communications

Incorporating CdTe Nanocrystals into Polystyrene Microspheres: Towards Robust Fluorescent Beads**

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Due to excellent optical properties such as size-tunable fluorescence and a narrow and symmetric emission profile with a broad excitation range, II–VI fluorescent quantum dots (Q-dots) have undergone intensive investigation as a new type of biolabeling material over recent years.[1] Although Q-dots have been proven to be superior to conventional dyes in many respects, the ultrasensitivity of their fluorescence to particle surface states,[2] and the release of highly toxic heavy-metal ions as a consequence of photodegradation are problems that remain to be solved for Q-dot-based biolabels with respect to both in vivo and in vitro application.[3] One of the possible solutions is to coat the fluorescent Q-dots with a polymer, since effective isolation can not only suppress the photodegradation[4] but can also impede the release of heavy-metal ions thereupon. Most importantly, the simultaneous integration of different types of fluorescent Q-dots into polymeric beads can lead to a useful encoding library for high-throughput biodetection.[5,6]

Different synthetic routes have been developed for incorporating preformed Q-dots into microbeads. For example, by directly assembling CdSe@ZnS dots into the voids of silica microspheres,[5] Nie and co-workers successfully obtained multiplexed optical encoding microbeads by simply manipulating the ratio of various Q-dots with different optical properties within the composite beads. Based on a similar idea, they also succeeded in encapsulating Q-dots by swelling polystyrene microbeads with an organic solution containing the Q-dots.[6] By utilizing the volumetric phase-transition properties of poly(*N*-isopropylacrylamide)

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(pNIPAM), Wang, Gao, and co-workers[7,8] also successfully incorporated water-soluble CdTe dots into pNIPAM hydrogel spheres. Although these investigations represent great progress towards a clear definition for the optical properties of the composite beads, in some chemical environments the incorporated Q-dots tended to be released due to the lack of strong interactions between the Q-dots and the polymeric matrices.[5,7] In addition, the use of porous beads may also enhance the nonspecific adsorption of analytes in bioanalytical applications. Furthermore, the diffusion of oxygen and water from the surrounding media into the composite beads will accelerate the photodegradation of the Q-dots.[9] Therefore, it is necessary to develop a new synthetic route for incorporating Q-dots within hydrophobic matrices possessing a compact structure. An easy way to achieve this goal is to directly polymerize hydrophobic radical monomer droplets containing organic soluble Q-dots.[10] However, two bottlenecks need to be overcome with this route, namely, the phase separation between the organic polymer and the aggregations of inorganic nanoparticles, and effective encapsulation of Q-dots within the polymeric beads. In addition, to preserve the fluorescence of the parent Q-dots in the resultant beads is also a challenging issue since some radicals can heavily quench the fluorescence of the Q-dots.[11]

Herein, we report a modified miniemulsion polymerization method for preparing multiplexed optical-encoding polystyrene beads with pH-independent fluorescence and excellent antisolvent properties. We also demonstrate that the use of polystyrene (PS) as a matrix can effectively enhance the photostability of the Q-dots within the PS beads.

The fluorescent CdTe dots used in this study were synthesized in an aqueous system using 3-mercaptopropionic acid (MPA) as a stabilizing agent.[12] Our previous research has demonstrated that MPA-stabilized CdTe Q-dots can be homogenously incorporated into a PS matrix to form a fluorescent bulk by using octadecyl-*p*-vinylbenzyldimethylammonium chloride (OVDAC) as a phase-transfer agent.[13] Since the vinyl group from OVDAC, which caps the surface of CdTe dots, can form covalent bonds with the PS backbone, the unwanted phase separation is consequently eliminated. Following this study, the fluorescent PS beads reported herein were prepared by the miniemulsion polymerization method using OVDAC and DVMAC (didecyl-*p*-vinylbenzylmethylammonium chloride) as an emulsifier and phase-transfer agent, respectively. Typically, DVMAC was used to transfer the MPA-stabilized CdTe dots into styrene, while modifying the surface of the CdTe dots with vinyl groups. Then, the resultant styrene solution was mixed with water under mechanical stirring. The immiscible system was subsequently emulsified by OVDAC with the help of a certain percentage of Triton X-100 (*t*-octylphenoxypolyethoxyethanol, Sigma). The polymerization reaction was initiated with 2,2′-azobisisobutyronitrile (AIBN) at 80 °C. The composite beads were finally obtained after being washed with water followed by centrifugation for at least three cycles.

Figure 1 presents fluorescence microscopic images of the PS beads incorporated with different-sized CdTe dots, with their photoluminescence (PL) peaking at 546 nm (green dots), 592 nm (yellow dots), and 634 nm (red dots). The con-
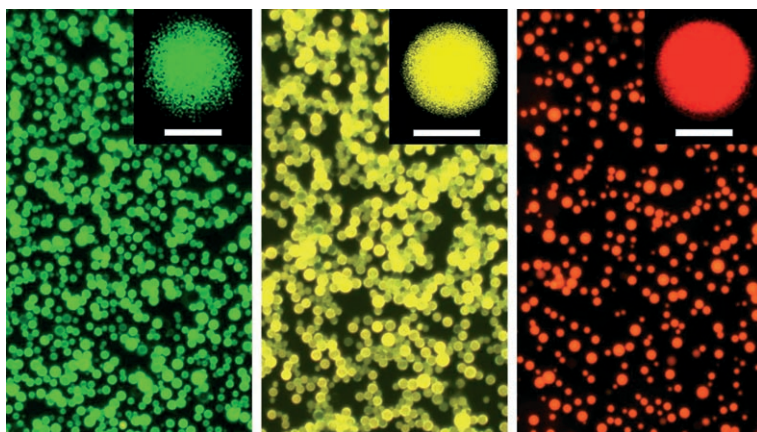
Figure 1. Fluorescence images of PS beads loaded with green (left), yellow (middle), and red CdTe dots (right). The insets are the corresponding confocal fluorescence images. The scale bars correspond to 2 μm.

dots show an emission profile mainly determined by their input ratio.

To clarify the importance of the polymerizable feature of the phase-transfer agent, didecyl-*p*-ethylbenzylmethylammonium chloride (DEMAC) was synthesized and used as a model surfactant for comparison with DVMAC. The fluorescent beads (containing 2 wt% divinylbenzene as a crosslinking reagent) obtained using DVMAC presented much better antisolvent properties; nearly
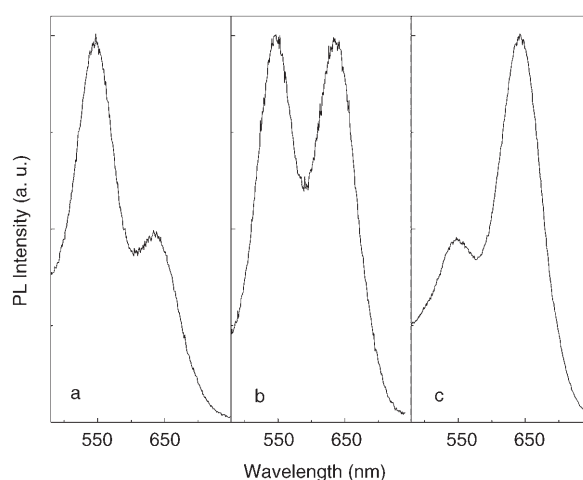
focal fluorescence microscopic images of the corresponding beads shown in the insets of Figure 1 suggest that the CdTe dots, irrespective of their size, are evenly distributed throughout the PS-bead matrices, which is also supported by the fact that there is nearly no change in the emission profile of the CdTe dots after they are encapsulated in the polystyrene beads (see Figure S1 in the Supporting Information), since a red-shifted PL would be expected if the CdTe Q-dots were aggregated within the polymer matrix.

Although further PL investigations revealed that the PL efficiency of the CdTe dots was reduced by a factor of 1.7–2.0 (slightly dependent on particle size), it remained at 8%, 10%, and 17% for the incorporated green, yellow, and red dots, respectively, throughout the whole preparative procedure, much higher than that reported in the literature (measurements on the PL efficiencies of various CdTe dots incorporated within polymeric beads are described in the Supporting Information).[14] Fluorescence-quenching effects were mainly caused by free radicals and strongly dependent on the presence of oxygen as well as the nature of the free radicals, especially those generated by initiators. It was observed that among all tested initiators almost all oxidants, such as benzoyl peroxide (BPO), $K_2S_2O_8$, and H_2O_2, exhibited strong fluorescence-quenching effects because they can heavily oxidize the CdTe dots. The fluorescence survived only when azo derivatives such as AIBN were used as an initiator. Although it remains unclear to what extent the fluorescence was quenched by radicals carried by radical monomers, the use of AIBN as an initiator could best preserve the fluorescence of the CdTe dots within the resultant PS beads.

In comparison with the PL efficiency, the PL peak position presented little variation after the CdTe dots were incorporated (see Supporting Information), which allows further application of the current synthetic approach to the preparation of multiplexed optical encoding beads. The feasibility was demonstrated by using green and red CdTe dots with PL emissions peaking at 546 nm and 634 nm, respectively. The results shown in Figure 2 demonstrate that composite beads simultaneously incorporating the two types of

Figure 2. Fluorescence spectra of PS beads incorporated with both green and red CdTe dots with their PL peak-intensity ratio being 2:1 (a), 1:1 (b), and 1:2 (c). The initial input ratio of green to red dots was 1:0.3 (a), 1:0.6 (b), and 1:1.2 (c).

no CdTe dots were extracted out of the beads after they were dispersed in toluene for 24 h. Although DEMAC is structurally very similar to DVMAC (except that its ethyl group is substituted by a vinyl group in DVMAC), 70% of the CdTe dots capped by DEMAC were extracted out of the beads into toluene, despite being prepared under exactly the same conditions (the experimental results are given in more detail in the Supporting Information). This further demonstrates that the covalent bonds formed between DVMAC and PS can firmly hold the CdTe dots inside the PS beads due to the formation of three-dimensional hybrid networks involving the CdTe dots.

The use of hydrophobic PS as a bead matrix was expected to enhance the photostability of the incorporated CdTe dots. Photobleaching experiments were performed by continuously illuminating the composite beads (suspended in O_2-aerated water) using ultraviolet light at 392 nm with a power density of $1.2\ W\,cm^{-2}$. The light spot at focus was

about 1 cm in diameter, nearly covering all of the solution within a 1 cm quartz cuvette. For comparison, the parent CdTe dots and the CdTe-dot-loaded pNIPAM hydrogel spheres were also subjected to the same irradiation. The results shown in Figure 3a indicate that the CdTe dots within
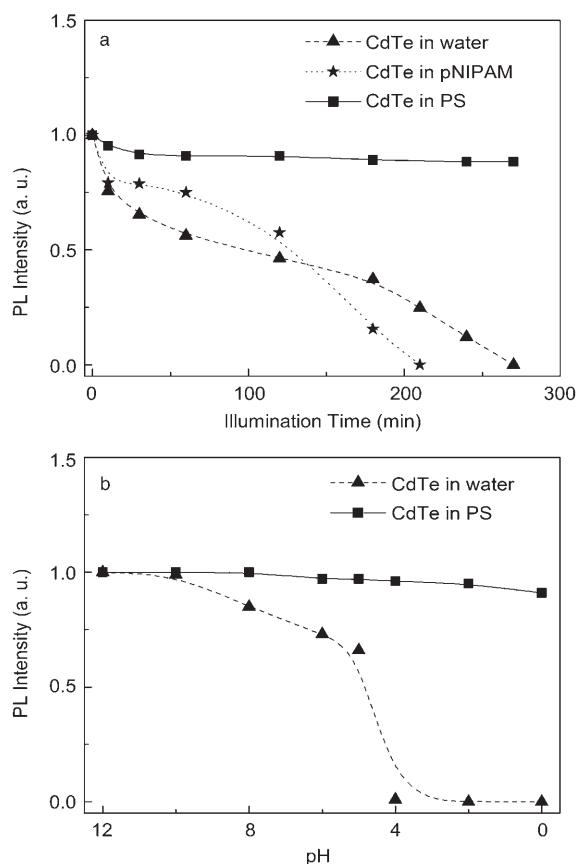

Figure 3. a) The photostability of red CdTe dots in water, in pNIPAM microspheres, and in PS beads. b) The pH dependency of the PL emissions of red CdTe dots in water and in PS beads.

the PS beads are much more stable than those in neutral water or in pNIPAM spheres, which suggests that the PS shield can effectively enhance the photostability of the CdTe dots, further implying that the hydrophobicity and compact structure of PS can effectively impede the diffusion of both oxygen and water from the surrounding environment into the inner part of the composite beads, since the photooxidation of Q-dots is strongly coupled with the presence of oxygen and water. Therefore, it is also reasonable to infer that the diffusion of heavy-metal ions generated by the photodegradation of the Q-dots can also be blocked by the PS shield. The early decrease in the PL intensity of the CdTe-dot-loaded PS beads may be attributed to the photodegradation of the dots in the vicinity of the shallow surface of the composite beads.

The compact structure of the PS matrix also affords the composite beads an excellent pH-independent PL, as shown in Figure 3b. The PL intensity of the composite beads presents nearly a constant intensity over a pH range of 2 to 12, with very little intensity loss being observed after the beads were dispersed in 1 M HCl for 30 min. In huge contrast, the fluorescence intensity of the parent CdTe dots decreased sharply when the pH drops below 6.0, and finally dies out when the pH is lower than 4.0.

In summary, fluorescent PS microbeads incorporated with CdTe dots were successfully prepared via the miniemulsion polymerization method using polymerizable surfactants as both emulsifier and phase-transfer agent. Systematic investigations revealed that this synthetic approach is also applicable for producing multiplexed optical encoding beads based on different types of fluorescent Q-dots. The hydrophobic nature and compact structure of PS allows the preparation of robust fluorescent beads whose PL intensity is nearly pH-independent. Capping the CdTe dots with a polymerizable surfactant (DVMAC) leads to an excellent fixation of the CdTe dots within the composite beads, due to the formation of covalent bonds between DVMAC and the polymer backbone. Therefore, the composite beads also present excellent antisolvent properties. In brief, the novel use of polymerizable surfactants as both emulsifier and phase-transfer agent successfully extends the miniemulsion polymerization method to the preparation of Q-dot-based fluorescent beads, which possess excellent photo- and chemical stability.

Experimental Section

Highly fluorescent CdTe nanocrystals were synthesized according to a previous report by employing 3-mercaptopropionic acid (MPA) as a stabilizing agent.[12] The size of the CdTe dots was controlled by the reflux time. The initial concentration of the CdTe dots was about 6.1×10^{-5} M, 4.7×10^{-5} M, and 2.1×10^{-5} M for the green, yellow, and red CdTe dots, respectively.[15] The surfactants octadecyl-*p*-vinylbenzyldimethylammonium chloride (OVDAC), didecyl-*p*-vinylbenzylmethylammonium chloride (DVMAC), and didecyl-*p*-ethylbenzylmethylammonium chloride (DEMAC) were prepared according to literature.[16]

The PS composite beads were prepared as follows: Typically, DVMAC (0.03 g) was dissolved in styrene (2 mL) and then mixed with 1 mL of an as-prepared CdTe dot solution under vigorous stirring. After the CdTe dots were transferred into the styrene phase, the organic solution was separated by decantation and AIBN (0.015 g) was introduced into the solution followed by 20 mL of water containing OVDAC (0.1 g) and Triton X-100 (0.1 g). Under mechanical stirring, the oil phase was emulsified. After 30 min of deaeration under N_2, the reaction mixture was heated in an oil bath at 80 °C and maintained at this temperature for about 6 h. After polymerization, the composite beads were washed with water followed by centrifugation. The cleaning procedures were normally repeated at least three times.

For antisolvent experiments, 2% divinylbenzene (DVB; with respect to the total weight of styrene) was fed as a crosslinking reagent to maintain the shape of the PS beads in nonpolar solvents. The fluorescent PS beads prepared using either DVMAC or DEMAC as the phase-transfer agent were dispersed in toluene under magnetic stirring for 24 h and then removed by centrifuga-

tion. The absorbance and photoluminescence spectra of the resultant supernatants were then recorded and compared.

True-color images were recorded with an inverted Olympus microscope (IX-70) by dropping aqueous dispersions of the fluorescent PS beads onto glass slides. Confocal fluorescence images were taken with a Leica confocal scanning system mounted to a Lecia Aristoplan and equipped with a 100× oil-immersion objective with a numerical aperture NA=1.4. UV/Vis absorption spectra were recorded with a Cary 50 UV/Vis spectrophotometer. Fluorescence spectra were obtained with a Cary Eclipse fluorescence spectrophotometer.

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