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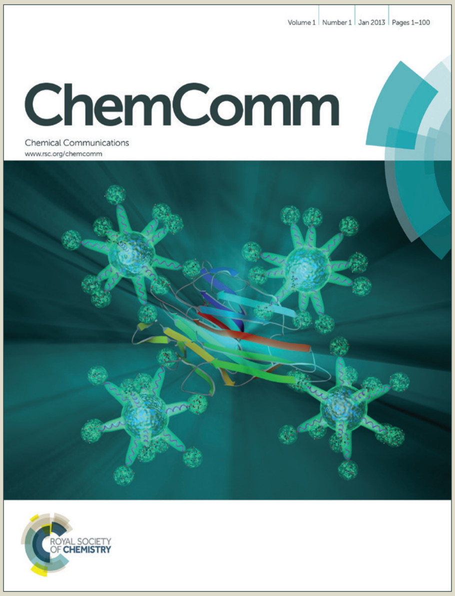

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# Synthesis of monodisperse nano colloidal microspheres with controlled size by vesicle bilayer templating

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**This study describes a new method to prepare monodisperse polystyrene (PS) colloidal nano particles with controlled size ranging from 60 nm to 140 nm by the direct polymerization of styrene and divinylbenzene inside the bilayers of the polydisperse hyperbranched polymer vesicles.**

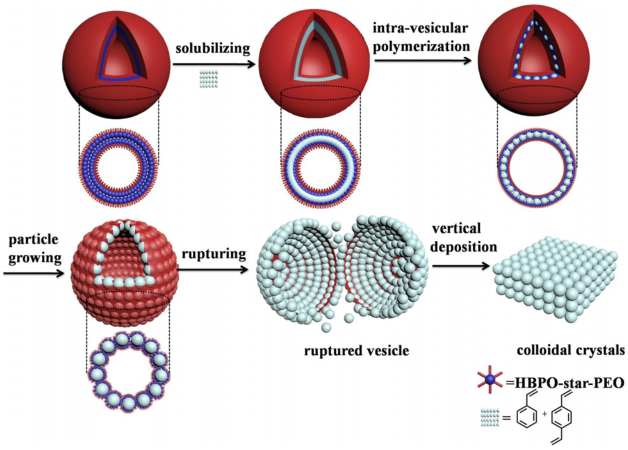
Monodisperse colloidal particles have attracted wide interest due to their extensive applications such as calibration standards, separation media, microelectronics, optical device, molecule-mimetic self-assembly and biotechnology.[1] Among them, monodisperse PS particles have been widely used due to the high quality, easy availability and controllable size. Several classical techniques, such as dispersion polymerization, seeded polymerization, emulsifier-free emulsion polymerization and conventional emulsion polymerization, have been developed in preparing uniform PS particles from 150 nm to tens of microns.[2] Microemulsion polymerization is capable to prepare PS particles below 150 nm, however the size distribution is not satisfactory.[3] Alternatively, "Polymerization Induced Self-assembly" of block copolymers can prepare small narrowly distributed micelles with PS shells.[4] Nevertheless, hitherto, it is still technologically challenging for facile and massive preparation of monodisperse PS spherical particles below 150 nm, especially below 100 nm, in spite of their unique applications such as in flexible visible transparent and UV shielding colloidal crystal films.[5] These films can efficiently block UVB region of the ultraviolet spectrum (from 280 nm to 315 nm) which may interfere with DNA replication and lead to cessation of cell proliferation.[6]

Herein, we report a novel method to prepare monodisperse PS particles around 60-140 nm in mass scale by using hyperbranched polymer vesicles as templates. The overall synthetic procedure is illustrated in Scheme 1. First, the hydrophobic monomers of styrene (St) and divinylbenzene (DVB) as well as photo-initiators of 2,2-dimethoxy-2-phenylacetophenone (DMPA) were solubilized (St:DVB =20:1 in molar ratio, DMPA is 0.2% in weight ratio) in the hydrophobic interiors of the bilayers of the hyperbranched polymer vesicles. Second, under UV light, intra-vesicular free-radical copolymerization of the oil-soluble monomers was initiated within the bilayers to obtain small particles like "seeds" in seeded emulsion polymerization. Third, PS particles grew continuously and uniformly from "seeds" to become bigger and bigger. At a certain stage, vesicle rupture happened due to the continuous increase of bilayer tension, leading to the release of uniform PS colloidal particles which could further self-assemble into colloidal crystals.

Generally, the applications of vesicles are mainly focused on encapsulation, drug delivery and mimic of cells (cytomimetic chemistry).[7] As a new application, the present work indicates vesicles are also promising in the preparation of monodisperse nanoparticles.

The polymer vesicles used here were previously reported by our group, and were obtained through direct aqueous self-assembly of amphiphilic hyperbranched multi-arm copolymers of HBPO-star-PEOs, where HBPO represents the hydrophobic hyperbranched poly(3-ethyl-3-oxetanemethanol) core and PEO represents the hydrophilic poly(ethylene oxide) arm (Fig. S2, ESI†). The HBPO-star-PEOs used here have a molar fraction of PEO segments ($f_{EO}$) of 73% and a number-average molecular weight of 7400 (Figs. S3-S5, ESI†). The as-prepared vesicles are named as "branched polymersomes" (BPs), and are around 1-10 µm in average diameter (Fig. S6, ESI†) and have a polydisperse size distribution and a bilayer structure. The detailed characterizations for the obtained BPs can be referred to our previous work.[8]

It is well-known that hydrophobic monomers can be solubilized in the hydrophobic domains of the vesicle bilayers. The same phenomena were observed in our experiments. St and DVB monomers are immiscible with water, however, a homogeneous milky solution was formed when stirring monomers and BPs together in water, indicating the successful solubilization. As the further evidence, the hydrophobic monomers were labeled with pyrene through co-mixing, followed by vigorous stirring with the suspension of BPs. The fluorescent image (Fig. 1a) clearly shows BPs have hollow lumens and green fluorescent skins, revealing the hydrophobic monomers are



**Scheme 1** Synthesis of monodisperse PS particles by vesicle bilayer templating. The hydrophobic HBPO layer of vesicles is in blue, the hydrophilic PEO coronae are in red, and the monomers and PS particles are in light blue.

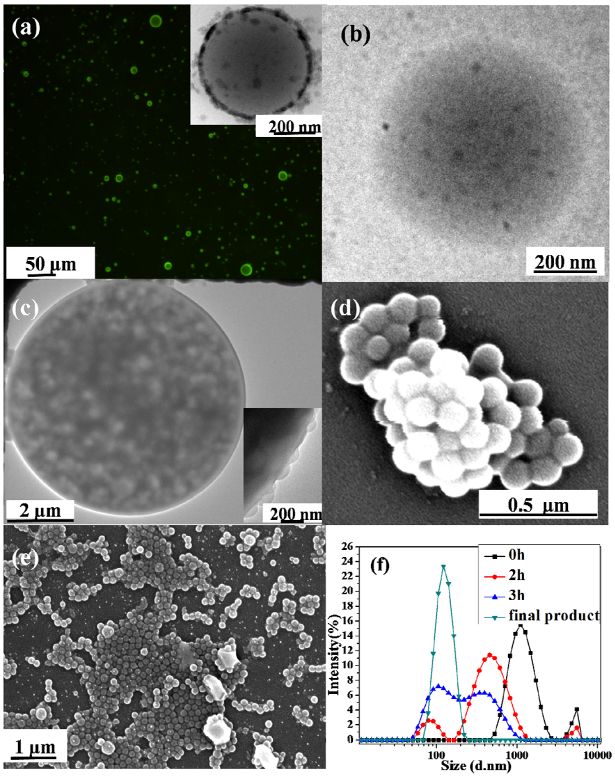


**Fig. 1** Characterizations of vesicles and PS colloidal particles. (a) The fluorescent image of the BPs encapsulated with monomers and pyrene, and the inset shows the typical TEM image of the vesicle loading with monomers; (b) The TEM image after polymerization in BPs for 0.5 h; (c) The TEM image after polymerization for 1 h, and the inset shows a magnified view of the boundary of the vesicle; (d) The SEM image of a rupturing vesicle after polymerization for 2 h; (e) The SEM image of the vesicle debris after polymerization for 2 h; (f) The intensity-weighted particle size distributions of the reaction supernatants after polymerization for different time. The feed compositions are as follows: St/DVB (molar ratio) =20:1, HBPO-star-PEO/St (mass ratio) =1:4.

transported into the vesicle bilayers. The transmission electron microscopy (TEM) image (inset in Fig. 1a) of the BPs after mixing with monomers also shows the black domains in the bilayer, which provides another direct evidence to support the solubilization process.

The initiation and intra-vesicular polymerization were carried out under nitrogen atmosphere in the quartz sleeve reactor by using an UV-lamp (250 W, $\lambda_{max}$=366 nm) as irradiation source (Fig.S1, ESI†). A small amount of reaction solution was collected at different time intervals through a syringe for the following tests. After reaction for 0.5 h, the TEM image (Fig. 1b) shows many black nuclei within the vesicle bilayers, and these nuclei are thought to functionalize like the “seeds” in seeded emulsion polymerization. After 1 h, many well-defined spherical particles on the vesicles could be discerned (Fig. 1c). After 2 hrs, vesicles with particles began to rupture, and Fig. 1d shows an intermediate of the rupturing vesicle with interconnecting particles according to the scanning electron microscope (SEM). In most case, the vesicles disintegrated into debris with different sizes consisting of uniform particles (Fig. 1e). It should be noted that the particles in the debris were still embedded into the vesicle membranes in this step, so a further sonication step was used to totally separate the particles from the vesicle templates after reaction for 3 hrs. The dynamic light scattering (DLS) analysis of different reaction stages is shown in Fig. 1f. At the beginning (polymerization for 0 h), two broad peaks centered on 1 μm and 5 μm were observed, indicating that the vesicles are highly polydisperse in size. After reacted for 2 hrs, a new peak centered on 100 nm emerged, which should be attributed to the formation of PS colloidal particles. In addition, the peak assigned to the original vesicles moved from 1 μm to 500 nm probably due to the rupture of vesicles into debris. After 3 hrs, the peak of PS colloidal particles increased with the consumption of vesicle debris, which showed that more PS particles escaped into water with the further disintegration of debris. Finally, after a further sonication step, PS particles with a nearly monodisperse size distribution (PDI=0.04) were obtained.

By using the same method, a series of uniform PS colloidal particles were produced in mass scale, and their size could be controlled from 60 nm to 143 nm according to DLS measurements by adjusting the mass ratio between HBPO-star-PEO and styrene from 1:1 to 1:6 (Table 1, Fig. S7 in ESI†). All the as-prepared PS particles have a very narrow size distribution according to the small PDI, and they tended to aggregate together into colloidal crystal structures according to the SEM images (Fig. 2). The insets in Fig. 2 also show the TEM images of the same PS particle samples under the staining by ruthenium tetraoxide. The particle size and size distribution measured by SEM and TEM are consistent with those characterized by DLS.

To our knowledge, narrowly distributed PS particles below 150 nm, like those shown in Fig. 2, are difficult to prepare and have been hardly reported. Especially, the very small but uniform PS particles around 60 nm, as shown in Fig. 2a, have not been observed before. A possible particle formation mechanism is illustrated in scheme 1 according to the data in Figs. 1 and 2.

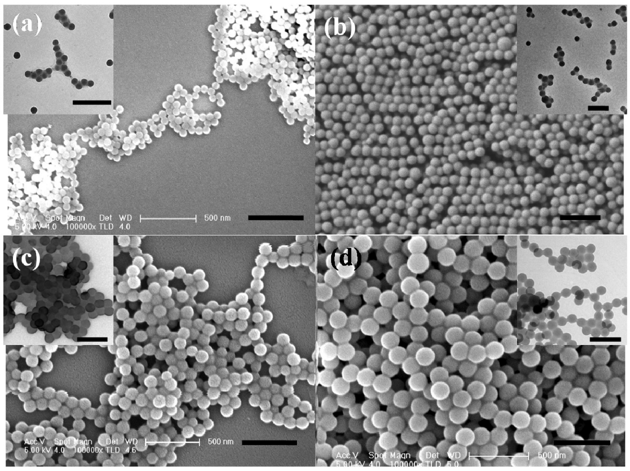


**Fig. 2** SEM and TEM images of PS colloidal particles obtained at different HBPO-star-PEO/St mass ratios of 1:1 (a); 1:2 (b); 1:4 (c) and 1:6 (d). Scale bars represent 500 nm.

**Table 1** Average size and PDI of different ratios of components

| St/DVB (molar ratio) | HBPO-star-PEO/St (mass ratio) | Average Size(nm) | PDI |
|---|---|---|---|
| 20：1 | 1:1 | 60 | 0.082 |
| | 1:2 | 106 | 0.046 |
| | 1:4 | 122 | 0.040 |
| | 1:6 | 143 | 0.072 |

The bilayer of the vesicles served as the templates for the nucleation and growth of PS particles. In fact, before us, some pioneering works have already been done on monomer polymerization in the bilayers of lipid vesicles (liposomes) or surfactant vesicles.[9] They obtained parachute-like or necklace vesicles with one or several polymer particles encapsulated in the bilayer of each vesicle. However, the yield of particles is very low, and particles are not uniform and have no size control. In contrast, herein, we realized mass production of uniform PS particles from the bilayer of hyperbranched polymer vesicles. We believe that such difference results from the special properties of hyperbranched polymer vesicles, such as thicker bilayers and very good fluidity, flexibility and stability.[10] The fluid and flexible BPs bilayer are the guarantee for the free diffusion of monomers and oligomers, which is very useful for the homogeneous nucleation and growth of PS particles.

There are another two important questions for the particle formation mechanism. The first one is: why are monodisperse PS particles formed from polydisperse BPs? Two factors are thought to contribute it. One factor is that the bilayer thickness is independent of the vesicle size and is highly uniform.[11] As a result, the size of the nuclei was governed by the uniform bilayer templates to get monodisperse. These nuclei are similar to “seeds” in seeded emulsion polymerization, and would induce homogeneous growth of PS particles with the continuous diffusion and polymerization of monomers within the bilayer. The other factor originates from the vesicle rupture process. It has been proved by dissipative particle dynamics (DPD) simulations that vesicle rupture is independent of vesicle size and will occur at the critical membrane properties such as tension increment.[12] Thus, for the vesicles with similar bilayers but different size, if the particles inside the bilayers grew into an similar critical size, the vesicles would exhibit similar membrane tension increment, leading to the rupture of vesicles. Meanwhile, the particle growth process was stopped due to the termination of polymerization in devoid of monomers with the rupture of vesicle templates, and thus uniform PS particles were obtained.

Another question for the particle formation mechanism is why the PS particle size can be controlled by the feed ratio between HBPO-star-PEO and styrene. It can also be attributed to the tension-induced vesicle rupture process. With the increase of feeded monomers, more monomers were solubilized into vesicles to get thicker vesicle membranes, which means smaller membrane tension.[12] In this case, the critical tension increment for vesicle rupture would be reached only if the PS particles grew into a larger size. Thus, the obtained PS particles increase with the decrease of HBPO-star-PEO/styrene mass ratio.

In summary, herein, we demonstrated a facile method for producing uniform PS colloidal particles with a controllable size from 60 to 150 nm and monodisperse size distribution by using the bilayers of hyperbranched polymer vesicle as templates. We anticipate that this technique may be a general method for the preparation of other monodisperse polymeric or hybrid particles with small size by changing the oil-soluble monomers, which are still unavailable by other techniques up to now. The related work is still in progress.

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## Notes and references

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