Fabrication of Multicolor-Encoded Microspheres by Tagging Semiconductor Nanocrystals to Hydrogel Spheres**

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Colloidal fluorescent spheres have attracted widespread interest because of their diverse applications, especially serving as markers for biological detection.[1] In order to generate fluorescent spheres, various organic dyes and metallic complexes have been embedded in monodisperse polymer and silica spheres.[2] Nevertheless, the unfavorable absorption and luminescent properties of these fluorescent molecules, such as narrow absorption band and broad emission profiles, restrict their applicability, particularly for multiplexed optical coding. Semiconductor nanocrystals (NCs) hold immense promise as alternatives to common dyes for fluorescence detection.[3] However, there is no reliable process to integrate nanocrystals with colloidal spheres. Based on electrostatic interactions, NC/polyelectrolyte multilayers have been layer-by-layer generated on colloidal spheres.[4] Due to the penetration of NCs through porous layers, however, the resulting NC-coated spheres have low luminescence efficiency and poor long-term stability.[4] There exist two major ways to incorporate NCs into colloidal spheres: one may either dope preformed NCs or prepare doped NCs in situ by doping the precursors in the precursor solutions of colloidal spheres.[5] The stability of both the precursor solution of colloidal spheres and the NCs limit the usage range of these two methods. The amount of doped NCs per sphere is hard to control. Meanwhile, using precursors to form NCs in situ inside colloidal spheres results in less control over the crystalline structure and size of the resulting NCs.

In this communication, we present an alternative way to incorporate NCs into colloidal spheres—utilizing hydrogel spheres to confine water-soluble NCs with different sizes with the help of their stimuli-responsive swelling properties. Our process is simple and versatile, leading to a uniform NC spatial distribution and control of the NC amount per sphere, and can be adapted to other systems. Stimuli-responsive hydrogel spheres are popular drug-delivery vehicles because they are biologically compatible and provide an aqueous environment.[6] The pore size of hydrogel spheres can be tuned by certain stimuli, such as pH, temperature, and ionic strength, allowing one to absorb and release drugs or proteins in a controlled fashion. In terms of size, NCs of a few nanometers are comparable to large molecular drugs or proteins. By swelling 1.2 μm polystyrene spheres in organic NC dispersions, Nie and co-workers have fabricated NC-tagged microspheres.[7] Theoretically, such NC-tagged spheres enable one to create a huge library for biological assays by varying the emission color and intensity combination. Herein, we employ *N*-isopropylacrylamide and 4-vinylpyridine copolymer (PNIPVP) spheres as colloidal carriers and confine water-soluble CdTe NCs of 2.5, 3, and 4 nm size within the collapsed gel network by varying the environmental pH. By absorbing different-sized NCs in one PNIPVP sphere, we also achieve multicolor-coded microspheres.

PNIPVP spheres were prepared by surfactant-free emulsion polymerization. A weight fraction of 4-vinylpyridine of 4 % and a crosslinking degree of 1 % were chosen. The poly(4-vinylpyridine) moieties render the hydrogel pH sensitive. The pH-dependent swelling behavior of PNIPVP spheres was investigated by dynamic light scattering (DLS). As shown in Figure 1a, the diameter of PNIPVP spheres reaches the maximum, 750 nm, at pH 3 and is reduced as the pH increases from 3 to 13. As suggested in a previous study,[8] when the pH of PNIPVP dispersions is lower than the apparent pK_a of 4-vinylpyridine, 5.39,[8] the pyridine groups become protonated, leading to internal charge repulsion between protonated pyridine groups, and thus causing an expansion of the PNIPVP spheres. Otherwise, the pyridine groups are less ionized and polymer–polymer interactions becomes dominant, leading to the collapse of PNIPVP spheres. The transmission electron microscopy (TEM) picture shows that PNIPVP spheres are monodisperse and 200 nm in diameter, much smaller than the diameter determined by DLS, due to drying (inset of Fig. 1a).

In this work we synthesized water-soluble CdTe NCs capped with thioglycolic acid, of 2.5, 3, and 4 nm in size,[9] and employed them as models to demonstrate our concept. Since PNIPVP spheres are pH sensitive the pore size of their gel network increases with the decrease of the pH of the environment due to protonation of the pyridine groups (Fig. 1a). When their pore size becomes comparable with or larger than that of the CdTe NCs used, PNIPVP spheres can uptake NCs. After the pH of the environment is increased, NCs are confined within the collapsed hydrogel network thanks to physical entrapment, as illustrated in Scheme 1.

In order to load 3 nm CdTe NCs we incubated them in PNIPVP dispersions for 5 min; the volume ratio of NC dispersion to PNIPVP dispersion was varied from 0.5 to 2. The diameter of the PNIPVP spheres was tuned by adding acetic acid. In our work, we found that PNIPVP spheres imbibe CdTe NCs only at around pH 3, at this time their pyridine

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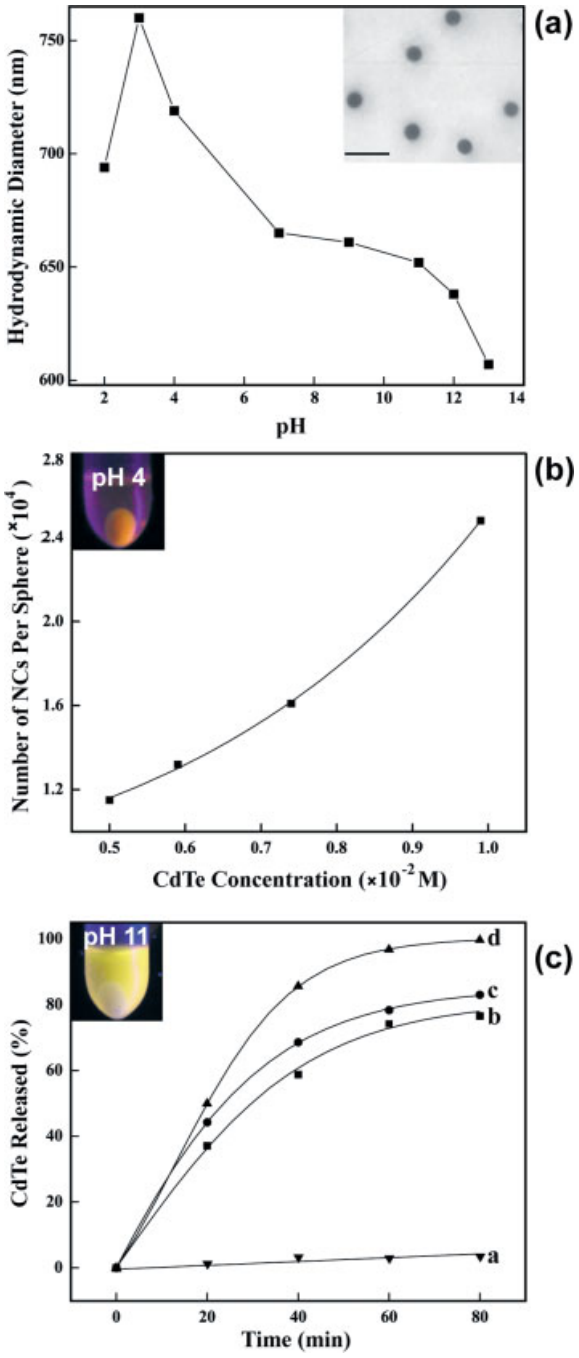
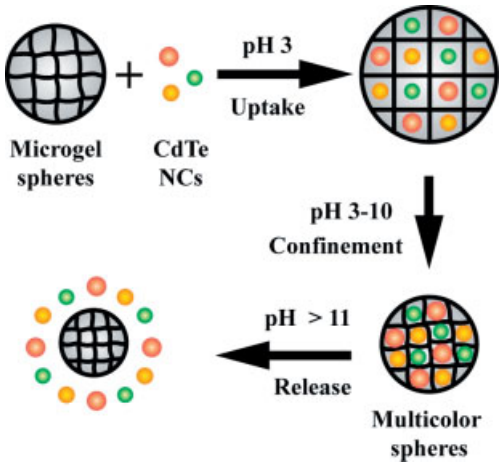


Figure 1. a) Plot of hydrodynamic diameter of PNIPVP spheres versus pH in water at 25 °C (Inset: TEM micrograph of PNIPVP spheres. The scale bar is 500 nm). b) Plot of the loading amount of 3 nm CdTe NCs per PNIPVP sphere versus the concentration of CdTe NCs added. (Inset: Fluorescence images of PNIPVP spheres loaded with 3 nm CdTe NCs at pH 4 after centrifugation at 2000 g for 10 min.) c) Plot of the amount of 3 nm CdTe NCs released from PNIPVP spheres versus time at pH 10 (a), pH 11 (b), pH 13 (c), and pH 13 with 0.05 M NaCl (d). (Inset: Fluorescence images of CdTe-PNIPVP spheres at pH 11 after centrifugation at 10 000 g for 1 min).

groups are fully ionized and their overall size is as large as 750 nm (Fig. 1a). The control experiment indicated that CdTe



Scheme 1. Schematic illustration of the loading of CdTe NCs in PNIPVP spheres and their controlled release by pH.

NCs are rather stable in an aqueous solution of acetic acid at pH 3. Upon decanting the supernatant containing excess CdTe NCs after centrifugation, PNIPVP spheres loaded with CdTe NCs (CdTe-PNIPVP) were redispersed in water of pH 7. After centrifugation, a yellow luminescence was observed exclusively in the sediment under irradiation by a UV lamp (inset in Fig. 1b), identifying the loading of NCs in the PNIPVP spheres. During this process, most of the NCs remain in the supernatant, which, however, can be reused. TEM pictures of CdTe-PNIPVP spheres are quite similar to those of PNIPVP spheres, in which one has difficulty identifying whether or not NCs agglomerate due to the poor electron contrast between NCs and gel matrices. We observed little change in the emission spectra of CdTe-PNIPVP spheres compared with those of CdTe NCs, suggesting that the hydrogel network protects CdTe NCs against aggregation. This emission behavior is consistent with that of hydrophobic NCs confined in microspheres observed by Nie et al.[7] The loading of CdTe NCs in PNIPVP spheres was analyzed by absorption spectroscopy. The loading amount of CdTe NCs, normalized by PNIPVP sphere numbers determined by single particle light scattering (SPLS),[10] is plotted in Figure 1b as a function of the concentration of CdTe NCs added. Upon increasing the CdTe NC concentration, one may increase the number of loaded NCs per gel sphere in a quite linear way, leading to control over the fluorescence intensity of CdTe-PNIPVP spheres. The volume faction of NCs in the gel spheres is about 0.1 %, which means that the average weight is hardly affected by loading. The average distance between CdTe NCs in a PNIPVP sphere is estimated in the range of 20–60 nm at pH 3–11, much larger than their Förster energy transfer radius of 5–8 nm.[7] This is consistent with the absence of a red-shift and narrowing of the emission spectra of CdTe-PNIPVP spheres, which are otherwise observed if an energy transfer to particles with a lower energy state occurs. The confocal fluorescence analysis of CdTe-PNIPVP spheres indicates that the population of the loaded CdTe NCs is not concentrated in the

outer zone of the sphere, the outer 25 % of the sphere's radius observed by Nie et al.,[7] but is quite uniform throughout the whole sphere. This may be attributable to the homogeneous swelling behavior of PNIPVP spheres.

To demonstrate the entrapment stability of CdTe NCs loaded in PNIPVP spheres and the interaction between NCs and the gel network, we conducted experiments to release the loaded NCs from the spheres. As shown in Figure 1a, PNIPVP spheres have a maximum size at pH 3; at either side of pH 3, PNIPVP spheres collapse. As the CdTe NCs used in our work are not stable in a highly acidic medium, the release experiments were implemented at high pH. After removal of CdTe-PNIPVP or PNIPVP spheres by centrifugation at 2000 g, the supernatant containing the released CdTe NCs were analyzed by UV-vis absorption spectroscopy. Figure 1c shows the release profile of 3 nm CdTe NCs at different pH and ionic strength. In our work, the loaded CdTe NCs can be maintained in gel spheres over a broad pH range of 3–10; no leakage of NCs was observed during storage for a few months, even in the presence of 0.154 M NaCl. When the pH jumps to 11, however, most of the loaded NCs (80 %) are squeezed out after 80 min. The inset in Figure 1c reveals that strong yellow luminescence is found exclusively in the supernatant and not in the sediment. A further increase of pH has only a small influence on the release of NCs. Since the apparent pK_a of 4-vinylpyridine is 5.39,[8] the loading stability and pH-triggered release behavior of the loaded CdTe NCs indicate that the entrapment of NCs in PNIPVP spheres is mainly due to the physical entanglement of the collapsed gel network. As shown in Figure 1c, the addition of 0.05 M NaCl allows us to release all of the loaded CdTe NCs (98 %) at pH 13. Since the ionic strength has little effect on the diameter of PNIPVP spheres at a pH higher than 8,[8] this influence of NaCl suggests that protonated pyridine groups exist in the CdTe-PNIPVP spheres at higher pH, which lead to electrostatic interactions with the negatively charged thioglycolate capped on the CdTe NCs. We are currently exploiting the influence of the pH of the environment on the protonation of pyridine groups in the CdTe-PNIPVP spheres.

In addition to CdTe NCs of 3 nm, those of 2.5 and 4 nm can also be loaded into PNIPVP spheres, rendering PNIPVP distinguishable emission colors: green (2.5 nm) and red (4 nm) (Fig. 2). The loading and release behavior of 2.5 and 4 nm CdTe NCs in PNIPVP spheres is similar to that of the 3 nm ones. However, the loading amount of 2.5 nm NCs is slightly smaller than those of the larger NCs. This suggests a broad size distribution of the pores in the PNIPVP spheres. These experiments encouraged us to simultaneously incorporate different-sized CdTe NCs into one PNIPVP sphere. In the present work, we incubated PNIPVP spheres with a mixture of 2.5 (green) and 4 nm (red) CdTe nanocrystals at pH 3. After removing excess NCs, as shown in the upper panel in Figure 2, the distinguishing emission color of the resulting CdTe-PNIPVP spheres can be obtained by optimizing the molar ratio of these two NCs. The lower panel in Figure 2 shows "emission spectral fingerprints" of the resulting spheres, indicating that the spectra of 2.5 and 4 nm CdTe NCs remain unchanged and no energy transfer between them was observed.

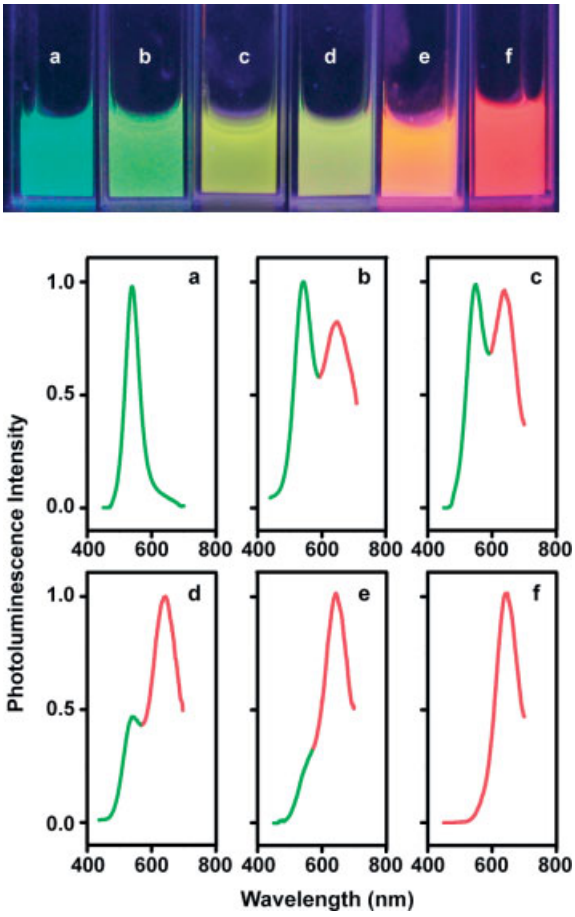


Figure 2. Upper panel: Fluorescence images of PNIPVP spheres embedded with 2.5 nm CdTe NCs (a), 4 nm CdTe NCs (f) and mixture of these two NCs with varied molar ratios of small to large NCs: 5:4 (b), 1:1 (c), 1:2 (d), and 1:3 (e). Lower panel: The corresponding photoluminescence spectra of these CdTe-PNIPVP spheres.

In addition to pH sensitivity, PNIPVP spheres have a volume phase transition at around 34 °C, as determined by DLS, which provides another method to confine CdTe NCs. Using this temperature sensitivity, however, we may confine NCs in PNIPVP spheres only at a temperature higher than 34 °C. The temperature entrapment of NCs is, therefore, not useful in the current system. Synthesis of hydrogel spheres with reversed temperature sensitivity[11] and the introduction of strong interactions between NCs and the gel network by varying the capping ligands of the NCs are currently being investigated.

In summary, we have demonstrated an efficient and facile protocol to generate uniform fluorescent microspheres by confining CdTe NCs into PNIPVP spheres. The physical entanglement of the collapsed gel network of PNIPVP spheres plays a dominant role in the NC loading process. The electrostatic interaction between the NCs and the gel network has a minor contribution to the NC entrapment in our system. The loading amount of CdTe NCs and fluorescence intensity level

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per sphere can be tuned by the concentration of NCs used. Multicolor-coded microspheres have also been realized by incorporating different-sized CdTe NCs into one sphere. As the concentration of NCs in the gel is too small to cause complicated excitonic or electronic interactions between them, the emission color of the resulting multicolor-coded spheres is mainly determined by the ratio of the different-sized NCs. Our preliminary experiments demonstrate that our protocol can be generalized to trap other water-soluble NCs, such as Au and γ-Fe_2O_3 within hydrogel spheres. To load NCs of different size and composition into hydrogel spheres is a topic of ongoing research in our laboratory. Due to the biocompatibility and the flexibility of the modification of surfaces of hydrogel spheres, such NC-loaded microspheres should hold promising prospects in biological applications. In addition, since the loaded CdTe NCs can be released from the PNIPVP spheres, triggered by pH, our protocol also provokes an opportunity for delivery of NCs and even their bioconjugates if considering NCs as a new sort of drug.[12]

Experimental

N-Isopropylacrylamide (250 mg), 4-vinylpyridine (10 μL), potassium persulfate (20 mg), and *N*,*N*′-methylenebisacrylamide (25 mg) were dissolved in 25 mL of water. The polymerization was conducted at 70 °C for 4 h under N_2. The as-prepared PNIPVP spheres were purified by centrifugation at 2000 g for 10 min and redispersed in water.

After incubating PNIPVP spheres with CdTe NCs solution at pH 3 for 5 min, the pH of the mixture was adjusted to pH 4. Upon decanting the supernatant containing excess CdTe NCs after centrifugation at 2000 g for 10 min, CdTe-PNIPVP spheres were redispersed in water of pH 7. To determine the amount of loaded NCs, the absorbance spectra of CdTe-PNIPVP spheres were recorded by using a Cary 50 UV-visible spectrophotometer. In our absorbance measurements, the diffuse reflectance mode was utilized to reduce the strong scattering of the gel spheres. The number of CdTe-PNIPVP spheres was determined by SPLS. Details of the SPLS experimental system and measurement have been described elsewhere [10].

Release experiments were conducted by incubating CdTe-PNIPVP spheres in aqueous solutions with pHs ranging from 4 to 13, adjusted by adding 1 M NaOH solution. After removal of CdTe-PNIPVP or PNIPVP spheres by 10 min centrifugation at 2000 g, the supernatants containing released CdTe NCs were analyzed by UV-vis absorption spectroscopy. The release period included the centrifugation time. Using a higher centrifugation speed of 10 000 g, one is able to release most of the loaded CdTe NCs in 1 min.

DLS measurements were implemented by a Malvern Zetasizer 3000HS. TEM images were obtained using a Philips CM 120 microscope operating at 80 kV. Luminescence spectra were obtained with a Spex Fluorolog 1680 spectrophotometer (the excitation wavelength is 400 nm).

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[1] a) B. J. Battersby, G. A. Lawrie, A. P. R. Johnston, M. Trau, *Chem. Commun*. **2002**, 1435. b) M. Trau, B. J. Battersby, *Adv. Mater*. **2001**, *13*, 975.

[2] a) R. J. Fulton, R. L. McDade, P. L. Smith, L. J. Kineker, J. R. Jr. Kettman, *Clin. Chem*. **1997**, *43*, 1746. b) B. J. Battersby, D. Bryant, W. Meutermans, D. Matthews, M. L. Smythe, M. Trau, *J. Am. Chem. Soc*. **2000**, *122*, 2138. c) F. J. Steemers, J. A. Ferguson, D. R. Walt, *Nat. Biotechnol*. **2000**, *18*, 94.

[3] a) H. Weller, *Angew. Chem. Int. Ed. Engl*. **1993**, *32*, 41. b) A. P. Alivisatos, *Science* **1996**, *271*, 933. c) A. P. Alivisatos, *Sci. Am*. **2001**, *285*, 66. d) W. C. W. Chan, D. J. Maxwell, X. Gao, R. E. Bailey, M. Han, S. Nie, *Curr. Opin. Biotech*. **2002**, *12*, 40. e) J. L. West, N. J. Halas, *Annu. Rev. Biomed. Eng*. **2003**, *5*, 285. f) M. Bruchez, M. Moronne, P. Gin, S. Wiess, A. P. Alivisatos, *Science* **1998**, *281*, 2013. g) W. C. Chan, S. Nie, *Science*, **1998**, *281*, 2015.

[4] D. Wang, A. L. Rogach, F. Caruso, *Nano Lett.* **2002**, *2*, 857.

[5] a) M. I. Martinez-Rubio, T. G. Ireland, G. R. Fern, J. Silver, M. J. Snowden, *Langmuir* **2001**, *17*, 7145. b) A. L. Rogach, D. Nagesha, J. W. Ostrander, M. Giersig, N. A. Kotov, *Chem. Mater*. **2000**, *12*, 2676.

[6] a) A. S. Hoffman, *J. Controlled Release* **1987**, *6*, 297. b) B. R. Sauders, B. Vincent, *Prog. Colloid Polym. Sci.* **1997**, *105*, 11. c) B. R. Saunders, B. Vincent, *Adv. Colloid Interface Sci*. **1999**, *80*, 1. d) N. A. Peppas, Y. Huang, M. Torres-Lugo, J. H. Ward, J. Zhang, *Annu. Rev. Biomed. Eng*. **2000**, *2*, 9.

[7] M. Han, X. Gao, J. Z. Su, S. Nie, *Nat. Biotechnol*. **2001**, *19*, 631.

[8] V. T. Pinkrah, M. J. Snowden, J. C. Mitchell, J. Seidel, B. Z. Chowdhry, G. R. Fern, *Langmuir* **2003**, *19*, 585.

[9] M. Y. Gao, A. L. Rogach, A. Kornowski, S. Kirstein, A. Eychmuller, H. Möhwald, H. Weller, *J. Phys. Chem. B* **1998**, *102*, 8360.

[10] H. Lichtenfeld, K. L. Napschinsky, C. Durr, H. Zastrow, *Prog. Colloid Polym. Sci*. **1997**, *104*, 148.

[11] P. Bouillot, B. Vincent, *Colloid Polym. Sci*. **2000**, *278*, 74.

[12] a) A. Jordan, R. Scholz, P. Wust, H. Fähling, R. Felix, *J. Magn. Magn. Mater*. **1999**, *201*, 413. b) L. R. Hirsch, R. J. Stafford, J. A. Bankson, S. R. Sershen. B. Rivera, R. E. Price, J. D. Hazle, N. J. Halas, J. L. West, *Proc. Natl. Acad. Sci*. **2002**, *100*, 13 549.

Synthesis of Skeletal-Structured Biporous Silicate Powders through Microcolloidal Crystal Templating**

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Porous materials possessing ordered pores with well-defined pore size distributions have been studied by many researchers for a long time, and have been applied to commercial processes in various fields such as catalysis, adsorption, absorption, and separation because of their attractive materials properties. As typical porous materials, natural and synthetic zeolites have micropores between 0.4–1.5 nm. Mesoporous inorganic materials such as the aluminosilicate FSM[1] and the silicate M41S[2] were first reported around 1990. These materials with very narrow pore size distributions and

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