Free radical polymerization of styrene in dioctadecyldimethylammonium bromide vesicles

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The novel radical polymerization of an unsaturated monomer in the hydrophobic part of vesicles is attempted using the experience gathered from kinetic and thermodynamic studies of the emulsion polymerization process. The influence of the monomer content of the system and also the initiation of the polymerization are discussed. A free radical polymerization of styrene in dioctadecyldimethylammonium bromide vesicles is performed. Evidence of the formation of polymer-containing vesicles is obtained by means of electron microscopy and the comparison of the diffusion rate of a paramagnetic probe over the bilayer of vesicles and polymer-containing vesicles.

(Keywords: vesicle; DODAB; emulsion polymerization; styrene; hollow polymer particle)

INTRODUCTION

The self-organization of amphiphilic molecules in water is a well known phenomenon. Depending on the structure of the amphiphilic molecule, different surfactant structures can be formed[1,2]. Organized surfactant structures are used in a wide range of applications such as drug carriers, solar energy conversion, catalysis and polymerization processes[3,4]. In the emulsion polymerization process, micelles are often used as the initial sites for the polymerization[5]. These polymerizations are of the free radical type. The polymer formed in an emulsion polymerization is present in the form of polymer particles stabilized with surfactant, also referred to as latex particles.

The aim of this investigation is to perform a free radical polymerization of an unsaturated monomer (styrene) within a surfactant structure, i.e. within the bilayer of dioctadecyldimethylammonium bromide (DODAB) vesicles. The motivation of this study is to 'freeze' the vesicle structure by addition of polymer, which will eventually lead to hollow polymer particles. Concomitantly, the most difficult problem is to ensure that the vesicle structure is not destroyed by the polymerization process. Therefore this feasibility study was performed to gain insight into the factors and parameters involved in this process.

The process of free radical polymerization within the hydrophobic part of vesicles has been attempted once before[6], but the aim of that investigation was not to create hollow polymer particles, i.e. not the study of the thermodynamic and kinetic factors that are involved in the polymerization process, but to investigate the influence of polymer on the physical properties of the vesicles, namely the lateral mobility of probe molecules in the plane of the bilayer and the diffusion of molecules across the bilayer.

THEORY

The theoretical guidelines for the polymerization of monomer in the hydrophobic part of vesicles must address at least two critical variables: (1) the monomer concentrations in the system, and (2) the initiator system. The possible occurrence of different nucleation mechanisms will be discussed below.

Monomer concentrations in the system

The total monomer content in the vesicle system prior to polymerization is a very crucial parameter. We distinguish two cases. In the first case the monomer partitions between the aggregates (vesicles) and the water phase. The monomer concentration in both phases is smaller than the saturation value of the monomer concentration in these two phases, therefore no separate monomer phase is present. A second possibility is that the monomer concentrations in both the water phase and the vesicles exceed the saturation value, resulting in the formation of a separate monomer phase.

Vesicle structures can normally swell with monomer to some saturation value and remain stable. However,

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during an emulsion polymerization, the reaction mixture is stirred to maintain thermal equilibrium, and hence any free monomer phase is dispersed as droplets. The surface area of the droplet phase will compete with vesicles for surfactant, and hence cause, to some extent, the break-up of the vesicle structures. Of the two cases described above, only the first is desirable, since the vesicle structure is maintained while the monomer concentration in the vesicles is close to its saturation value. The saturation value of styrene in the water phase at different temperatures is well documented in the literature (e.g. ref. 7). On the other hand, the saturation value of styrene in DODAB vesicles is a very difficult parameter to determine. Therefore we have used an approximation based on the results of the statistical mechanical model developed by Gruen[8,9] which described the extent by which a planar lipid is swollen by different hydrocarbons.

The initiator system

Different mechanisms are involved in the nucleation stage of the emulsion polymerization process. First, Harkins' mechanism for emulsion polymerization considered that the major source of particle nucleation was the monomer-swollen micelles (micellar nucleation)[10]. Smith and Ewart attempted to quantify the Harkins micellar entry mechanism[11]. An alternative nucleation mechanism proposed in surfactant-free systems involves homogeneous nucleation[12,13], which was first quantified by Fitch and Tsai[14] and became known as the 'HUFT' (Hansen–Ugelstad–Fitch–Tsai) theory[15]. The coagulative nucleation theory is a straightforward extension of the homogeneous nucleation theory[16]. It postulates that the species first formed by precipitation, or even micellar nucleation, are colloidally unstable and undergo coagulation until colloid stability is attained. A consideration of the presence of a separate monomer phase is critical to the success of the attempted polymerization of monomer entirely within the hydrophobic part of vesicles. As previously stated, the presence of a separate monomer layer in a stirred vessel may imply vesicle break-up. Hence any polymerization will be by homogeneous or coagulative nucleation, forming normal latex particles.

From studies on nucleation mechanisms in emulsion polymerization it is known that water-soluble initiators greatly increase the risk of homogeneous and/or coagulative nucleation[17], while oil-soluble initiators, which are mainly present in the organic phase, will almost exclusively lead to micellar and monomer droplet nucleation. The choice of the initiator system is restricted to the requirement that during the polymerization process the monomer-swollen vesicle structure is retained and the formation of new particles is prevented, i.e. only vesicular nucleation occurs. This means that all the monomer-swollen vesicles should be polymerized 'simultaneously', resulting in polymer-containing vesicles. If this is not the case, monomer and surfactant from non-reacting monomer-swollen vesicles will diffuse to polymerizing vesicles. This can be prevented by creating as many radicals as possible in a short period of time so that all monomer-swollen vesicles are initiated to form polymer-containing vesicles. This approach is known for microemulsion polymerization[18]. We used a redox initiator system consisting of cumenehydroperoxide (CHP) and iron(II)[19]. This initiation system satisfies the major requirements, i.e. it is oil-soluble (CHP) and it produces many radicals in a short period of time.

EXPERIMENTAL

Materials

Styrene (Merck) was distilled at reduced pressure under nitrogen and stored at 4°C. DODAB (Fluka), CHP ($C_9H_{12}O_2$, Fluka), iron(II)sulfate ($FeSO_4$, Fluka), ethylenediaminetetraacetic acid (EDTA, Fluka), ascorbic acid ($C_6H_8O_6$, Merck), tris(hydroxymethyl)aminomethane (TRIS, Janssen Chimica) and 2,2,6,6-tetramethylpiperidinooxy free radical (TEMPO, Janssen Chimica) were all used as received.

Preparation of vesicles

Typically 10^{-2} M solutions of DODAB in water were sonificated for 3–4 h at 55°C in a thermostatted Branson 3200 sonification bath (90 W). Before use, all vesicle solutions were filtered over a 0.45 μm Millipore filter. The influence of the sonification power and time on the vesicle formation will be discussed elsewhere[20].

Free radical polymerization of styrene in DODAB vesicles

The polymerizations were all performed in a 100 ml glass reactor thermostatted at 50°C and stirred with a magnetic stirrer. The vesicles were swollen with the monomer for 24 h at 50°C, so that equilibrium was attained between the water phase and the monomer-swollen vesicles, after which the initiator (CHP/Fe(II)) was added to the system. Fe(II) was present in the form of the Fe(II)–EDTA complex to ensure that no precipitation/oxidation of the Fe(II) by water could occur. The Fe(II)–EDTA solution was set at pH 4–5 with sulfuric acid[19]. Typical quantities and concentrations are listed in *Table 1*. (In this typical case the monomer concentration in the vesicles was calculated to be approximately 1.7 mol styrene per litre swollen vesicle, assuming an average diameter of the vesicles of 50 nm, bilayer thickness of 5 nm (twice the extended chain of the hydrophobic part of the surfactant[1,2]) and a headgroup area[21] of 0.55 nm^2. The volume fraction of polymer in the vesicles after polymerization was approximately 0.2 at 80% monomer conversion.)

Electron microscopy

Transmission electron microscopy (TEM) was performed on a Jeol FX-2000 microscope while scanning electron micrsocopy (SEM) was carried out on a Cambridge Stereoscan 200 microscope. The samples used for TEM were prepared according to the following procedure. A droplet of the sample was applied to a strip of Parafilm. A 400 mesh carbon-coated grid was placed on top of this droplet for 1 min. The residual sample was removed from the grid with a tissue. The same procedure was followed

Table 1 Free radical polymerization of styrene in DODAB vesicles

Ingredient	Amount
DODAB	0.630 g
Water	100 g
Styrene	1 g
CHP	0.11 M[a]
Fe(II)	10^{-7} M[b]

[a] Concentration in monomer phase
[b] Concentration in aqueous phase

for the negative staining with a 2% uranylacetate solution. The freeze-fracture samples were prepared as described elsewhere[22]. The samples used in cryo-electron microscopy were dealt with as described in reference 23.

Characterization of vesicles and polymer-containing vesicles

The vesicle and polymer-containing vesicle solutions were prepared in the presence of the paramagnetic probe TEMPO ([TEMPO] = 10^{-3} mol l^{-1})[24–26]. It was assumed that the paramagnetic probe was exclusively present in the water phase and that the concentrations of the probe in the internal and external water volume were equal. The ascorbate reducing solution contained 0.15 mol l^{-1} of ascorbic acid after setting the pH at 5 with 0.05 mol l^{-1} TRIS buffer. The intensity of the electron spin resonance (e.s.r.) signal of a 1.5 ml aliquot was measured (Bruker ER 200, centre field 0.35 T, modulation amplitude 1.3×10^{-4} T) before adding the reductant and after the addition of 15 μl of the reducing solution. The intensity of the signal was followed as a function of time.

RESULTS AND DISCUSSION

Following polymerizations as described in the previous section, several different techniques were used to determine the presence of polymer in the vesicles. These are discussed below.

Freeze-fracture electron microscopy

The freeze-fracture process splits the bilayer into two monolayers. The fracture plane is determined by the hydrophobic interface. It runs between the ends of the hydrocarbon chains through the middle of the bilayer. Although the freeze-fracture technique seems very successful in the study of vesicles and polymerized vesicles[27], it is very difficult to use in the case of polymer-containing vesicles because the polymer in the vesicles sticks to the replica and is very hard to remove.

Cryo-electron microscopy

With this technique it should be possible to measure the bilayer thickness of vesicles. Due to the relatively low volume fraction of polymer in the vesicles, the difference in bilayer thickness between vesicles and polymer-containing vesicles is very small. Concomitantly it was very difficult to distinguish between vesicles and polymer-containing vesicles with this technique.

The cryo-electron microscopy and freeze-fracture technique were not successful in distinguishing between vesicles and polymer-containing vesicles due to the specific problems outlined above.

Transmission electron microscopy

When a vesicle solution is applied to a grid and subsequently dried, the vesicles lose their specific structure, i.e. they collapse (*Figure 1*). However, when the vesicles contain a low volume fraction of polymer, it is assumed that during the drying process structures are formed with a morphology comparable to erythrocytes (red blood cells)[28]. The expected intensity patterns in TEM and SEM are identical although they have different origins. Before the samples were dried they were negatively stained, as described in the Experimental section. It is expected that the observed intensity profile of the transmitted electrons closely resembles the pattern shown in *Figure 1*. *Figure 2* shows a TEM micrograph of a typical polymer-containing vesicle. When normal vesicles are stained using the described procedure a totally different pattern is observed (*Figure 3*).

Scanning electron microscopy

When dried polymer-containing vesicles are studied by SEM, one can expect a scattering of electrons according to *Figure 1*, i.e. more scattering of electrons at the edges and less in the middle of the dried structures. In *Figure 4* an SEM micrograph of polymer-containing vesicles is shown.

In both the electron microscopy techniques the observed intensity patterns are identical to those expected, which could indicate that there are polymer-containing vesicles and that they exhibit a behaviour different to that of vesicles. Electron microscopy techniques have a strong tendency to be subjected to artifacts which could lead to incorrect interpretation of the micrographs. Therefore, in the following, we describe an independent method for distinguishing between vesicles and polymer-containing vesicles.

Encapsulation of a paramagnetic probe by vesicles and polymer-containing vesicles

We determined the diffusion behaviour of a paramagnetic

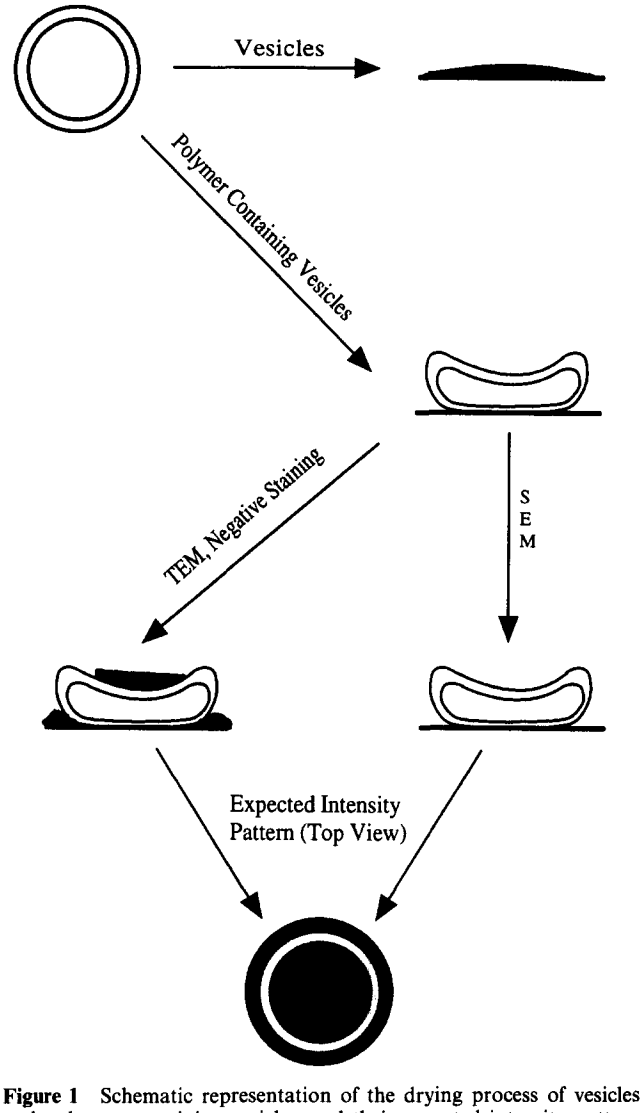

Figure 1 Schematic representation of the drying process of vesicles and polymer-containing vesicles, and their expected intensity pattern in the SEM and TEM experiments

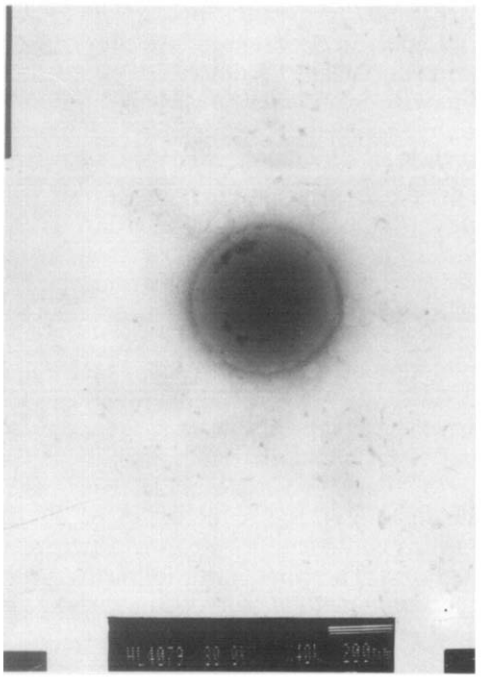

Figure 2 TEM micrograph of typical negatively stained polystyrene-containing DODAB vesicle (original magnification 40 000×; bar indicates 200 nm)

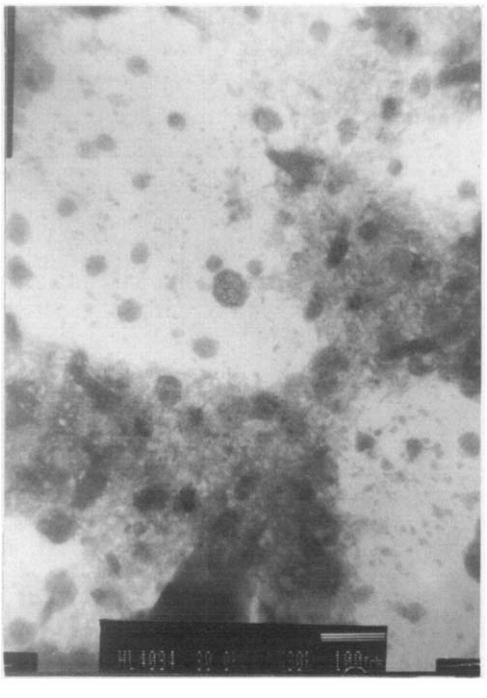

Figure 3 TEM micrograph of negatively stained DODAB vesicle (original magnification 80 000×; bar indicates 100 nm)

probe over the bilayer of vesicles and polymer-containing vesicles in order to distinguish between these two. It is assumed that the non-diffusive paramagnetic probe (see below) is only present in the water phase. This was verified by measuring the e.s.r. signal of the paramagnetic probe (TEMPO) in the presence and absence of vesicles. There were no detectable differences in the observed peak widths and heights of the e.s.r. signals. To ensure that this encapsulation behaviour is typical for so-called hollow structures, a polystyrene latex, with approximately the same solids content as the polymer-containing vesicles, was used in an encapsulation experiment. After the addition of reductant no measurable e.s.r. signal was obtained, indicating the absence of voids and also the absence of probe in the polymer phase. The bilayer composition of vesicles and polymer-containing vesicles may differ strongly. It is assumed that the different bilayers exhibit different diffusion behaviours for the same paramagnetic probe. This can be illustrated by *Figure 5*. At t_0 the reductant is added and the e.s.r. signal of the paramagnetic probe is followed as a function of time. In the case of vesicles the paramagnetic probe can diffuse through the bilayer, while in the case of polymer-containing vesicles this diffusion is more restricted. In

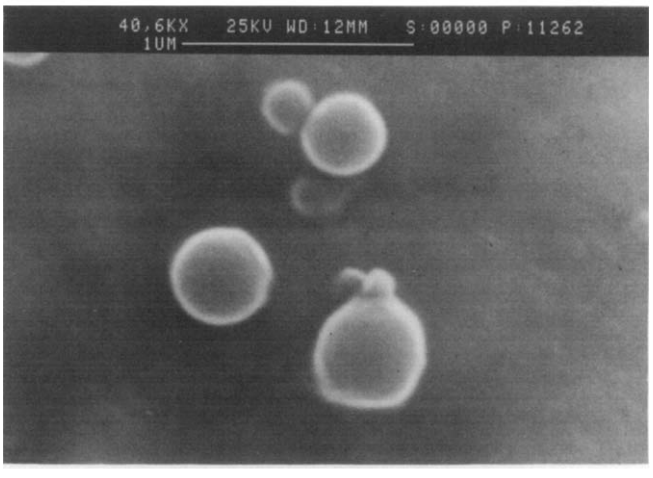

Figure 4 SEM micrograph of polystyrene-containing DODAB vesicle (original magnification 40 600×; bar indicates 1 μm)

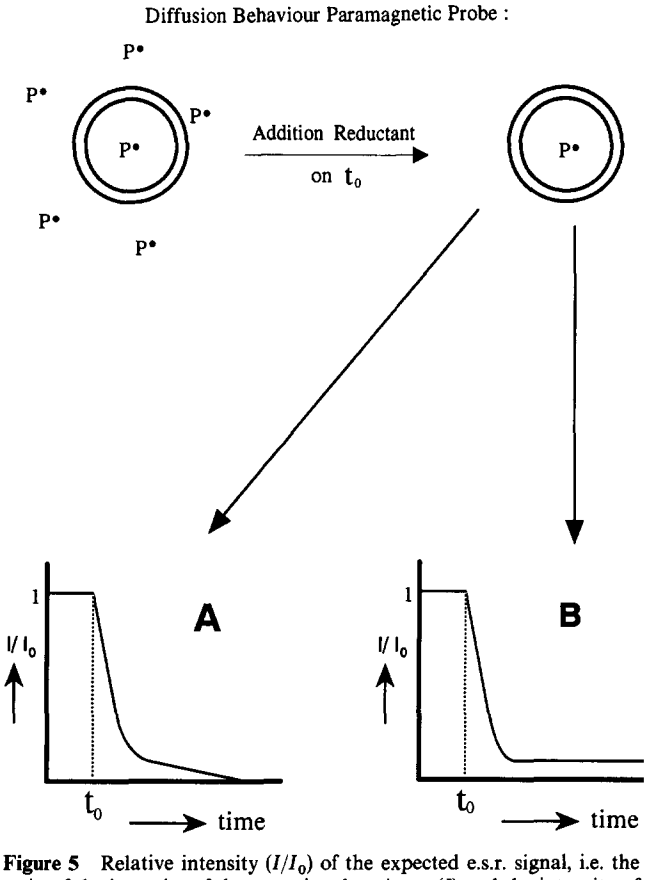

Figure 5 Relative intensity (I/I_0) of the expected e.s.r. signal, i.e. the ratio of the intensity of the e.s.r. signal at time t (I) and the intensity of the e.s.r. signal at t_0 (I_0), of a diffusive paramagnetic probe ($P^\bullet$) as a function of time, for vesicles (A) and polymer-containing vesicles (B)

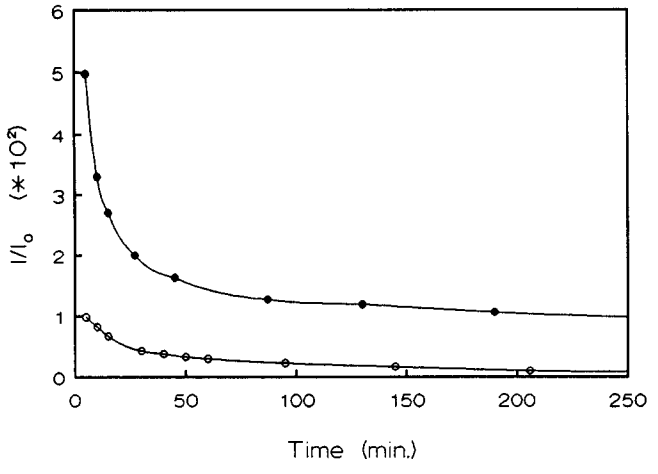

Figure 6 Relative intensity (I/I_0) of the measured e.s.r. signal, i.e. the ratio of the intensity of the e.s.r. signal at time t (I) and the intensity of the e.s.r. signal at t_0 (I_0), as a function of time. Diffusion rate measurement of the diffusive paramagnetic probe TEMPO over the bilayer of DODAB vesicles (○) and polystyrene-containing DODAB vesicles (●)

Figure 6 a plot is shown of the diffusion rate measurement of the paramagnetic probe TEMPO through the bilayer of vesicles and polymer-containing vesicles. The observed curve for vesicles (*Figure 6*, open circles) resembles that predicted in *Figure 5*, i.e. a steep drop of the relative intensity caused by the reduction of the paramagnetic probe in the external water phase, with a slow decrease of the relative intensity in the latter part of the curve due to the diffusion of the probe through the bilayer and subsequent reduction in the water phase. In the case of the polymer-containing vesicles, much slower diffusion takes place through the polymer-containing bilayer, resulting in a non-zero slope of the diffusion-controlled part of the curve. The reason why some diffusion is detected is that TEMPO can diffuse through the polymer, albeit at a reduced rate. The measured diffusion rate of the paramagnetic probe through the bilayer of polymer-containing vesicles is a combination of the two limiting cases outlined above, i.e. of vesicles and polymer-containing vesicles.

We conclude that the difference in diffusion behaviour of the paramagnetic probe through the bilayer of vesicles and polymer-containing vesicles is due to the presence of polymer in the polymer-containing vesicles.

CONCLUSIONS

The aim of this investigation was to perform a free radical polymerization of an unsaturated monomer within the hydrophobic part of a vesicle. DODAB vesicles were swollen with styrene and subsequently the monomer contained therein was polymerized, which led to the formation of polymer in the hydrophobic part of these DODAB vesicles. The stability of the system was maintained, i.e. no coagulation occurred. There was no evidence that normal latex particles were formed using the described process strategy. Evidence of polymer-containing vesicles was obtained from independent methods, i.e. by electron microscopy (SEM, TEM) and by the measurement of the diffusion rate of a paramagnetic probe through the different bilayers, where the polymer-containing vesicles showed the lowest rate of diffusion of this paramagnetic probe over the bilayer.

Future work will be attempted to increase the volume fraction of polymer in the vesicles so that one can truly speak of hollow polymer particles. Our eventual goal is an alternative preparation route for hollow polymer particles, in which the internal diameter and the polymer layer thickness of the hollow polymer particles can be accurately controlled.

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