A general approach for encapsulating aqueous colloidal particles into polymeric microbeads

Yunhua Yang, Chifeng Tu and Mingyuan Gao*

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Herein we report a general synthetic approach, relying on the use of polymerizable surfactants as both phase transfer agents and emulsifiers, for encapsulating various types of aqueous colloidal NPs, independent of their chemical composition, in polystyrene microbeads.

Effectively encapsulating inorganic nanoparticles (NPs) with size-dependent properties in polymeric beads will not only offer protection to NPs against different harsh chemical environments,[1] but also provide unique physical properties to the resultant beads which could be useful for photonic applications,[2,3] sensors,[4] and high through-put bioassays for clinical applications as well as life sciences.[5–7] Several preparations have been reported by generating NPs *in situ* within microbeads,[8] or by loading preformed NPs into microspheres *via* electrostatic interactions,[9] hydrogen bonding[10] or hydrophobic interactions.[11] However, the former method lacks the ability to control the properties of the resultant NPs. Although the latter methods offer a better definition for the physical properties of the composite beads, in some harsh chemical environments the incorporated NPs tend to be released due to the lack of strong enough interactions between NPs and matrices.[9,11] Although the (mini)emulsion polymerization method has already been adopted in preparing polymeric beads doped with various NPs,[2–3,12] the beads obtained present poorly defined composite structures since the NPs aggregated and tended to fall out of the beads. Recently, through a ligand exchange followed by suspension or dispersion polymerization, robust beads were obtained with NPs covalently linked to polymer matrices.[13] Nonetheless, the polymerizable ligand and its exchange with that capped on the particle surface need to be specific for different types of NPs.

In contrast to all these methods, the synthetic route we report herein, as shown in Scheme 1, has been demonstrated to be a more general approach for encapsulating different types of aqueous colloidal NPs into polymeric beads with high glass temperature and hydrophobic nature, *i.e.*, polystyrene (PSt). The hydrophobicity of the bead matrix is expected to prevent various aqueous species as well as oxygen from diffusing into the composite beads and contacting the NPs incorporated, and consequently offers effective protection to them. To demonstrate the feasibility of our approach, fluorescent CdTe dots, superparamagnetic Fe_3O_4 and Au nanoparticles were chosen as the representatives of semiconductor, metal oxides and noble metal NPs, respectively.

Experimental section

The aqueous colloidal CdTe and Au NPs were synthesized according to previous reports.[14a,15] Fe_3O_4 NPs were synthesized using α,ω-dicarboxyl-terminated poly(ethylene glycol) as the surface stabilizing agent instead of monocarboxyl-terminated poly(ethylene glycol).[16] The recipes for CdTe and Fe_3O_4 particles were the same as those reported in references.[14a,16] The preparation of Au NPs was slightly modified. Firstly, 0.226 mL aqueous solution of $HAuCl_4$ (7.77×10^{-2} mol L^{-1}) was introduced into 60 mL water under stirring. Then, the resultant solution was boiled. Subseqently, 0.9 mL aqueous solution of citrate acid (7.74×10^{-2} mol L^{-1}) was added dropwise. After the reaction system was cooled down to room temperature, a colloidal sultion of Au particles was finally obtained and used directly in the following preparations. The average sizes of CdTe dots, Au and magnetic Fe_3O_4 particles were of about 3.8 nm,[14b] 13.1 ± 1.0 (s. d.: standard deviation) nm, and of 15.8 ± 2.0 (s. d.) nm, respectively.

Two polymerizable surfactants, *i.e.*, octadecyl-*p*-vinylbenzyldimethylammonium chloride (OVDAC) and didecyl-*p*-vinylbenzylmethylammonium chloride (DVMAC), were synthesized according to literature methods[17] and used in the synthesis of NP-doped polystyrene beads. Taking the synthesis of OVDAC for example, 2 mmol *N*,*N*-dimethylocatadecylamine and 2 mmol 4-vinylbenzyl chloride were firstly mixed in 5 mL acetone and then the resultant solution was maintained under stirring at 40 °C for 4 h. After that, the reaction mixture was cooled down to room temperature followed by precipitation and washing procedures using acetone. OVDAC was finally obtained by subjecting the white powder obtained in vacuum to a re-crystallization process using chloroform as the solvent and acetone as the precipitator, respectively. By similar procedures, DVMAC and a model surfactant, didecyl-*p*-ethylbenzylmethylammonium chloride (DEMAC), were also synthesized. The molecular structures of DVMAC and DEMAC were confirmed by ^{1}H NMR. The molecular structures of all surfactants except for Triton® X-100 are shown in Scheme 1.

The preparation of NPs@PSt (polystyrene beads incorporated with inorganic nanoparticles) beads followed the general procedures described in Scheme 1. DVMAC was chosen as the phase transfer agent. Typically, 0.03 g, 0.04 g and 0.001 g DVMAC were used to transfer the as-prepared CdTe, Fe_3O_4 and Au particles from 1 mL aqueous solution into 2 mL

Key Laboratory of Colloid, Interface Science and Chemical Thermodynamics, Institute of Chemistry, Chinese Academy of Sciences, Zhong Guan Cun, Bei Yi Jie 2, Beijing 100080, China.
E-mail: gaomy@iccas.ac.cn; Fax: (+86) 10-82613214;
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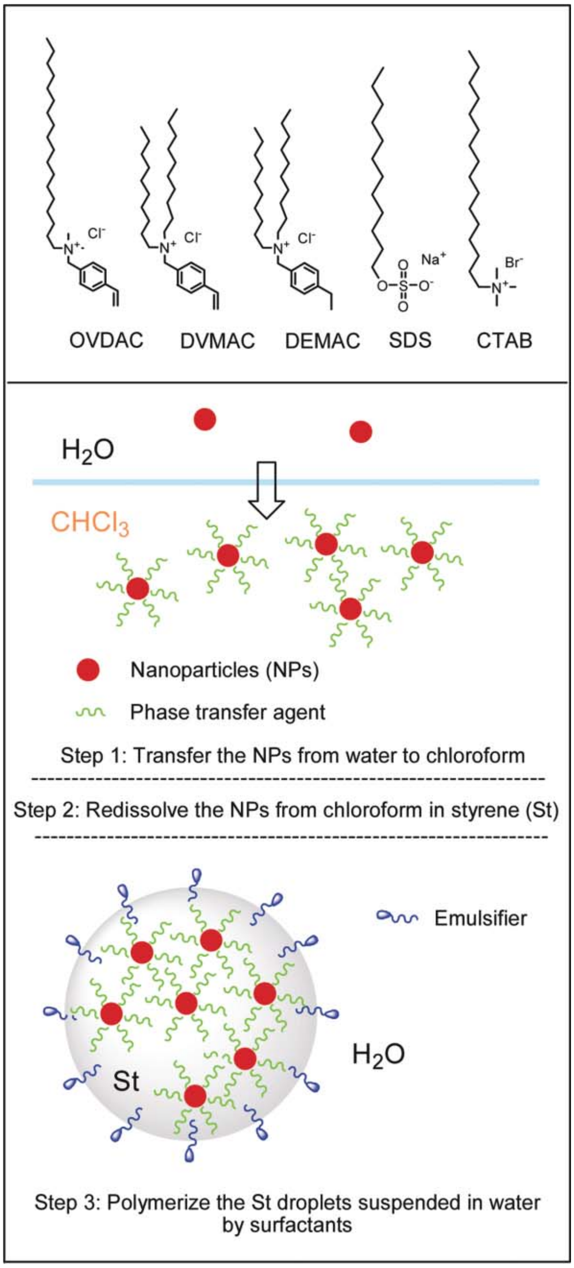

Scheme 1 The top frame shows the molecular structures and abbreviations for the surfactants used in the current investigations. The bottom frame depicts the general procedures for preparing nanoparticle-loaded polymeric beads by a miniemulsion polymerization method.

chloroform, respectively. By evaporating the chloroform, a solid of NPs was obtained and then re-dissolved in 2 mL styrene containing 0.015 g AIBN. Subsequently, 20 mL water containing 0.1 g OVDAC serving as emulsifier and 0.1 g Triton® X-100 as co-emulsifier were introduced. Under mechanical stirring, the oil phase was emulsified. After 30 min of deaeration by N_2, the reaction mixture was subjected to an oil bath at 80 °C and maintained at this temperature for about 6 h. After the polymerization, the polymeric NPs@PS microbeads were washed with water followed by centrifugation. The cleaning procedures were normally repeated more than 3 times.

The UV-vis absorption spectra were recorded with a Cary 50 UV-vis spectrophotometer. The fluorescence spectra were obtained with a Cary Eclipse fluorescence spectrophotometer. TEM images were recorded by a JEM-100CX II microscope operating at 100 kV. The confocal fluorescence image was 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) of 1.4. To better reveal the distribution of the NPs within the polymeric beads, the resultant beads were suspended in methyl methacrylate containing AIBN as an initiator. Upon polymerization the NPs@PS beads were embeded in poly(methyl methacrylate) (PMMA). The cross-sectional TEM samples were prepared by cutting the beads supported by PMMA into slices of about 50 nm by a microtome (LEICA ULTRACUT R). The morphology of the bead samples were characterized with a JEOL JSM-6700F field emission scanning electron microscope (SEM).

Results and discussions

Fig. 1 presents both absorption and fluorescence spectra of the CdTe dots and transmission electron microscopy (TEM) images of Au and Fe_3O_4 particles used in the current investigations.

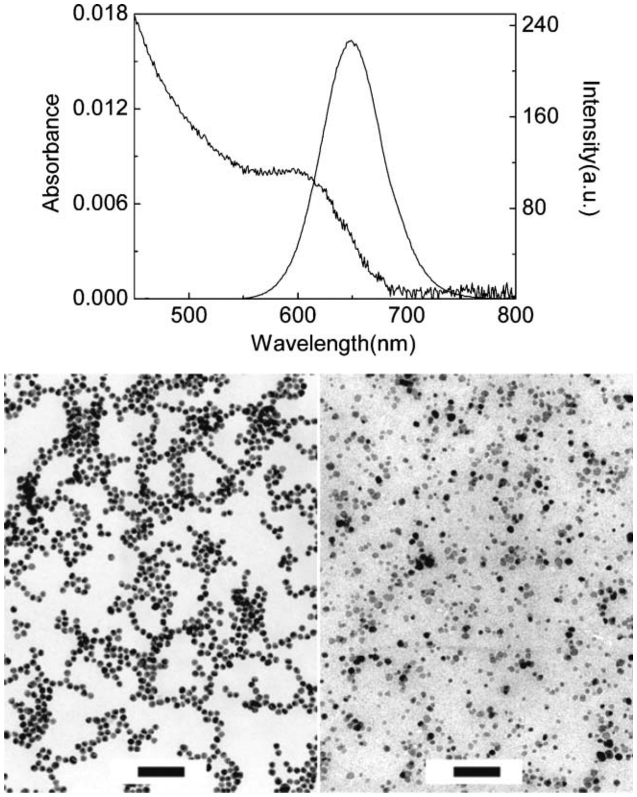

Fig. 1 Top: absorption and fluorescence spectra of the as-prepared CdTe dots in aqueous solution. The excitation wavelength was 400 nm for the fluorescence measurement. Bottom: TEM images of Au particles (left) and Fe_3O_4 particles (right) used for the preparation of Au@PSt and Fe_3O_4@PSt beads, respectively. The scale bar corresponds to 100 nm.

The top left image in Fig. 2 shows three typical aqueous dispersions of NPs@PSt beads, *i.e.*, CdTe@PSt, Au@PSt, and Fe_3O_4@PSt, prepared using DVMAC (didecyl-*p*-vinylbenzylmethylammonium chloride) as the phase transfer agent and OVDAC (octadecyl-*p*-vinylbenzyldimethylammonium chloride) as the emulsifier, respectively. Normally Triton® X-100, a nonionic surfactant, was used as the co-emulsifier of OVDAC to better emulsify the styrene/water immiscible system. The CdTe@PSt beads prepared in this way presented very strong fluorescence from the CdTe dots; the Fe_3O_4@PSt beads suspended in water exhibited strong magnetic response to a permanent magnet; the Au@PSt beads show the typical pink colour of the Au nanoparticles.

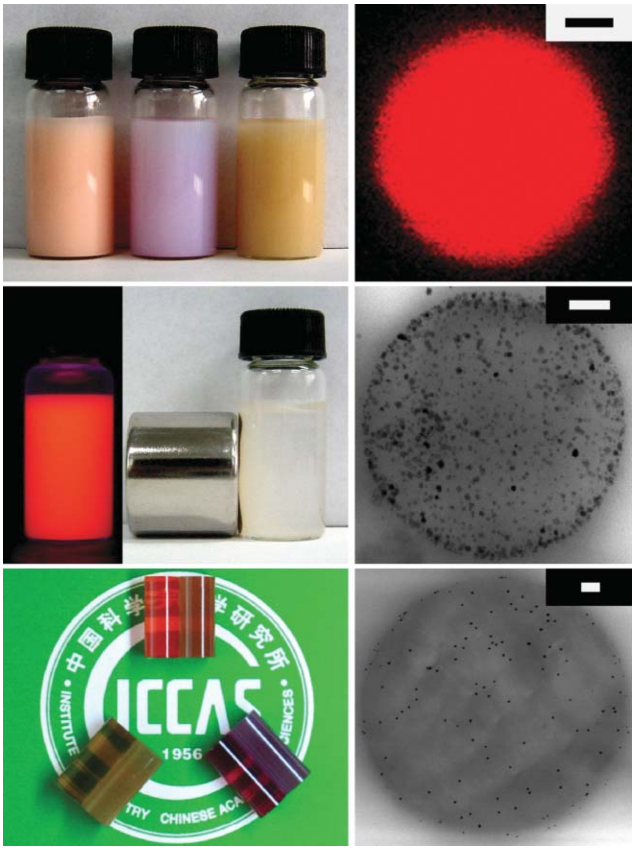

Fig. 2 The top left image was taken from aqueous dispersions of CdTe@PSt (left), Au@PSt (middle), Fe_3O_4@PSt (right) beads. The middle left images were taken from the CdTe@PSt bead solution under UV light (left) as well as the Fe_3O_4@PSt bead solution after it was put near to a permanent magnet for minutes (right). The bottom left image presents three pairs of NPs/PSt bulks prepared using DVMAC (left) and DEMAC (right) as phase transfer agents, respectively. The bulks shown clockwise are CdTe/PSt (top), Au/PSt and Fe_3O_4/PSt. The top right image was taken from a single CdTe@PSt bead by confocal fluorescence microscopy. The fluorescence efficiency of the CdTe@PSt bead was estimated to be 17%. The middle right and bottom right photographs are cross-sectional TEM images taken from thin slices of Fe_3O_4@PSt and Au@PSt beads, respectively. The scale bar in the right-hand images corresponds to 100 nm. The initial concentrations of CdTe, Fe_3O_4 and Au particles in the styrene solutions were 6.4×10^{-5} M, 1.1×10^{-6} M and 1.8×10^{-7} M, respectively. The concentrations of NPs were controlled on purpose in such a large range for testing the validity of the current synthetic route.

The internal structures of these beads were investigated by different methods. Due to the low electron density contrast between CdTe dots and the PSt matrix, as well as the smaller size of the CdTe dots in comparison with Au and Fe_3O_4 particles, the CdTe@PSt beads were firstly characterized by confocal fluorescence microscopy while the Fe_3O_4@PSt and Au@PSt beads were characterized by TEM. A typical confocal fluorescence image shown on the right-hand side of Fig. 2 suggests that the CdTe dots are evenly distributed throughout the PSt matrix. The cross-sectional TEM measurements reveal rather similar morphologies for both Fe_3O_4@PSt and Au@PSt beads although there is a certain percentage of NPs located near the bead surface.

A cross-sectional TEM image of the CdTe@PSt sample is shown in Fig. 3. A relatively small bead was selected for better showing the morphology of the "NPs in bead". However, due to the mechanical sheer force introduced by the microtome, the slice of the CdTe@PSt bead does not appear in a perfectly round shape. Nevertheless, the CdTe dots present a homogenous distribution within the bead matrix. The fluorescence of the resultant CdTe@PSt beads was measured and compared with that of the mother CdTe NPs. The results shown in Fig. 4 demonstrate that the emission position was not altered after CdTe NPs were incorporated in the polystyrene beads.

The general morpholgy of the CdTe@PSt, Au@PSt, and Fe_3O_4@PSt beads were also characterized by scanning electron microscopy. The SEM images together with the histograms of the resultant beads are shown in Fig. 5.

In general, to effectively incorporate inorganic NPs into polymeric beads two bottlenecks need to be overcome. The

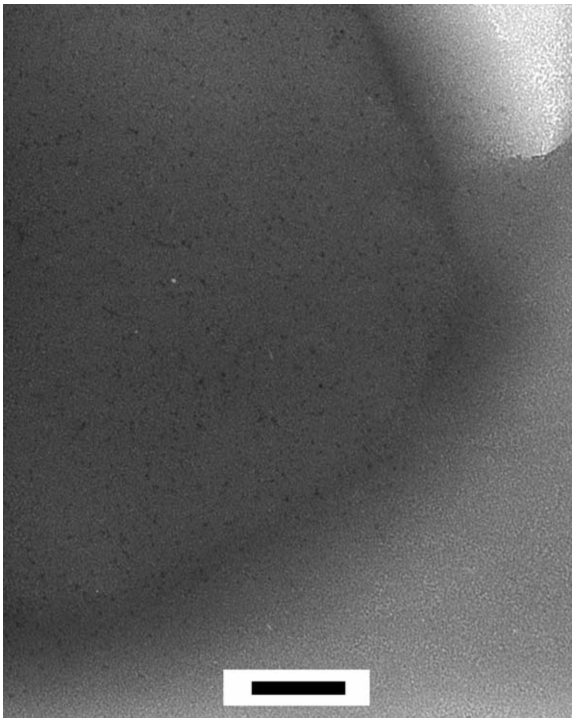

Fig. 3 Cross-sectional TEM image of a CdTe@PSt bead. The sample was prepared by cutting a CdTe@PSt bead embedded in a PMMA matrix into slices of about 50 nm. The scale bar corresponds to 100 nm.

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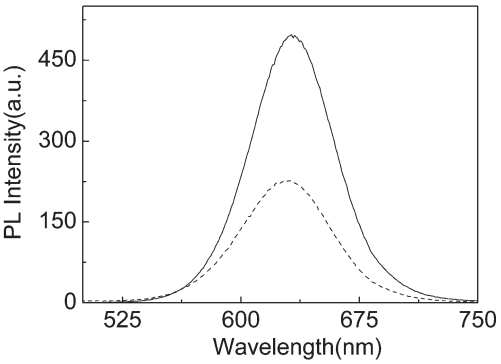

Fig. 4 Fluorescence spectra of an aqueous dispersion of CdTe@PSt beads (dashed line) together with the corresponding mother CdTe NPs (solid line) in water. The spectra were recorded by exciting the samples at 400 nm.

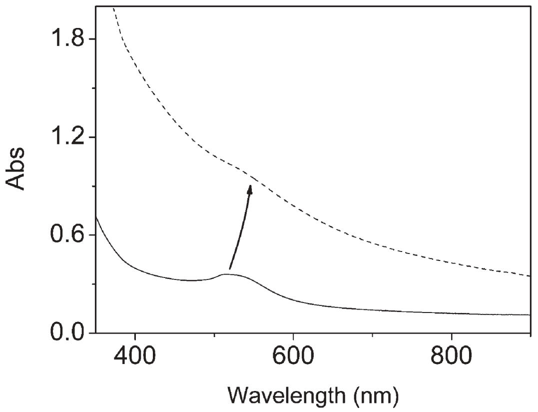

Fig. 6 Absorption spectra of the Au/PSt composite bulks prepared using either DVMAC (solid line) or DEMAC (dashed line) as the phase transfer agent. The absorption spectra were recorded in transmission mode using air as reference. The bulk samples for the current measurements were prepared by cutting the monoliths, shown in Fig. 2, into 1 mm thick disks, followed by a fine polishing on both sides.

first one is the aggregation of NPs in the polymer matrix. Our previous work has demonstrated that the use of polymerizable surfactant as a phase transfer agent can lead to uniform distribution of CdTe dots in the polymer matrix,[18] which is not only beneficial for preserving the fluorescence of the CdTe dots, but also necessary for preserving the physical properties of the Au and Fe_3O_4 particles. For example, the composite bulks obtained by polymerizing the styrene solution of NPs capped by DVMAC appear transparent as shown in Fig. 2 (bottom left image). Their colours generally resemble those of their mother aqueous solutions. In huge contrast, if non-polymerizable surfactant DEMAC was used instead of polymerizable DVMAC, opaque bulks were obtained due to obvious phase separation, although DEMAC is structurally similar to DVMAC except that its ethyl group is substituted by a vinyl group in DVMAC. Such phase separation is not only unfavourable for the fluorescence from CdTe dots, but also leads to a colour variation for the Au/PSt bulks. For further characterizing this effect, the composite Au/PSt monoliths shown in Fig. 2 were cut into 1 mm thick disks followed by a fine polishing on both sides. The absorption spectra of the Au/PSt disks were presented in Fig. 6. The sample prepared using DVMAC presents a sharp plasma absorption peak at around 511 nm. In contrast, the sample prepared using DEMAC presents broadened and a red-shifted plasma absorption peak, indicating the aggregation of Au NPs within the PSt matrix. In addition, due to the phase separation, it shows a very strong scattering effect in the non-absorption region of the Au NPs. These differences strongly support that phase separation could effectively be avoided using polymerizable surfactants as a phase transfer agent, which is important for preserving the physical properties of the NPs incorporated.

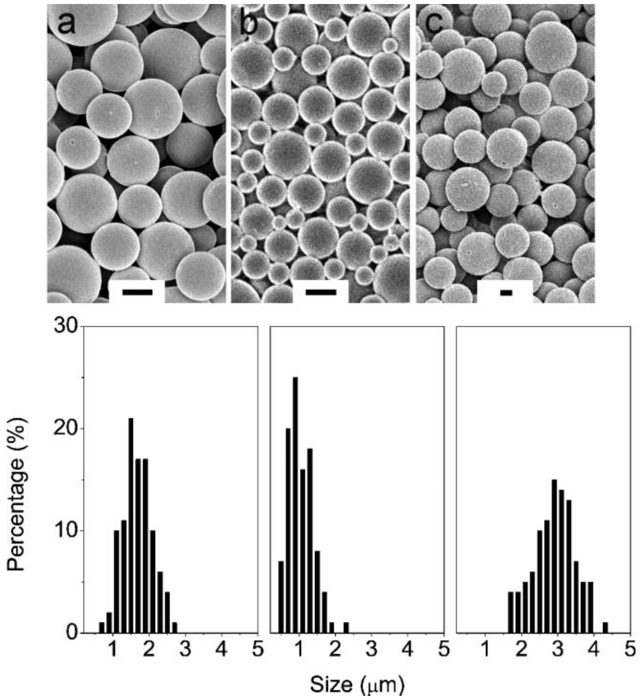

Fig. 5 Scanning electron micrographs of CdTe@PSt (a), Fe_3O_4@PSt (b) and Au@PSt (c) beads together with the corresponding histograms. The scale bar corresponds to 1 μm.

The second bottleneck for incorporating inorganic NPs into polymeric beads is to effectively encapsulate the NPs within the resultant beads. The use of polymerizable surfactants as emulsifier plays a very crucial role in this respect, which was demonstrated by the following experiments. To better illustrate the role of the polymerizable emulsifier, in the following experiments two conventional surfactants such as sodium dodecyl sulfate (SDS) and cetyltrimethylammonium bromide (CTAB) were used as the emulsifier, respectively, in combination with OVDAC serving as the phase transfer agent. However, the fluorescence of CdTe dots died out quickly in the SDS system shortly after initiation, which was quite possibly caused by the quenching effects of some intermediates generated upon reaction between free radicals and SDS. In contrast, the CdTe dots remained highly fluorescent in the

CTAB system, but almost all of them fell out of the oil droplets after the polymerization reaction was completed. Since the phase transfer agent OVDAC has a certain degree of solubility in water, it was supposed that OVDAC was redistributed between the aqueous and oil phases during the reaction. As a result, the number of OVDAC molecues assembled on the surface of the CdTe NPs decreased, consequently the CdTe dots were driven out of the oil phase. If this hypothesis was correct, reducing the aqueous solubility of the phase transfer agent would help the fixation of the CdTe dots in the beads. However, nearly no improvements were achieved when OVDAC was replaced by the less water soluble DVMAC in the CTAB system.

To better understand this failure, CdTe dots which fell out of the oil droplets emulsified by CTAB were carefully collected during polymerization and then cleaned three times with water. After drying under vacuum, the resultant solid was re-dissolved in chloroform and compared with those dots directly transferred from water to chloroform by DVMAC. As shown in Fig. 7, the absorption shoulder appearing at 614 nm for both samples can be assigned to the first excitonic transition of the CdTe dots, while the absorption band around 293 nm can be assigned to the absorption of the phenyl group in DVMAC. Therefore the absorbance ratio between the phenyl group in DVMAC and the CdTe dots can be used to estimate the number of surfactant molecules capped on each single CdTe dot. A quantitative comparison shows that this ratio drops to 62% for the CdTe dots falling out of the oil droplets in comparison with those dots directly transferred into chloroform, suggesting that the amount of DVMAC molecules caping on the surface of CdTe NPs decreased during polymerization and further indicating that DVMAC was partly replaced by CTAB at elevated temperature. As a result, most CdTe dots partly capped by CTAB aggregated during the polymerization and finally were expelled out of the styrene droplets. These results suggest that the polymerizability of the emulsifier DVMAC played a critical role in blocking the NPs from being expelled out of the oil droplets, which is also supported by the fact that there remain a certain percentage of NPs located near the bead surface, as shown in Fig. 2. However, those NPs remained firmly fixed within the composite beads as no NPs were extracted even after the exhausting washing procedures with water.

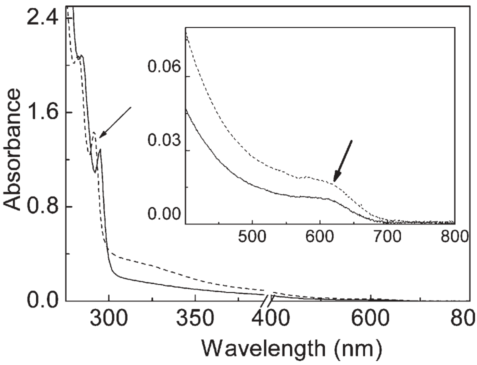

Fig. 7 Absorption spectra of chloroform solutions of CdTe dots transferred from water to chloroform using DVMAC (solid line) and those dots falling out of styrene droplets during polymerization (dashed line). Parts of these two spectra are enlarged in the inset to better show the first excitonic transition of the CdTe dots.

In summary, herein we report a general synthetic approach, relying on the use of polymerizable surfactants as both phase transfer agent and emulsifier, for encapsulating various types of aqueous colloidal NPs, independent of their chemical composition, into polymeric microbeads upon miniemulsion polymerization. Systematic investigations reveal that the use of polymerizable surfactants as the emulsifier plays the most important role in effectively encapsulating the aqueous colloidal particles into polymeric beads. The use of polymerizable surfactants as the phase transfer agents guarantees homogenous distribution of inorganic nanoparticles within the polystyrene matrix, and the consequent preservation of the native properties of the NPs within the polymeric beads. In brief, the current investigations have paved a very general route for creating functional polymeric beads whose properties can be tailored by the inorganic nanoparticles incorporated. Therefore, by encapsulating preformed high quality NPs, composite beads with clearly defined properties can certainly be expected. To place these results in a larger perspective, libraries of multifuncational beads, achieved by combinations of different types of inorganic nanocrystals within the beads, may find new applications in various fields.

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