Different dispersion polymerization strategies influence the quality of fluorescent poly (St-*co*-GMA) microspheres

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ABSTRACT: The polymerization strategy plays a vital role in the preparation of functional microspheres. In this work, fluorescent poly (styrene-*co*-glycidyl methacrylate) (PSt-GMA) microspheres were synthesized via one-stage and two-stage dispersion polymerization with 4-Bis(5-phenyl-1,3-oxazol-2-yl)benzene (POPOP) as fluorescence agent. SEM and DLS were adopted to characterize the properties of prepared microspheres. The UV-vis and fluorescence spectroscopy were used to analyze the mechanisms of two-stage dispersion polymerization. The experimental results showed that the size distribution and fluorescence intensity of prepared microspheres could be improved by two-stage dispersion polymerization compared to one-stage dispersion polymerization. In addition, according to UV-vis, the interactions between POPOP and Poly (N-vinyl pyrrolidone) (PVP) as well as POPOP and Glycidyl methacrylate (GMA) could affect the particle size and its distribution. UV-vis and fluorescence spectra implied that the POPOP existed outside of the particle's core via two-stage strategy. The monomer conversion of styrene was similar at the beginning of reaction; however, the monomer conversion of styrene by two-stage strategy was higher than that of by one-stage strategy. In a word, two-stage dispersion polymerization could prepare fluorescent microspheres with the monodispersion micrometer-size and high quality. © 2015 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2015**, *132*, 41927.

INTRODUCTION

As kind of special functional microspheres, fluorescent microspheres with narrow distribution and size control have a variety of applications in biomedical and biochemical fields.[1–3] Particularly, fluorescent microspheres are the core of support materials in scintillation proximity assay (SPA, an assay platform of high throughput screening). The normal matrix of SPA beads can be divided into inorganic microspheres (YOx and YSi) and polymeric microspheres (polyvinyl tolune, PVT and polystyrene, PS).[4–6] Polymeric microspheres have a lower density than inorganic beads, so they can remain longer in suspension, which are beneficial for automated high throughput screening (HTS). Polymeric microspheres with different range sizes can be synthesized through various polymerizations, such as emulsion polymerization,[7,8] precipitation polymerization,[9,10] seed polymerization,[11,12] and dispersion polymerization,[10,13–15] etc. Dispersion polymerization is an attractive method for preparing monodisperse polymeric microspheres with diameters in the range of 0.1–15 μm;[15] and the ideal diameter of the SPA bead is

approximate 5 μm,[4] so dispersion polymerization is the optimal strategy.

Dispersion polymerization, originated from Osmond and coworkers at ICI in the 1970s,[15] undergoes the transformation from an initially homogeneous mixture to a turbid heterogeneous latex. Dispersion polymerization consists of two major stages: the nucleation (particle formation) stage and the particle growth stage.[15,16] Briefly, the length of polymer chain increases along with the polymerization processed, and then reaches a critical point to form the nuclei; lastly, aggregates with others to form the bigger latex particles. At the second stage, no new nucleus generates and the particle growth occurs within the particles by adsorption of monomers and polymer radicals from the continuous phase. It has been known that the nucleation stage during the particle formation is very complex and sensitive, and is vital for obtaining the monodisperse particles.

Up to now, the hot topic points for dispersion polymerization include: (1) to prepare functional microspheres with various biomaterials and functional molecules;[3,17,18] (2) to use all kinds

Table I. The Recipes for the Dispersion Polymerization of Styrene with GMA, POPOP in Ethanol

Materials	Amounts (g)		
	One-stage reaction	Two-stage reactions	
		1st stage	2nd stage
Styrene	13.6	6.8	6.8
PVP	0.4	0.4	
POPOP	0.05		0.05
Ethanol (mL)	60	30	30
AIBN	0.2	0.2	

of stabilizers, initiators, solvent system, and monomers;[19–22] (3) to combine the other polymerization methods,[23–26] such as RAFT, ATRP, TEMPO, etc.; (4) to change the non-aqueous to aqueous dispersion polymerization.[27–29] For functional microspheres, its uniform and shape could be influenced by adding co-monomers into 'one-pot' polymerization. To resolve this problem, one strategy is to delay the addition of functional monomers in the end of the nucleation stage; the other strategy is to use two-stage dispersion polymerization. The two-stage dispersion polymerization was developed by Winnik et al.[3,16,30–32] for avoiding the disturbance of functional material added in the nucleation stage. This method prepared functional monodisperse microspheres by delaying the addition of various functional molecules to reaction until 3% monomer conversion for dispersion polymerization.

In this work, we investigated how the preparation strategy affects the quality of fluorescent microspheres. Fluorescent poly (St-co-GMA) microspheres were synthesized by one-stage and two-stage dispersion polymerization, respectively. The purposes of this research are to analyze the polymerization systems by the UV-vis and fluorescent spectrum and to explain the reason, i.e., why the fluorescent microspheres with narrow size distribution were obtained by two-stage polymerization.

MATERIALS AND METHODS

Materials
Styrene (St, Xilong Chemicals, Guangdong, China) was distilled under reduced pressure to remove the inhibitor hydroquinone completely, and then stored in a refrigerator at 4°C. Other reagents were used as received without further purification. 2, 2′-Azobisisobutyronitrile (AIBN), poly (N-vinyl pyrrolidone) (PVP, Mw=30,000), and absolute ethyl alcohol purchased from the kelong chemicals were used as initiator, the stabilizer, and solvent, respectively. Glycidyl methacrylate (GMA, 98%) was obtained from Jiyuan Chemicals Co. (Shanghai, China). POPOP (scintillation grade, MW = 364.39) was purchased from Acros Organics (Morris Plains, NJ, USA). Deionized water was used in all experiments. The rest of reagents were analytical grade.

One-Stage and Two-Stage Dispersion Polymerization
The standard protocol for the one-stage and two-stage dispersion polymerization of fluorescent PSt-GMA microspheres are listed in Table I. The reaction process of one-stage dispersion polymerization was described: firstly, 15 mL styrene, 0.2 g AIBN, 0.4 g PVP, 60 mL ethanol and 0.05 g POPOP were simultaneously added into a 100-mL four-neck round-bottom flask. Nitrogen gas was blown in for 30 min to eliminate atmosphere. Then, the polymerization was conducted at 70°C for 5 h with an agitation speed of 250 rpm. Thereafter, 15 mL ethanol solutions containing 0.5 g GMA was slowly introduced into the system at a rate of 6 mL/h; and the copolymerization reaction underwent copolymerization at 70°C for another 7 h with the agitation speed of 300 rpm (total reaction time 12 h). Finally, the obtained particles were fined through a gauze filter to remove the sediment before they were purified by successive centrifugation, decantation and re-dispersion with ethanol and double-distilled water for at least 3 times, respectively.

The reaction process of two-stage dispersion polymerization was similar to one-stage dispersion polymerization. Briefly, half of styrene and ethanol, the total of AIBN and PVP were added into a 100-mL four-neck round-bottomed flask equipped with a mechanical stirrer, nitrogen inlet, and reflex condenser. After being pre-polymerized for 2 h at 70°C, the POPOP pre-warmed to 70°C containing the other half of styrene and ethanol was introduced into the polymerization systems. The rest of procedure was identical to the one-stage dispersion polymerization.

Particle Size and Morphology Analysis
Particle size and morphology of prepared microspheres were observed via scanning electron microscopy (SEM, Leitz-AMR-1000) and Dynamic light scattering (DLS). The prepared microspheres were dispersed into phosphate buffer solution (PBS, 0.1 M, pH 7.0), then measured the particle size and size distribution in 2 mg/mL dispersions by DLS. All samples were measured in triplicate.

Determination of the Epoxy Group Concentration of Microspheres
A reaction between the oxirane ring and sodium thiosulfate ($Na_2S_2O_3$) was designed to determine the concentration of epoxy groups for prepared microspheres.[33] The chemical formula was following:
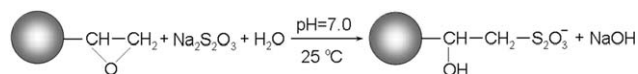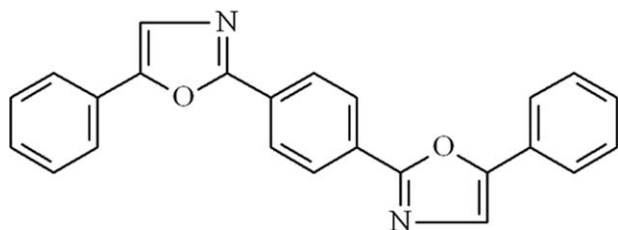

Generally, 0.6 g microspheres dispersed in 20 mL of methanol with 40 mL $Na_2S_2O_3$ solution (pH 7.0) were shaken for 30 min in conical flask. Then, hydrochloric acid was added to neutralize the released OH^- and the epoxy content on the surface of particles was calculated from the used amount of hydrochloric acid. One mole of OH^- produced corresponds to one mole of epoxy groups converted.

UV-Vis and Fluorescence Spectroscopy
With the same medium of the polymerization system, the UV spectra of samples were obtained from U-2010 spectrophotometer (Hitachi, Japan). At ambient temperature, the sample was put into the quartz of vessel with medium as reference. The scanning region was in 190–360 nm.

The prepared microspheres were re-dispersed into ethanol and measured by fluorescence spectroscopy. The quantum yields of

Scheme 1. Chemical structure of fluorescein POPOP.

Figure 1. Comparison of appearance of non-fluorescent and fluorescent microspheres exposed under UV light: Without POPOP (left) and with POPOP (right). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

prepared microspheres were calculated via the following equation:

$$\phi_u = \phi_o \frac{F_u A_s}{F_s A_u} \qquad (2)$$

where ϕ_u, ϕ_o were the quantum yields of measured and reference standard substance respectively; F_u and F_s were integral fluorescence intensity of measured and reference standard substance, respectively; A_u and A_s were the absorbance of the incident light of measured and reference standard substance respectively.

Determination of Monomer Conversions

According to the description as in prepared the microspheres, the conversions of monomer (St) at various times or temperatures were determined. Under the protection of nitrogen gas, a sample was taken out from the polymerization system. The conversion was calculated according to the following equation:

$$x = \frac{m_p}{m_m} \times 100\% \qquad (3)$$

where x is the conversion of monomer, m_p the mass of polymer produced in the given time, and m_m the total mass of monomer containing in the sample.

RESULTS AND DISCUSSION

Characterization of PSt-GMA Fluorescent Microsphere

Compared to 2,5-diphenyloxazole (DPO), POPOP possesses higher fluorescence efficiency and is excited easily. According to our previous study,[17] POPOP is a better choice for preparation

of fluorescence microspheres as the SPA carriers. The chemical structure and properties of POPOP are shown in Scheme 1.

A UV-visible light detector was used to provide the UV excitation energy of the fluorescent microspheres. Under the irradiation of UV light, the fluorescent microspheres could be excited and simultaneously released redundant energy in the form of blue fluorescence. Figure 1 shows the transmitted fluorescence from the fluorescent microspheres dispersed in aqueous solution. From Figure 1, it can be seen that the fluorescent microspheres showed the blue-light; however, the non-fluorescent microspheres did not. The spectra band range of POPOP is broad (380–650 nm), so it is easy to be detected by the scintillation read-outer, flow cytometry, fluorescent detector, etc.

The particle diameter by dispersion polymerization is in the range of 0.1–15 μm. Although the morphology of microspheres could be observed by the light microscope, the definition of the optical picture was low. The SEM images of fluorescent PSt-GMA microspheres prepared by two-stage dispersion polymerization are given in Figure 2, indicating that the fluorescent microspheres had a good sphericity and well-distribution [shown in Figure 2(a)]. In order to further obverse the shape
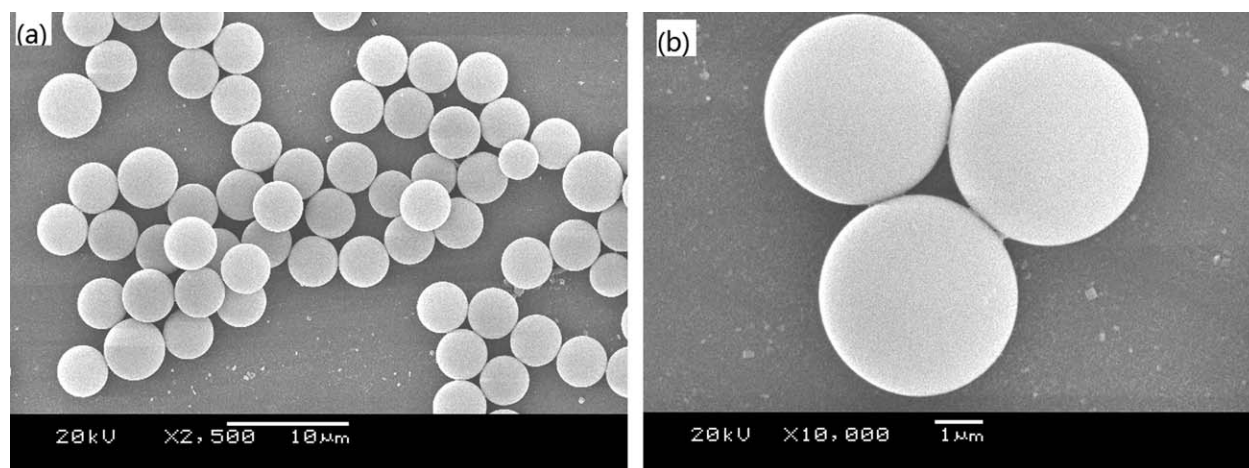

Figure 2. SEM images of fluorescent microspheres: (a) 2500× and (b) 10,000×.
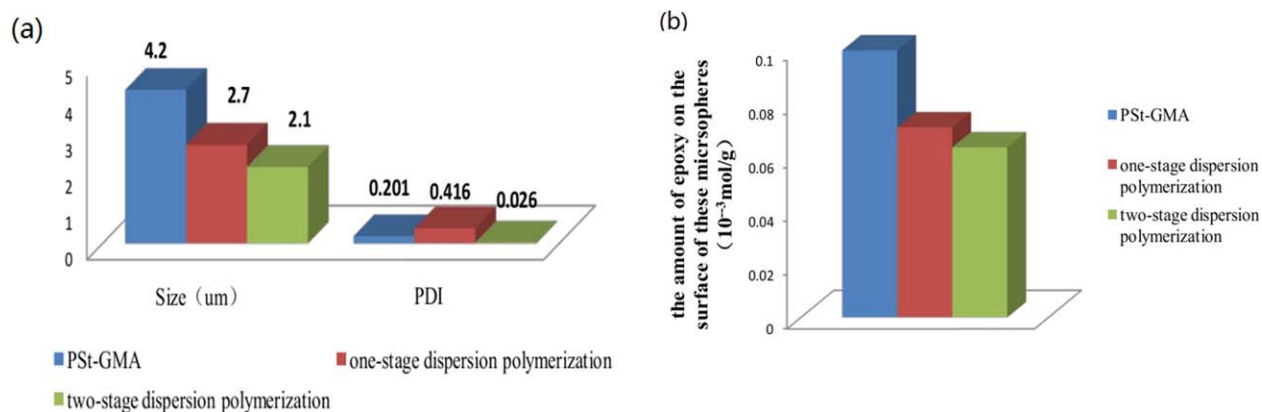
Figure 3. The particle size, size distribution (a) and the amount of epoxy (b) on the surface of microspheres: the particles were PSt-GMA and fluorescent microspheres via one-stage dispersion polymerization and two-stage dispersion polymerization, respectively. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

and apparent form of fluorescent microsphere, an arbitrary chosen region was enlarged 10,000 times [Figure 2(b)]. It can be seen that these particles were distinctly globular particle, smooth surface, no damage and no defect.

However, it can be found that some microspheres adhered to each other due to the adhesion action of the stabilizer, PVP. According to the coagulative and aggregative nucleation theories of dispersion polymerization, the effect of PVP on the dispersion polymerization is not only physically adsorbed but also chemically grafted onto a colloid to provide the particle with stability. The physically adsorbed of PVP was reversible, so the amount of PVP on the particle was decreased after washing; however, the grafted PVP was irreversible and it cannot be removed by washing. It had reported that the adhesion between these particles was decreased after the washing of microspheres; moreover, the absorption peak of PVP was found in the FT-IR of microspheres after washed many times,[34,35] so physically adsorbed and chemically grafted PVP were acted together on the stable of microspheres.

One-Stage and Two-Stage Dispersion Polymerization

The particles were PSt-GMA and fluorescent microspheres via one-stage dispersion polymerization and two-stage dispersion polymerization, respectively.

Because the particle size distribution of the fluorescent microspheres was broad by one-stage dispersion polymerization, the two-stage dispersion polymerization was used to deal with this problem. The particle size and size distribution of prepared microspheres as well as the amount of epoxy on the surface of these microspheres are shown in Figure 3. Compared to PSt-GMA, the particle size of fluorescent microspheres was decreased; their size distribution was broader and the amount of epoxy on the surface was decreased via one-stage dispersion polymerization. However, the size distribution of fluorescent microspheres was narrower via two-stage dispersion polymerization. The similar result was reported that micro-size fluorescent PMMA microspheres were prepared by dispersion polymerization based on MMA and DPO.[36] There are two stages in the dispersion polymerization: the nucleation and the particle

growth stage. The essential hypothesis is that the nucleation stage is short-lived, complex and very sensitive, whereas the particle growth stage is more robust. If coagulum or no secondary particles are formed during the particle growth stage, the particle size distribution and number are determined by the nucleation stage. However, the particle size distribution and number could change when other functional material is added during the nucleation stage. Fluorescent reagent and monomer will become the nucleating centers when fluorescent reagent was added in the nucleation stage. Compared to the monomer (St), POPOP is inert compounds and is hard to form free radical, so POPOP would not form the nucleating center. Therefore, the influence of POPOP in dispersion polymerization could be the interactions between POPOP and the other materials include styrene, PVP, PSt-GMA and GMA in the polymerization systems.

The UV-vis was used to detect the interactions between POPOP and GMA, PVP, PSt-GMA, and St, respectively. From Figure 4, it is known that there is a change in adsorption peak for GMA or PVP in the presence of POPOP, but there is no change for PSt-GMA or St in the presence of POPOP, suggesting that POPOP had reacted with the GMA or PVP. Although the POPOP did not form the nucleation center when the fluorescent PSt-GMA microspheres were prepared by one-stage dispersion polymerization, POPOP could be linked onto the surface of nuclei or embedded in nuclei [Scheme 2(a)], so it could influence the formation and growth of nuclei particles resulting in a broader particle size distribution. Despite of POPOP was difficult to react with St and PSt, the interactions between POPOP and PVP could change the critical chain length, which resulted in decreasing the particle size. In addition, the interactions between POPOP and GMA affected the properties of fluorescent microspheres.

It has been proved that the nucleation stage of dispersion polymerization occurs early during the reaction process. The nuclei with a narrow range of sizes formation at the beginning of polymerization would result in a narrow size distribution for gained particles.[34,35] Yasuda et al.[34] had reported that the nucleation stage of styrene dispersion polymerization was finished at
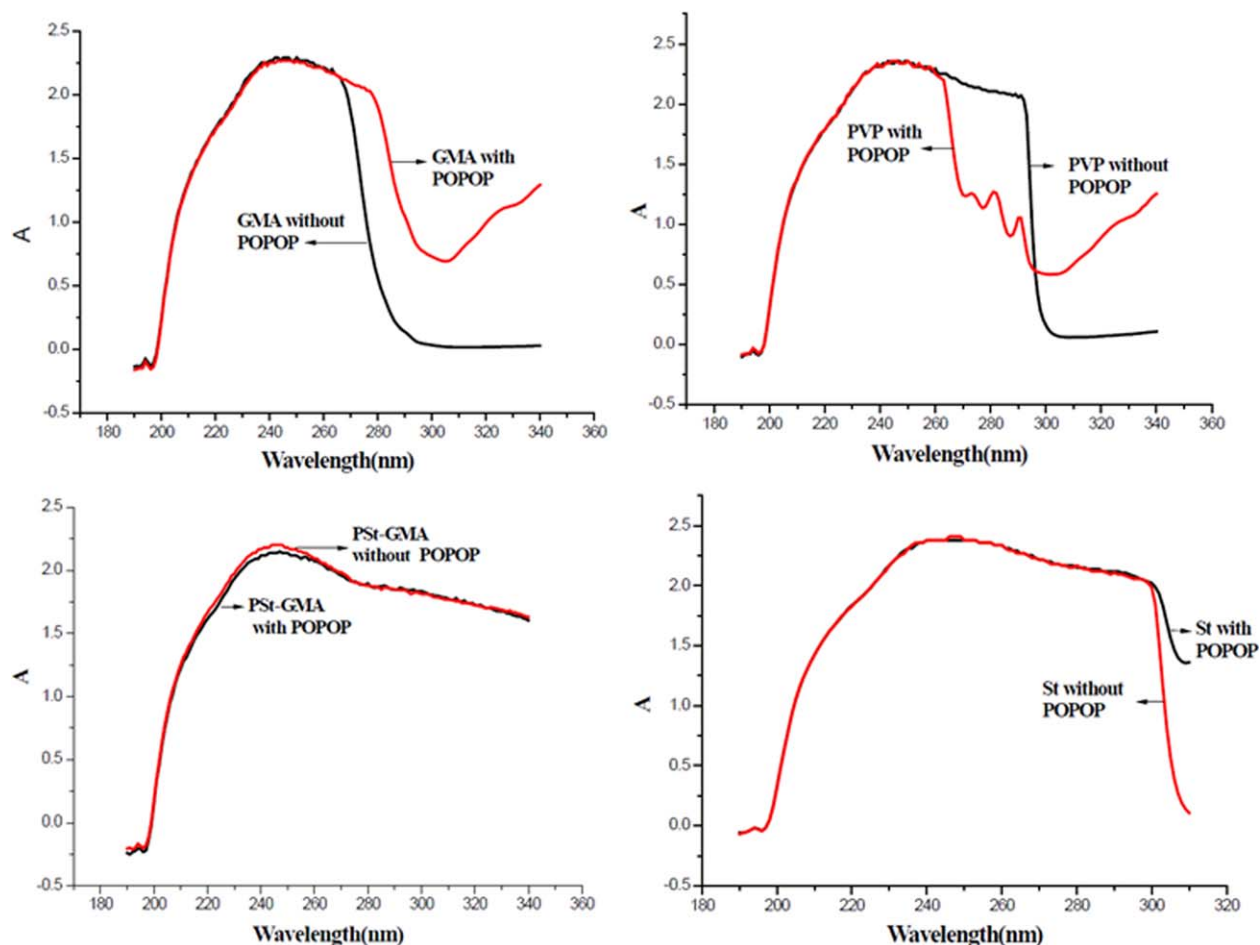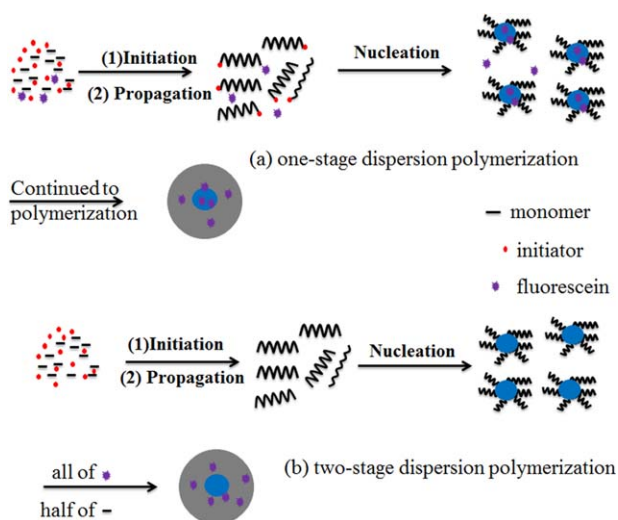
Figure 4. UV–vis spectrum of GMA, PVP, PS-GMA, St with/without POPOP. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

less than 1% styrene conversion and 15 min reaction time under 70°C and using ethanol as the solvent. While if the fluorescent PSt-GMA microspheres were prepared by two-stage dis-

Scheme 2. Schematic representation of one-stage and two-stage dispersion polymerization. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

persion polymerization, the nucleation stage was completed 2 h late after POPOP was added in; therefore, POPOP was linked onto the surface of nuclei particle, i.e., the middle between the shell and core, which could effectively control the particle size distribution. In addition, the interactions between POPOP and PVP or GMA would influence critical chain length, resulting in the decrease of particle size.

Epoxy group might exist on the surface of the fluorescent microspheres, or encapsulated in the microspheres; however, the epoxy group measured by neutralization titration was proved on the surface of the fluorescent microsphere. As mentioned, the interactions between POPOP and GMA could influence the position of GMA in the microspheres and the amount of epoxy group on microspheres, so it can be understood why the amount of epoxy group on the surface of particle was decreased.

In order to further investigate the trait of one-stage and two-stage dispersion polymerization, the fluorescent spectra of these microspheres were measured and shown in Figure 5. It is known that the maximum adsorption peak didn't shift, but the strength of adsorption peak for fluorescent microspheres by two-stage dispersion polymerization was higher than that by one-stage dispersion polymerization. The quantum yields of
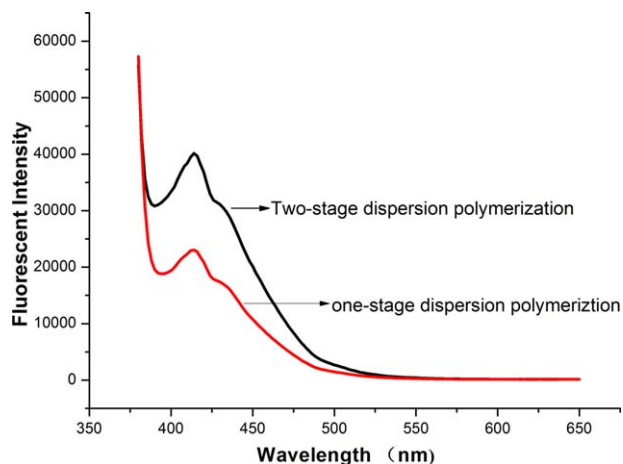
Figure 5. Fluorescent spectra of fluorescence microspheres prepared by different processes. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

prepared microspheres were calculated via eq. (2). The ratio of the quantum yields of prepared microsphere via one-stage and two-stage dispersion polymerization was 2.906. There indicated that the fluorescent reagent was located in the different position with different polymerization strategies. The POPOP in the core was shielded by the out shell via one-stage dispersion polymerization [Scheme 2(A)], which weakened the strength of the adsorption peak. However, POPOP existed on the outside of core by two-stage dispersion polymerization [Scheme 2(B)], so the shield content for adsorption peak of fluorescent microspheres was lower, and the strength of adsorption peak was higher.

The Effect of POPOP on the Styrene Conversion

The effect of POPOP on the styrene conversion is shown in Figure 6. As can be observed from Figure 6(a), at the beginning of polymerization, the styrene conversion was similar for all polymerization conditions. Then the styrene conversion was higher

in two-stage dispersion polymerization than that in one-stage dispersion polymerization after 4 h reaction. Alike, the styrene conversion in one-stage dispersion polymerization was lower than in the preparation of PSt-GMA without POPOP. Also, it was gained that the styrene conversion in two-stage dispersion polymerization was higher than that in one-stage dispersion polymerization according to Figure 6(b). Obviously, the polymerization rate of styrene was little influenced by added POPOP and dispersion polymerization strategy. In Figure 4(D), the UV-vis spectra of styrene almost didn't change both in the presence or absence of POPOP. It implied that the interaction between POPOP and styrene was extremely weak, so the added POPOP affected little on the physicochemical properties of styrene in the polymerization system. Therefore, in theoretical discussion, the interaction between POPOP and styrene was not the key factor for the change of polymerization rate of the styrene. According to the theory of free radical polymerization, free radical was generated after the decomposition of initiator, and then combined the monomer to generate a polymeric chain increasing the length during the reaction; finally, the chain termination was occurred. Obviously, the properties of intermediate free radical, particularly for the stabilizer, significantly influenced the dispersion polymerization system during reaction process. As seen in Scheme 1, POPOP has highly electronic conjugated system, so its conjugated properties inevitably influence the stability of intermediate free radical. The change of the stability of intermediate free radical would affect the addition activity of free radical and monomer, resulting in the alteration of the polymerization rate. The reactivity of free radical was lower when adding the POPOP, which would lead to lower the polymerization rate and monomer conversion. Similar results had reported.[36]. However, if the fluorescent microspheres were prepared by two-stage dispersion polymerization, which reduce the influence of intermediate free radical due to the strategy of adding the POPOP, so it could improve the polymerization rate and monomer conversion.
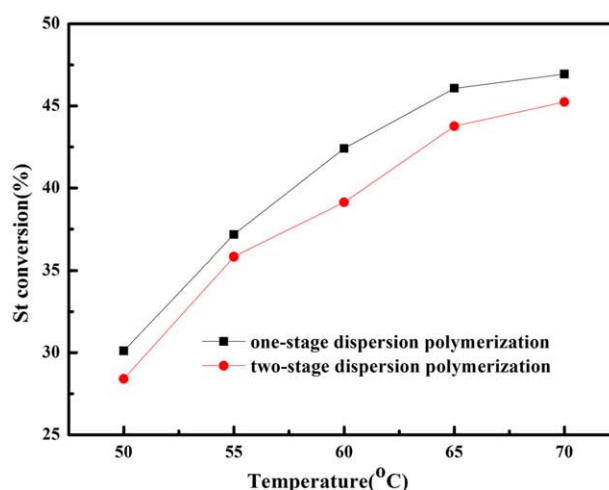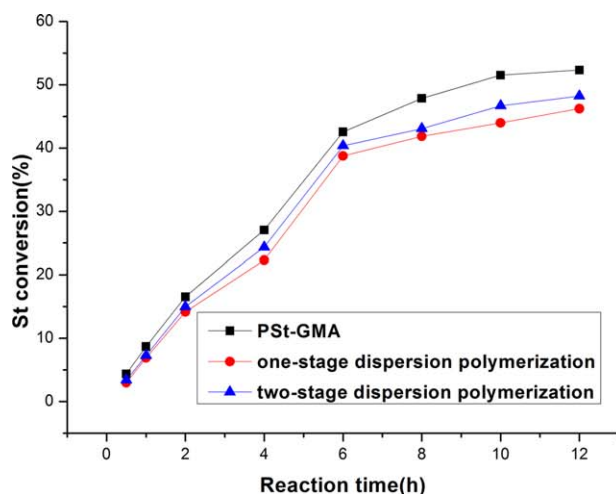

Figure 6. St conversion (a) under different reaction times (70°C), (b) under different polymerization temperatures. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

CONCLUSION

In summary, we prepared fluorescent PSt-GMA microspheres by one-stage and two-stage dispersion polymerization. The fluorescent microspheres showed a more perfect sphericity and better size distribution by two-stage dispersion polymerization. The interactions between POPOP and GMA as well as POPOP and PVP could influence the particle size, size distribution, and the amount of epoxy group on the surface of microsphere according to UV-vis and fluorescence spectra. Although the styrene conversion was alike at the beginning of different polymerization under 70°C, the styrene conversion decreased because of adding POPOP. Furthermore, the styrene conversion in two-stage method was higher than that in the one-stage method. In a word, the information obtained from our present work could be adopted for preparation of fluorescent polymeric carriers that would be used in radioactive immunoassay, especially in high throughput screening through SPA method.

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