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Four strategies for water transfer of oil-soluble near-infrared-emitting PbS quantum dots

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Abstract The successful transfer of oil-soluble quantum dots (QDs) into water is critical for many of their bioapplications. In this paper, the impacts of four various strategies (i.e., via micelles, nanohydrogels, amphiphilic polymers and water-soluble thiol small molecules) on the phase transfer of oil-soluble oleic acid-capped NIR-emitting PbS QDs into water were evaluated systematically. It was found that the process of water transfer and the optical property of the resulting water-soluble QDs highly hinge on the type of the phase transfer agents used due to their different interactions with QD surface. Among all these phase transfer agents, SOC micelles and glutathione (thiol) molecules are more favorable for retaining the optical property of the initial oil-soluble PbS QDs. As a result, the obtained water-soluble QDs show strong NIR fluorescence (PL QY $>$ 30% in water). However, in the case of nanohydrogel and amphiphilic polymers, the corresponding water-soluble ones display relatively weak fluorescence emission. These results suggest fully that "correct" phase transfer agents should be selected in order to obtain high-quality water-soluble PbS QDs. The possible reasons for this obvious difference were further analyzed and revealed. Besides, the preliminary results obtained also indicate that the NIR-emitting PbS QDs will be a potential probe in the in vivo biomedical imaging of small animals.

Abbreviations

NIR	Near-infrared
QDs	Quantum dots
OA	Oleic acid
SOC	*N*-succinyl-*N*′-octyl chitosan
P(NIPA-co-AAm)	Poly(*N*-isopropylacrylamide-co-acrylamide)
PAA	Poly(acrylic acid)
GSH	Glutathione
PL QY	Photoluminescence quantum yield

1 Introduction

Optical imaging methods are particularly interesting for the molecular medicine due to its non-radioactive, easy operation, better temporal resolution and relative low cost, besides the traditional silos of imaging specialties such as computed tomography (CT), magnetic resonance imaging (MRI), single photon emission tomography (SPECT), positron emission tomography (PET), and ultrasound imaging [1–3]. In the last 10 years, the development of new NIR-emitting (700–1000 nm) semiconductor quantum dots (QDs) has attracted considerable attention, owing to their potentials for applications in noninvasive biomedical imaging [1–3]. Compared with organic dyes commonly used as imaging probes, inorganic QDs have several

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advantages, such as size-tunable fluorescence emission, broad excitation spectrum, large Stokes shifts, high photoluminescence quantum yield (PL QY), high resistance to photobleaching, and so on [4, 5]. As such, a series of various high quality QDs with tunable emission in the NIR region have been synthesized, including CdTe, CdHgTe, HgTe, InAs, InP, $CuInSe_2$, $CuInS_2$, etc., in aqueous or organic media [6, 7]. Among them, PbS is of particular interest because of the small band gap (0.41 eV) and large exciton Bohr radius (18 nm), which can offer excellent tunable emission across the NIR region with relatively large size [8, 9]. Meanwhile, it also provides a chance to produce air-stable NIR-emitting QDs with inexpensive and relatively safe synthetic precursors. Hence, the NIR-emitting PbS QDs are being developed as a new NIR fluorescent probe in biomedicine [10, 11].

To date, several methods have been developed to synthesize high quality NIR-emitting PbS QDs [8, 11–18]. Most of them are achieved in a non-polar organic solvent (PL peak > 1,000 nm), in the presence of organic ligands such as oleylamine (OLA), oleic acid (OA), and OA/trioctylphosphine (TOP). Until recently, Yu et al. and our group have succeeded in synthesizing small-sized PbS QDs with <1000 nm of NIR fluorescence emission [11, 16]. However, unfortunately, these QDs all are insoluble in water due to the use of the above-mentioned hydrophobic ligands. Hence, to be used in biological systems, PbS QDs are first required to have good water solubility and biocompatibility. To our knowledge, while a number of techniques have been applied to yield water-soluble visible-emitting QDs (e.g., CdSe and CdTe QDs) [19–22], relevant reports on transferring NIR-emitting PbS QDs (PL peak: 700–1000 nm) into water are still quite limited.

Currently, there are three main and common strategies for the water transfer of oil-soluble QDs, where thiol small molecules, amphiphilic molecules, and nanospheres (e.g., silica nanoparticles, micelles, liposomes, or hydrogels) were used as phase transfer agents, respectively [23]. Recently, hydrophilic thiol-containing small molecules and amphiphilic molecules have been applied to transfer PbS QDs (PL peak > 1300 nm) from organic phase into water [24–28]. However, these phase transfer methods all result in significant decreases in PL QY, even almost no fluorescence emission after water solubilization [25], as this property is very sensitive to the surface chemistry of QDs. So finding other more efficient methods for water transfer of oil-soluble PbS QDs remains a worthy challenge.

In this study, we focus on the water transfer of oil-soluble NIR-emitting ODs, by using OA-capped PbS QDs as a model system. Particularly, four kinds of different materials, that is, SOC micelles, P(NIPA-co-AAm) nanohydrogels, PAA-based amphiphilic polymers and water-soluble thiol small molecules, were used as phase transfer agents. The experimental results show that when using SOC micelles or glutathione (GSH) as phase transfer reagents, no significant decrease in fluorescence emission was observed. The resulting water-soluble PbS QDs have PL QY of 30% over the NIR imaging window, which is much higher than that (QY, ~10%) of aqueous PbS QDs synthesized directly in water (although the aqueous synthesis is very facile) [10, 17]. However, in the case of nanohydrogels and amphiphilic polymer, the PL emission of the as-prepared water-soluble QDs is relatively poor. Thus, the results from these studies suggest fully that the "correct" phase transfer agents should be chosen to yield highly fluorescent water-soluble PbS QDs. At last, here, these observations are believed to be interesting for future bioapplications of oil-soluble NIR-emitting PbS QDs.

2 Materials and methods

2.1 Materials

All chemicals used are commercially available and were used as received. Main chemicals used are as follows: lead(II) acetate trihydrate (≥99%), oleic acid (99%), $Na_2S{\cdot}9H_2O$ (≥98%), thioacetamide (TAA, 99.5%), chitosan with deacetylation degree of 90% (100 kDa, ≥95%), succinic anhydride (98%), octaldehyde (99%), *N*-isopropylacrylamide (NIPA, ≥99%), acrylamide (AAm, ≥99%), *N*,*N*′-methylene-bis-acrylamide (BIS, ≥99%), potassium persulfate (KPS, ≥99.5%), sodium dodecyl sulfate (SDS, 99.5%), poly(acrylic acid) (PAA), octylamine (99%), GSH (≥98%). Apart from these, the water used in all experiments had a resistivity higher than 18.2 MΩ cm.

2.2 Synthesis of oil-soluble OA-capped PbS QDs

Oil-soluble OA-capped NIR-emitting PbS QDs were synthesized according to our two-phase method, where water-soluble differently reactive Na_2S and thioacetamide were used as sulfur sources, respectively [11]. The typical synthetic procedure can be divided into two steps: first, the Pb-precursor solution was prepared; then, the n-decane solution of lead precursor was mixed with an aqueous solution of sulfur precursor, such as Na_2S and TAA, at the appointed temperature. As a result, high quality oil-soluble NIR-emitting PbS QDs were achieved, where both nucleation and growth of QDs occur at the interface of the two liquid phases, i.e., n-decane and water (see details in Ref. [11]).

2.3 Transfer of PbS QDs into water with micelles

In this section, *N*-succinyl-*N*′-octyl chitosan (SOC) micelles (the phase transfer agent) were firstly synthesized according to the method reported by us recently [29]:

Chitosan (100 KD) (1.0 g) was dissolved in 4.8% succinic acid solution, and then suspended in 80 mL methanol under stirring at room temperature. Afterward, the succinic anhydride acetone solution (0.18 g/mL) was added dropwise. After stirring for 48 h, the reaction solution was neutralized with sodium hydroxide. The precipitate was filtered and repeatedly washed with alcohol. The product, *N*-succinyl chitosan (SC) was dried under vacuum at 60°C. To obtain the SOC micelles, 1.0 g SC was suspended in 100 mL acetic acid solution and then added 1.02 g octaldehyde. After stirring for 4 h, $NaBH_4$ (0.16 g) dissolved in 1.6 mL water was slowly added to the solution. After a further 12 h continuous stirring, the reaction solution was neutralized. Next, the product was dialysis (MWCO 10,000) against distilled water. Finally the product was lyophilized to obtain the SOC powder for subsequent research.

Before QD phase transfer into water with micelles, the QDs obtained above were precipitated with ethanol or acetone to remove excess unreacted precursors and were redispersed in chloroform. Here, SOC micelles were used as the phase transfer reagent of ODs: 0.2 mL monodisperse PbS QDs in chloroform (10 mM) was added dropwise to the SOC (1 mg/mL) solution with sonication in ice-water bath. Next, the mixture was stirred at room temperature until the chloroform was evaporated completely. The obtained solution was then centrifuged and the resultant PbS QDs-loaded SOC solution was kept in the room temperature for further research.

2.4 Transfer of PbS QDs into water with nanohydrogels

P(NIPA-co-AAm) nanohydrogels were synthesized using our previously described free-radical precipitation polymerization method [30, 31]. In detail, monomer NIPA (1000 mg), AAm (25–200 mg), cross-linker BIS (27 mg) and surfactant SDS (50–200 mg) were added to 100 mL of double distilled water and with a magnetic stirrer. The solution was nitrogen-purged for 40 min at room temperature. And then the polymerization was initiated by adding 75 mg of KPS and lasted for 4 h under nitrogen atmosphere at a temperature of 70 ± 1°C. The resultant nanohydrogels were cooled down to room temperature and then dialyzed (molecular weight cut off 10 kDa) against double distilled water for 5 days. The dialyzed aqueous solution of nanohydrogels was then lyophilized to obtain dried powder for subsequent research. Their diameters and lower critical solution temperature (LCST) could be controlled by modulating the amounts of SDS and AAm, respectively.

Similar to that describe in Sect. 2.3, the QD chloroform solution was initially obtained before the QD water transfer. Then, the QD chloroform solution was dropwise added to the nanohydrogel solution (5 mg/mL) at room temperature and mixed in air under dark conditions. After the chloroform was evaporated completely, the mixture was centrifuged to remove unloaded QDs, and thus, the supernatant solution with nanohydrogel-encapsulated QDs was obtained.

2.5 Transfer of PbS QDs into water with amphiphilic polymer

Many previous studies have indicated that 40% amine-modified PAA might be an effective amphiphilic polymer to impart water solubility to hydrophobic QDs [23, 32]. So in this section, the amphiphilic 40% amine-modified PAA was also synthesized by a procedure modified from those reported previously [23, 32]: briefly, first, 0.6 g (0.2 mmol) of commercially available 3,000 MW dry PAA powder was diluted into 10 mL of dry *N*,*N*-dimethylformamide (DMF). Next, 0.644 g (3.36 mmol) of EDCI was dissolved into the same solution, stirring for 0.5 h followed by the addition of 0.555 mL (3.36 mmol) of octylamine. The solution was stirred at room temperature overnight before reduction of the solvent under vacuum. Next, distilled water was added to precipitate the polymer which was subsequently isolated by centrifugation; the excess water was discarded. Next, an aqueous solution of NaOH (1 mol/L) was added, and the solution was shaken to resolubilize the polymer in water. Then, a dilute solution of HCl was added to the aqueous layer until the pH was below 5 to precipitate the polymer again. The solid product was collected by centrifugation and dried under vacuum.

Amphiphilic polymer encapsulation was carried out by mixing 30 mg of poly(acrylic acid)-octylamine polymer and 1 mg of QDs in chloroform. The mixture was vortexed for 5 min, and the solvent was removed under vacuum. The dried film was dissolved in 50 mM borate buffer (pH 8.4), sonicated in ice-water bath, and centrifuged to yield a clear supernatant.

2.6 Transfer of PbS QDs into water with hydrophilic thiol ligands

As reported previously [19–22], specific thiol ligands only work with specific dots without inducing excessive quenching, although the mechanism is unknown. Hence, in this study, five various thiol ligands [i.e., 3-mercaptopropionic acid (MPA), L-cysteine (L-cys), *N*-acetyl-L-cysteine (NAC), GSH, dihydrolipoic acid (DHLA)] were selected and tested for this phase transfer, according to our recent study [10]. Here, we only describe the typical synthesis of water-soluble PbS QDs capped with GSH, as follows. In brief, firstly, the QDs were precipitated with acetone or ethanol to remove excess oleic acid and were redispersed in chloroform. Then, ~2 mg of PbS QDs in chloroform was

added dropwise to 2 mL of GSH solution ([GSH] = 150 mM, pH = 6). Next, the mixture was shaken at room temperature for about 10 min. Upon shaking, the QDs were transferred gradually into the water phase. At this time, the surfaces of the QDs are negatively charged. At last, the supernatant containing PbS QDs was collected and stored at room temperature in the dark for further research. The similar method was used in the synthesis of the other types of thiol-coated water-soluble PbS QDs.

2.7 Characterization

An S2000 eight-channel optical fiber spectrographotometer (Ocean Optics corporation, America), and a broadband light source (X-Cite Series 120Q, Lumen Dynamics Group Inc., Canada) were utilized for the detection of fluorescence spectra ($\lambda_{ex} = \sim 610$ nm). A 754-PC UV–Vis spectrophotometer (JingHua technological instrument corporation, Shanghai, China) was used for the measurement of UV–Vis spectra. All optical measurements were performed at room temperature. PL quantum yield of PbS QDs in chloroform was calculated by comparing their integrated emission to that of a solution of cypate in 20% aq. DMSO (the absorption and PL emission peaks of cypate are at 790 and 810 nm, respectively; the PL QY is 12%) supplied by prof. Samuel Achilefu (Department of radiology, Washington University at St. Louis, USA) [33]. A JEM-2100 transmission electron microscope (JEOL, Japan) was used to evaluate the QD morphology operating at 200 kV. The hydrodynamic diameter and distribution of micelles and nanohydrogels were measured by dynamic light scattering (DLS) (Mastersizer 2000 Laser Particle Size Analyzer, Malvern, British).

3 Results and discussion

This manuscript addresses intensively four strategies to transfer oil-soluble QDs into water using near-infrared (NIR) emitting PbS QDs as a model system; thus, the Results and Discussion mainly consist of five parts. In the first part (Sect. 3.1), we describe briefly the two-phase synthesis of oil-soluble OA-capped NIR-emitting PbS QDs in n-decane, by using differently reactive water-soluble Na_2S and thioacetamide as sulfur precursors. Next, we attempted four various strategies for the phase transfer of QDs from organic to aqueous solution. The results are presented in detail, in a sequence of micelle-based QD water transfer (Sect. 3.2), nanohydrogel-based QD water transfer (Sect. 3.3), amphiphilic polymer-based QD water transfer (Sect. 3.4), and thiol-based QD water transfer (Sect. 3.5). Here, this study focused on investigating the influence of phase transfer on the fluorescence properties of the QDs by fluorescence spectrometer and NIR imaging system.

3.1 Synthesis of oil-soluble NIR-emitting OA-capped PbS QDs

In our recent report [11], high quality oil-soluble PbS QDs have been synthesized via a facile two-phase approach. As shown in Fig. 1a, by changing the reactivity of water-soluble sulfur sources used, the resulting oil-soluble QDs could show tunable photoemission throughout the NIR region (~750–1,000 nm): (1) when using highly reactive Na_2S as sulfur source, PbS QDs with a small size and a narrow size distribution were prepared (PL peak position can be tuned between 750 and 880 nm; the maximum PL quantum yield up to 40% estimated by using NIR dye Cypate (QY 12%) as reference standard). (2) When using medium reactive TAA as sulfur source, the resulting PbS QDs with larger size show the long-wavelength emission (PL peak position can be tuned from 800 to 980 nm; the maximum PL quantum yield up to 35%) at the reaction temperature of 60–65°C. Meanwhile, in the experiment, the reaction temperature and the precursor Pb/S/OA molar ratios also were observed to play important roles in

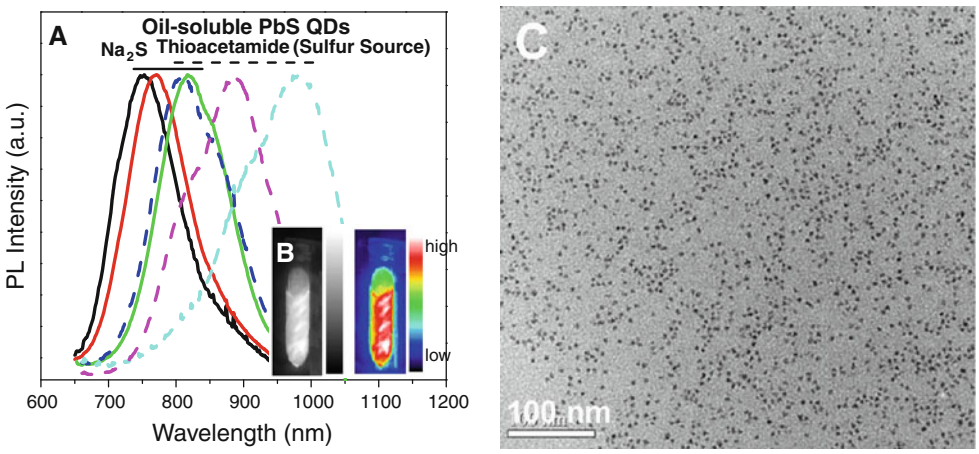

Fig. 1 **a** The normalized PL spectra of the as-prepared oil-soluble OA-capped PbS QDs, using water-soluble differently reactive Na_2S (*solid line*) and thioacetamide (*dashed line*) as sulfur sources, respectively. **b** The fluorescence image of PbS QDs in Eppendorf tube excited by a laser light ($\lambda_{max} = 765.9$ nm) (inset of **a**, the right is the corresponding pseudocolor image of the left). **c** Typical TEM image of oil-soluble PbS QDs ($\lambda_{em} = 800$ nm) by using Na_2S as sulfur source

controlling the optical properties of the resulting OA-capped PbS QDs (see details in Ref. [11]). Next, the fluorescence images of these oil-soluble OA-capped PbS QDs were acquired further by a NIR imaging system and shown in Fig. 1b. As shown, under the radiation of NIR laser light ($\lambda_{max} = 765.9$ nm), these oil-soluble QDs emit bright NIR fluorescence. The TEM image in Fig. 1c indicates that oil-soluble NIR-emitting PbS QDs produced here have a very uniform size distribution and regular shape (the average size is ~4 nm). Hence, these data all indicate that the quality of the synthesized oil-soluble NIR-emitting PbS QDs is comparable or higher than those reported in previous studies [12–16], indicating that they are very promising for in vivo biomedical imaging as fluorescence probe.

3.2 Water transfer of PbS QDs with SOC micelles

As described above, high-quality NIR-emitting PbS QDs have been synthesized by a facile two-phase method. However, these QDs are insoluble in water due to the use of the hydrophobic ligand-oleic acid. To be applied in biomedical systems, QDs are required to have good water solubility and biocompatibility as well as to exhibit high PL quantum yields and photostability. To our knowledge, the water transfer of visible light-emitting QDs has been investigated intensively in previous studies [19–22], whereas relevant reports on transferring NIR-emitting PbS QDs are still quite limited [24–28]. For this reason, the present study focused primarily on the water transfer of oil-soluble NIR-emitting PbS QDs.

In the first part, SOC micelles were used as the phase transfer reagent of oil-soluble NIR-emitting PbS QDs (here, SOC micelles were considered as a model). Before water transfer, the QDs obtained above were precipitated with ethanol or acetone to remove excess unreacted precursors and were redispersed in chloroform. Then, the monodisperse QDs in chloroform and the SOC micelles in water were mixed, sonicated and stirred; chloroform was gradually removed by evaporation at room temperature; the clear and colored solution of water-soluble QDs with SOC micelles were obtained by centrifugation and decantation for further research. Detailed scheme for the formation of micelles and water transfer of oil-soluble PbS QDs with micelles was shown in Fig. 2a. As we expected, after chitosan is chemically modified using succinic acid and octaldehyde, the amphiphilic polymer molecules are formed and will spontaneously self-assemble into a spherical micelle having a hydrophobic core and a hydrophilic shell [29]. The average dynamic size of the SOC micelles measured by DLS was ~200 nm in diameter. Meanwhile, PbS QDs capped by one monolayer of OA molecules (due to the coordination interaction between the carboxyl groups and Pb cations) are hydrophobic. Thus, the hydrophobic interaction may entrap spontaneously the QDs into the hydrophobic core of the micelles, where the loading efficiency of SOC micelles for hydrophobic QDs

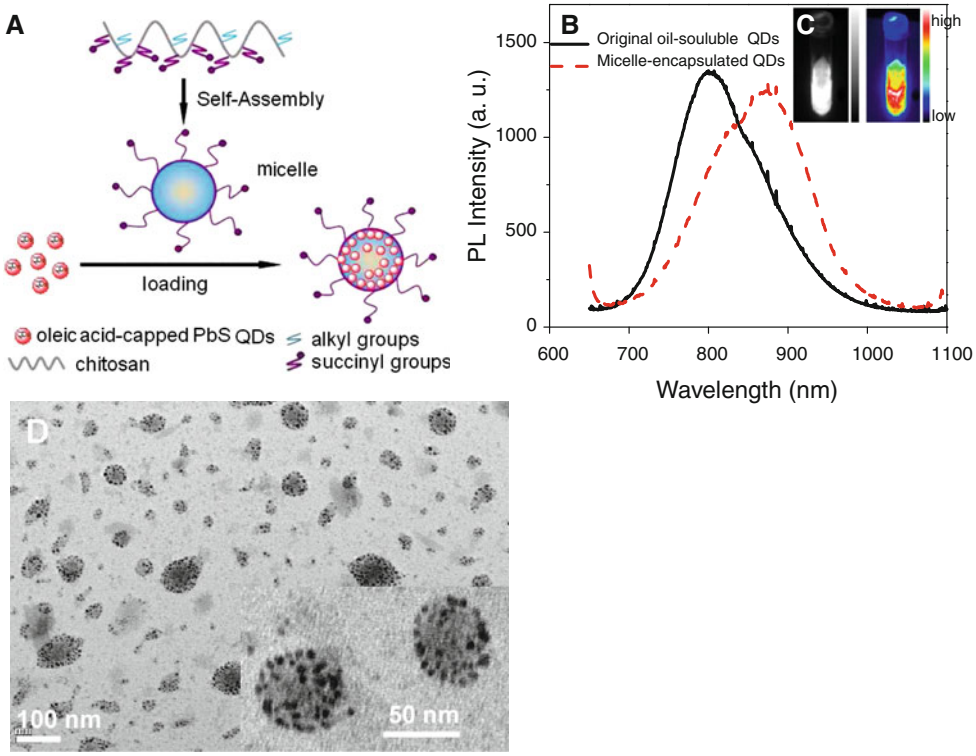

Fig. 2 **a** Detailed scheme for the formation of SOC micelles and the corresponding phase transfer of oil-soluble PbS QDs into water. **b** PL spectra of PbS QDs before and after water transfer via SOC micelles. **c** NIR fluorescence images of micelle-encapsulated PbS QDs under radiation of NIR laser light (765.9 nm) (inset of **b**, the right is the corresponding pseudocolor image of the left). **d** Typical TEM image of PbS QDs after SOC micelle encapsulation and transfer into water

could be up to ~50% (here, the loading efficiency = amount of QDs encapsulated in SOC micelles/amount of SOC micelles added × 100%).

The PL (and absorption) spectra of PbS QDs prior to and following transfer into water via SOC micelles were measured and presented in Fig. 2b. Compared with initial oil-soluble PbS QDs, the encapsulation (or water transfer) of QDs with micelles results in a red shift of ~70 nm in fluorescence emission peak. The spectral red-shift could be attributed to the stabilization interaction of QDs after micelle entrapment due to the interior hydrophobic microenvironment of micelles, which can decrease the transition energy of PbS QDs [30]. Here, it should be noted that after water transfer with SOC micelles, no significant decrease in the PL emission intensity of QDs is observed. In the meantime, the experimental result in Fig. 2c from fluorescence imaging also supports the former spectral analysis, indicating that using SOC micelles as a phase transfer agent, oil-soluble OA-capped PbS QDs could be transferred successfully into water and retain the initial strong NIR fluorescence emission (PL QY, >30%). Besides, the TEM characterization (Fig. 2d) confirms clearly the formation of the QD/micelle nanocomposites, where the hydrophobic PbS QDs were located in the hydrophobic core of the micelles. Based on the above characteristics, we find that the encapsulation of QDs into micelles with a large hydrophobic core (>100 nm) seems to not disturb the surface ligands, and thus the initial high PL QY can be retained [24–26]. On the other hand, these data also suggest that the highly fluorescent QDs produced here might be used for tracing the micelles in vivo by NIR imaging system.

3.3 Water transfer of PbS QDs with nanohydrogels

In this part, nanohydrogels were used further as the phase transfer reagent of oil-soluble NIR-emitting PbS QDs into water. Due to biological compatibility and feasibility for conjugating with biological molecules, nanohydrogels have been widely employed as delivery vehicles for drugs, proteins, and genes [30, 31]. Therefore, the incorporation of NIR-emitting QDs into hydrogel spheres is interesting and could provide promising fluorescent probes for biological detection [34]. However, to our knowledge, up to now, only limited efforts have been made on these relevant studies. Here, temperature-responsive P(NIPA-co-AAm) nanohydrogels were synthesized using our previously described free-radical precipitation polymerization method (see details in Ref. [30]), and used as a model system. Detailed scheme for the formation of nanohydrogels and the corresponding water transfer of oil-soluble PbS QDs was displayed in Fig. 3a. It is well known that the P(NIPA-co-AAm) chains generated in the reaction solutions (at a temperature higher than their LCST) may be self-cross-linked to form gel nanospheres, in the presence of BIS. In this part, the nanohydrogels with the average DLS diameter

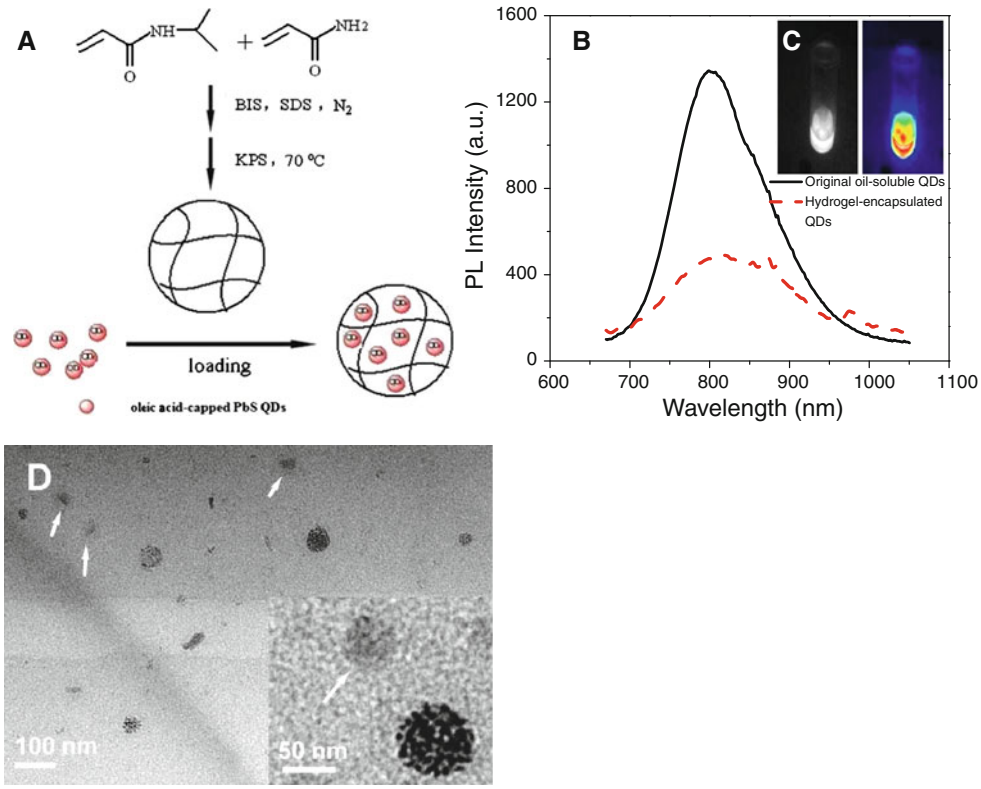

Fig. 3 **a** Detailed scheme for the formation of nanohydrogels and the corresponding phase transfer of oil-soluble PbS QDs into water. **b** PL spectra of PbS QDs before and after water transfer via nanohydrogels. **c** NIR fluorescence images of nanohydrogel-encapsulated PbS QDs under radiation of NIR laser light (inset of (**b**)). **d** TEM image of PbS QDs after nanohydrogel encapsulation and transfer into water (the nanohydrogels marked by *white arrows* are empty, without containing any hydrophobic QDs)

of ~100 nm were chosen because the nanoparticles smaller than 200 nm are capable of escaping from the capture by reticuloendothelial system (RES), and thus have greater potential for use in biomedical drug delivery. The networks in nanohydrogels might be used to load hydrophobic QDs by hydrophobic association effect between the isopropyl groups from the P(NIPA-co-AAm) gel networks and hydrophobic OA ligands capped on QDs. Here, it should be mentioned that in our previous studies, the networks in thermally responsive nanohydrogels have been used successfully to load physically hydrophobic NIR dye [30, 31].

The PL (and absorption) spectra of PbS QDs before and after water transfer via P(NIPA-co-AAm) nanohydrogels were measured and shown in Fig. 3b. Compared with initial oil-soluble PbS QDs, the encapsulation of QDs with nanohydrogels leads to a significant drop in the PL emission intensity, and with a small red shift of ~15 nm in PL peak. The significant decrease in the PL emission might be attributed to the fact that the hydrophobic interaction between the gel networks (i.e., the isopropyl groups) and the OA-capped QDs is relatively weak, as compared to that between the SOC micelles (i.e., the octyl groups) and the OA-capped QDs, which could break the integrity of the QD surface structure. However, as presented in Fig. 3c, the resulting aqueous solution of QDs with nanohydrogels as a phase transfer agent still show relatively favorable NIR fluorescence emission under the radiation of NIR laser light (PL QY, close to 15%), although a significant decrease in the luminescence occurred. To reveal further the reason of the reduction in the PL QY, TEM was further employed to characterize the morphology of PbS QDs after water transfer via nanohydrogels. The result from TEM imaging (Fig. 2d) shows clearly that in comparison with SOC micelles, the loading efficiency of nanohydrogel for hydrophobic PbS QDs is low (~10%); that is to say, only a part of nanohydrogels were loaded with hydrophobic QDs. Hence, based on the preliminary data described above, we consider that indeed, hydrophobic NIR-emitting PbS QDs can be loaded into nanohydrogels to form the QD/nanohydrogel nanocomposites, due to the hydrophobic interaction between the gel networks and the OA-capped QDs, whereas this interaction is relatively weak, which results in a low loading efficiency of QDs and an accompanied decrease in the PL QY.

3.4 Water transfer of PbS QDs with amphiphilic polymers

The use of amphiphilic polymers in water transfer of QDs appears to be more promising, as it is believed that encapsulating QDs into amphiphiles seems not to disturb the surface ligands and thus the initial high quantum yield can be retained. Recently, relevant reports on NIR-emitting PbS QDs have been carried out. However, unfortunately, these phase transfer processes have been found to be less efficient, resulting in a ~50% drop in PL QY [24, 25], even almost no fluorescence emission after transfer into water for 5 min [26]. The mechanism for this phase transfer process of oil-soluble QDs via amphiphilic polymers was schemed in Fig. 4a, where the hydrophobic ends of the polymer were supposed to interleave with but not

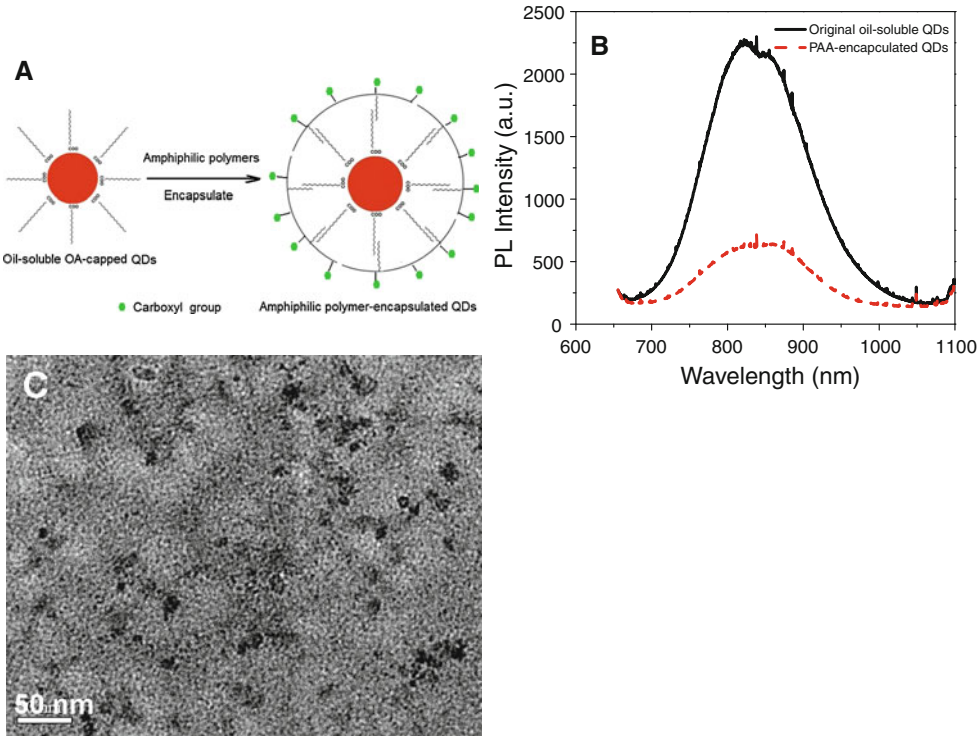

Fig. 4 **a** Detailed scheme for the phase transfer of oil-soluble PbS QDs into water via amphiphilic polymers. **b** PL spectra of PbS QDs before and after water transfer via PAA-based amphiphilic polymers. **c** TEM image of PbS QDs after amphiphilic polymer encapsulation and transfer into water

replace the initial capping ligands of QDs. In this section, the water transfer of oil-soluble NIR-emitting PbS QDs was explored briefly, by using poly(acrylic acid)-octylamine polymer as phase transfer agent (the PAA-based amphiphilic polymers have been used successfully to transfer oil-soluble CdSe/ZnS core–shell QDs into water). As shown in Fig. 4b, similar to previous relevant reports [24–26], after transfer into water, a ~75% loss in the PL intensity of QDs was observed, as compared to the initial value in chloroform, accompanied by a remarkable redshift (~40 nm). And several hours later, the PL peak will totally disappear, as it is highly sensitive to surface states. The result in Fig. 3d from TEM imaging indicates that after water transfer, the QDs are not stable and tend to irregularly aggregate together, which is a possible reason for the decrease in PL emission. Thus, combining with those reported in previous studies, a conclusion can be draw safely that as compared to micelle-based (or nanohydrogel-based) phase transfer methods described above, the phase transfer method via PAA-based amphiphilic polymers might be difficult in yielding highly fluorescent water-soluble PbS QDs, apart from using the core–shell-structured PbS/CdS QDs [24] or changing the hydrophobic ligand of initial oil-soluble QDs [25].

3.5 Water transfer of PbS QDs with hydrophilic thiols

In previous studies, the technique of ligand-exchange via hydrophilic thiols has been applied to yield high quality water-soluble visible-emitting QDs (e.g., CdSe and CdTe QDs) [19–22], whereas relevant reports on transferring NIR-emitting PbS QDs into water are quite limited. For instance, recently, ligand exchange method has been applied to transfer the high quantum-yield PbS QDs from the organic phase into water [27, 28]. However, the quantum yield is found to decrease by 30–60% after transfer due to the stripping of initial surface ligands during the ligand exchange process. Hence, in this section, we further tried to find new more efficient hydrophilic thiol-containing small molecules for water transfer of oil-soluble PbS QDs. As we known, the high affinity of the thiol group for the surface Pb atoms with respect to the carboxyl will drive the exchange of the carboxyl (oleic acid) by the thiol. Detailed scheme for this phase transfer process was shown in Fig. 5a. Here, five thiols with different functional groups: MPA, DHLA, L-cys, NAC and GSH were selected and used as the phase transfer reagents, based on the fact that the ligand exchange process was very complicated and ligand-dependent (that is to say, specific thiol ligands only work with specific dots without inducing excessive quenching) [27, 28]. The typical synthetic procedures for water-soluble PbS QDs were shown in Sect. 2.

In the QD phase transfer experiments, the thiol ligands—DHLA, L-cys and GSH are observed to be more efficient in transferring oil-soluble PbS QDs into water than MPA and NAC. Next, the PL and absorption spectra of the as-prepared water-soluble PbS QDs were further measured. The results show that highly fluorescent water-soluble PbS

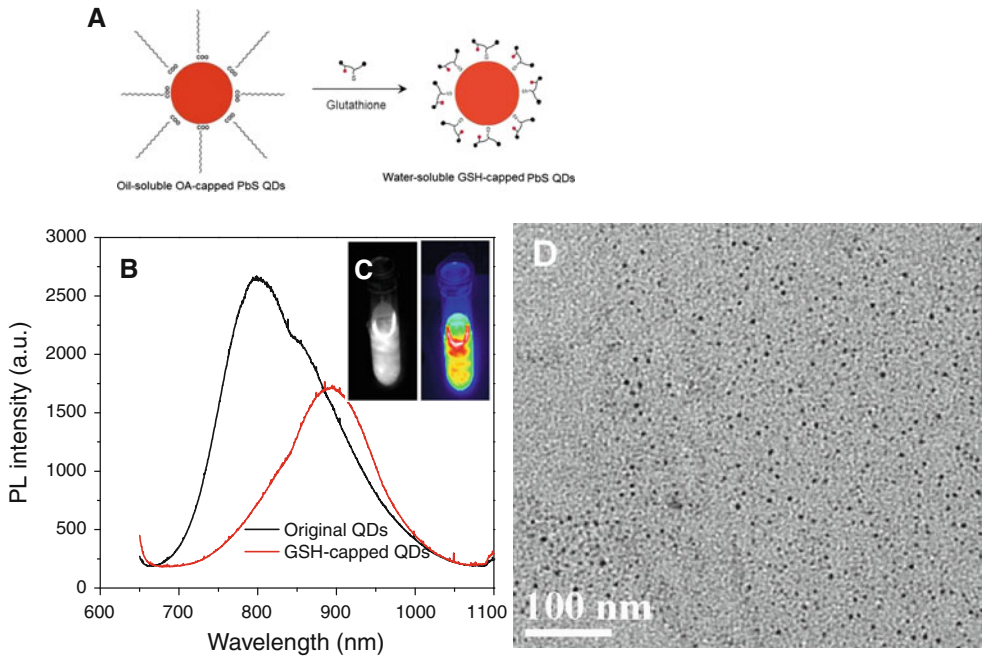

Fig. 5 **a** Detailed scheme for the phase transfer of oil-soluble PbS QDs into water via hydrophilic GSH. **b** PL spectra of PbS QDs before and after water transfer via GSH. **c** NIR fluorescence images of the resulting GSH-capped PbS QDs under radiation of NIR laser light (inset of (**b**)). **d** Typical TEM image of the resulting GSH-capped PbS QDs

QDs (PL QY, ~30%) could be obtained by using GSH as a surface-modifying agent (with L-cys, PL QY, ~5%), whereas with MPA, DHLA, or NAC, the resulting aqueous PbS QDs showed very weak or almost no fluorescence signal. Here, only the PL spectra of the original oil-soluble and the as-prepared water-soluble GSH-capped PbS QDs were shown in Fig. 5a. As shown, the emission intensity of QDs with GSH only decreased ~30%, as compared with that of the original organic dots; an obvious red shift in the emission peak (from initial 800 to 890 nm) also was observed. Meanwhile, the fluorescence images (Fig. 5c) of the resulting GSH-capped PbS QDs acquired by a NIR imaging system also supports the above spectral analysis, indicating that GSH is an appropriate agent for water transfer of OA-capped oil-soluble PbS QDs. Besides, TEM was employed further to characterize the morphology of PbS QDs prior to and following transfer into water via GSH. The result obtained shows that as compared to the initial oil-soluble PbS QDs, these aqueous ones only show a slightly poor size distribution and with a slight size increase (from 4 nm to 4.5 nm). These data might suggest that during the ligand exchange between initial surface oleic acid ligands and GSH molecules, the major structure of PbS QDs was maintained, i.e., few surface defects might be generated, although the mechanism is not known fully. Here, it should be mentioned that during storage at room temperature, the fluorescence intensity of QDs in water will recover to close to their initial value in chloroform within a week. Hence, GSH should be more efficient for water transfer of NIR-emitting PbS QDs than other thiol-containing small molecules reported in previous literatures [27, 28].

As described above, our results illuminate further that the surface ligands do play an important role in the process of water transfer of PbS QDs, and "correct" ligand (GSH) should be selected to yield highly fluorescent water-soluble PbS QDs. To explain the possible reason for this discrepancy, we compare their molecular structures, and observe that GSH has a different functional terminus compared to MPA, DHLA and NAC. These structural differences may be used to elucidate the vital effect of GSH on the water transfer of PbS QDs. Previous studies have reported the possibility of the secondary coordination between the carbonyl oxygen and (or) amino nitrogen atoms of the capped thiol ligands and the metal ion sites on QDs [35]. In this case, the carbonyl oxygen and amino nitrogen atoms all might participate in the secondary coordination with the surface Pb sites, decreasing the dangling bonds at QD surfaces to prevent nonradiative recombination at surface sites. However, here, in order to keep PbS QDs water-soluble and stable, a part of the carboxyl groups must be free and do not coordinate with Pb ions. Thus, amino nitrogen atoms seem to play a more crucial role in maintaining the original fluorescent property of QDs. Hence, the resulting water-soluble PbS QDs wrapped with GSH (or L-cys) would show more favorable fluorescence properties, compared to use of MPA, DHLA and NAC, because they two contain simultaneously the carboxyl and amino groups, although the mechanism is not known fully. Here, it should be mentioned that in the case of the QD transferred in water by the use of chitosan micelles, thiol-exposing molecules, etc., the stability of QDs might be decreased upon in protein-containing solutions, because thiol and amino functionality will facilitate the interactions of the QD with proteins present in the body fluids [2–5].

Caution: Although we did not observe the obvious biotoxicity of the PbS QDs for the organism when the injection dose is low, the in vivo imaging experiments of these QDs should be limited to the mice or other animal, and need further investigation in the integral cell toxicity.

4 Conclusion

Four different protocols for transferring oil-soluble NIR-emitting PbS QDs into water have been explored. Optical characterizations display that when using SOC micelles or GSH as phase transfer reagents, the resulting water-soluble PbS QDs show strong NIR fluorescence (PL QY more than 30%), whereas, in the case of nanohydrogels and amphiphilic polymers, the corresponding PL emission became relatively weak, especially for the PAA-based amphiphilic polymer-encapsulated PbS QDs. These experimental results indicate clearly that the "correct" phase transfer agents should be chosen to yield high quality water-soluble NIR-emitting PbS QDs. Meanwhile, the features of these PbS QDs studied are promising for NIR fluorescence imaging. Hence, the observations obtained here should be attractive for future bioapplications of oil-soluble NIR-emitting PbS QDs in preclinical medicine. That is, NIR-emitting PbS QDs could be used to in vivo trace drug-loaded micelles and nanohydrogels or image the bioactive molecules in small animals.

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