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Fast and facile one-step synthesis of monodisperse thermo-responsive core-shell microspheres and applications

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Highly monodisperse PMMA microspheres covered with thermo-responsive shell were synthesized in a single step by means of photoinitiated RAFT dispersion polymerization at room temperature. Thermo-responsive Macro-RAFT agents (P(mPEGA-*co*-MEA)-TTCs) with different lower critical solution temperatures (LCST) were used as stabilizers in the process. Particle yields of over 95% were achieved within 45 min of UV irradiation, showing a fast process. The microspheres obtained were well-dispersed in water at a temperature below the LCST, and precipitated from the aqueous dispersion at a temperature above the LCST, exhibiting a reversible thermo-responsive property. The particle size can be precisely controlled by adding the monomer to the reaction in batches, and the microspheres maintained their high uniformities during the reaction. The thermo-responsive PMMA microspheres can be effectively conjugated to proteins via the carboxyl groups on the Macro-RAFT chains. A thermo-responsive PMMA/ZIF-8 core-shell structure was also synthesized by using P(mPEGA-*co*-MEA)-*b*-PAA stabilized PMMA microspheres as the template, and exhibited good adsorption properties for methyl blue.

Introduction

Functional polymeric microspheres continue to be an important class of polymeric microspheres and have attracted increasing attention over the past years.[1–6] This immense interest lies in their broad applications such as molecular imprinting, [7,8] antibacterial agents,[9,10] protein separation,[11,12] and emulsion,[13,14] which arise from their unique surface property. Thermo-responsive polymeric microspheres are of particular interest for catalyst carrier, biomolecule separation and cell culture scaffolds, and various methods have been developed to synthesize these particles, such as emulsion polymerization, [15–17] precipitation polymerization [5,7,8] and dispersion polymerization. [18,19]

As a facile and single-step strategy, dispersion polymerization is an attractive method to synthesize monodisperse polymeric microspheres in the range of 1 – 15 μm.[20,21] In dispersion polymerization, stabilizers play an important role in the synthesis of monodisperse polymeric microspheres.[20] The obtained microspheres are covered with the stabilizer, and disperse readily in solution. Therefore, using thermo-responsive polymers as the stabilizer in dispersion polymerization is a straight-forward method to prepare polymeric microspheres with a thermo-responsive shell. Chen et al.[22] used a poly(*N*-isopropylacrylamide) (PNIPAM) macromonomer as the stabilizer for dispersion polymerization of styrene in an ethanol-water mixture. They added silver nitrate at the beginning of the reaction and near monodisperse PNIPAM-coated polystyrene microspheres with silver nanoparticles on the surface were prepared. Fujii et al. [23] used a macroinitiator as the stabilizer and initiator for dispersion polymerization of styrene, and hairy polystyrene particles with poly[2-(diethylamino)ethyl methacrylate] (PDEA) on the surface were prepared. Mckee et al. [18] successfully synthesized thermo-responsive polystyrene microspheres based on alcoholic dispersion polymerization by using a series of well-defined poly (*N*-isopropylacrylamide) macromonomers as the stabilizers. In these cases, thermo-responsive macromonomers or macroinitiators were used as the stabilizers, giving a thermo-responsive shell to the microspheres. However, the size uniformity of the obtained particles was limited and the reaction time was long.

The use of Macro-RAFT agents as the stabilizer is another strategy to synthesize surface-functional polymeric microspheres by dispersion polymerization. However, because the nucleation stage of dispersion polymerization is very sensitive, it will be disturbed in the presence of the RAFT agent, leading to a broad particle size distribution. [18,24–26] Recently, we have developed a photoinitiated RAFT dispersion polymerization process which overcomes the problems stemming from the high sensitivity of the nucleation stage by combining the RAFT process with photoinitiation technique. [27] One of the attractive features of this approach is that Macro-

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RAFT agents can be directly used as stabilizers without consideration of the sensitive nucleation stage.[28] Thus, it is expected that monodisperse thermo-responsive microspheres can be synthesized by photoinitiated RAFT dispersion polymerization through the use of a thermo-responsive Macro-RAFT as the stabilizer.

In photoinitiated RAFT dispersion polymerization, both photoinitiation and RAFT control are crucial for obtaining highly uniform microspheres. Photoinitiated polymerization is widely used in the fields of coating, ink and adhesive, but scarcely applied to the large-scale synthesis of polymer materials due to the limited penetration depth of UV light. In view of the very fast reaction rate and the good flowability of the dispersion, photoinitiated dispersion polymerization can be designed as a tubular continuous process, so as to realize the large-scale synthesis of polymeric microspheres. However, the reaction rate was greatly slowed down when the RAFT process was introduced to the system; reaction time of 4h was normally needed for achieving high conversions.[27,28] In this case, the large-scale synthesis with a continuous process becomes difficult.

The present study was performed in an effort to synthesize highly monodisperse thermo-responsive microspheres under a high reaction rate. A series of thermo-responsive Macro-RAFT agents were prepared and used as the stabilizers in photoinitiated RAFT dispersion polymerization of methyl methacrylate (MMA). Highly monodisperse PMMA microspheres with thermo-responsive shells were synthesized at room temperature, and a particle yield of over 95% was achieved within 45 min of UV irradiation. We have also found that the microspheres were effectively conjugated to proteins via the carboxyl groups on the Macro-RAFT chains, which is critical for biomolecule separation. Furthermore, a thermo-responsive core-shell PMMA/zeolitic imidazolate frameworks-8 (ZIF-8) structure was prepared, which has potential applications in water treatment.

Experimental

Materials

Methyl methacrylate (MMA, Tianjin Kermel Chemical Reagents Development Center) was purified by distillation under reduced pressure and stored in a refrigerator prior to use. 4, 4′-Azobis(4-cyanovaleric acid) (ACVA, Aladdin), methoxy polyethylene glycol (350) acrylate (mPEGA, Sartomer), 2-methoxyethyl acrylate (MEA, Aladdin), Acrylic acid (AA, Aladdin), 2-methylimidazole (Aladdin), $Zn(NO_3)_3 \cdot 6H_2O$ (Aladdin), 2,2-dimethyl-2-phenyl-acetophenone (Darocur 1173, Ciba), *N*-Hydroxysuccinimide (NHS, Aladdin), fluorescein isothiocyanate conjugated bovine serum albumin (FITC-BSA, Sigma-Aldrich), and *N*-(3-Dimethylaminopropyl)-*N*'-ethylcarbodiimide hydrochloride (EDC, Aladdin) were used as received. The RAFT agent S-1-dodecyl-S′-(α,α'-dimethyl-α''-acetic acid) trithiocarbonate (DDMAT) was synthesized using the published procedure.[29]

Synthesis of thermo-responsive P(mPEGA-*co*-MEA)-TTCs

In a typical experiment for the synthesis of the Macro-RAFT agent, P(mPEGA-*co*-MEA)-TTC-2, a three-neck round bottom flask was charged with DDMAT (1.194 g, 3 mmol), ACVA (0.098 g, 0.6 mmol), mPEGA (30 g, 75 mmol), MEA (39 g, 300 mmol), and dissolved in 60 g ethanol. The sealed reaction flask was purged with nitrogen for 60 min and placed in a preheated oil bath at 70 °C for 24 h. The disappearance of ^{1}H NMR double band signals for the reaction mixture reveals all the monomers were consumed in the polymerization (Fig. S1). The Macro-RAFT agent was precipitated by excess diethyl ether, washed several times, and dried in a vacuum oven for 24 h. The obtained product was characterized by ^{1}H NMR (Fig. S2). Other thermo-responsive Macro-RAFT agents were synthesized by the same procedure using the ratios of mPEGA/MEA listed in Table 1.

Table 1. Thermo-responsive Macro-RAFT agents with different lower critical solution temperatures (LCST) synthesized by RAFT solution polymerization.

Macro-RAFT agents	n (mPEGA): n (MEA)	LCST (°C)	Mw	PDI
P(mPEGA-co-MEA)-TTC-1	35: 65	21	14000	1.46
P(mPEGA-co-MEA)-TTC-2	20: 80	43	17000	1.56
P(mPEGA-co-MEA)-TTC-3	10: 90	61	40000	1.67

Synthesis of P(mPEGA-*co*-MEA)-*b*-PAA-TTC

P(mPEGA-*co*-MEA)-*b*-PAA-TTC was synthesized by a one-pot reaction. In this case, after the synthesis of P(mPEGA-*co*-MEA)-TTC-2 as described above, a degassed solution contained AA (4.2 g, 58 mmol), ACVA (0.034 g, 0.12 mmol) and ethanol (6.0 g) was injected into the three-neck flask. The reaction was continued for another 8 h. The Macro-RAFT agent was precipitated by excess diethyl ether, washed several times, and dried in a vacuum oven for 24 h.

Photoinitiated RAFT dispersion polymerization using Macro-RAFT agents

In a typical experiment, an ethanol/water (7.2 g/10.8 g) mixture with a weight ratio of 40/60 was introduced into the reactor as the reaction medium, 10 wt% of monomer (MMA, 2 g) relative to the system, 15 wt% of the stabilizer (Macro-RAFT, 0.3 g) relative to MMA, 0.25 wt% of small molecular RAFT agent (DDMAT, 0.005 g) relative to MMA and 3 wt% of the photoinitiator (Darocur 1173, 0.06 g) relative to MMA were dissolved into the medium. The mixture was purged with N_2 for 15 min, sealed, and then irradiated by a 365 nm LED lamp (light intensity 15 mW/cm^2) from the top of the reaction cell at 25 °C for 1 h. The reaction mixture became slightly turbid after 40 s, and milky-white after 5 min. The product was precipitated by centrifugation, rinsed with an ethanol/water mixture (40/60, w/w) and centrifuged repeatedly. The washed product was dried in a vacuum oven for 24 h to give a fine powder, and then weighed for the reaction conversion calculations.

Photoinitiated RAFT dispersion polymerization in batches

The recipe and procedure of the first batch were the same as described above. After 1 h of UV irradiation, a mixture of reactants containing 1 g MMA, 0.03 g photoinitiator, 1.6 g ethanol and 2.4 g water was added to the reaction mixture as the second batch, and the system was irradiated for another 1 h. This procedure was repeated for the next batch. Samples were withdrawn at the end of each stage for SEM characterization.

Attachment of FITC-BSA to the microspheres

An aliquot of the PMMA particle (Entry 2) dispersion (20 µL, solids content 7.5 wt%) was washed twice with 600 µL MES buffer (pH 5.5, 100 mM), and then redispersed in 500 µL MES buffer. Then a solution of EDC and NHS in MES buffer (100 µL, containing 8 mg EDC and 22 mg NHS) was added to the vial with gentle vortexing for 25 min, and washed twice with 500 µL PBS buffer. The activated particles were redispersed in 500 µL PBS buffer, then 20 µL FITC-BSA solution (1 mg/mL) was added to the vial, and the sample was incubated with gentle vortexing for 3 h. The sample was then washed three times with PBS buffer.

As a control experiment, an aliquot of the PMMA particle (Entry 2) dispersion (20 µL, solids content 7.5 wt%) was redispersed in 500 µL PBS buffer, then a FITC-BSA solution (20 µL, 1 mg/mL) was added, and the sample was incubated with gentle vortexing for 3 h. The sample was washed three times with PBS buffer.

Synthesis of core-shell PMMA/ZIF-8 structures

A precursor solution was prepared by mixing 2-methylimidazole (0.83 g) and $Zn(NO_3)_3{\cdot}6H_2O$ (0.30 g) in an ethanol/water (16 g/21 g) mixture. A 3 g dispersion of P(mPEGA-*co*-MEA)-*b*-PAA stabilized PMMA microspheres (solids content 10 wt %) was then added to the precursor solution. The resulting mixture was dispersed by ultrasonic for 2 min and then placed in a pre-heated oil bath (70 °C) for 2 h. The product was precipitated by centrifugation, rinsed with an ethanol/water mixture (40/60, w/w) and centrifuged repeatedly.

Characterization

Morphological analysis was carried out on a JSM-6330F Field Emission Scanning Electron Microscope (FE-SEM) at 10 kV. Samples were dispersed in water and a drop of the solution was placed on a clean glass film. The samples were gold-coated prior to take the measurement. Image-Pro Plus 5.1 (Media Cybernetics) was used to analyze the SEM micrographs to determine particle diameters (number–average diameter d_n and weight–average diameter d_w) and size distributions (PDI = d_w/d_n). Transmission electron microscope (TEM) observations were carried out on a FEI Tecnai G2 Spirit instrument operated at 120 kV.

The molecular weight and polydispersity were determined by a Waters 1515 GPC instrument with tetrahydrofuran as the mobile phase. The flow rate of tetrahydrofuran was 1 mL/min. Narrow distribution linear polystyrenes were used as the standard to calibrate the apparatus, and the molecular weight of the samples were measured using a universal calibration.

The light transmittance of aqueous solutions of the Macro-RAFT agents was monitored at a wavelength of 500 nm on a PGENERAL TU-1901 UV/vis spectrophotometer. The operation was conducted from 18 to 60 °C at a heating rate of 0.2 °C/min.

The confocal fluorescence microscopy images of the polymer particles were obtained with a Leica TCS SP2 confocal laser scanning microscope.

XRD pattern was recorded on a Rigaku D/max-2200 X-ray diffractometer with Cu-Kα radiation (λ = 1.5406 Å)

Thermogravimetric Analysis (TGA) was performed using a TG-209/Vector-22 instrument under a stream of nitrogen. The dried PMMA/ZIF-8 particles were heated from 25 to 800 ^{o}C at a scan speed of 10 ^{o}C/min.

Results and discussion

Synthesis of thermo-responsive P(mPEGA-*co*-MEA)-TTCs

Although poly(N-isopropylacrylamide) (PNIPAM) is a representative thermo-responsive polymer and has been widely used in many research areas, it has some inherent disadvantages, such as significant hysteresis during the phase transition of PNIPAM. [30] Furthermore, hydrogen-bonding between the amide groups of PNIPAM and other polyamides, such as proteins, may limit the bioapplication of PNIPAM.[31] Moreover, the affinity of PNIPAM to alcohol/water mixtures is not good due to the cononsolvency property of PNIPAM.[32] Therefore, PNIPAM is not an efficient stabilizer in alcohol/water mixtures, especially in a fast process. For these reasons, we selected another thermo-responsive system in the present research. As reported by Lutz,[30] nonlinear PEG analogues (i.e., macromolecules constructed from oligo(ethylene glycol) macromonomers) can be made thermo-responsive by varying the length of oligo(ethylene glycol) side chains or by copolymerizing two monomers of different side chain lengths, and LCST of such polymers can be adjusted easily from 20 °C to 80 °C. This is a potential polymer material to be used in different areas including drug delivery, separation nanogels, and emulsions.[33–36] One of the attractive properties of the thermo-responsive PEG analogues is their very good affinity to alcohol/water mixtures which is beneficial to the fast synthesis of highly monodisperse microspheres via photoinitiated RAFT dispersion polymerization. In the present work, we synthesized a series of thermo-responsive Macro-RAFT agents via RAFT copolymerization of 2-methoxyethyl acrylate (MEA) and methoxy polyethylene glycol acrylate (mPEGA) using DDMAT as the RAFT agent. According to ^{1}H NMR, complete conversions were observed in all cases, thus the obtained macro-RAFT agents should have the same composition as expected. By varying the molar ratio of the two monomers, Macro-RAFT agents with different LCSTs were obtained (Fig. 1 and Table 1). It is noteworthy to mention that the obtained polymers have relatively broad molecular weight distributions, which may ascribe to the large scale of the RAFT solution polymerization and the steric constraint of mPEGA. We also found that the relatively high polydispersities of macro-RAFT agents have no effect on the preparation of uniform microspheres.

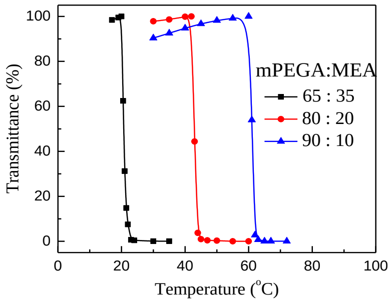

Fig. 1 Plots of transmittance as a function of temperature measured for aqueous solutions (10 mg/mL) of P(mPEGA-*co*-MEA)-TTCs.

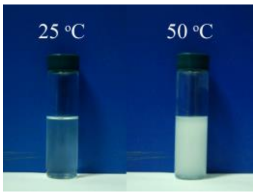

Fig. 2 Visual appearance of a 1.0 wt% aqueous solution of P(mPEGA-*co*-MEA)-TTC-2 at 25 °C and the same solution heated up to 50 °C.

The thermo-responsive behavior of a 1.0 wt% aqueous solution of P(mPEGA-*co*-MEA)-TTC-2 (LCST 43 °C) was briefly observed over temperature ranging from 25 to 50 °C. As shown in Fig. 2, the dilute aqueous solution of P(mPEGA-*co*-MEA)-TTC-2 was transparent when the temperature was below the LCST, and became turbid at a temperature higher than the LCST due to the precipitation of the polymer.

Synthesis of thermo-responsive PMMA microspheres

Thermo-responsive PMMA microspheres were synthesized by photoinitiated RAFT dispersion polymerization of MMA in the presence of a thermo-responsive Macro-RAFT agent. Considering that the Macro-RAFT agent contains a certain amount of RAFT groups, we first tried the procedure without the addition of any small molecular RAFT agent. Under UV irradiation, colloidally stable particles were obtained in a short period without any observable coagulum, and the particle yield reached 90% within 30 min of irradiation, exhibiting a very fast process. However, the size distribution of the final particles was broad (Fig. 3a), which is similar to what has been observed for conventional photoinitiated dispersion polymerization.[27,37] This may be attributed to an insufficient number of RAFT groups which leads to poor control during the nucleation stage.[27,28] To address this problem, a certain amount of small molecular RAFT agents was added into the reaction mixture to compensate for the low RAFT agent concentration. As shown in Fig. 3b, highly monodisperse PMMA microspheres with smooth surfaces were prepared in the presence of 15 wt% P(mPEGA-*co*-MEA)-TTC-2 and 0.25 wt% DDMAT. The results suggest that the RAFT control process is crucial for the procedure.

We then carried out the same procedure except that the stabilizer was replaced by another two Macro-RAFT agents with different LCSTs, P(mPEGA-*co*-MEA)-TTC-1 and P(mPEGA-*co*-MEA)-TTC-3, and highly monodisperse PMMA microspheres were also obtained (Fig. 3c and 3d). These results suggest that this method is a facile and versatile platform to synthesize highly monodisperse thermo-responsive polymeric microspheres with well-defined LCSTs.

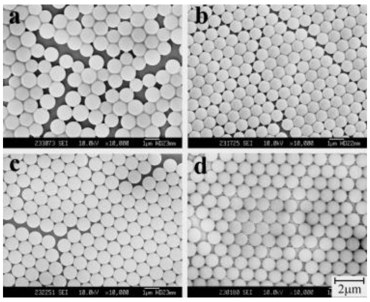

Fig. 3 SEM images of PMMA microspheres prepared by photoinitiated RAFT dispersion polymerization with (a) 15 wt% P(mPEGA-*co*-MEA)-TTC-2 (without DDMAT), (b) 15 wt% P(mPEGA-*co*-MEA)-TTC-2 and 0.25 wt% DDMAT, (c) 15 wt% P(mPEGA-*co*-MEA)-TTC-1 and 0.25 wt% DDMAT, and (d) 15 wt% P(mPEGA-*co*-MEA)-TTC-3 and 0.25 wt% DDMAT.

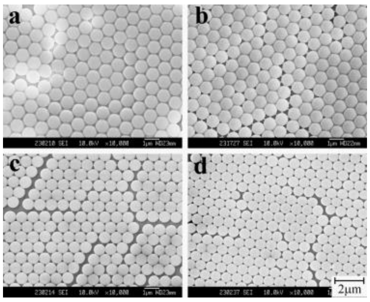

Fig. 4 SEM images of PMMA microspheres obtained by photoinitiated RAFT dispersion polymerization of MMA with (a) 10 wt%, (b) 15 wt%, (c) 20 wt% and (d) 25 wt% P(mPEGA-*co*-MEA)-TTC-2 in the presence of 0.25 wt% DDMAT.

Fig. 4 shows the SEM images of the PMMA microspheres prepared by photoinitiated RAFT dispersion polymerization with P(mPEGA-*co*-MEA)-TTC-2 concentrations ranging from 10 wt% to 25 wt% (Table 2, Entries 1-4). Monodisperse PMMA microspheres were obtained in all cases, indicating that one can easily tune up the thermo-sensitive layer of the microspheres in a wide range without disturbing the uniformity of the microspheres. Fig. 5 shows that the particle diameter decreased exponentially with increasing Macro-RAFT agent concentrations. This tendency is well-known for dispersion polymerization. A higher stabilizer concentration induces faster adsorption of the stabilizer, which reduces the aggregation of the particle nuclei, and results in a smaller final particle size. An exponent of -0.5 for the dependence of particle diameter on stabilizer concentration was predicted by Paine's theoretical analysis.[38] The exponent of -0.23 obtained in this work is obviously lower than the theoretical predicted value, suggesting that the particle diameter is not as sensitive to the stabilizer as it is in conventional dispersion polymerization. One possible factor related to this characteristic is the prolonged nucleation stage in photoinitiated RAFT dispersion polymerization. A longer nucleation period will give the nuclei more time to absorb the stabilizer, and thus results in a lower dependence on the stabilizer concentration.

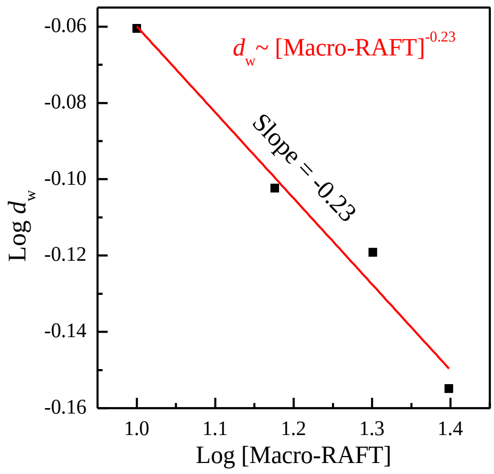

Fig. 5 Double logarithmic plot of d_w versus P(mPEGA-*co*-MEA)-TTC-2 concentration ([Macro-RAFT]) for PMMA microspheres obtained by photoinitiated RAFT dispersion polymerization of MMA in the presence of 0.25 wt% DDMAT.

Table 2. Recipes and particle size data for photoinitiated RAFT dispersion polymerization of MMA (10 wt% relative to the system) in the presence of DDMAT (0.25 wt% relative to MMA) and P(mPEGA-*co*-MEA)-TTC-2.

Entry	Macro-RAFT agent[a], wt%	Darocur 1173[a], wt%	Ethanol/water ratio, wt / wt	d_w, µm	CV[b], %
1	10	3	40/60	0.87	2.4
2	15	3	40/60	0.79	2.0
3	20	3	40/60	0.76	2.6
4	25	3	40/60	0.70	3.0
5	15	1	40/60	0.75	2.1
6	15	6	40/60	0.94	2.5
7	15	9	40/60	1.02	1.7
8	15	3	35/65	~	~
9	15	3	45/55	0.83	3.0
10	15	3	50/50	0.96	2.7
11[c]	15	3	40/60	0.97	5.7
12[d]	15	3	40/60	0.91	2.2
13[e]	15	3	40/60	0.91	0.7

[a] The concentration of Macro-RAFT agents and photoinitiator presented in the table is relative to monomer. [b] CV = $(1/n)\sum^{n}_{i=1}|(d_i - d_{av})|/d_{av}$, where d_{av} is the average diameter of all particles; [c] Without DDMAT; [d] P(mPEGA-*co*-MEA)-TTC-1 was used as the Macro-RAFT agent; [e] P(mPEGA-*co*-MEA)-TTC-3 was used as the Macro-RAFT agent

Formation Process of thermo-responsive PMMA microspheres

In a thermal initiated dispersion polymerization process, a reaction period up to 24 h is commonly used for achieving a high particle yield and forming uniform particles. [26,39–41] This time length was shortened to 3~4 h in our previous work by using the photoinitiated RAFT dispersion polymerization procedure.[27,28] And in the present research, we tried to further speed up the reaction via stronger UV irradiation (15 mW/cm^2 light intensity). With this change, particle yields of over 90% were achieved within 1 h in most cases, and the resulted microspheres were highly uniform. Therefore, it is valuable to investigate the formation process of the particles in such a fast procedure.

Nucleation and particle growth. In dispersion polymerization, the formation of particles goes through two stages: a nucleation stage and a particle growth stage.[21,27,39,42] During the growth stage, the particle size increases while the particle number keeps constant.[26,38,40,42] Therefore we can figure out the time length of the nucleation stage via monitoring the variation of particle size and number (Fig. 6). In the absence of DDMAT, the particle number underwent no significant change from 5 min to 60 min (Fig. 6a), indicating that the nucleation period is less than 5 min. In this case, the obtained particles were not uniform during the whole process (Fig. S3), which is similar to conventional photoinitiated dispersion polymerization with poly(N-vinyl-2-pyrrolidone) (PVP) as a stabilizer.[27,37]. When 0.25 wt% DDMAT was added into the reaction, the particle number increased at first, and then leveled off at 20 min (Fig. 6b), suggesting that the nucleation period of this procedure is about 20 min. The PDI (D_w/D_n) value changed from 1.16 at 5 min to 1.006 at 20 min, then slightly decreased to 1.001 at 60 min, showing that the particles were polydisperse during the nucleation stage, and became highly monodisperse during the particle growth stage. The result suggests that even though the high UV intensity induces a high reaction rate, the addition of small molecular RAFT agents can prolong the nucleation stage to an appropriate time scale, which is one of the features of photoinitiated RAFT dispersion polymerization.[27,28].

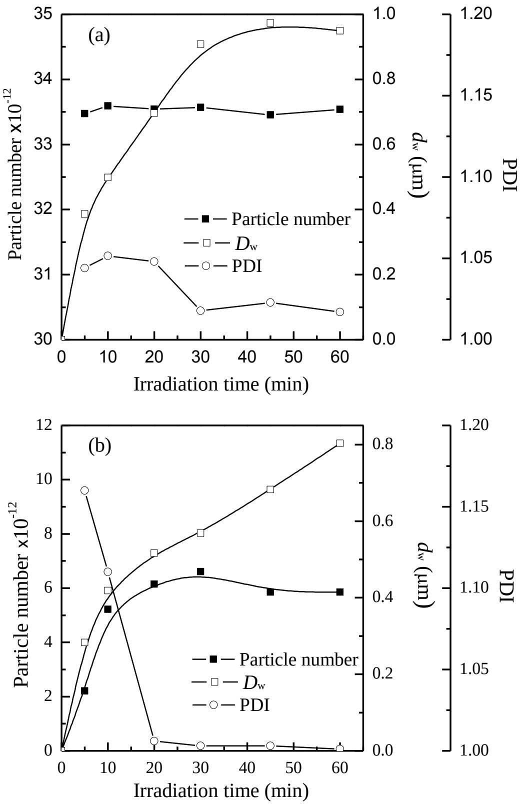

Fig. 6 Plots of particle number, weight-average diameter (d_w) and PDI versus irradiation time for PMMA microspheres prepared by photoinitiated RAFT dispersion polymerization in the presence of 15 wt% P(mPEGA-*co*-MEA)-TTC-2 without DDMAT (a) or with 0.25 wt% DDMAT (b).

Fig. 7 shows that some large spheres were formed at nucleation stage and then disappeared (recovered to a normal size) at growth stage. This is another feature of photoinitiated RAFT dispersion polymerization on particle formation and growth.[27,28] The large spheres are primary composed of polymer chains with a shorter chain length, and act as a short-chain reservoir at the nucleation stage, providing a buffering effect to the nucleation process.[27,28] They play an important role in obtaining uniform particles. The above results reveal that the procedure still kept the two crucial feathers even at a high polymerization rate, ensuring the formation of highly monodisperse microspheres. This encourages us to further accelerate the reaction.

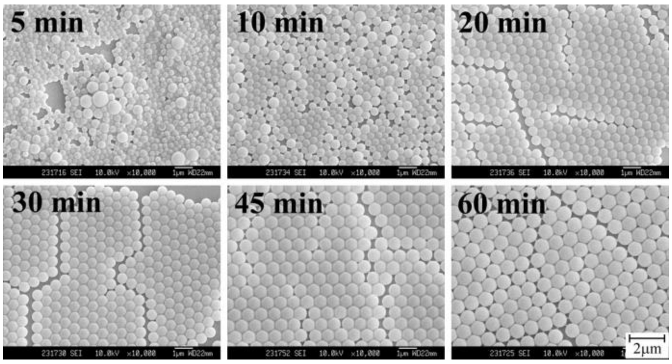

Fig. 7 SEM images of PMMA particles prepared by photoinitiated RAFT dispersion polymerization of MMA with 15 wt% P(mPEGA-*co*-MEA)-TTC-2 and 0.25 wt% DDMAT at irradiation time marked on the images.

GPC traces and control/"livingness" characteristic of the procedure. We have further followed the reaction process by GPC (Fig. 8). The samples were withdrawn at different reaction times and washed several times with ethanol/water (40/60, w/w) mixtures. The GPC curves are unimodal at the beginning of the reaction (5 min and 10 min). These signals can be assigned to homo-PMMA chains since their molecular weights are lower than that of the Macro-RAFT agent. At this stage, the block copolymer has a higher medium affinity and poorer anchoring ability on the particles due to the short PMMA block, and thus they can be washed out easily from the particles during the separation and purification processes. Therefore, no GPC signal for the block copolymer was found in this period. During the particle growth stage, the length of the PMMA blocks in P(mPEGA-*co*-MEA)-*b*-PMMA becomes longer, and the block copolymers can be adsorbed effectively by the particles. In this case, the PMMA homopolymers and the P(mPEGA)-*b*-PMMA block copolymers propagate synchronously in the particles. Correspondingly, the GPC curve shows a peak and a shoulder in the latter period, and eventually becomes bimodal, giving a broad molecular weight distribution (Table 3). The signal of the left shoulder may be attributed to the block copolymer, while the signal of the peak on the right should be derived from the homopolymer, since the molecular weight of the block copolymer is expected to be higher than that of the homopolymer.

Table 3 shows that the molecular weight polydispersity index (M_w/M_n) of the particles was higher than 2.0 in most cases during the process. This result suggests that the polymerization was not fully controlled by the RAFT agent. In addition to the presence of both small RAFT agent and macro-RAFT agent in the system, the low RAFT agent/initiator ratio is another primary reason for the broad molecular weight distribution. Although the RAFT control process is crucial for obtaining uniform microspheres, a high RAFT agent concentration is unfavorable because excessive control of the process will lead to low particle yields and polydisperse microspheres.[27] This is different from other RAFT process, e.g. polymerization-induced self-assembly (or named RAFT dispersion polymerization), in which high RAFT agent/initiator ratio is normally needed for obtaining highly controlled polymer chains and thus achieving desired product morphologies such as micelles, vesicles and nanogels.[43–45]

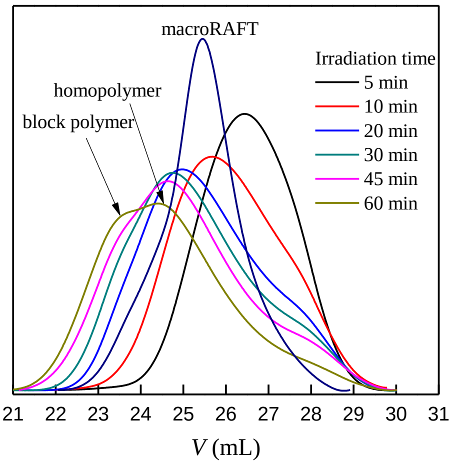

Fig. 8 GPC traces for photoinitiated RAFT dispersion polymerization of MMA in the presence of 15 wt% P(mPEGA-*co*-MEA)-TTC-2 and 0.25 wt% DDMAT.

Table 3 GPC results for photoinitiated RAFT dispersion polymerization of MMA in the presence of 15 wt% P(mPEGA-co-MEA)-TTC-2 and 0.25 wt% DDMAT at different UV irradiation time.

Irradiation time	M_n	M_w	M_w / M_n
5 min	5700	9000	1.59
10 min	6400	12700	1.98
20 min	8300	19400	2.32
30 min	9300	23700	2.55
45 min	10200	29500	2.86
60 min	13600	36900	2.72

Further speeding up the reaction

As mentioned above, the photoinitiated RAFT dispersion polymerization process was greatly sped up by using a strong UV irradiation, and the microspheres obtained in such a fast manner were still highly uniform. We were interested in what would happen if we further sped up the procedure. Increasing free radical content is a direct way to speed up free radical polymerization, which can be achieved by increasing photoinitiator concentration or by enhancing UV light intensity. For traditional dispersion polymerization, however, the initiator concentration is often limited to a narrow range if monodisperse polymeric microspheres are desired. Low initiator concentrations usually lead to low monomer conversions, while high initiator concentrations usually result in broad particle size distributions, and even secondary nucleation which leads to the formation of some small particles.[25,37,39,46]

In the present study, we varied photoinitiator concentrations ranging from 1 wt% to 9 wt% in photoinitiated RAFT dispersion polymerization under UV irradiation with a 15 mW/cm^2 light intensity. As shown in Fig. 9(a~d), highly monodisperse PMMA microspheres were obtained in all cases, and no small particles deriving from secondary nucleation was observed even at high photoinitiator concentrations. Similarly, the microspheres still kept highly uniform at a UV light intensity up to 80 mW/cm^2, as shown in Fig. 9f.

Fig. 10 shows that the polymerization rate was obviously enhanced at a higher photoinitiator concentration. In the cases of 6 wt% and 9 wt% photoinitiator, particle yields of over 95% were achieved within 45 min of UV irradiation. This is a fast rate for the synthesis of monodisperse polymeric microspheres.

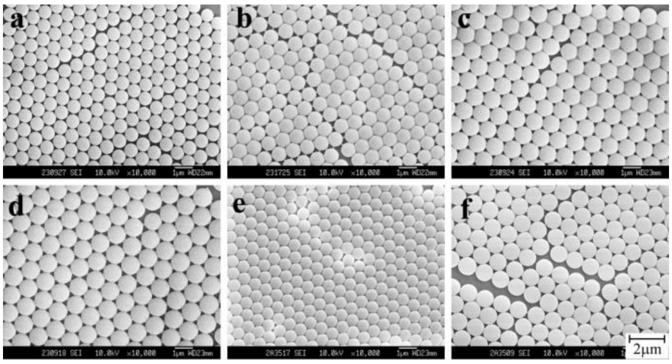

Fig. 9 SEM images of PMMA microspheres obtained by photoinitiated RAFT dispersion polymerization of MMA in the presence of 15 wt% P(mPEGA-*co*-MEA)-TTC-2 and 0.25 wt% DDMAT with photoinitiator concentration of (a) 1 wt%, (b) 3 wt%, (c) 6 wt%, (d) 9 wt%, (e) 3 wt% and (f) 3 wt%, and with UV intensity of (a)~(d) 15 mW/cm^2, (e) 1.8 mW/cm^2 and (f) 80 mW/cm^2.

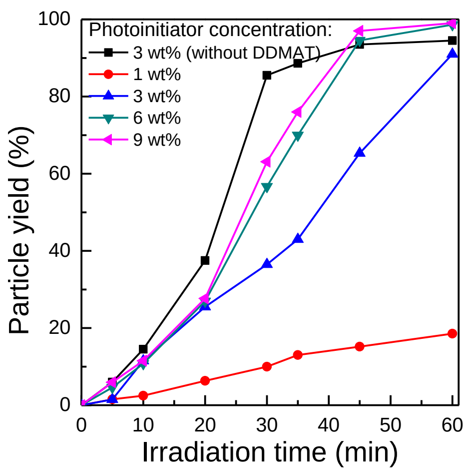

Fig. 10 Plots of particle yields versus time for photoinitiated RAFT dispersion polymerization of MMA in the presence of 15 wt% P(mPEGA-*co*-MEA)-TTC-2 and 0.25 wt% DDMAT with photoinitiator concentration marked on the figure.

Particle size control

As discussed above, by virtue of the insensitive nucleation process in photoinitiated RAFT dispersion polymerization, the crucial reaction parameters such as Macro-RAFT agent concentrations and photoinitiator concentrations could be adjusted in a wide range without disturbing the particle uniformity. Meanwhile, the particle size also became insensitive to the reaction parameters in comparison to traditional dispersion polymerization (Fig. 5 and Fig. S4). As a result, the diameter control via adjusting the reaction parameters will be limited.

Increasing monomer concentrations should be a direct way to increase the final diameter of the microspheres. However, the monomer concentration can only be adjusted in a narrow window (Fig. S5). To address this problem, we tried adding the monomer to the reaction in batches. It is expected that the particle volume should increase proportionally with the addition of monomers from batch to batch if no new particle is formed. As shown in Fig. 11, the microspheres maintained their high uniformity during the reaction, and the particle volume increased linearly with the total amount of MMA (Fig. 12). Thus, one can control the particle size precisely by adding the monomer in batches.

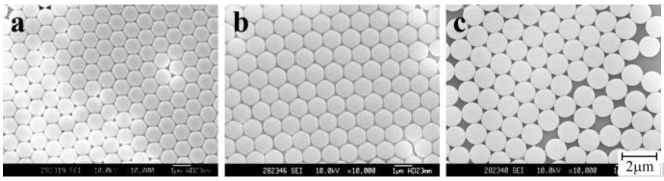

Fig. 11 SEM images of PMMA microspheres prepared by photoinitiated RAFT dispersion polymerization in an ethanol/H_2O mixture (40/60 wt/wt) with 15 wt% P(mPEGA-*co*-MEA)-TTC-2 and 3 wt% Darocur 1173. MMA was added in batches: (a) 2 g MMA (concentration 10 wt% relative to the system), irradiation 1 h; (b) 1 g MMA additional to the first batch, one additional hour of irradiation; (c) 1 g MMA additional to the second batch, one additional hour of irradiation.

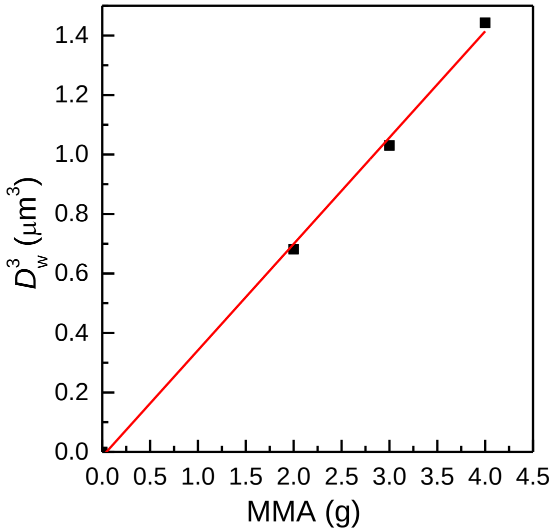

Fig. 12 Effect of the total amount of MMA added in photoinitiated RAFT dispersion polymerization on the final particle volume.

Thermo-responsive property of the microspheres.

In the dispersion polymerization process with a Macro-RAFT agent, the particles are stabilized by the block copolymers derived from the Macro-RAFT agents. When a thermo-responsive Macro-RAFT agent is employed, the obtained particles will be covered with a thermo-responsive layer (Fig. 13a). We have briefly investigated the thermo-responsive properties of the obtained microspheres in water, and the PMMA microspheres prepared with P(mPEGA-*co*-MEA)-TTC-2 (LCST, 43 °C) were selected as the example. As shown in Fig. 13b, the microspheres were well-dispersed in water at 25 °C. When the temperature was increased to 50 °C, the microspheres precipitated from the aqueous dispersion due to the collapse of P(mPEGA-*co*-MEA) polymers on the particle surface. This thermally-induced microsphere aggregation is fully reversible, since the sedimented aggregates could be redispersed completely at 25 °C. This reversible thermo-responsive property is very useful in the area of biotechnology, such as biomolecule separation.

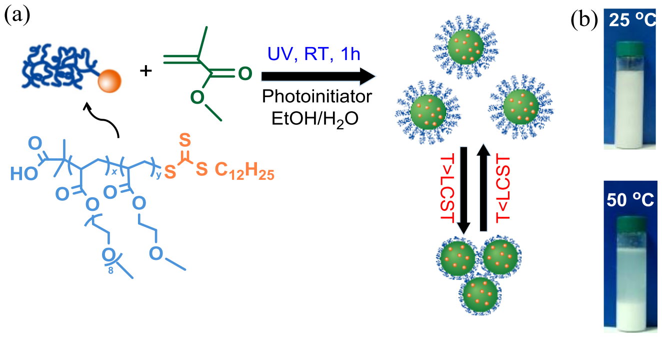

Fig. 13 (a) Formation of core-shell thermo-responsive PMMA microspheres via photoinitiated RAFT dispersion polymerization, (b) Visual appearance of 5.0 wt% aqueous dispersion of P(mPEGA-*co*-MEA)-TTC-2 stabilized PMMA microspheres at 25 °C and the same solution heated up to 50 °C.

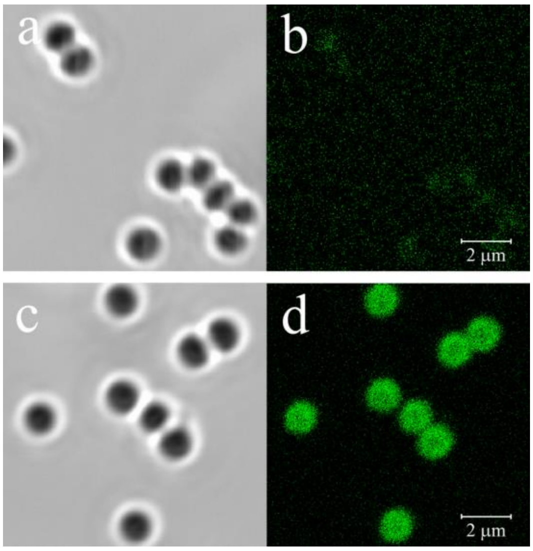

Fig. 14 Optical (a) and CFM (b) images of the particles after mixing with FITC-BSA without EDC activation; Optical (c) and CFM (d) images of the particles after mixing with FITC-BSA with EDC activation.

Applications of the microspheres

Protein attachment. RAFT polymerization is one of the most attractive methods to functionalize polymer chains by varying the functional end-group of the RAFT agent. In this case, DDMAT was used to prepare thermo-responsive Macro-RAFT agents, and the carboxyl group that was introduced to the end of the polymer chain can be used as the reactive site for bioconjugation.

In the present work, we tried to attach a fluorescent labelled protein, fluorescein isothiocyanate conjugated bovine serum albumin (FITC-BSA), to the surface of the thermo-responsive microspheres. When the microspheres were mixed directly with FITC-BSA, only weak fluorescence was observed, suggesting that only a few proteins were absorbed (Fig. 14b). When the carboxyl groups on the microsphere surface were activated with EDC [47] and then the microspheres were mixed with FITC-BSA, strong fluorescence was observed (Fig. 14d), showing that the protein molecules were effectively attached to the particle surface. These results suggest that the thermo-responsive PMMA particles prepared by photoinitiated RAFT dispersion polymerization can be surface-functionalized with biomolecules (e.g. antibodies, streptavidin and oligonucleotides), which has potential applications in bioassay and biomolecule separation.

Thermo-responsive core-shell PMMA/ZIF-8 particles. ZIF-8 is one of the most studied zeolite imidazolate frameworks, which has extensive applications in catalysis, water treatment and gas separation.[48–52] In the present research, we tried to synthesize thermo-responsive core-shell PMMA/ZIF-8 particles by using thermo-responsive microspheres as the template.

As a ZIF-8 template, the particles must be covered with carboxyl groups on the surface. This can be achieved by using a block copolymer P(mPEGA-*co*-MEA)-*b*-PAA-TTC as the stabilizer in the photoinitiated RAFT dispersion polymerization process. Fig. 15a shows the SEM image of PMMA microspheres prepared by photoinitiated RAFT dispersion polymerization with 15 wt % P(mPEGA-*co*-MEA)-*b*-PAA-TTC as the stabilizer. The obtained PMMA microspheres have a very narrow particle size distribution. These microspheres exhibit both pH- and temperature-responsive properties in water. Fig. 15(b~d) shows the SEM and TEM images of the obtained PMMA/ZIF-8 particles. It can be clearly seen that a large amount of nanoparticles were attached on the particle surface. The composition of the resulting particles were then analysed by XRD and TGA. Fig. 16a shows the XRD pattern of the core-shell PMMA/ZIF-8 particles, which clearly exhibited the sodalite topology of ZIF-8.[53] Fig. 16b shows the TGA result of the product performed under nitrogen. The weight loss of 7% in the range of 25 to 200 °C corresponds to the remove of guest molecules and water. A large weight loss in the range of 200 to 450 °C is ascribed to the decomposition of PMMA core. The weight loss in the range of 450 to 800 °C is ascribed to the decomposition of ZIF-8. Based on this result, we can know that the weight percentage of ZIF-8 in the core-shell structure is about 30%.

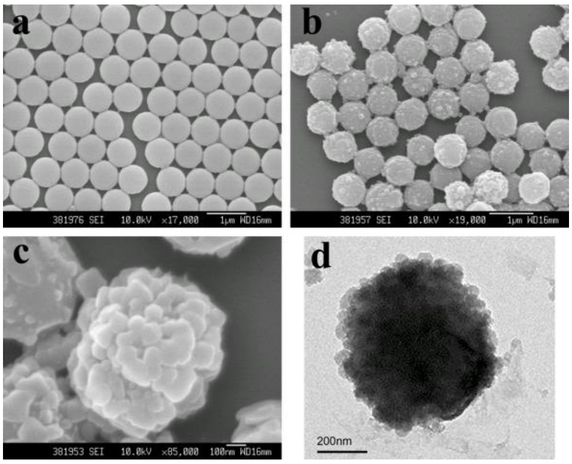

Fig.15 (a) SEM image of PMMA microspheres prepared by photoinitiated RAFT dispersion polymerization with 15 wt % P(mPEGA-*co*-MEA)-*b*-PAA-TTC as the stabilizer; (b) and (c) SEM images of PMMA/ZIF-8 particles; (d) TEM image of PMMA/ZIF-8 particles.

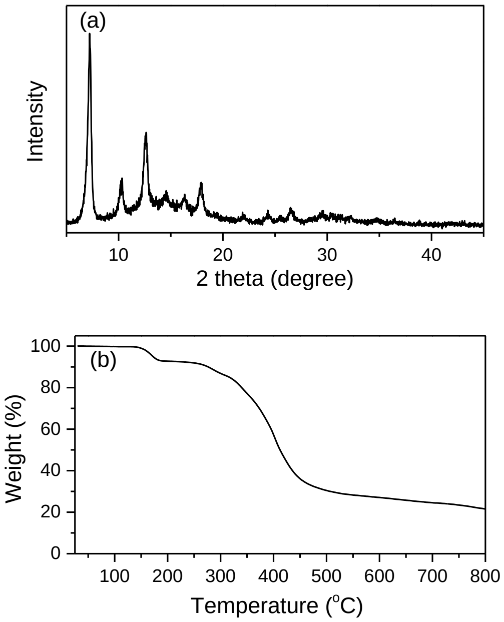

Fig 16. (a) XRD patterns of PMMA/ZIF-8 particles; (b) TGA curve for the PMMA/ZIF-8 particles.

The potential application of thermo-responsive PMMA/ZIF-8 particles in water treatment was also studied. Methylene blue (MB) is a typical organic contaminant in wastewater, which is usually used as a chemical dye in industry. ZIF-8 has been proved as an excellent adsorbent for the remove of MB in water. In this case, a certain amount of PMMA/ZIF-8 particles were directly added into the MB aqueous solution (10 mg/L), and stirred magnetically for 6 h at room temperature. Fig. 17a shows the visual appearances of the resulted solution, and the particles were relatively stable though a few aggregates were observed. The thermo-responsive property was briefly investigated by increasing the temperature from 25 ^{o}C to 60 ^{o}C, as shown in Fig 17b. It can be clearly seen that the particles precipitated from the solution and the supernatant was almost clear, which indicates the effective adsorption of MB by the PMMA/ZIF-8 particles. Bare PMMA particles were used as the control experiment, and the results are shown in Fig. 17c~d. Due to the presence of PAA polymer chains, the particles could only precipitate from the aqueous solution at 60 ^{o}C with a pH value of 3.0. And we found that the resulted supernatant was still blue, which suggests the limit adsorption of MB by bare PMMA particles. These results indicate that the obtained core-shell PMMA/ZIF-8 particles have potential applications for wastewater treatment, which can be easily isolated from the solution by just increasing the temperature. It is noteworthy to mention that the colloidal stability of these particles need to be further improved, e.g. longer P(mPEGA-*co*-MEA) block or shorter PAA block.

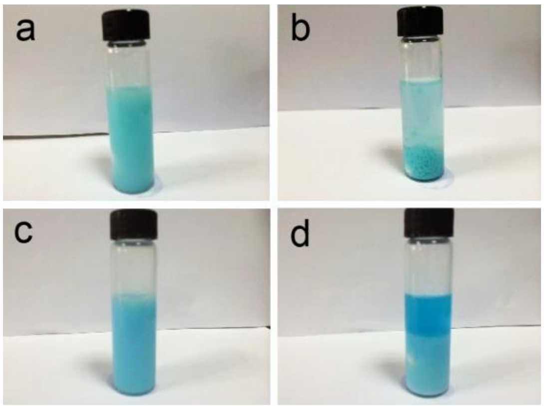

Fig 17. Visual appearance of (a) 10 mg/L aqueous solution of MB magnetically mixed with PMMA/ZIF-8 for 6h at 25 ^{O}C and (b) the same solution heated up to 60 ^{O}C; and a 10 mg/L aqueous solution of MB magnetically mixed with bare PMMA particles for 6h with a pH value of 3.0 at (c) 25 and (d) 60 ^{o}C.

Conclusions

Highly monodisperse PMMA microspheres with reversible thermo-responsive properties have been synthesized by photoinitiated RAFT dispersion polymerization using PEG-based thermo-responsive Macro-RAFT agents as the stabilizer. Particle yields of over 95% were achieved within 45 min of UV irradiation, demonstrating an ultra-fast procedure. The microspheres retained their uniformity within a wide range of Macro-RAFT agent concentrations and photoinitiator concentrations. The particle diameter can be adjusted precisely by adding the monomer in batches; the particle volume increased linearly with the total amount of MMA. It was also found that the microspheres could be effectively functionalized with proteins via the carboxyl groups on the Macro-RAFT chains, which is critical for further applications (e.g. bioassay or biomolecules separation). Thermo-responsive core-shell PMMA/ZIF-8 particles were also synthesized by using P(mPEGA-*co*-MEA)-*b*-PAA stabilized PMMA particles as the template, which could be used in watertreatment (e.g. remove of organic dyes). Based on features of photoinitiation including low reaction temperature, low energy cost and high reaction rate, this strategy is a promising alternative for the synthesis of bio-functional microspheres.

Acknowledgements

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Table of contents entry

Fast and facile one-step synthesis of monodisperse thermo-responsive core-shell microspheres and applications

Jianbo Tan, Mingguang Yu, Xin Rao, Jianwen Yang and Zhaohua Zeng*

Highly monodisperse PMMA microspheres covered with thermo-responsive shell were synthesized in a single step by means of photoinitiated RAFT dispersion polymerization at room temperature.