

# Monodisperse, controlled micron-size dye-labeled polystyrene particles by two-stage dispersion polymerization

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## Abstract

Monodisperse controlled micron-sized colored polystyrene particles have been prepared by two-stage dispersion copolymerization with polymerizable dyes. These polymerizable dyes have a methacrylate moiety for copolymerization with styrene and suitable spacers between the dye nucleus and the methacrylate function to promote the solubility of the dye. In the past, various research groups have found that when they added a dye-comonomer to a traditional dispersion polymerization recipe, they obtained poor results. The final particle size changed significantly; and the size distribution became broader. In the two-stage method, we defer the addition of the dye-comonomer until the nucleation stage is complete. These dyes become incorporated into the particles without disturbing the final particle size and size distribution.

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## 1. Introduction

Colored copolymer particles with micrometer diameters, particularly fluorescent particles, can be used in medical diagnostic applications. One approach to the preparation of this type of particle involves the synthesis of crosslinked polymer particles, which are subsequently swollen in an organic solvent containing free dye molecules in solution. When the solvent is evaporated or the particles are deswollen by changing the polarity of the medium, the dyes become physically trapped inside the particle [1]. An alternative approach to the preparation of this type of particle involves synthesis of colored polymer particles in which dyes are covalently bound to the polymer molecules that comprise the particles. Covalent attachment can prevent dye aggregation that would cause the quenching of fluorescent emission. For many of these applications, one needs particles with a narrow size distribution and diameters in the range of 2–10  $\mu\text{m}$ .

Micron-sized uniform particles can be prepared by the successive seeded emulsion polymerization developed by Vanderhoff [2] and by the activated swelling and suspension polymerization methods developed by Ugelstad [3].

The ‘dynamic swelling method’ developed by Okubo is also possible [4]. It is difficult for these methods to incorporate monomers bearing hydrophobic dyes into these polymer particles because of difficulties with transport of water-insoluble species from the monomer droplets to the growing particles. And all of these above processes for making micrometer diameter particles are complex and difficult to implement on a large scale.

Traditional dispersion polymerization is a very attractive method for preparing micrometer-sized monodisperse polymer particles [5–10]. Dispersion polymerization is defined as a polymerization reaction in which the monomer is soluble, but the polymer is not. The polymer begins to precipitate as it is formed. Dispersion polymerization differs from precipitation polymerization in that it is carried out in the presence of a second soluble polymer as a steric stabilizer. The steric stabilizer becomes attached to the surface of the precipitating polymer formed in the reaction, and forces the precipitate to form micron-sized particles. The starting reaction mixture of dispersion polymerization system is a clear, single-phase solution. The polymerizable dye is also soluble in the reaction mixture. The diffusion of hydrophobic dye from the medium to the growing particles is no longer a problem.

Winnik and Ober [11] reported dispersion polymerization of styrene in the presence of polymerizable anthraquinone dyes. They found that the final particle sizes changed significantly and the particle size distributions became very broad. Horák et al. [12] studied the dispersion polymerization of styrene

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and butyl methacrylates in the presence of nonpolymerizable dyes. They found that with increasing concentration of the black dye Nigrosin (even only 0.4 wt% based on the total monomers), the particle size increased and the particle size distribution broadened significantly. As we describe below, our attempts to incorporate a benzothioxanthene dye into PS particles by traditional dispersion polymerization encountered similar problems.

Other functional monomers (polar monomers, crosslinking agents) have also been found to interfere with the course of a dispersion polymerization reaction. Some authors carried out seeded dispersion polymerization reactions in an attempt to overcome these problems [13]. In this approach, one prepares particles by dispersion polymerization, and then introduces these particles into a second reaction. In contrast to traditional seeded emulsion polymerization, where the seed latex comprises a very small fraction (2–10%) of the final particle mass, in seeded dispersion polymerization, the polymer formed in the second step represents typically half of the particle mass. In this way Okubo and coworkers were able to introduce chloromethyl groups [14,15] and vinyl groups [16,17] into the shell of particles produced. The El-Aasser group [18] has used this approach to prepare structured particles by employing a different monomer in the second step.

Other authors introduced a long delay between the start of the reaction and the addition of a crosslinking agent to a dispersion polymerization reaction. Thomson et al. [19] and Zhang et al. [20] used this type of approach to prepare PS particles with divinylbenzene as the crosslinker. This method was successful only when the crosslinking agent was added at high monomer conversions. Horák et al. [21,22] prepared crosslinked poly(2-hydroxyethyl methacrylate) (PHEMA) particles using ethylene glycol dimethacrylate (EGDMA) as a cross-linking agent. They found that they could obtain a relatively narrow distribution of particle sizes by delaying addition of the EGDMA until the reaction had run for 2 h.

In a recent communication, we described a new strategy for carrying out dispersion polymerization reactions in alcoholic media that allows one to overcome many of these problems. The fundamental hypothesis is that the nucleation step in dispersion polymerization reactions is short-lived but very sensitive to perturbation, whereas the particle growth step is more robust. The particle number and particle size distribution are determined by the nucleation stage if no secondary particles or coagulum are formed during particle growth stage. Problematic reagents such as fluorescent dye-comonomers or cross-linking agents were added to the reaction at low conversion, but after the end of the nucleation stage. We call this process two-stage dispersion polymerization [23,24]. We recently showed that this method could be used to synthesize carboxyl-functionalized particles [25]. Our full paper describing the synthesis of crosslinked particles [26] has recently appeared.

In this paper, we provide full details of the reaction conditions and explore some of the differences between the traditional one-stage and our two-stage dispersion

polymerization reaction for synthesizing dye-labeled particles. We were able to prepare monodisperse dye-labeled polystyrene (PS) particles.

## 2. Experimental

### 2.1. Reagents

All organic reagents were used without further purification, including styrene (Aldrich), methanol and ethanol, polyvinylpyrrolidone (PVP,  $M_w = 55,000$  g/mol, Aldrich), Triton X-305 (70% solution in water, Aldrich), Triton N-20 (TCI), azobisisobutyronitrile (AIBN, Aldrich), 2,2'-azobis(2-methylbutyronitrile) (AMBN, Wako Pure Chemical Industries Ltd).

2-(8-Methacryloyloxy-3,6-dioxaoctyl)-thioxantheno[2,1,9-dej]isoquinoline-1,3-dione (HYE3OMA) and 2-(6-methacryloyloxyhexyl)-thioxantheno[2,1,9-dej]isoquinoline-1,3-dione (HYC6MA) were samples provided by Liu and were synthesized as described by Tronc [27]. *N*-[2,5-Dichloro-4-(1,3-dioxo-1H-benz[de]isoquinolin-2(3H)-yl)phenyl]-2-methyl-propenamide (dye-NSA-DCAR-MMA) was provided by Graciet at Clariant.

### 2.2. One-stage dispersion polymerization

The recipes for the one-stage dispersion polymerization of styrene, and for the copolymerization of styrene and dye-comonomer, in ethanol are listed in Tables 1 and 2. The following procedure was used: to a three neck 150 mL round bottom flask equipped with a condenser and a gas inlet, HYE3OMA (0.018 g, 0.46 wt% to styrene) was dissolved in ethanol (26.1 g). PVP (1.27 g), Triton N-20 (0.32 g) and styrene (1.1 g) were added to the flask and then the flask was placed in an oil bath. In the homopolymerization reaction, no dye-comonomer was added. The solution was deoxygenated by bubbling nitrogen, heated to the reaction temperature 80 °C,

Table 1  
Recipes for the dispersion polymerization of styrene in the presence of HYE3OMA (numbers indicated for each reagent are amounts in grams)

Run	1	2	3	4
Styrene	2.0	3.15	3.15	3.2
HYE3OMA	0.014 (0.46) <sup>a</sup>	0.012 (0.38) <sup>a</sup>	0.014 (0.43) <sup>a</sup>	0.018 (0.46) <sup>a</sup>
Solvent	MeOH 19.8	MeOH 19.8	EtOH 19.8	EtOH 26.1
PVP	0.75	0.75	0.75	1.27
Triton N-20	0.25	0.25	0.25	0.32
AIBN	0.051	0.097	0.10	0.15
<i>T</i> (°C) <sup>b</sup>	65	65	80	80
Conversion (%)	65	82	85	88
[Dye] in PS (wt%)	0.29	0.30	0.40	0.44
[Dye] conversion (%)	63	79	93	95
$M_{\text{peak}}^c$	39,000	43,000	80,000	68,000

<sup>a</sup> Wt% based on styrene.

<sup>b</sup> Reaction temperature.

<sup>c</sup> Estimated molecular weights by GPC.

Table 2  
Recipes for the polymerization of styrene in ethanol with different dye content in the feed

Run	5	6	7	8	9
Styrene	12.5	2.0	15.0	12.5	0.9
HYE3OMA	0.056 (0.45) <sup>a</sup>	0.03 (1.5) <sup>a</sup>	0.26 (1.75) <sup>a</sup>	0.25 (2.0) <sup>a</sup>	0.036 (4.0) <sup>a</sup>
EtOH	79	23.7	118.5	118.5	19.8
PVP	3.0	0.20	5.0	1.5	0.1
Triton N-20	1.0	0.06	0.9	0.42	0.03
AIBN	0.51	0.11	0.75	0.75	0.06
Conversion (%)	95	92	93	90	40
[Dye] in PS (wt%)	0.44	1.35	1.55	1.85	8.1
[Dye] conversion (%)	95	91	90	93	–
$M_{\text{peak}}^b$	69,000	42,000	85,000	52,000	36,000

The reaction temperature was 80 °C (numbers indicated for each reagent are amounts in grams).

<sup>a</sup> Wt% based on styrene.

<sup>b</sup> Estimated molecular weights by GPC.

and stirred mechanically at 100 rpm. When the reaction mixture reached the reaction temperature, a solution of AIBN (0.15 g) dissolved in styrene (2.1 g, AIBN/styrene = 7 wt%) was deoxygenated and added to the reaction flask. The reaction was run for 24 h. The monomer conversion was monitored gravimetrically by removing aliquots during the polymerization.

### 2.3. Two-stage batch dispersion polymerization

The standard recipe for the two-stage dispersion copolymerization of styrene and dye-comonomer in ethanol is listed in Table 3. The following procedure was used: all of the stabilizer (PVP), the co-stabilizer (Triton X-305) and initiator (AMB N), and half of the monomer and ethanol were added to a 250 mL three-neck reaction flask equipped with a condenser and a gas inlet. After a homogeneous solution formed at room temperature, the solution was deoxygenated by bubbling nitrogen gas at room temperature for at least 30 min. Then the flask was placed in a 70 °C oil bath and stirred mechanically at 100 rpm. The dye was dissolved in the remaining styrene plus ethanol at 70 °C under nitrogen. After the dye had dissolved and the polymerization reaction had run for 1 h, the hot dye solution was added into the reaction flask. The reaction was run for 24 h. The monomer conversion was determined gravimetrically by removing aliquots during the polymerization.

### 2.4. Determination of the dye content in polymer particles

The extent of covalent dye incorporation into the polymer was determined by UV–vis measurements of solutions in chloroform using the extinction coefficient ( $\lambda_{\text{max}} = 460 \text{ nm}$ ,  $\epsilon = 17,900 \text{ L/mol cm}$ ) of HYE3OMA as a reference. Polymer samples were prepared as follows: a sample of particles was centrifuged at 2000 rpm for 10 min. The precipitated particles were redispersed into fresh ethanol and centrifuged again. This process was repeated three times to remove the free stabilizer, unreacted dye and monomer. After the particles were dried, they were dissolved in chloroform. UV–vis spectra were

recorded with a Perkin–Elmer Lambda 25 UV/vis spectrometer.

### 2.5. Particle characterization

The particle size was examined by both optical microscopy (Olympus, BX41) and scanning electron microscopy (SEM, Hitachi S-5200). To prepare samples for optical microscopy, the final polymer particle suspension was diluted with water, and a drop was placed on a clean glass microscope slide. The SEM samples were prepared with a drop of diluted suspension on a mica film. There was no need to purify the particles by centrifugation and resuspension in alcohol to obtain clear images. The particle size distributions were determined by SEM. A particle-size histogram was constructed from measurements of 200–300 individual particles from the electron micrographs.

The molecular weights and molecular weight distributions of the constituent polymers were determined by gel permeation chromatography (GPC) in tetrahydrofuran (THF) using polystyrene calibration standards. GPC measurements were performed on a Waters liquid chromatograph equipped with a Waters 480 tunable UV–vis absorbance (UV) detector and a Waters R410 differential refractometer (RI) detector. HY-labeled polymer samples for GPC analysis were prepared as follows: the particles were centrifuged at 2000 rpm for 10 min. We note that for polymer samples prepared in runs 3–5, 7 and S-2, the continuous phase showed only a trace of fluorescent

Table 3  
Standard recipe for the dispersion copolymerization of styrene with dye-comonomer in ethanol (amounts in grams) by two-stage method

Materials		Two-stage method	
		1st stage	2nd stage
Monomer	Styrene	6.25	6.25
Dye	HYC6MA	–	0.125
Medium	Ethanol	18.75	18.75
Stabilizer	PVP	1.0	
Co-stabilizer	Triton X-305	0.35	
Initiator	AMB N	0.25	

yellow color, suggesting essentially complete dye incorporation into the particles. The precipitated particles were redispersed into fresh ethanol and centrifuged again. This process was repeated three times. After the particles were dried, they were dissolved in chloroform, and the solution was passed through a 0.45  $\mu\text{m}$  filter before injecting it into the GPC column. The UV–vis signal at 460 nm was used to monitor the HY-dye incorporation into the polymer.

A simpler method was used to check for free dye in the reaction solution when the dye NSA-DCAR-MMA was used. Here an aliquot of the PS particle suspension in ethanol was directly dissolved in THF. Because PVP is not soluble in THF, PVP (and possibly PVP-grafted PS) precipitated. The filtered solution was injected into the GPC column for analysis. The presence of unreacted dye could be detected by the UV signal (350 nm) at retention volumes corresponding to low molar mass species.

### 3. Results and discussion

It is well known, since the pioneering work of Lok and Ober [9], that the dispersion polymerization of styrene in ethanol in the presence poly(vinyl pyrrolidone) (PVP) as a stabilizer produces beautiful particles with micrometer dimensions and a very narrow size distribution. Unfortunately, the nucleation stage of this reaction is extremely sensitive to small variations in reaction conditions [19]. For example, even though one can obtain monodisperse particles repeatedly in a series of reactions run under seemingly identical conditions, there is a run-to-run variability in mean particle diameter. Various other problems with this reaction have been noted. For example, comonomers that can be employed without problem in traditional emulsion polymerization, such as polar monomers, fluorescent dye derivatives, and cross-linking agents, interfere seriously with the dispersion polymerization reaction. If these species are present at the start of the reaction, even in only tiny amounts, problems arise. The particle size and shape change, the size distribution broadens, and/or the system loses colloidal stability. We refer to these species as problematic reagents, and we refer to batch reactions in which all the reagents are present at the start of the reaction as a one-stage reaction. To place our results on the effects of dyes in context, we begin by describing the one-stage reaction of

styrene plus PVP in ethanol in the presence of a tiny amount of dyes, along the lines initially reported by Winnik and Ober [11].

#### 3.1. Properties of the dye-comonomers

To incorporate dye-comonomers into particles, they have to be soluble in the monomers or soluble in the reaction mixture. This concept applies to miniemulsion polymerization as well as dispersion polymerization. Dyes with high melting points commonly exhibit low solubility in any medium because the crystal packing energy is too strong to be disrupted by exposure to solvents. One way to lower the melting point of the dye and enhance its solubility in various solvents is to introduce substituents or vary the spacer group connecting the dye to the polymerizable function.

In this work, we used three polymerizable dyes, whose structures are shown in Chart 1. The two benzothioxanthene dyes differ in the length and nature of the spacer between the dye nucleus and the polymerizable function. HYE3OMA has a  $-(\text{CH}_2\text{CH}_2)_6-$  chain as the spacer, and HYE3OMA has a  $-(\text{CH}_2\text{CH}_2\text{O})_2-\text{CH}_2\text{CH}_2-$  chain as the spacer. Both dyes are more soluble in common solvents than the corresponding dyes with only two  $\text{CH}_2$  groups as a spacer (HYC2MA) [27]. The melting point of the dye monomers decreases dramatically with the increase of the spacer chain. HYE3OMA has a melting point of 104  $^\circ\text{C}$  and is much more soluble at room temperature in styrene. HYE3OMA is an oil at ambient temperature. HYE3OMA has the lowest solubility in styrene due to the hydrophilic oxyethylene chain. It does have enhanced solubility in alcohol solvents as compared with HYE3OMA and HYE2MA.

#### 3.2. Copolymerization of styrene with HYE3OMA by one-stage method

Several years ago, we attempted to prepare PS particles labeled with HYE3OMA by dispersion polymerization. We carried out several reactions with ca. 0.5 wt% dye according to the recipe in Table 1. Fig. 1 shows the SEM images of these particles. The particles were spherical and relatively uniform in size, but the particle size varied from 0.9 to 3.1  $\mu\text{m}$  with the same dye content at different polymerization conditions.

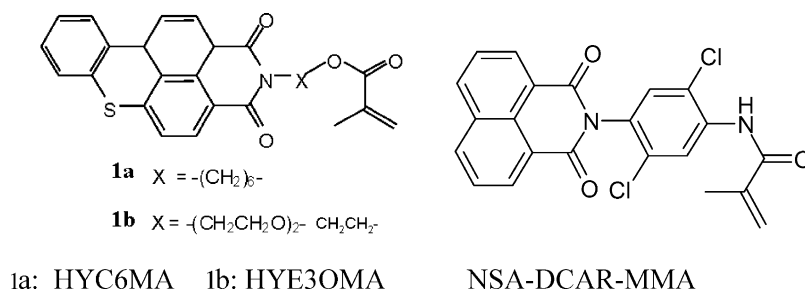


Chart 1.

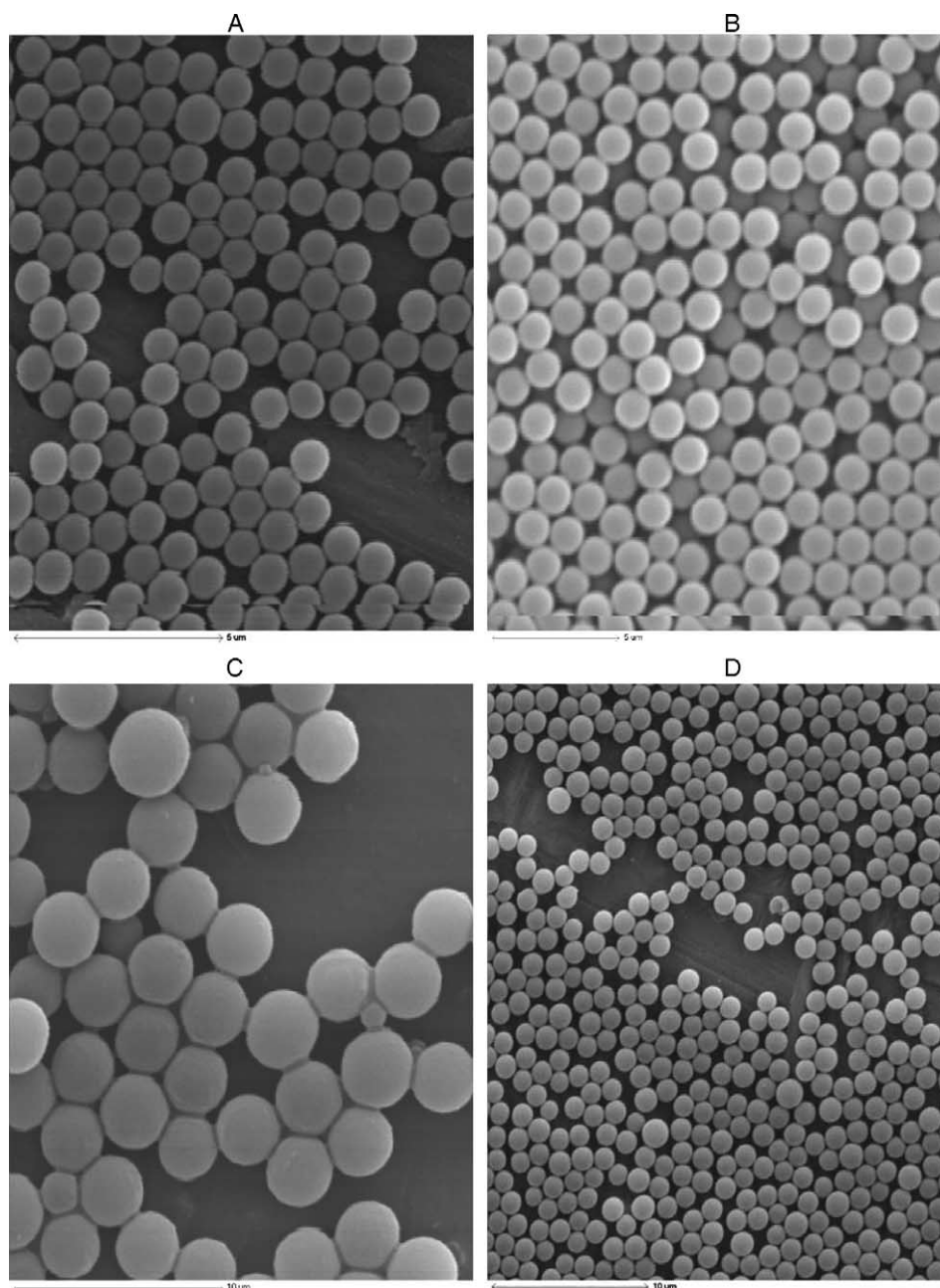


Fig. 1. SEM images of 0.5 wt% HYE3OMA labeled polystyrene particles prepared by the traditional method according to the recipes in Table 1. Average particle diameters for each run were (A) run 1, 0.9  $\mu\text{m}$  (B) run 2, 1.3  $\mu\text{m}$ , (C) run 3, 3.1  $\mu\text{m}$  and (D) run 4, 1.3  $\mu\text{m}$ .

When we carried out dispersion polymerization of styrene in ethanol with a higher dye content in the feed using the recipes described in Table 2, polydisperse particles were obtained. With 4 wt% dye, the particles have an irregular surface. SEM images (Fig. 2) show that the amount of dye in the feed seems to have a significant influence on the particle size, the size distribution and the morphology of the particles.

Tables 1 and 2 also present the results on the dye content determined by UV–vis absorbance measurements. One can see that the dye content in the polymer is very similar to that of dye in the feed when the dye feed ratio was less than 2 wt%, indicating a high yield of dye incorporation. When a reaction

was run with a higher ratio of dye to styrene in the feed (4 wt%), we found 8 wt% dye in the polymers. We speculate that this surprising value is related to the low styrene conversion (40%) in the reaction.

### 3.3. Copolymerization of styrene with HYC6MA by the two-stage method

In dispersion polymerization of styrene in ethanol, the nucleation stage is short-lived. Recent evidence [28] suggested that nucleation was complete at less than 1% monomer conversion. Operationally, the end of the nucleation step was

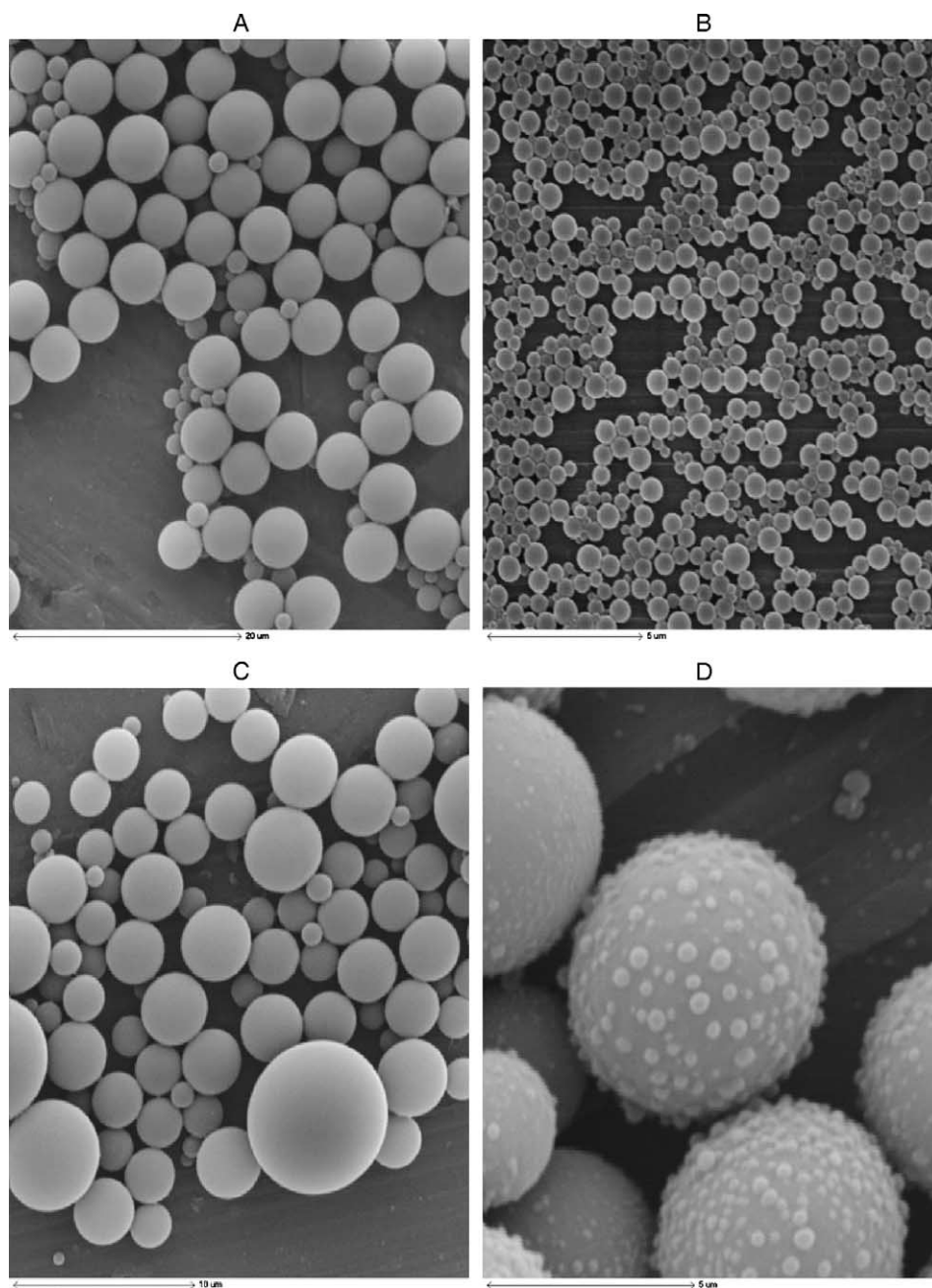


Fig. 2. SEM images of polystyrene particles labeled with different wt% of HYE3OMA prepared by conventional dispersion polymerization following the recipes in Table 2 (A) run 6, 1.5 wt%, 0.8–5  $\mu\text{m}$ , (B) run 7, 1.75 wt%, 0.25–1  $\mu\text{m}$ , (C) run 8, 2.0 wt%, 1–8  $\mu\text{m}$  and (D) run 9, 4 wt%, 2–6  $\mu\text{m}$ .

signaled by the onset of turbidity. In our experiments, turbidity was detected at less than 1% monomer conversion. We found that the reagents that would interfere with particle nucleation had a much smaller effect if added to the reaction following the onset of turbidity.

In our two-stage procedure, we chose a recipe and a set of reaction conditions appropriate for a conventional dispersion polymerization. We reserved half of the monomer and solvents. The dye was dissolved in the reserved styrene plus ethanol at 70 °C under nitrogen. After the dye had dissolved and the polymerization reaction had run for 1 h, the hot dye

solution was added into the reaction flask. The conversion was still low; less than 5% of the initial monomer had reacted. One reason is that the initiator was added into the solution before heating the system and about 15 min was needed for the temperature of the solution to increase from room temperature to 70 °C. Another reason is that the polymerization rate at the beginning of dispersion polymerization is low due to the solution polymerization mechanism, and the autoacceleration effect is not evident at low conversions.

When dispersion polymerization of styrene in ethanol with 1 wt% of HYE3OMA (based on total styrene) was carried out by

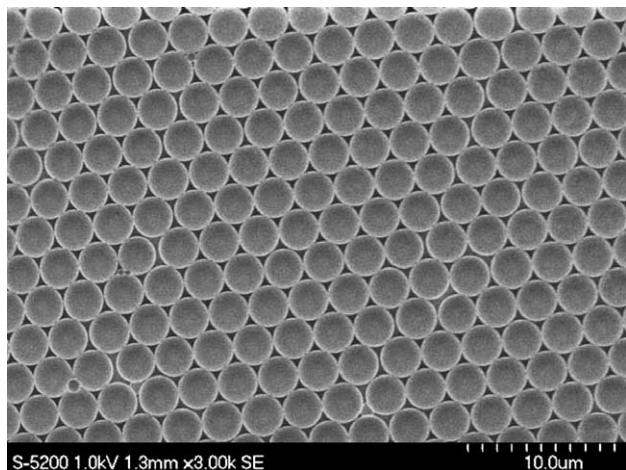


Fig. 3. SEM image of HY-labeled polystyrene particles prepared by the two-stage method (containing 1.0 wt% of HYC6MA based on styrene) according to the recipe in Table 3.

the two-stage method according to the recipe in Table 3, highly monodisperse particles were obtained. The result is shown in Fig. 3, where one can see that the presence of dye no longer perturbs the particle size and particle size distribution.

Fig. 4 shows the GPC spectrum of the polymer obtained in this reaction. The elution of the polymer was monitored by the refractive index (RI) detector, whereas the presence of the dye was monitored by its absorbance of light at 460 nm. The two peaks overlap, indicating covalent incorporation of the dye into the polymer. The small peak at 16 mL elution volume is not due to unreacted dye. This peak corresponds to a small amount of ethanol-soluble oligomers formed in the reaction.

Fig. 5 compares UV-vis spectra of HY-labeled polystyrene (PS, 1 wt% dye) prepared by the two-stage method with that of HYC6MA itself in  $\text{CHCl}_3$ . One can see that the covalently-bound HYC6MA and the free HYC6MA possess very similar absorption spectra. The free radical copolymerization of HYC6MA with styrene did not change the wavelength and shape of the absorbance.

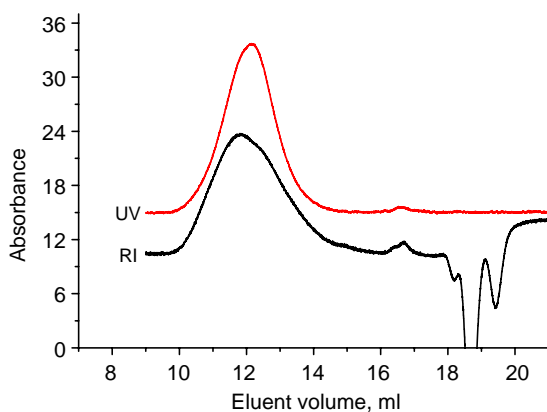


Fig. 4. GPC spectrum of HY-labeled polystyrene particles prepared by the two-stage method (containing 1.0 wt% of HYC6MA based on styrene). UV absorbance was monitored at 460 nm.

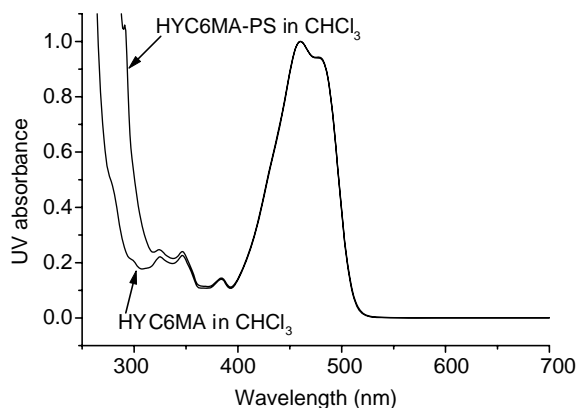


Fig. 5. UV-vis spectra of free HYC6MA and PS-bound HYC6MA (prepared by the two-stage method) in  $\text{CHCl}_3$ . The spectra have been arbitrarily normalized at the peak absorption of HYC6MA at 460 nm.

### 3.4. Copolymerization of styrene with NSA-DCAR-MMA dye by the two-stage method

NSA-DCAR is a non-fluorescent dye that we are exploring as a potential energy-transfer acceptor for phenanthrene as a donor. To explore the breadth of the two-stage dispersion polymerization, we carried out a synthesis of NSA-DCAR-labeled PS particles. Using a recipe analogous to that in Table 3 with 1 wt% of this dye based on styrene, we obtained 2.5  $\mu\text{m}$  diameter particles (see Fig. 6) with a coefficient of variation (CV) of 0.4%.

Because the solubility of NSA-DCAR-MMA in ethanol/styrene is limited, it was not possible to increase the dye content without modifying the recipe. We attempted some reactions with 3 wt% of NSA-DCAR-MMA based on styrene. In one reaction, 60 wt% (based on the total weight of styrene) of dichlorobenzene was added into the remaining ethanol/styrene mixture to dissolve the dye, and then the solution was added 1 h after the start of the reaction. Monodisperse dye-labeled particles were also obtained

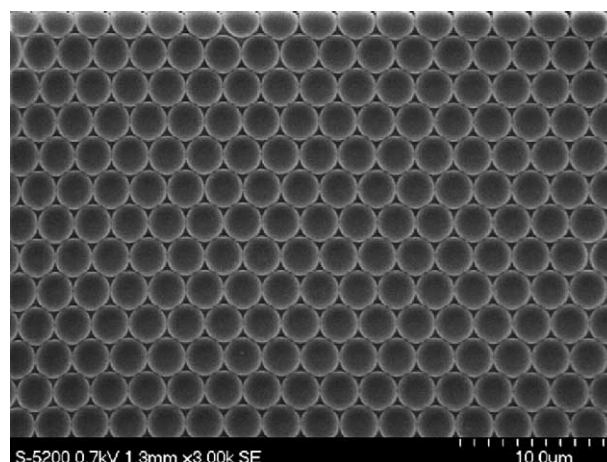


Fig. 6. SEM image of dye-labeled polystyrene particles prepared by the two-stage method (containing 1.13 wt% of NSA-DCAR-MMA based on styrene).

Table 4  
Effect of dye-comonomer on the particle size and size distribution by two-stage method

Sample	Comonomer	Dye (wt%)	$D$ ( $\mu\text{m}$ )	CV (%)	$M_w(\times 10^{-4})$	$M_w/M_n$	Method	Remark
S-0 <sup>a</sup>	No	–	2.0	<1	12.1	3.0	One-stage	Mono-disperse
S-1	No	–	2.5	<1	–	–	Two-stage	Mono-disperse
S-2	HYC6MA	1	2.5	<1	14.7	4.3	Two-stage	Mono-disperse
S-4	NSA-DCAR-MMA	1	2.5	<1	21.1	3.7	Two-stage	Mono-disperse
S-5 <sup>b</sup>	NSA-DCAR-MMA	3	3.2	3.2	12.4	3.1	Two-stage	Mono-disperse

$CV = \frac{1}{n} \sum_{i=1}^n |D_i - D_{av}| / D_{av}$ ,  $D_{av}$ , the average diameter of all particles.

<sup>a</sup> PVP = 16 wt% to St, all the ingredients were added at the beginning.

<sup>b</sup> AMBN dissolved in styrene and added after the system was heated to 70 °C. 60 wt% (based on the total weight of styrene) of dichlorobenzene was added in the remaining ethanol/styrene to help to dissolve the dye and then the mixture was heated at 70 °C before added into the flask at the second stage.

( $D=3.2 \mu\text{m}$  and  $CV=3.2\%$ , shown in Table 4), but this size distribution is not as narrow as that of particles synthesized in the presence of 1 wt% of NSA-DCAR-MMA dye. When less dichlorobenzene was added, some of the dye was not soluble and was not able to be incorporated into polymer particles. In order to increase the amount of the dye incorporated into the particles, a dye with better solubility in the reaction medium should be used.

A simple method was used to check for free dye in the reaction solution, when the dye NSA-DCAR-MMA was used. Here an aliquot of the PS particle suspension in ethanol was directly dissolved in THF. Fig. 7 presents typical GPC signals obtained for the polystyrene particles with 1.1 and 3.1 wt% of NSA-DCAR-MMA. The elution of the polymer was monitored

by the refractive index (RI) detector, whereas the presence of the dye was monitored by its absorbance of light at 350 nm. There is nearly no free dye in the solution, which means this dye copolymerized very well with styrene. The small UV peak at about 7.5 mL elution volume corresponds to the dye attached to oligomers that formed in the reaction and remained in solution.

Fig. 8 compares the UV–vis spectra of NSA-DCAR-MMA-labeled polystyrene (PS) prepared as described above containing 1.1 wt% of NSA-DCAR-MMA with that of free NSA-DCAR-MMA in  $\text{CHCl}_3$ . Both the covalently-bound NSA-DCAR-MMA and the free NSA-DCAR-MMA have very similar UV–vis absorbance spectra. This dye can be incorporated into PS without perturbation of its absorption spectrum.

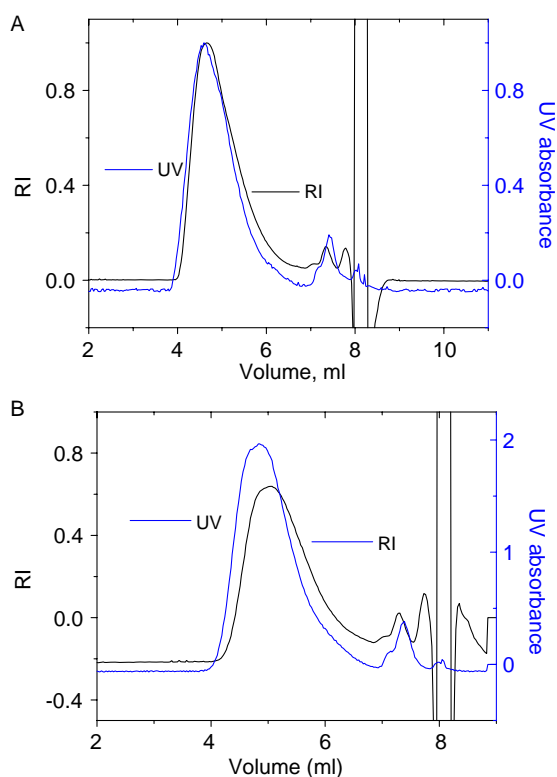


Fig. 7. GPC spectra of NSA-DCAR-MMA-labeled PS particles prepared by the two-stage method. UV absorbance was monitored at 350 nm. ((A) 1 wt% of NSADCAR-MMA and (B) 3.1 wt% NSA-DCAR-MMA based on PS).

#### 4. Conclusions

The use of polymerizable dyes in two-stage dispersion polymerization offers a straightforward route to prepare monodisperse micron-sized dye-labeled PS particles. GPC spectra show that essentially all of the dye is covalently incorporated into the PS particles, and the UV–vis spectra show that the covalently-incorporated dyes possess the same spectra as the free dye. A small amount of solvent-soluble dye-containing oligomer is produced in the reaction. This oligomer

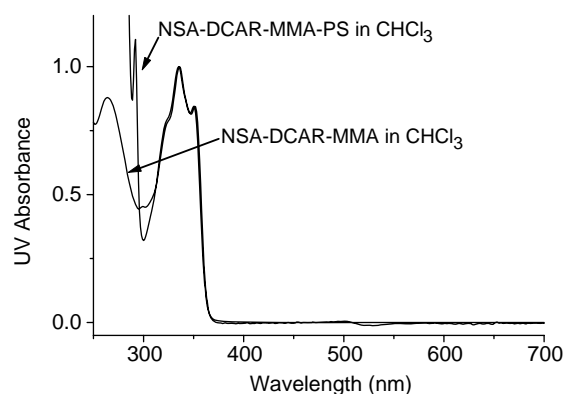


Fig. 8. UV–vis spectra of free NSA-DCAR-MMA and PS-bound NSA-DCAR-MMA (prepared by the two-stage method) in  $\text{CH}_3\text{Cl}$ . The spectra have been arbitrarily normalized at the peak absorption of NSA-DCAR-MMA at 350 nm.



can be removed by centrifuging the particles and redispersing them in ethanol or water.

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