorescent spheres. As shown in Figure 1b, the loading amount of CdTe NCs drops dramatically against the concentration of MBA used in the preparation. When the feed concentration of MBA is higher than 3 wt %, it is hard to trap CdTe NCs into the PNIPAM spheres. This is likely due to the fact that the pore size of the PNIPAM becomes smaller than the size of CdTe NCs at a relatively high cross-linking degree. Hence, the PNIPAM spheres used further in the current work are the self-cross-linking ones.

Figure 2 reveals TEM images of the PNIPAM and CdTe−PNIPAM spheres formed at different incubation temperatures, where one may observe a decreasing size and an increasing contrast of the resultant CdTe−PNIPAM spheres with increasing incubation temperature. Since the electron density contrast in the TEM pictures of CdTe−PNIPAM spheres mainly arises from the inorganic NCs, this suggests that the amount of NCs loaded within PNIPAM spheres increases with incubation temperature.

Due to drying and especially collapsing of the gel network in the high-vacuum TEM chamber, it is difficult to determine the real size of the hydrogel spheres. DLS was therefore employed to determine the hydrodynamic diameters ($\langle D_h \rangle$) of the PNIPAM spheres and those loaded with CdTe NCs in solution for investigating their temperature-responsive swelling behavior. As shown in Figure 3a the PNIPAM spheres used in the present work have an LCST around 33 °C. Their $\langle D_h \rangle$ is about 870 nm at room temperature, declines with an increase of the environmental temperature, and reaches a plateau, 320 nm, when the temperature is above

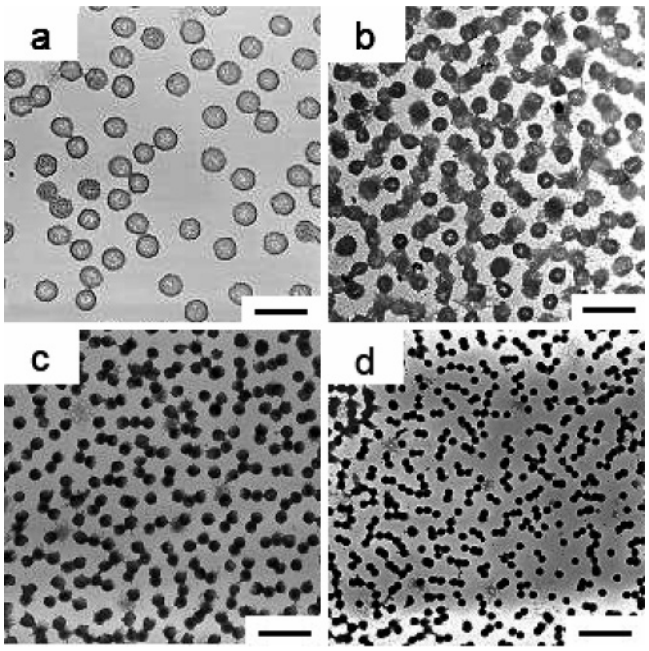


Figure 2. TEM images of blank PNIPAM spheres (a) and those loaded with 2.5 nm CdTe NCs, formed by incubating gel spheres and NCs at varying temperatures of 25 (b), 38 (c), and 45 (d) °C. The scale bar is 1.2 μm.

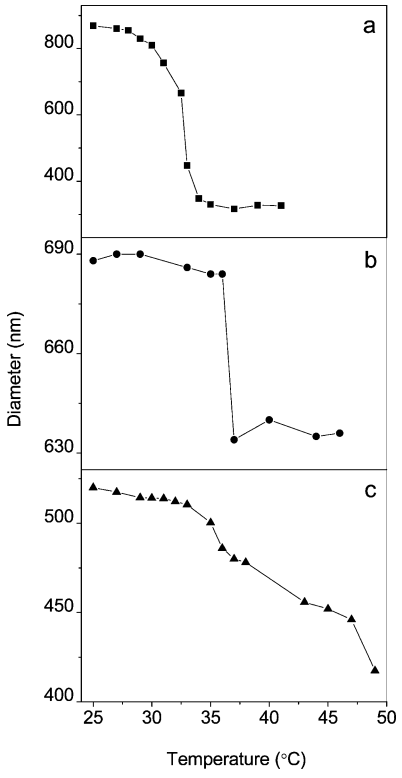


Figure 3. Profiles of temperature-dependent hydrodynamic diameters of PNIPAM spheres (a) and those loaded with 2.5 nm CdTe NCs formed by incubating NCs and gel spheres at 30 (b) and 45 (c) °C.

35 °C. In contrast, the CdTe-loaded PNIPAM spheres obtained at an incubation temperature of 30 °C present a higher LCST, around 37 °C. Their $\langle D_h \rangle$ turns out to be smaller at room temperature (∼700 nm) but bigger at a temperature above the LCST (∼630 nm) than that for the blank PNIPAM spheres. It was demonstrated that further increasing the incubation temperature blurs the temperature-induced transition in sphere size. As shown in Figure 3c,

the $\langle D_h \rangle$ of the CdTe-loaded PNIPAM spheres, formed at an incubation temperature of 45 °C, turns out to be much smaller, 520 nm at room temperature, and gradually declines as the environmental temperature increases. For hydrogel spheres, the increase in the LCST and decrease in $\langle D_h \rangle$ are usually associated with an increase in the cross-linking degree of the gel networks. Therefore, it can be deduced that the loaded CdTe NCs act as cross-linking agents since single CdTe nanocrystals can form multiple hydrogen bonds with the gel network. This is consistent with data derived from absorption spectra and TEM images. In the case of blank PNIPAM spheres, as suggested in the literature,[28,29] most amide groups within the gel spheres are strongly associated with water via hydrogen bonds below the LCST and dissociate with water above the LCST; incubation at high temperature may therefore favor the entrapment of CdTe NCs within the PNIPAM gel network through hydrogen bonding.

Parts a and b of Figure 4 show typical fluorescence micrographs of PNIPAM microspheres loaded with 2.5 and 3.5 nm CdTe NCs, obtained by incubating TGOL/TGA-capped NCs with the gel spheres at 45 °C. The emission spectra of these fluorescent spheres are rather similar to those of the initial CdTe NCs in solution. The fwhm (full width at half-maximum) of NC emission remains little changed (Figure 5a,b). It is therefore expected that the gel network may efficiently prevent the aggregation of the NCs embedded, consistent with previous reports.[13] This therefore provides the opportunity to incorporate differently sized CdTe NCs in one PNIPAM sphere for realizing the multiplex optical encoding. Figure 4c shows a typical fluorescence micrograph of CdTe−PNIPAM spheres obtained by incubating both 2.5 and 3.5 nm NCs and the gel spheres at 45 °C. Figure 5c reveals that the PNIPAM spheres doped with 2.5 and 3.5 nm NCs possess the PL characteristics of both of these two types of NCs. The emission of the 3.5 nm NCs remains nearly unchanged, while that of the 2.5 nm NCs exhibits a blue shift of about 10 nm in comparison with its PL in aqueous solution. This is not fully understood at this moment.

Combining the $\langle D_h \rangle$ of the CdTe−PNIPAM spheres determined by DLS and the loading amount of CdTe NCs within the gel spheres determined by absorption spectroscopy, the average separation distance between two neighboring CdTe NCs within PNIPAM spheres, prepared under different conditions mentioned above, was estimated to be 11−20 nm, which is larger than that required for Förster energy transfer between NCs, 4−9 nm.[30] In the case of CdTe−PNIPAM spheres obtained at an incubation temperature of 45 °C, the separation distance between the adjacent CdTe NCs is in the range of 11−13 nm at room temperature, depending on the size of the NCs, which is close to the Förster energy transfer distance. As shown in Figure 3c, the $\langle D_h \rangle$ of these CdTe−PNPAM spheres decreases with increasing environmental temperature. At 45 °C, their $\langle D_h \rangle$ is reduced by 13.5%; thus, the separation distance of neighboring NCs in the gel spheres falls into the range of 8−11 nm, which partly intersects the spatial distance range for Förster energy transfer. One may therefore expect the occurrence of energy transfer between CdTe NCs embedded in the PNIPAM spheres upon a further increase of the environmental temperature as depicted in Scheme 1 (bottom). Figure 6 shows temperature-dependent emission spectra of the PNIPAM spheres loaded with both 2.5 and 3.5 nm CdTe NCs (in a molar ratio of 5:1), formed by incubation at 45 °C. As the environmental temperature increases, obviously, the emission peaks of the NCs, either 2.5 or 3.5 nm in size, shift slightly to the red and the emission of 2.5 nm NCs gradually declines in intensity; meanwhile that of 3.5 nm NCs increases simultaneously. This suggests that the energy transfer between NCs, and particularly between small and large NCs, is enhanced as the environmental temperature increases. The inset in Figure 6 presents an eye-detectable variation in emission color of these composite gel spheres when the environmental temperature increases from 25 to 65 °C. One realizes that the energy transfer is increased with

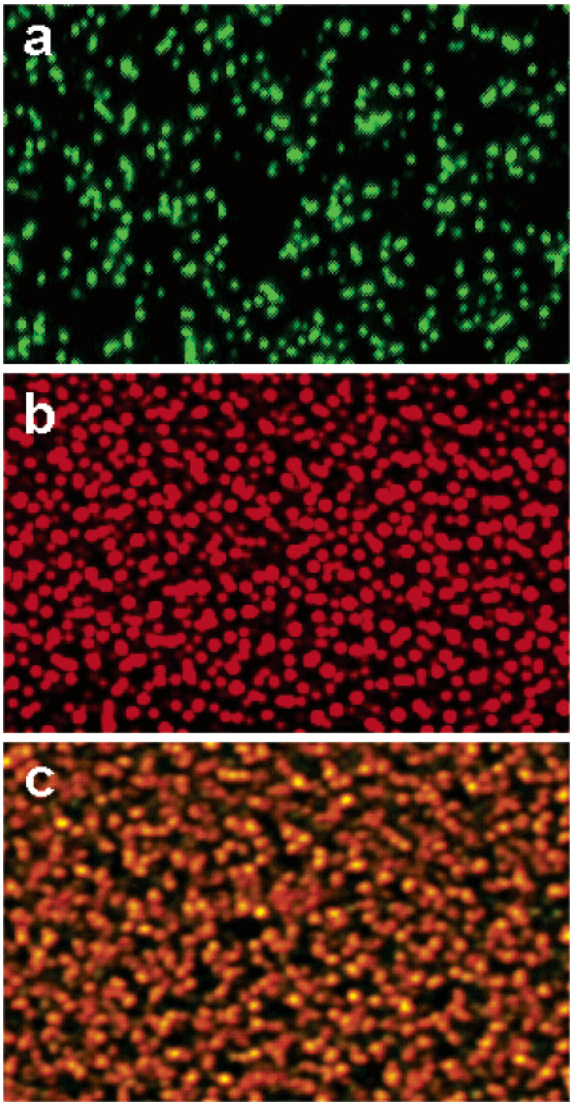


Figure 4. Fluorescence images of PNIPAM spheres loaded with 2.5 nm CdTe NCs (a), 3.5 nm CdTe NCs (b), and their mixture with a molar ratio of 5:1 of 2.5 nm NCs to 3.5 nm NCs (c). These spheres were prepared by incubating NCs and gel spheres at 45 °C. The fluorescence images were captured at room temperature.

(28) Cho, E. C.; Lee, J.; Cho, K. *Macromolecules* **2003**, *36*, 9929−9934.
(29) Woodward, N. C.; Chowdhry, B. Z.; Snowden, M. J.; Leharne, S. A.; Griffiths, P. C.; Winnington, A. L. *Langmuir* **2003**, *19*, 3202−3211.
(30) Mićić, O. I.; Jones, K. M.; Cahill, A.; Nozik A. J. *J. Phys. Chem. B* **1998**, *102*, 9791−9796.

Table 1. Fluorescence Peak Intensities of 2.5 nm (CdTe I) and 3.5 nm (CdTe II) CdTe NCs Incorporated into a PNIPAM Microgel versus Environmental Temperature[a,b]

	25 °C		35 °C		45 °C		65 °C	
	CdTe I	CdTe II	CdTe I	CdTe II	CdTe I	CdTe II	CdTe I	CdTe II
first heating	602	851	143	272	98	232	54	149
first cooling	428	750	107	272	81	219		
second heating	513	840	129	268	68	204	48	165
second cooling	350	681	90	272	72	206		

[a] Different from those shown in Figure 6, the data presented here are not normalized. [b] Note: the second heating procedure was started 24 h after the first round of experiments was completed.

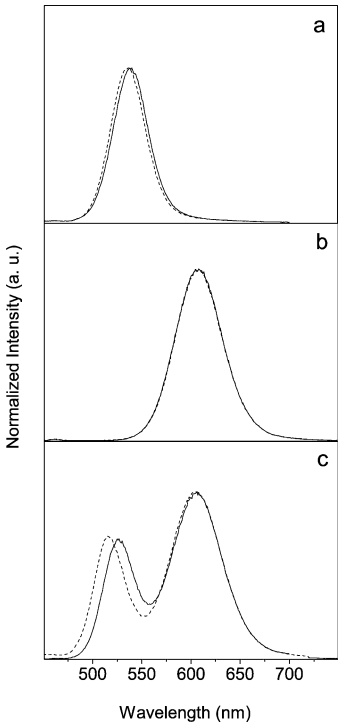


Figure 5. Fluorescence spectra (dashed line) of PNIPAM spheres loaded with 2.5 nm NCs (a), 3.5 nm NCs (b), and their mixture with a molar ratio of 5:1 (c) and those of the initial CdTe NCs in aqueous solutions (solid line). These spheres were prepared by incubating NCs and gel spheres at 45 °C. These emission spectra were recorded at room temperature.

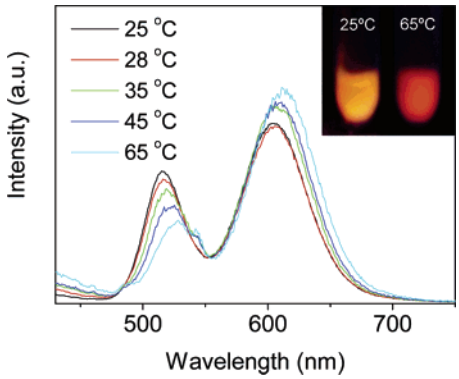


Figure 6. Fluorescence spectra of PNIPAM spheres loaded with both 2.5 and 3.5 nm CdTe NCs against environmental temperature. All spectra presented were normalized by taking the temperature effects into account since both emissions from 2.5 and 3.5 nm CdTe decrease with increasing temperature, but by a rather similar factor. The CdTe−PNIPAM spheres were obtained by incubating NCs and gel spheres at 45 °C. The molar ratio of 2.5 to 3.5 nm NCs is 5:1. The loading amount of NCs is around 4.0×10^4 NCs (2.5 nm + 3.5 nm) per gel sphere. The inset shows the fluorescent pictures of these spheres under UV irradiation at 25 and 65 °C, respectively.

temperature by about a factor of 2. This is probably not due to a change of the excited-state lifetime since this may be reduced with temperature, thus decreasing the transfer efficiency. Most probably it is caused by a reduction of the interparticle distance, and the fact that the change is so strong can be explained by the R^{-6} distance dependence. It is worth mentioning that this variation in emission spectra and color with temperature is reversible, suggesting that this variation arises from the energy transfer between differently sized NCs rather than the aggregation of NCs. More detailed results on the reversibility data on the temperature-dependent fluorescence of the PNIPAM spheres loaded with both 2.5 and 3.5 nm CdTe NCs are given in Table 1. Note that the temperature effects on the PL emissions of both 2.5 and 3.5 nm CdTe NCs were not subtracted.

Conclusion

We have fabricated fluorescent microspheres by incorporating preformed CdTe NCs, stabilized with a mixture of TGOL and TGA, into preformed PNIPAM spheres. The successful incorporation of CdTe NCs is achieved by hydrogen bonding between the NC capping agents and the gel network. The higher the temperature to incubate the NCs and the gel spheres, the higher the loading amount of NCs. Since the average separation distance of neighboring NCs is generally beyond the Förster energy transfer distance, the emission of the NCs embedded within the gel spheres remains nearly unchanged in comparison with that of their parent NCs in aqueous solution. Thus, the emission color of the resulting fluorescent spheres can easily be tuned by embedding differently sized NCs. Multiplex optical encoding has then been realized by loading differently sized NCs into one gel sphere. The DLS experiments indicate that the spatial distance between NCs embedded within the gel networks can be tuned by the environmental temperature. The fluorescent microspheres with temperature-responsive emission color are therefore obtained by manipulating the energy transfer between NCs and especially from small to large NCs. The current investigations should certainly pave a facile and efficient way for creating a new generation of fluorescent markers, in particular stimulus-responsive ones, which should hold immense promise in biological and clinical detection.

Acknowledgment. Funding was provided by an 863 project (Grant 2002AA302201) and NSFC projects (Grants 20225313, 90206024, and 90201006). Y.G. acknowledges the China Postdoctoral Science Foundation for financial support.

CM047932C