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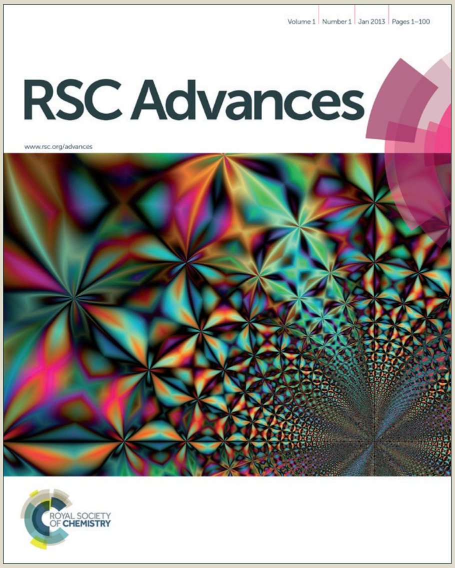

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Graphical abstract

Monodisperse Highly Cross-linked “Living” Microspheres Prepared via Photoinitiated RAFT Dispersion Polymerization

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

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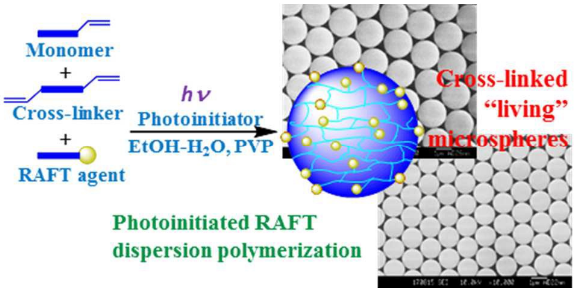

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ARTICLE

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

Cross-linked PMMA microspheres have been synthesized by photoinitiated RAFT dispersion polymerization with dipropyleneglycol diacrylate (DPGDA) or trimethylolpropane triacrylate (TMPTA) as the cross-linker. Monodisperse microspheres were obtained in the presence of 0.5 wt% S,S′-bis(α,α′-dimethyl-α″-acetic acid) trithiocarbonate (BDMAT, a RAFT agent) with DPGDA concentration up to 5 wt% or TMPTA concentration up to 2 wt%. The particle formation process was investigated, and the results reveal that the cross-linking reaction was retarded to a certain extent by the RAFT process, which is crucial for the formation of uniform particles under high cross-linker concentrations. The phase diagrams for the methodology have been built up, and show that the procedure could only give polydisperse microspheres at a low DPGDA concentration or coagulum at a high DPGDA concentration in the absence of a RAFT agent, and monodisperse microspheres were obtained at DPGDA concentration up to 5 wt% when the RAFT agent concentration reached an appropriately high level, e.g. 0.5 wt% BDMAT or 0.75 wt% DDMAT. By adjusting the reaction conditions, we have prepared monodisperse cross-linked PMMA microspheres with 10 wt% DPGDA added at the beginning of the reaction. Furthermore, we have functionalized the cross-linked microspheres by adding a functional comonomer to the system at the beginning of polymerization, or by surface-initiated RAFT polymerization via the RAFT groups attached on the particle surface.

Introduction

Monodisperse cross-linked polymeric microspheres have been widely used in a variety of applications, such as column packing materials for chromatography, growing seeds for seed emulsion polymerization, biomedical and biochemical analysis.[1–6] These microspheres are commonly synthesized by precipitation polymerization in the presence of a large amount of cross-linkers.[4,7–10] Precipitation polymerization does not require stabilizer nor surfactant, thus the obtained cross-linked polymeric microspheres have a "clean" surface. However, a low monomer concentration (less than 3 %) is usually required in precipitation polymerization, and the particle yield is low even with a long reaction time. In addition, a large amount of cross-linker is required to obtain monodisperse polymeric microspheres, which is not desired for some applications.[2] These drawbacks greatly limit the application of precipitation polymerization.

Dispersion polymerization is an attractive method to prepare monodisperse polymeric microspheres with high monomer concentrations, and has been given great interest over the past two decades.[11–17] However, the synthesis of cross-linked polymeric microsphere is still one of the major challenges for dispersion polymerization.[18–21] Tseng et al[18] reported a dispersion polymerization of styrene in ethanol with a small amount of divinylbenzene (DVB) added at the beginning of the reaction. When the amount of DVB was higher than 0.3 wt%, the particle size distribution became broad, and further increasing the amount of DVB resulted in coagulation. Two problems restrict the synthesis of cross-linked polymeric microspheres by dispersion polymerization: (i) the nucleation is easily disturbed by even a tiny amount of cross-linkers due to the sensitive nucleation stage of dispersion polymerization, resulting in particles with broad size distribution and even flocculation; (ii) the rapid consumption of cross-linker leads to the rapid formation of cross-linked network, which could restrict the growing particles to be swollen by monomer and thus disturbs the particle growth stage.[22]

For obtaining monodisperse cross-linked polymeric microspheres by dispersion polymerization, Choi et al.[23] carried out dispersion polymerization of styrene in an isopropyl alcohol/toluene mixture in the presence of cross-linkers. In this case, toluene was used to improve the solubility of polystyrene in the reaction medium and thus to achieve greater solvency for the growing particles in the reaction medium. However, monodisperse polymeric microspheres could only be obtained under precise conditions, and the concentration of cross-linker was limited to a low level. Winnik's group[22,24] developed a two-stage dispersion polymerization to synthesize monodisperse cross-linked polystyrene microspheres, in which the cross-linker was added after the nucleation stage so as to avoid the disturbance of cross-linkers to the nucleation, and the cross-linker concentration can reach 2 wt%. Further increase the cross-linker concentration was found to be difficult even using the two-stage procedure, because the fast formation of cross-linked network would restrict the growth of the particles via being swollen by monomers. To overcome this problem, they developed a multibatch or semi-continuous process in which the cross-linker was added gradually into the reaction system over 12 h after the nucleation stage. This method yielded monodisperse polystyrene microspheres containing up to 6 wt% cross-linkers. Several research groups have since used the two-stage method to synthesize various kinds of cross-linked microspheres. Wang et al[25] developed a two-stage dispersion polymerization of styrene by using a photoreactive cross-linker, and monodisperse photoreactive core-shell cross-linked/hollow microspheres were obtained based on this method. Lee et al.[26,27] have done extensive research on the preparation of highly cross-linked poly(methyl methacrylate) (PMMA) microspheres by two-stage batch and semi-continuous dispersion polymerization. Ke et al.[28] synthesized monodisperse "living" cross-linked polystyrene microspheres by using a two-stage atom transfer radical (ATRP) dispersion polymerization, and the obtained microspheres that can be further functionalized by surface-initiated ATRP polymerization. Peng et al.[29] synthesized monodisperse fluorescent cross-linked PMMA colloids in a water/methanol mixture based on a two-stage dispersion polymerization by adding cross-linkers and fluorescent dyes in the second stage. Sonawane et al.[30] synthesized a series of cross-linked fluorescent polystyrene microbeads with narrow size distribution by using perylene bismide (PBI) or oligo(p-phenylenevinylene) (OPV) acrylic cross-linker. Overall, a disadvantage of the two-stage dispersion polymerization is its relatively complicated procedure, and the particles are not homogenously cross-linked, which may limit its practical application. In addition, a long reaction period is required to obtain monodisperse cross-linked microspheres.

Reversible addition fragmentation chain transfer (RAFT) dispersion polymerization is an attractive method to prepare various functional polymer nanoparticles.[31–34] Recently, we have introduced RAFT radical polymerization into photoinitiated dispersion polymerization to control the nucleation stage, and monodisperse functional PMMA microspheres were obtained.[35,36] One of the attractive features of this approach is that the nucleation stage becomes robust; functional reagents can be added at the beginning without disturbing the nucleation. Furthermore, the slow chain growth of RAFT polymerization gives chains sufficient time for relaxation and diffusion, which facilitates the reaction centers in the polymerization system to contact with each other. This favours intermolecular cross-linking and suppresses the gelation.[37–41] Thus, the photoinitiated RAFT dispersion polymerization strategy is a possible approach to overcome the two problems for traditional dispersion polymerization on the synthesis of cross-linked microspheres as described above. It is more attractive that the RAFT groups on the cross-linked particles prepared by this strategy will give the particles "living" characteristic, and can be used to further modify the particles via surface-initiated RAFT polymerization. The preparation of various surface-functional polymeric microspheres based on "living" cross-linked polymeric microspheres has special applications in molecular imprinting area.[5,6,8,42,43]

In this paper, we focused on the synthesis of monodisperse cross-linked PMMA microspheres based on photoinitiated RAFT dispersion polymerization with cross-linkers added at the beginning of the reaction. Monodisperse PMMA microspheres with the cross-linker concentration up to 10 wt% were obtained in a single step at room temperature. The particle formation process was investigated, and the phase diagrams for the methodology have been built up. We have also carried out functionalization of the cross-linked PMMA microspheres via copolymerization of functional monomers or by surface-initiated RAFT polymerization.

Experimental Section

Materials

Methyl methacrylate (MMA, Tianjin Kermel Chemical Reagents Development Center) was purified by distilling under reduced pressure and stored in a refrigerator prior to use. Glycol methacrylate (GMA, Aladdin), methacrylic acid (MAA, Tianjin Kermel Chemical Reagents Development Center) and 2-hydroxyethyl methacrylate (HEMA, Aladdin) were purified by passing through a column of basic alumina prior to storage under refrigeration at 4 °C. RAFT agents S-1-dodecyl-S′-(α,α′-dimethyl-α″-acetic acid) trithiocarbonate (DDMAT) and S,S′-bis(α,α′-dimethyl-α″-acetic acid) trithiocarbonate (BDMAT) were synthesized using previously described methods[44], and their chemical structures are shown in Fig. 1. Poly (vinylpyrrolidone) (PVP, K30, number–average molecular weight 40,000; Shanghai BoAo Biologic Technology), 2-Hydroxy-2-methylpropiophenone (Darocur 1173, Ciba), dipropyleneglycol diacrylate (DPGDA, Sartomer), trimethylolpropane triacrylate (TMPTA, Sartomer), polyethylene glycol diacrylate (PEGDA200, Sartomer), and ethanol were used without further purification. All other chemicals used were analytical grade and used without further purification.

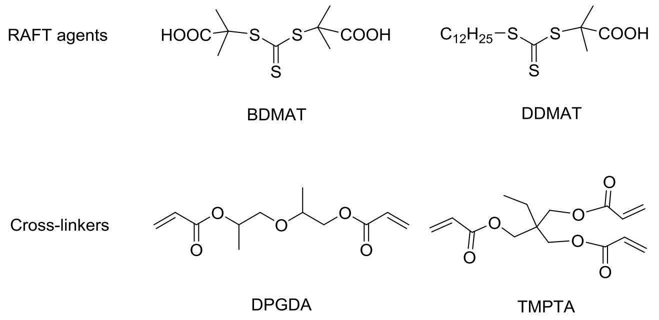

Fig. 1. Chemical structures of the RAFT agents and the cross-linkers used.

Synthesis of cross-linked PMMA microspheres based on photoinitiated RAFT dispersion polymerization

In a typical reaction, all reactants including stabilizer (PVP, 0.30 g, 15 wt% based on the total MMA), photoinitiator (Darocur 1173, 0.04 g, 2 wt% based on the total MMA), cross-linker (DPGDA, 5 wt% based on the total MMA), RAFT agent (BDMAT, 0.01 g, 0.5 wt% based on the total MMA) and monomer (MMA, 2 g) were dissolved in an ethanol/water (7.2 g/10.8 g) mixture to form a homogenous solution. The reactants were purged with nitrogen for 15 min and sealed. A 3-W LED lamp (365 nm, light intensity 0.8 mW/cm^2) was employed to irradiate the mixture from the top of the reaction cell. The mixture became turbid at about 2 min of UV irradiation, and the reaction period was proceeded for 4 h. The product was precipitated by centrifugation, rinsed with ethanol/water (40/60, w/w) mixtures, and centrifuged repeatedly to remove excess stabilizers. Then the products were dried in a vacuum oven for 24 h to gain fine powder, and weighed for calculating the reaction conversion. For the typical reaction of DDMAT, we added 4 wt% Darocur 1173 to ensure high reaction yields, and other reaction conditions are the same as described above. Table 1 lists the reaction conditions employed in this paper, and the particle sizes and coefficients of variation.

Table 1. Synthesis and characterization results for the polymeric microspheres prepared by photoinitiated RAFT dispersion polymerization of MMA under different reaction conditions.

Entry	RAFT agent^a	Cross-linker^a	PVP^a (wt%)	Darocur 1173^a(wt%)	Ethanol/H_2O ratio	D_{av}, µm	CV^b (%)
1	0.5 wt% BDMAT	1 wt% DPGDA	15	2	40/60	1.11	2.3
2	0.5 wt% BDMAT	2 wt% DPGDA	15	2	40/60	1.03	1.2
3	0.5 wt% BDMAT	5 wt% DPGDA	15	2	40/60	0.97	2.7
4	0.5 wt% BDMAT	10 wt% DPGDA	15	2	40/60	1.21	7.8
5	0.75 wt% DDMAT	1 wt% DPGDA	15	4	40/60	1.07	1.1
6	0.75 wt% DDMAT	2 wt% DPGDA	15	4	40/60	1.28	0.8
7	0.75 wt% DDMAT	5 wt% DPGDA	15	4	40/60	1.39	0.9
8	0.75 wt% DDMAT	10 wt% DPGDA	15	4	40/60		
9	0.5 wt% BDMAT	1 wt% TMPTA	15	4	40/60	0.89	1.6
10	0.5 wt% BDMAT	2 wt% TMPTA	15	4	40/60	0.86	4.9
11	0.5 wt% BDMAT	4 wt% TMPTA	15	4	40/60		
12	0.75 wt% DDMAT	1 wt% TMPTA	15	4	40/60	1.27	1.9
13	0.75 wt% DDMAT	2 wt% TMPTA	15	4	40/60	1.36	1.9
14	0.75 wt% DDMAT	4 wt% TMPTA	15	4	40/60		
15	0.75 wt% DDMAT	5 wt% DPGDA	5	4	40/60	1.87	3.9
16	0.75 wt% DDMAT	5 wt% DPGDA	10	4	40/60	1.64	1.1
17	0.75 wt% DDMAT	5 wt% DPGDA	20	4	40/60	1.85	1.4
18	0.75 wt% DDMAT	5 wt% DPGDA	25	4	40/60	1.13	2.5
19	0.75 wt% DDMAT	5 wt% DPGDA	15	2	40/60		
20	0.75 wt% DDMAT	5 wt% DPGDA	15	6	40/60	1.79	3.2
21	0.75 wt% DDMAT	5 wt% DPGDA	15	8	40/60	1.76	2.2
22	0.75 wt% DDMAT	5 wt% DPGDA	15	4	35/65	1.39	0.9
23	0.75 wt% DDMAT	5 wt% DPGDA	15	4	45/55	1.98	2.6
24	0.75 wt% DDMAT	5 wt% DPGDA	15	4	50/50		

^{a}The concentration of RAFT agents, cross-linkers, stabilizers and photoinitiators presented in the table is relative to monomer. ^{b} CV = $(1/n)\sum_{i=1}^{n}|(D_i - D_{av})|/D_{av}$, where D_{av} is the average diameter of all particles

Grafting polymer brushes onto the cross-linked microspheres via surface-initiated RAFT polymerization

Poly(2-hydroxyethyl methacrylate) brushes were grafted onto the PMMA microspheres based on the following procedure: 100 mg "living" cross-linked PMMA microspheres prepared by the typical recipe as described above (entry 7, Table 1) were dispersed in a 5 mL ethanol/water (40/60, w/w) mixture, then 0.9 g HEMA and 0.01 g AIBN were added to the reaction mixture. The reaction mixture was purged with nitrogen for 30 min to remove oxygen and then heated to 70 °C for 24 h. The obtained product was separated by centrifugation and washed with ethanol for three times.

Fluorescence labeling of polymeric microspheres with PHEMA brushes

The fluorescent labeling of polymeric microspheres with PHEMA brushes was done by esterification between the hydroxyl group of HEMA and the carboxyl group of Rhodamine B dye. 20 mg "living" cross-linked PMMA microspheres with PHEMA brushes were dispersed in 5 mL THF. Then 20 mg Rhodamine B, 8.57 mg DCC, and 1.71 mg DMAP were added to the dispersion. The mixture was stirred at room temperature for 24 h. The obtained product was separated by centrifugation and washed with THF for three times.

Gel content determination

The synthesized particles (weighed W_0) were immersed in THF and the mixture was stirred gently at room temperature for 24 h. The resulting mixture was then centrifuged, and the precipitate was collected, dried and weighed (W_1). The gel content was calculated from the expression: Gel content (%) = $(W_1/W_0) \times 100$

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 dropped onto a clean glass slide. The diameters and size distribution of microspheres were determined by analyzing SEM micrographs using Image-Pro Plus 5.1 (Media Cybernetics).

The fluorescence microscopy images of the polymeric particles were obtained with an inverted fluorescence microscope (Axio Observer Z1).

RESULTS AND DISCUSSION

Synthesis of cross-linked microspheres with a bifunctional cross-linker

Monomers containing two polymerizable double bonds are the most common used cross-linker for the synthesis of cross-linked polymeric microspheres. In the present work, we employed a typical bifunctional monomer, dipropylene glycol diacrylate (DPGDA), as the cross-linker in photoinitiated RAFT dispersion polymerization with 0.5 wt% BDMAT as the RAFT agent. The procedure was carried out under UV irradiation at room temperature, and the cross-linker was added at the beginning of the reaction. As shown in Fig. 2, highly monodisperse PMMA microspheres were obtained with DPGDA concentrations ranging from 1 wt% to 5 wt% (entry 1 to entry 3, Table 1). When the DPGDA concentration further increased to 10 wt% (entry 4, Table 1), the monodispersity of PMMA microspheres became poorer with a CV value of 7.8%. The results suggest that this one-step method allows us to prepare monodisperse cross-linked polymeric microspheres with the bifunctional cross-linker concentration up to 5 wt%. In a traditional one-step dispersion polymerization, however, the concentration of bifunctional cross-linkers is limited to a low level (normally less than 0.5 wt%) for obtaining monodisperse cross-linked polymeric microspheres. As a control experiment, photoinitiated dispersion polymerization without adding any RAFT agent was carried out with different amounts of DPGDA added at the beginning of the reaction. Polydisperse PMMA microspheres were obtained at low DPGDA concentrations (Fig. S1), and coagulum formed as the DPGDA concentration further increased to 5 wt%. It was concluded that the RAFT agent is crucial in the formation of monodisperse microspheres.

Synthesis of cross-linked microspheres with a trifunctional cross-linker

In principle, trifunctional monomer is a more effective cross-linker in comparison with bifunctional monomer. In dispersion polymerization, however, trifunctional monomer is rarely used as a cross-linker for preparing cross-linked particles, since it can induce fast formation of cross-linked network and thus seriously disturb the nucleation as well as the particle growth. In the present study, we tried to synthesize cross-linked PMMA microspheres by photoinitiated RAFT dispersion polymerization using TMPTA as the cross-linker. As shown in Fig. 3, highly monodisperse PMMA microspheres were obtained at TMPTA concentration up to 2 wt% (entry 9 and entry 10, Table 1). This is a very high content of trifunctional cross-linker used in a one-step dispersion polymerization process while forming monodisperse microspheres. When the TMPTA concentration increased to 4 wt%, only polydisperse PMMA microspheres were obtained with rough surfaces.

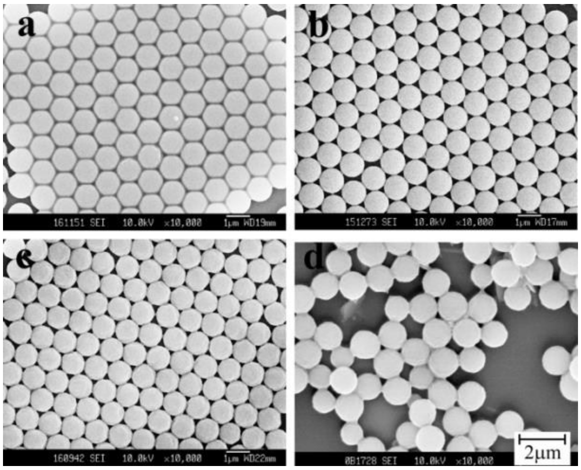

Fig. 2 SEM images of PMMA microspheres prepared by photoinitiated RAFT dispersion polymerization with 0.5 wt% BDMAT and with DPGDA concentrations of (a) 1 wt%, (b) 2 wt%, (c) 5 wt% and (d) 10 wt%. Other conditions were the same as the typical experiment.

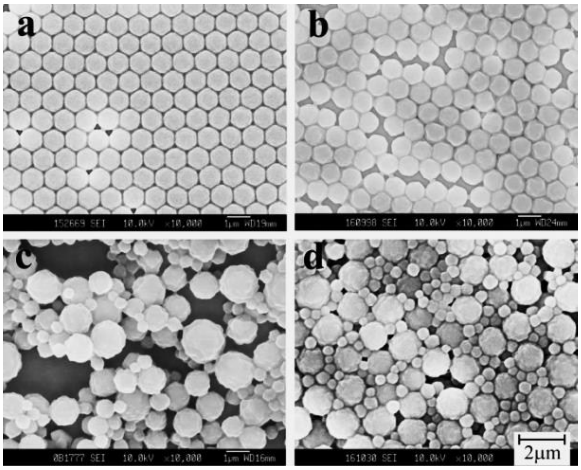

Fig. 3 SEM images of PMMA microspheres prepared by photoinitiated RAFT dispersion polymerization with 0.5 wt% BDMAT and with TMPTA concentrations of (a) 1 wt%, (b) 2 wt% and (c) 4 wt% and (d) 5 wt% (with 1.0 wt% BDMAT). Other conditions were the same as the typical experiment.

Formation of cross-linked particles

The above results indicate that photoinitiated RAFT dispersion polymerization is a facile one-step approach to synthesize monodisperse cross-linked polymeric microspheres with high cross-linking densities. We are interested in understanding how the particles form in the procedure at a high cross-linker concentration. We employed FE-SEM to monitor the morphologies of the polymeric microspheres obtained in the presence of 0.5 wt% BDMAT and 5 wt% DPGDA, and the results are shown in Fig. 4. At early stage, small particles and some extremely large spheres were formed. As the reaction proceeded, the big spheres disappeared and the particles tended to be uniform. This phenomenon was also observed in photoinitiated RAFT dispersion polymerization in the absence of cross-linkers.[35] The nucleation stage in photoinitiated RAFT dispersion polymerization involves the formation of pseudo-nuclei and real nuclei. The pseudo-nuclei, which formed from the initiator-derived polymer chains, absorb the RAFT controlled polymer chains and form large spheres. These large spheres act as a short-chain reservoir and provide a buffering effect to the nucleation process. They disappear at the end of nucleation stage. Meanwhile, controlled polymer chains reach the critical chain length and precipitate to form the real nuclei. In the case of photoinitiated RAFT dispersion polymerization with cross-linker, the occurrence and disappearance of the big spheres tell us that the cross-linking density of the polymer chains was low at the early stage of the reaction. Importantly, this reveals that the cross-linking reaction was retarded to a certain extent by the RAFT process. In the RAFT system, the cross-linking reaction occurs more randomly at early stage due to the lower chain length and higher concentration of primary chains. This condition leads to a more homogeneous solution,[45] and consequently prevents the cross-linker from disturbing the nucleation process. The time point of the big spheres disappearance (20 min in this case) can be regarded as the end of nucleation stage and the beginning of the growth stage.[35]

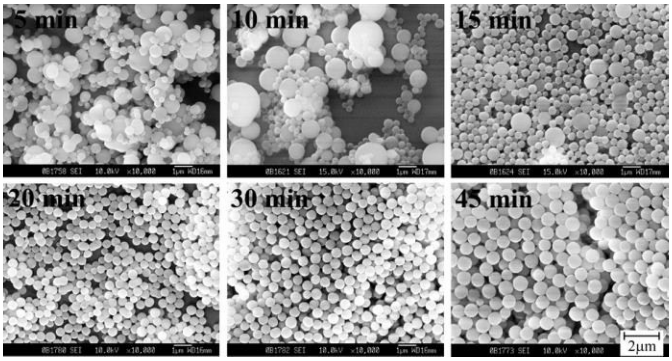

Fig. 4 SEM images of PMMA microspheres obtained by photoinitiated RAFT dispersion polymerization of MMA with different reaction times (marked in the image 5 min, 10 min, 15 min, 20 min, 30 min, 45 min). The reaction conditions were the same as the typical experiment with 0.5 wt% BDMAT and 5 wt% DPGDA.

The cross-linking degree of polymers can be expressed by the gel content as described in the experimental section. We have tested the gel content of the particles prepared by photoinitiated RAFT dispersion polymerization with 0.5 wt% BDMAT and 5 wt% DPGDA at different irradiation times. As shown in Fig. 5, the gel content was very low during the nucleation stage, supporting that the cross-linking reaction was effectively retarded by the RAFT process, as mentioned above. When the process went into the growth stage at 20 min of irradiation, the gel content increased immediately to a high level and then reached 100 wt% at 30 min, suggesting that the particles were fully cross-linked in the growth stage. It is noticed that the particles still grew steadily and tended to be uniform under such a high degree of cross-linking. This may attribute to the more homogeneous networks, and thus higher swelling ability. Due to this, monomers and propagating chains are able to enter the growing particles to form uniform particles. Fig. 6 shows the plots of particle yield versus reaction time for the photointiated RAFT dispersion polymerization of MMA with 0.5 wt% BDMAT in the presence of different amounts of DPGDA. In comparison with the procedure without adding DPGDA, the reaction rate of the procedure with 2 wt% DPGDA slightly increased. This may ascribe to the faster chain extension in the presence of cross-linkers, since the acrylate group in DPGDA cross-linker has a higher polymerization reactivity compare to the methacrylate group in MMA monomer, and the presence of cross-linkers also facilitate the formation of polymer networks. Meanwhile the reaction rate decreased when the concentration of DPGDA increased to 5 wt%. This may be attributed to the high cross-linking density of the particles at a high cross-linker concentration. In this case, the particles cannot be swollen effectively by monomers during the particle growth stage, and the polymerization is more likely to proceed in the reaction medium. In general, the polymerization in the reaction medium is slower than that in the monomer-swollen particles due to the lower monomer concentration of the former. Therefore the reaction rate decreased as the DPGDA concentration further increased to 5 wt%.

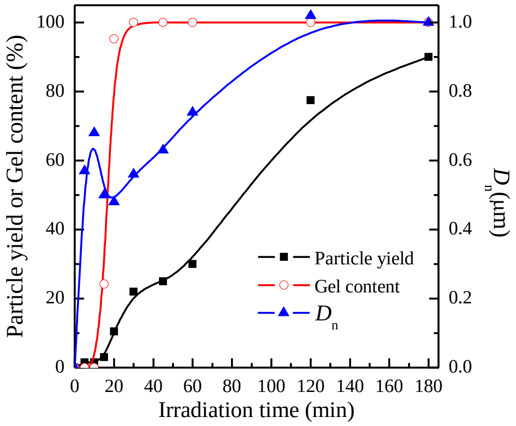

Fig. 5 Plots of gel content of PMMA microspheres versus time and conversion versus time for photoinitiated RAFT dispersion polymerization of MMA with 0.5 wt% BDMAT and 5 wt% DPGDA

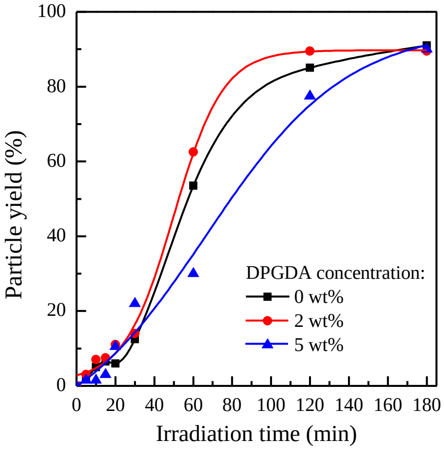

Fig. 6 Plots of MMA versus irradiation time for the photoinitiated RAFT dispersion polymerization of MMA in the presence of 0.5 wt% BDMAT with different DPGDA concentration (marked in the graph), and the other reaction conditions were the same as the typical experiment.

Phase diagrams of photoinitiated RAFT dispersion polymerization in the presence of cross-linkers

Phase diagram based on BDMAT. The above results show that monodisperse cross-linked microspheres can be prepared via photoinitiated RAFT dispersion polymerization by adding cross-linkers at the beginning. In some cases, however, the procedure can only produce polydisperse particles or even coagulum. The morphology of the product is primarily affected by the RAFT agent concentration and the cross-linker concentration. Therefore we constructed a phase diagram based on the variations of BDMAT concentrations and DPGDA concentrations (Fig. 7). It shows that photoinitiated dispersion polymerization of MMA without adding any RAFT agent could only give polydisperse microspheres at low DPGDA concentrations, or coagulum at high DPGDA concentrations. When 0.25 wt% BDMAT was used in the procedure, monodisperse microspheres were obtained at a DPGDA concentration of 2 wt% or lower, and polydisperse particles or even coagulum were formed at a high DPGDA concentration (5 wt%). When the BDMAT concentration increased to 0.5 wt%, monodisperse microspheres were obtained at a DPGDA concentration up to 5 wt%. These results strongly demonstrate that the RAFT agent is crucial for keeping the system stable and forming uniform particles, especially at a high cross-linker concentration.

When the BDMAT concentration reached 1 wt%, polydisperse microspheres were obtained at high DPGDA concentrations (5 wt% and 10 wt%). This may be related to the effect of RAFT process on the formation of the graft copolymer PVP-*g*-PMMA. In the procedure, PVP is grafted with some PMMA chains and then acts as a stabilizer to stabilize the particles. In the presence of RAFT agents, the propagation of PMMA chains will slow down, and thus the chain length of grafted PMMA on PVP will be shortened compared to the same scenario using a conventional dispersion polymerization. As a result, the absorption of PVP-g-PMMA by the particles will be more difficult, affecting the stabilization of PMMA particles. This phenomenon will be more obvious at a high RAFT agent concentration and a high cross-linker concentration. According to our previous research on photoinitiated RAFT dispersion polymerization,[36] the PVP content on the particle surface increased with increasing BDMAT concentration and reached maximum at 0.5 wt% BDMAT, and then decreased as the amount of BDMAT further increased to 1.0 wt%. This suggests that the particles have the best stability at 0.5 wt% BDMAT.

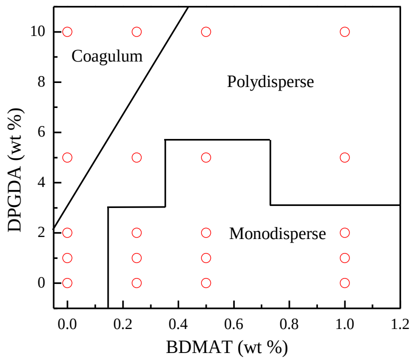

Fig. 7 Phase diagram for cross-linked PMMA products obtained by photoinitiated RAFT dispersion polymerization at various DPGDA concentrations and BDMAT concentrations. The other reaction conditions were the same as the typical experiment. The corresponding SEM images are shown in Fig. S3.

Phase diagram based on DDMAT. We then constructed another phase diagram of PMMA particles synthesis via photoinitiated RAFT dispersion polymerization based on another RAFT agent, S-1-dodecyl-S′-(α,α′-dimethyl-α″-acetic acid) trithiocarbonate (DDMAT). As shown in Fig. 8, the phase diagram at low RAFT concentrations was analogous to the case of BDMAT; low DPGDA concentrations led to monodisperse or polydisperse microspheres depending on DDMAT content, and coagulum was observed at high DPGDA concentrations. In the cases of high RAFT agent concentrations (0.75 wt% and 1.0 wt% DDMAT), monodisperse microspheres were obtained at the DPGDA concentration up to 5 wt%.

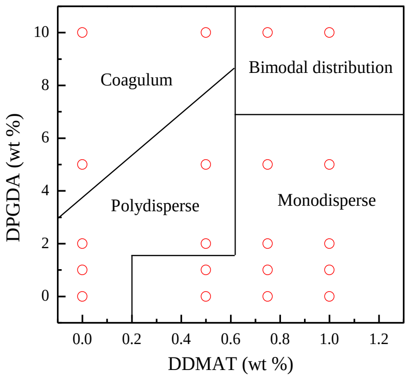

Fig. 8 Phase diagram for cross-linked PMMA products obtained by photoinitiated RAFT dispersion polymerization with 4 wt% Darocur 1173 at various DPGDA concentrations and DDMAT concentrations. The other reaction conditions were the same as the typical experiment. The corresponding SEM images are shown in Fig. S4.

It is noticed that particles with bimodal size distribution were formed at high DPGDA concentrations and high DDMAT concentrations, which is different from the case of BDMAT. This can be attributed to the structural difference between these two RAFT agents. BDMAT contains two carboxyl groups, and thus has good affinity to ethanol/water mixture. In particle growth stage, some BDMAT molecules stay in the medium and control the propagation of the polymer chains generated in continuous phase, avoiding the precipitation of these chains. In contrast, DDMAT tends to be absorbed by the particles in particle growth stage due to the hydrophobic dodecyl group. In this case, the propagation of the polymer chains generated in the continuous phase cannot be controlled effectively. This will lead to the precipitation of these polymer chains especially at high cross-linker concentrations, resulting in secondary nucleation and thus the formation of small particles in the final product.

Coagulation problem. The phase diagrams show that coagulum was formed in some cases, which is not desired. We have tried to figure out the coagulation problem under some reaction conditions. For example, in a photoinitiated RAFT dispersion polymerization procedure with 0.5 wt% DDMAT and 10 wt% DPGDA, the system lost its stability and coagulated after 60 min of UV irradiation (Fig. 9b). Meanwhile, a sample taken from the reaction at 30 min of UV irradiation showed that the procedure gave particles with a narrow size distribution as well as rough surface at this time point (Fig. 9a). It is obvious that the nucleation stage of photoinitiated RAFT dispersion polymerization is still well-controlled under this reaction condition. We presume that the coagulation occurred at 60 min is related to the rough surface of particles. During the particle growth stage of dispersion polymerization, the particles are swollen by monomers and the polymerization mainly carries out inside the monomer-swollen particles. At the same time, the short polymer chains formed in the reaction medium are absorbed to the particle surface and then diffuse into the monomer-swollen particles. Therefore, the growing particles can keep stable with spherical morphology during the polymerization. In the cases of high cross-linker concentrations and low RAFT agent contents, however, the swelling of the particles is limited due to the high cross-linking density, thus the short polymer chains deposited on the particle surface is difficult to diffuse into the particles, resulting in rough surfaces. In fact, particles prepared by photoinitiated RAFT dispersion polymerization with high cross-linker concentrations always showed rough surface (Fig. 3c and 3d, Fig. 10 and Fig. S2). This rough surface seems to be a characteristic of highly cross-linked particles prepared by dispersion polymerization.[22,23,26,27] When the amount of deposited chains reaches a high level, the stability of the particles will be affected since the amount of stabilizer covered on the particle surface will be limited. An extreme case is the occurrence of coagulation, as shown in Fig. 9b.

In view of the above discussion, one strategy to avoid coagulation under a high cross-linker concentration is the sufficient control of cross-linking process, which can be achieved by increasing the RAFT agent concentration. As shown in Fig. 10, when 1 wt% BDMAT was used, stable particles were obtained at 10 wt% DPGDA. This is a very high cross-linker concentration for the preparation of cross-linked particles by dispersion polymerization even using the two-stage process. However, the particle size distribution is relatively broad in this case. As mentioned above, because of the low propagation rate of PMMA chains in a RAFT process, the chain length of grafted PMMA on PVP will be greatly shortened at a high RAFT agent concentration, which affects the stabilization of PMMA particles and in turn the particle uniformity. This can be compensated by increasing the photoinitiator concentration since a higher photoinitiator concentration results in a faster polymerization rate. Thus we increased the concentration of Darocur 1173 to 3 wt%, and monodisperse microspheres were obtained even with 10 wt% DPGDA (Fig. 10b). These results suggest that in addition to RAFT agents and cross-linkers, other reaction parameters must be taken into account for preparing uniform cross-linked microspheres.

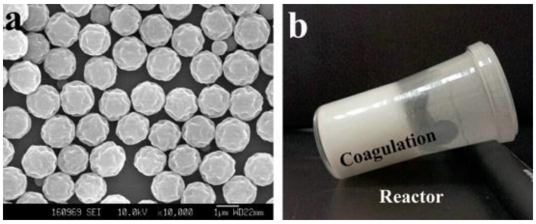

Fig. 9 (a) SEM image of PMMA microspheres prepared by photoinitiated RAFT dispersion polymerization with 0.5 wt% DDMAT and 5 wt% DPGDA (sample was taken from the reaction after 30 min irradiattion); (b) optical image of the reaction cell after 60 min irradiation.

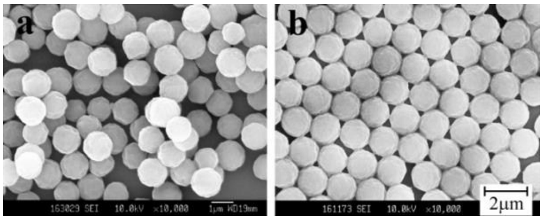

Fig. 10 SEM image of cross-linked PMMA microspheres prepared by photoinitiated RAFT dispersion polymerization with 1 wt% BDMAT and 10 wt% DPGDA, using (a) 2 wt% Darocur 1173 and (b) 3 wt% Darocur 1173 as photoinitiator. The other conditions were the same as the typical experiment.

Other reaction parameters

In dispersion polymerization, the morphology and particle size of the microspheres are affected by reaction parameters including stabilizer concentrations, initiator concentrations, reaction medium and monomer concentrations. In the present study, we have simply investigated the effect of these factors on the morphology and particle size of the cross-linked particles prepared by photoinitiated RAFT dispersion polymerization with 0.75 wt% DDMAT and 5 wt% DPGDA.

Fig. 11 shows the SEM images of PMMA microspheres prepared by photoinitiated RAFT dispersion polymerization with different photoinitiator concentrations. Monodisperse cross-linked PMMA microspheres were formed at photoinitiator concentrations ranging from 4 wt% to 8 wt% (entry 7, entry 20 and entry 21, Table 1). At a low photoinitiator concentration (2 wt%, entry 19, Table 1), the reaction system lost its stabilization and the obtained polymeric microspheres were polydisperse with rough surface (Fig. 11a). A similar phenomenon was observed and discussed in the above section (Fig. 10). The results suggest that a high photoinitiator concentration is beneficial to synthesize uniform cross-linked particles by photoinitiated RAFT dispersion polymerization.

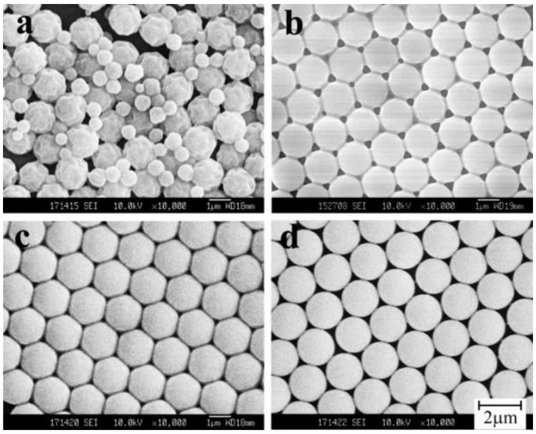

Fig. 11 SEM images of PMMA microspheres prepared by photoinitiated RAFT dispersion polymerization of MMA in the presence of 0.75 wt% DDMAT and 5 wt% DPGDA with Darocur 1173 concentration of (a) 2 wt%, (b) 4 wt%, (c) 6 wt% and (d) 8 wt%.

We also found that the particle size had little change in a wide range of photoinitiator concentrations (4 wt% to 8 wt%). As described before,[35] in photoinitiated RAFT dispersion polymerization, the real nuclei are formed by the precipitation of controlled polymer chains. This nucleation process is mainly affected by the RAFT process. As a result, the effect of photoinitiator concentrations on particle size and morphology is limited. Therefore, by changing the photoinitiator concentration, one can speed up or slow down the reaction easily without affecting the particle size and size distribution.

Fig. 12 shows the SEM images of PMMA microspheres prepared by photoinitiated RAFT dispersion polymerization with different PVP concentrations. Monodisperse cross-linked PMMA microspheres were formed with PVP concentrations ranging from 10 wt% to 20 wt% (entry 16, entry 7 and entry 17, Table 1). The particle size distribution became a little broad at 5 wt% PVP (entry 15, Table 1) due to the insufficient stabilization. The particle diameter has no significant change in a wide range of PVP concentrations. This may attribute to the long nucleation period of photoinitiated RAFT dispersion polymerization. During the elongated nucleation period, the nuclei and growing particles have enough time to adsorb the stabilizer. This results in decreased dependence of particle size to stabilizer concentration.[36]

Fig. 13 shows the SEM images of PMMA microspheres prepared by photoinitiated RAFT dispersion polymerization with different ethanol/water ratios. Highly monodisperse PMMA microspheres were obtained with ethanol/water ratios (wt/wt) ranging from 35/65 to 45/55 (entry 22, entry 7 and entry 23, Table 1). As the ethanol/water ratio increases, the diameter increased from 1.39 μm to 1.98 μm. This tendency is consistent with the traditional dispersion polymerization.[13] The higher solubility of PMMA in ethanol than in water allows the critical chain length to increase with increasing ethanol content in the reaction medium. Consequently, there is a decrease in the number of nuclei and thus an increase in the final size of particles. Another factor is that the adsorption of stabilizer in a good solvent is relatively difficult. This will enhance the coalescence of the primary nuclei and lead to larger particles, but fewer particles will form.[46] In the case of 50/50 ethanol/water ratio (entry 24, Table 1), the coalescence became so severe that only polydisperse particles with extra-large microspheres were obtained (Fig. 13d). Analogously, due to the high solubility of PMMA in MMA, the increase of MMA concentrations also led to large particles with a broad particle size distribution (Fig. S5).

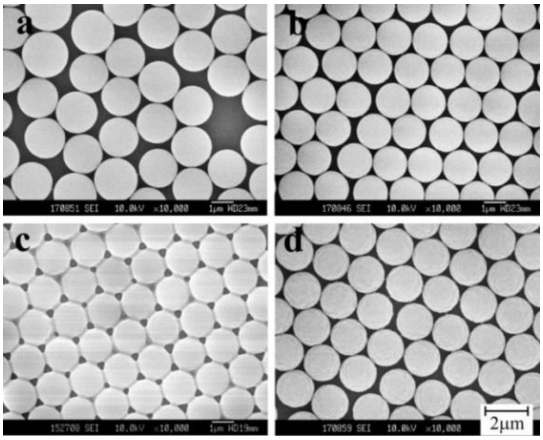

Fig. 12 SEM images of PMMA microspheres prepared by photoinitiated RAFT dispersion polymerization of MMA in the presence of 0.75 wt% DDMAT and 5 wt% DPGDA with PVP concentration of (a) 5 wt%, (b) 10 wt%, (c) 15 wt% and (d) 20 wt%.

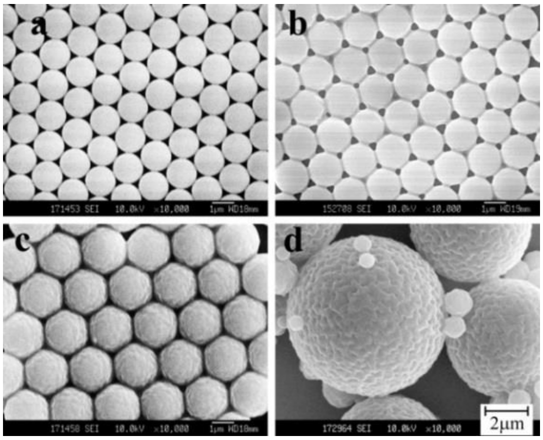

Fig. 13 SEM images of PMMA microspheres prepared by photoinitiated RAFT dispersion polymerization of MMA in the presence of 0.75 wt% DDMAT and 5 wt% DPGDA with ethanol/water ratio (wt/wt) of (a) 35/65, (b) 40/60, (c) 45/55 and (d) 50/50.

Functionalization of the cross-linked PMMA microspheres

Body Functionalization. Owing to the insensitive nucleation stage of photoinitiated RAFT dispersion polymerization, functional comonomers can be added to the system at the beginning without disturbing the nucleation. This produces copolymer microspheres with functional groups embedded in the body.[35] In the present study, we introduced epoxy groups or carboxyl groups into the cross-linked PMMA microspheres by adding both cross-linker and functional comonomer (6 wt% GMA or 6 wt% MAA) into the system at the beginning of the reaction. And monodisperse copolymer microspheres were obtained, as shown in Fig. 14. These cross-linked copolymer microspheres can be well swollen in a good solvent, therefore the functional groups embedded in the microspheres will be the effective active centers for further modification or for some applications, e.g. bead-based immunoassay and heavy metal ion recovery.

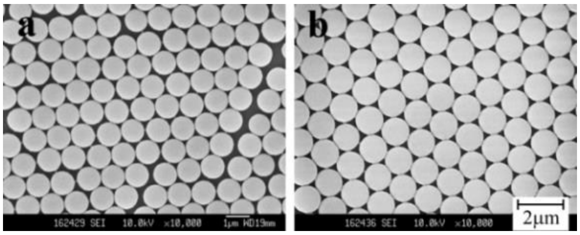

Fig. 14 SEM images of PMMA microspheres prepared by photoinitiated RAFT dispersion polymerization of MMA in the presence of 0.5 wt% BDMAT with (a) 2 wt% DPGDA and 6 wt% GMA; (b) 2 wt% PEGDA200 and 6 wt% MAA.

Surface Functionalization. It is well-known that RAFT polymerization can prepare well-defined polymers with "living" end groups, which can be further extended. One advantage of the cross-linked microspheres prepared by photoinitiated RAFT dispersion polymerization is the presence of RAFT groups on the surface which give the particles "living" characteristic and can be used to further modify the particles via surface-initiated RAFT polymerization. Such surface-functional cross-linked microspheres can be applied in various research areas such as molecular imprinting and bioassay.[1,5,43] As an example, we modified the cross-linked PMMA microspheres by grafting poly(2-hydroxyethyl methacrylate) (PHEMA) brushes onto the particle surface via the surface-initiated RAFT polymerization. The polymerization was carried out in an ethanol/water (40/60, w/w) mixture at 70 °C for 24 h as described in experimental section. In this case, the microspheres could be prevented from swelling in the reaction medium because ethanol/water (40/60, w/w) mixture is a poor solvent for PMMA, and thus the reaction would primarily occur on the surface of the PMMA microspheres. We found that the resulted particles still kept spherical (Fig. 15a), and the particle size increased from 1.39 μm to 1.46 μm after the polymerization. The particles were then labeled with Rhodamine B (a fluorescent dye) via esterification between the hydroxyl group on the grafted PHEMA brush and the carboxyl group of Rhodamine B. The fluorescence microscopy image of the Rhodamine B labeled PMMA microspheres is shown in Fig. 15b. The result demonstrates the successful surface functionalization of the cross-linked PMMA microspheres via surface-initiated RAFT polymerization.

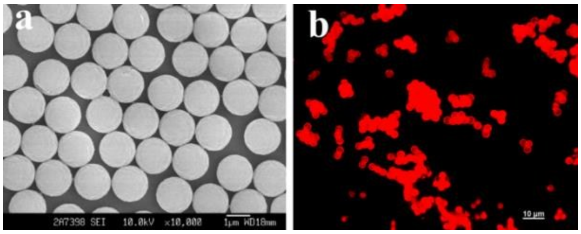

Fig. 15 (a) SEM image of cross-linked PMMA microspheres (prepared by the typical dispersion polymerization with 0.75 wt% DDMAT) after suspended in DMF for 24 h; (b) Fluorescence microscopy image of the Rhodamine B-labeled PMMA microspheres with PHEMA brushes (the color used here is not the real fluorescent color).

Conclusion

Monodisperse cross-linked PMMA microspheres have been synthesized by means of photoinitiated RAFT dispersion polymerization in the presence of a bifunctional cross-linker or a trifunctional cross-linker. The investigation of particle formation process revealed that the cross-linking reaction was retarded to a certain extent by the RAFT process, which is crucial for the formation of uniform particles under high cross-linker concentration. The phase diagrams for the methodology of the polymerization products showed that the procedure could only give polydisperse microspheres at a low DPGDA concentration or coagulum at a high DPGDA concentration in the absence of a RAFT agent, and monodisperse microspheres were obtained at DPGDA concentration up to 5 wt% when the RAFT agent concentration reached an appropriately high level, e.g. 0.5 wt% BDMAT or 0.75 wt% DDMAT. By adjusting the reaction conditions, we have prepared monodisperse cross-linked PMMA microspheres with 10 wt% DPGDA. Furthermore, the cross-linked microspheres have been functionalized by adding a functional comonomer to the system at the beginning of polymerization, or by surface-initiated RAFT polymerization via the RAFT groups attached on the particle surface.

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Notes and references

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† Electronic Supplementary Information (ESI) available: Schematic diagram of the equipment for photoinitiated dispersion polymerization,

additional research results, recipes and particle size data. See DOI: 10.1039/b000000x/

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