Preparation of Macroporous Replica Particles Using Stereocomplex of isotactic Poly(methyl methacrylate) and syndiotactic Poly(methacrylic acid)

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ABSTRACT

Macroporous silica gel was employed for layer-by-layer (LbL) film preparation of isotactic- (it-) poly(methyl methacrylate) (PMMA) and syndiotactic- (st-) poly(methacrylic acid) (PMAA). The extraction of macroporous silica gel by fluorine hydride resulted in macroporous replica particles, confirmed by SEM. The further extraction of st-PMAA from their particles gave the macroporous replica consisting of porous it-PMMA films. The obtained porous it-PMMA films as macroporous replica particles showed the re-incorporation of st-PMAA. Therefore, the structure of the porous it-PMMA film was stabilized by the macroporous particle structure, although they did not include substrate. The physically crosslinked network structures maintained the molecular porous structures, without a chemically crosslinked reaction.

KEYWORDS: isotactic poly(methyl methacrylate), syndiotactic poly(methacrylic acid), stereocomplex, replica particles.

1. Introduction

The stabilization of layer-by-layer (LbL) films are one of the important techniques due to their application as nanofilms and hollow particles. For example, UV irradiation to LbL films of diazoregin and poly(styrene sulfonate) produced covalent bonded hollow capsules which encapsulated enzymes.[1] A simple thermal treatment to crosslink the polyelectrolytes for stabilization has been reported.[2] The permeability of polyelectrolyte capsules were also investigated after various annealing conditions.[3] Crosslinking reaction by oxidation was one of the approaches used to stabilize hollow particles.[4,5] The disulphide was utilized for poly(methacrylic acid) and poly(vinylpyrrolidone) for the formation of crosslinking.[6]

Several examples of ester formation were made by the use of carbodiimide.

Hydroxypropylcellulose and poly(acrylic acid) formed covalent bonding by *N*-(3-dimethylaminopropyl)-*N'*-ethyl-carbodiimide hydrochloride are known.[7] Similarly, the click reaction is also applicable for stabilization of particles.[8,9] Chemically crosslinking with some compounds such as diamine[10] and glutaraldehyde[11] were the possible other options.

On the other hand, the polymer structure of natural substances such as DNA is controlled precisely[12] and this polymerization system is based on a template polymerization mechanism. Template polymerization of methyl methacrylate and methacrylic acid in the presence of stereoregular polymers has been reported.[13,14] That template polymerization system was achieved by LbL assembled thin film[15] and the subsequent stereoregular pore film preparation[16], by stereocomplex formation as a driving force between isotactic (*it*-) poly(methyl methacrylate) (PMMA) and syndiotactic- (*st*-) poly(methacrylic acid) (PMAA). *It*-PMMA/*st*-PMMA stereocomplex were initially reported by Fox.[17] The structure of stereocomplexes were investigated by XRD[18] and AFM[19] to reveal stranded helix structures.

Since the LbL technique was first reported by Decher,[20] many kinds of materials have been prepared by various polymer-polymer interactions as reviewed.[21-24]. It is considered that the *it*-PMMA/*st*-PMAA stereocomplex formation was formed by weak polymer-polymer interaction, such as Van der Waals interaction.

Recently, we have investigated the porous *it*-PMMA films as a template polymerization field.[25] The LbL film prepared with *it*-PMMA/*st*-PMAA by stereocomplex formation on substrate and the subsequent extraction of *st*-PMAA produced a porous *it*-PMMA film, however, the porous nano space of the LbL film could be lost by the crystallization of *it*-PMMA under specific conditions, such as the addition of acetonitrile,[26] and at above 70 °C in water.[27] The dynamic change of the porous *it*-PMMA thin films prepared by the extraction of *st*-PMAA from *it*-PMMA/*st*-PMAA stereocomplex LbL films have been investigated under various conditions.[28] In order to stabilize the porous film, the crosslinking approach was applied, resulting in the very limited available condition for nanospace.[29] During the series of the study regarding porous *it*-PMMA thin film, the substrate for LbL preparation was found to be important for retaining the film structure.[30] At the same time, it has been reported that the porous structure was retained under nanoparticles as a substrate, for the controlled drug release.[31] We motivated to stabilize the porous *it*-PMMA film for the possible template polymerization by the different approach.

In this study, we used macroporous silicagel as substrate for *it*-PMMA/*st*-PMAA stereocomplex LbL preparation,[32] and the successive removal of silica gel by hydrogen fluoride gave the macroporous replica particles. The structurally featured stereocomplex particles were investigated both by extraction and re-incorporation of *st*-PMAA.

2. Experimental Section.

2.1. Materials.

Macroporous silicagel (diameter: 7 μm , pore: 100 nm) was purchased from Daiso Co., Ltd. Methyl methacrylate (MMA) (Tokyo Chemical Industry Co., Ltd.), methacrylic acid (MAA) (Tokyo Chemical Industry Co., Ltd.), trimethylsilyl methacrylate (Aldrich), toluene (Tokyo Chemical Industry Co., Ltd.) were distilled with CaH_2 before use. *it*-PMMA (*mm:mr:rr*=99:1:0, M_n =52700) and *st*-PMAA (*mm:mr:rr*=0:3:97, M_n =89000) for LbL assembly in this study were synthesized by anionic polymerization of MMA in toluene at -78°C for 5 days with *tert*-BuMgBr [33] and trimethylsilylmethacrylate with *tert*-BuLi/bis(2,6-di-*tert*-butylphenoxy)methyl aluminium,[34] respectively.

2.2. Measurements and observation.

FT-IR spectra were obtained with a Spectrum 100 FT-IR spectrometer (Perkin-Elmer, USA). The interferograms were co-added 8 times and Fourier-transformed at a resolution of 4 cm^{-1} . The XRD patterns were measured by a Rigaku RINT2000. $\text{CuK}\alpha$ ($\lambda = 0.154\text{ nm}$) was used as the X-ray source and operated at 40 kV, 200 mA with a Ni filter (Rigaku ultraX18). The samples were examined at $2\theta = 5$ to 25° at a scan rate of $0.5^\circ/\text{min}$.

Scanning electron microscope (SEM) images were observed by a JSM-6701F (JEOL, Akishima, Japan) at an acceleration voltage of 5 kV after osmium tetroxide was spattered onto the surfaces of particles on plastic plates.

Confocal laser scanning microscopy (CLSM) was used for particle observation. The rhodamine 6G water solution (0.1 wt%) was used to disperse the particles. After 4 hr immersion, the

particles were recovered by centrifugation at 1000 rpm for 2 min, then washed repeatedly with ultrapure water. The particle dispersion in water was dropped onto a glass substrate for CLSM observation.

2.3. LbL assembled particles.

The macroporous silicagels (SP-1000-7, 180 mg) were immersed into *it*-PMMA solution in acetonitrile (40 mL, 1.7 mg/mL) at room temperature for 15 min using a rotator. After centrifugation (3000 rpm, 3 min.) and washing with acetonitrile twice, the silicagels were then immersed into *st*-PMAA solution in acetonitrile/water (4/6, v/v, 40 mL, 1.5 mg/mL) at room temperature for 15 min. Similarly, the silicagels were washed with the same solvent, acetonitrile/water (4/6, v/v) twice. The above mentioned cycle was repeated 60 times, and the aliquot was reserved every 10 cycles for FT-IR/ATR and SEM analyses (**Figure 1**).

Figure 1. Schematic illustration of LbL stereocomplex film, replica particle of stereocomplex film, and replica particle of porous film.

2.4. Macroporous replica particles composed of *it*-PMMA/*st*-PMAA stereocomplex films.

The interior silicagels moieties from LbL assembled particles were removed by immersing into fluorine hydride solution (2.3 wt%) at room temperature for 12 hr. After the centrifugation, the particles were repeatedly washed with ultrapure water to confirm that the supernatant showed pH 6,^[31] and then the prepared macroporous replica particles were analyzed by FT-IR, XRD, and SEM.

2.5. Macroporous replica particles composed of porous *it*-PMMA films.

st-PMAA was then removed from the macroporous replica particles, which was composed of *it*-PMMA/*st*-PMAA stereocomplex, using 0.1M NaOH aq. at room temperature for 24 hr. After centrifugation, the macroporous particles composed of porous *it*-PMMA were washed with ultrapure water until the pH was 6. The obtained macroporous replica particles composed of porous *it*-PMMA were confirmed by FT-IR/ATR and XRD. Furthermore, the molecular porosity was confirmed by re-incorporation of *st*-PMMA, immersing particles into *st*-PMAA in acetonitrile/water (4/6, v/v) for 12 h. The re-incorporation was confirmed by FT-IR/ATR and XRD.

3. Results and Discussion.

Figure 2 shows the observation of macroporous silicagel as substrate (Figure 2a and 2d), LbL assembled particles (Figure 2b and 2e), and macroporous replica particles composed of *it*-PMMA/*st*-PMAA stereocomplex films (Figure 2c and 2f). After LbL assembly onto the

macroporous silica gels (Figure 2e), the 100 nm pores of silicagels (Figure 2d) appear to have disappeared from the surfaces. The surface does not look as smooth as expected, suggesting that

Figure 2. The SEM images of mesoporous silica particles (a), stereocomplex on silica particles (b : TU2-68), and stereocomplex after HF etching (c : TU2-85) at 550 magnification. The SEM images of mesoporous silica particles (d) stereocomplex on silica particles (e) and stereocomplex after HF etching (f) at 10000 magnification.

the partial heterogeneous layers generated on the macroporous silicagels, which were different from nano sized films[16] and particles.[31]

After the treatment of fluorine hydride, the partial shrunken moieties were observed from the particle surfaces (Figure 2c and 2d), however, the change could be due to the porous space inside

particles from the vacuum dry procedure for SEM observation. In order to clarify the hypothesis, we next used CLSM for the observation of hollow space inside the macroporous replica particles (Figure 3).

Figure 3. The fluorescein microscopy images of mesoporous silicagel (a and b), stereocomplex on silicagels (c and d) and stereocomplex after HF etching of silicagels (e and f) with different magnifications.

Rhodamin 6G was selected to dye the particles. At first, macroporous silicagels themselves were treated with Rhodamin 6G as a control, using electrostatic interaction (Figure 3a and 3b). The red fluorescence was reflected on the macroporous structures and many pore structures were observed (Figure 3b). Then, the LbL assembled particles by *it*-PMMA/*st*-PMAA stereocomplex films were treated with Rhodamin 6G (Figure 3c and 3d). Since both silica and stereocomplex

moieties were absorbed with Rhodamin 6G, the whole particles show fluorescence in this case (Figure 3d). After the extraction of silica moieties by fluorine hydride (Figure 3e and 3f), the porous spaces in particles were clearly observed (Figure 3f). The results displayed the successful preparation of macroporous replica particles composed of *it*-PMMA/*st*-PMAA stereocomplex films. In order to investigate the structure of the macroporous replica particles, we next conducted FT-IR/ATR and XRD measurements.

Figure 4 shows the results of FT-IR/ATR spectra of particles. As comparisons, spectral patterns of *it*-PMMA (Figure 4a) and *st*-PMAA (Figure 4b) were shown, bearing characteristic peaktops at 1727 cm^{-1} and 1694 cm^{-1} , respectively. Stereocomplex formation of *it*-PMMA/*st*-PMAA on the substrate was confirmed by the appearance of a peak top of 1717 cm^{-1} (Figure 4c).[16] The conformation and structural preservation of stereocomplex after removing silica moieties were confirmed by the same peaktop of carbonyl parts of the polymers (Figure 4d). The disappearance of absorbance from silica moieties was also confirmed by FT-IR-ATR around 1060 cm^{-1} , suggesting the removal of silica gel inside and the successful preparation of macroporous replica particles (Supporting Information, Figure S1 and S2).

Furthermore, *st*-PMMA was removed from the above mentioned macroporous replica particles composed of *it*-PMMA/*st*-PMAA stereocomplex films. The FT-IR/ATR spectrum of the resultant particles is shown in Figure 4e. The clear shift from 1717 cm^{-1} to 1727 cm^{-1} was observed, suggesting the porous *it*-PMMA thin film and the successful removal of *st*-PMAA.[16] It is noteworthy that re-incorporation of *st*-PMAA was also achieved into the macroporous replica particles composed of the porous *it*-PMMA thin films, returning the peaktop to 1717 cm^{-1} (Figure 4f). In our last report, the substrate of the porous *it*-PMMA was required to keep the nanoporous space,[30] however, the shape of the substrate for LbL in this study was not flat but

macroporous silicagel. The three dimensional shape structure[31] contributed to maintaining porosity for the incorporation of *st*-PMAA. We also clarified the particle and porosity structures by XRD.

Figure 4. FT-IR/ATR spectra of *it*-PMMA (a), *st*-PMAA (b), stereocomplex on silica particles (c), and stereocomplex after HF etching (d), after HF etching and immersion into NaOH aq. (e), and the precipitation after reincorporation of *st*-PMAA (f).

Figure 5 shows the results of XRD patterns of particles. As a comparison, the XRD pattern of macroporous silica gels is shown (Figure 5a). The LbL assembled *it*-PMMA/*st*-PMAA stereocomplex particles showed two characteristic peaks ($2\theta=12$ and 15° , $d=0.74$ and 0.59 nm, respectively).[35] After the removal of silica moieties to prepare macroporous replica particles

composed of *it*-PMMA/*st*-PMAA stereocomplex films, these peaks remained whereas the peaks disappeared due to the silica (Figure 5c). Then the subsequent extraction of *st*-PMAA from *it*-PMMA/*st*-PMAA stereocomplex films was achieved. These particles do not possess silica gel substrate, however, the characteristic peaks of the porous *it*-PMMA films was observed ($2\theta=13^\circ$, $d=0.67$ nm) (Figure 5d). Compared with the our last reports on flat substrate[30] and particles[31], the result suggested that the nanoporous space was maintained when the stereocomplex films were prepared in the three dimensional nanostructure, such as particle and macroporous replica particles while the flat films were not applicable.[30]

Figure 5. XRD patterns of silica particles (a), stereocomplex on silica particles (b), stereocomplex after HF etching (c), after HF etching and immersion into NaOH aq. (d), and the precipitation after reincorporation of *st*-PMAA (f).

Furthermore, the particles were used for re-incorporation of *st*-PMAA to form a stereocomplex again. The two peaks ($2\theta=12$ and 15° , $d=0.74$ and 0.59 nm, respectively) recovered suggest that

stereocomplex formation occurred. At the same time the new peaks were also observed ($2\theta=9$ and 14° , $d=0.96$ and 0.62 nm, respectively), that shows the *it*-PMMA formed double stranded crystals during the re-incorporation of *st*-PMAA.[36,37]

Figure 6 shows the SEM images to compare particle morphology. The macroporous replica particles composed of *it*-PMMA/*st*-PMAA stereocomplex films maintain their spherical shapes (Figure 6a), while the squeezed particles were observed in the macroporous replica particles composed of porous *it*-PMMA films after removal of *st*-PMAA by NaOH aq. (Figure 6b). On the other hand, the spherical shapes were still present after re-incorporation of *st*-PMAA into the macroporous replica particles.

Figure 6. SEM images of stereocomplex after HF etching (a), the precipitation after NaOH aq. immersion (b), and the precipitation after re-incorporation of *st*-PMAA (c).

These observations also supported the mechanical strength difference between the macroporous replica particles with *it*-PMMA/*st*-PMAA stereocomplex films and the porous *it*-PMMA films.

4. Conclusions.

Macroporous silica gels have been successfully employed to prepare the macroporous replica particles composed of *it*-PMMA/*st*-PMAA stereocomplex films. The porosity of the particles was confirmed by CLSM with Rhodamin 6G absorbents. The further removal of *st*-PMAA moieties yielded macroporous replica particles composed of porous *it*-PMMA films, which maintained the nanospace of *st*-PMAA, evidenced by FT-IR/ATR and XRD. The three dimensional spherical structure, which was provided by the pores of macroporous silicagel, contributed to the stabilization of the porous *it*-PMMA films, although the stabilization was difficult on a plate substrate as reported. This obtained porous *it*-PMMA thin films as macroporous replica particles are expected as not only the efficient reaction field of stereoregular template polymerization but also the stable storage of organic compounds possessing the specific shaped nanospace.

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