



Key techniques to control porous microsphere morphology in S/O/W emulsion system

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ABSTRACT

A solid-in-oil-in-water (S/O/W) emulsion system has been developed to prepare porous polymeric microspheres. The obtained microspheres showed unique **core-shell structure with a dense core and a surface porous layer**. The emulsion system has two processes. In the first process, S/O/W₁ viscous emulsion is prepared by dropping of S/O phase in the first water phase (W₁). In the second process, the S/O/W₁ emulsion is poured to another water phase (W₂) as S/O/W₁/W₂ emulsion. During the process, S/O/W₁ droplet becomes microsphere after organic medium completely diffusion. Emulsion techniques have various effective combinations such as additive and process conditions to design microsphere morphology. With regards to the proposed S/O/W system, addition of the solid phase in the system is a key factor to form the porous structure. **When the medium diffusion starts, the solid makes W₁ phase kept inside the S/O/W₁ droplet. The remained W₁ phase changes surface porous layer after purification.** Affinity between the solid and oil phase should be adjusted as well. In this study, an optimization of the emulsion system was attempted considering solubility parameter and polarity. Additionally, it is found that process conditions could help to design microsphere morphology such as pore size and porous layer thickness.

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1. Introduction

We have much interest in morphology design of porous microspheres of biopolymer which in turn find applications in protein loading/release [1], as bone substitutes [2], and in bone tissue recovery [3]. In order to obtain these functionalities, it is necessary to control their physical properties such as pore size, porosity, and microsphere size [4,5]. Various emulsion routes have been developed to fabricate such porous materials because there are lots of available modified techniques by combining emulsion types, organic medium, additives, process conditions, and so on [6–9].

Hydrophobic microspheres are prepared using oil-in-water (O/W) type emulsions, in which oil droplets disperse in an outer water phase. Polymeric substance which dissolves in the droplet solidifies to be the microspheres with beginning of organic medium diffusion. Affinity between organic medium and outer water phase changes microsphere size. Nano-sized particle can be formed by the usage of water miscible solvent because its fast diffusion makes the polymer solidification quickly [10]. Emulsifiers with amphiphilic

property are usually added in outer phase to stabilize droplets by adsorbing at the surface. Polyvinylalcohol (PVA) works as an effective stabilizer in O/W types because of high water solubility and having hydrophobic main chain and hydrophilic side chain. Murakami et al. [11] suggested that the PVA adsorbing ability is affected by its hydrolyzation and polymerization degrees. Process condition is an important factor which operates microsphere formation as well. Stirring time and rate during emulsification decides droplet size [12]. As one of the ways to obtain fine uniform microspheres, membrane emulsification techniques using glass membrane have been reported [13].

As modified techniques to prepare porous microspheres, removal of pore formation agent (called as porogen) which is added in the droplet seems to be the most effective way. Porosity and pore size are easily changeable by porogen size and loading amount [14,15].

Generation of gas inside the droplet caused by the chemical reaction of added gas forming agent has been reported by Kim et al. The generated gas remains as fine pores after polymer solidifies [16]. He et al. developed pore formation route by water phase shift between inside and outside the droplet using water-in-oil-in-water (W₁/O/W₂) emulsion system. By keeping lower osmotic pressure of inner water phase than of outer phase, the inner phase leaves though the microsphere shell and vice versa [17].

Previously, solid-in-oil-in-water (S/O/W) emulsion technique has been developed to prepare porous microspheres with a dense

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core and a surface porous layer [18]. The emulsion system consists of two processes. In the first process, S/O phase is prepared by dispersing of solid phase in polymer solution as oil phase. Then the S/O phase is dropped in the first water phase (W_1) and stirred vigorously to obtain S/O/ W_1 emulsion. In the second process, the S/O/ W_1 phase is poured in the second water phase (W_2) to prepare S/O/ W_1 / W_2 phase.

In this system, dispersed S/O/ W_1 droplet turns porous microsphere. When organic medium in S/O/ W_1 droplet starts to diffuse to W_2 phase, polymeric substance solidifies with W_1 phase. After completely medium diffusion, the remained W_1 phases form porous layer on the microsphere surface. The solid phase in the oil phase could make W_1 phase kept inside the S/O/ W_1 droplet against diffusion flow. Any experimental condition without the solid phase never induces pore formation.

The unique pore formation caused by additive is quite interesting. However, it is true that lots of factors which affect each other make the emulsion system complicated. In this study, the emulsion system was optimized considering affinity between solid and oil phases. The results that showed high affinity between solid-polymer and polymer-medium, low affinity between solid-medium are the best combination to obtain porous microspheres. To control medium diffusion rate, stirring time needs to be adjusted. Additionally, control of pore size and porous layer thickness has been achieved in the optimized emulsion system.

2. Materials and methods

As solid phase, hydroxyapatite (HAp, Taihei Chemical Industrial Co., Ltd.) and silicon dioxide (SiO_2 , JGC Catalysts and Chemicals, Ltd.) granules with primary size of approximately several nano meters, as polymeric substance, poly(lactic-co-glycolic acid) (PLGA, lactide:glycolide copolymer ratio of 50:50, molecular weight of 10,000, Wako Pure Chemical Industries, Ltd.), poly methyl methacrylate (PMMA, molecular weight of 15,000, Acros Organics N. V.) and polystyrene (PSt, molecular weight of 35,000, Sigma-Aldrich Corporation), as organic medium, chloroform (CF, Kanto Chemical Co., Inc.), dichloromethane (DCM, Kanto Chemical Co., Inc.) and acetone (AC, Kanto Chemical Co., Inc.) were used. At first, polymeric substance was dissolved in organic medium to prepare 6.0% (w/v) oil phase solution. Solid phases were dispersed in the oil phase as 6.5% (w/v) S/O suspension. The 2 ml of S/O suspension was dropped in 6 ml of the first water phase (W_1) with 2.5% (w/v) polyvinyl alcohol (PVA, hydrolyzation degree of 87–89%, molecular weight of 88,000–97,000, Alfa Aesar) to obtain S/O/ W_1 emulsion. The S/O/ W_1 was poured into the second water phase (W_2) with 0.5% (w/v) PVA and 0.5 M sodium chloride (NaCl, Tomita Pharmaceutical Co., Ltd.). After gentle 2 h stirring, the precipitants in the emulsion were separated and dried under vacuum pressure.

For the investigation of affinities, solubility parameters were calculated from chemical structures [19].

3. Results and discussion

Formation of porous microspheres can be roughly explained as follows [18]. In the first step, stable S/O/ W_1 emulsion was prepared during vigorous stirring by dropped of S/O phase in W_1 phase. In the second step, the S/O/ W_1 emulsion droplets which become microspheres were dispersed in W_2 phase by poured of S/O/ W_1 in W_2 phase. The W_1 phases which are hold inside the S/O/ W_1 droplet change into porous layer after purifications. In order to obtain porous microspheres, W_1 phase holding ability is the most important by the control of affinities among each phase in the S/O/ W_1 droplet.

Affinities among solid, polymer, and organic medium in the S/O/W system have been considered using various phase systems.

As solid, hydroxyapatite (HAp) and silicon dioxide (SiO_2), as polymer, poly(lactic-co-glycolic acid) (PLGA), poly methyl methacrylate (PMMA), polystyrene (PSt), as organic medium, chloroform (CF), dichloromethane (DCM), and acetone (AC) were selected.

With focus on organic medium, HAp-PLGA-CF, HAp-PLGA-DCM and HAp-PLGA-AC in as solid-polymeric substance-organic medium have been considered. CF, DCM and AC showed good dissolving abilities for PLGA due to their similar high solubility parameters as 22.10 (CF), 21.71 (DCM), 21.06 (AC), and 16.71 (PLGA). On the other hand, they have different dispersing abilities for HAp because of their different polarities. HAp settled down immediately in CF and DCM while good dispersion showed in AC. Because CF and DCM have low polarity (relative permittivity of 4.8 and 8.4, respectively), they work as poor dispersing medium for HAp which is slightly hydrophilic caused by its hydroxyl groups. Meanwhile, AC with high relative permittivity of 20.6 can be good dispersing medium.

Interestingly, HAp dispersability has been improved when PLGA was dissolved in CF and DCM. It can be thought that PLGA works as like good dispersant by adsorbing at HAp surface due to chemical interaction between ester group of PLGA and calcium (Ca) site of HAp surface [20]. No remarkable dispersion improvement was observed in PLGA-AC.

Difference in polymer dispersant ability for HAp has been discussed by HAp-PLGA-DCM, HAp-PMMA-DCM and HAp-PSt-DCM systems. Fig. 1 shows optical microscopic observations of S/O phase of (a) HAp-PLGA-DCM, (b) HAp-PMMA-DCM and (c) HAp-PSt-DCM. In these photos, black colored are HAp particles. Similar high HAp dispersion can be seen in HAp-PLGA-DCM (Fig. 1(a)) and HAp-PMMA-DCM (Fig. 1(b)) systems while large aggregation was observed in HAp-PSt-DCM (Fig. 1(c)). As mentioned above, ester groups in PLGA main chain contribute good interaction with HAp. PMMA also has ester groups in its side chain so that it seems to be good dispersant for HAp in the system. PSt without ester groups showed no dispersing ability.

The prepared S/O phase with high HAp dispersion is dropped in W_1 phase to form white pale viscous S/O/ W_1 emulsion in the first process. The S/O/ W_1 emulsion is poured in W_2 phase to prepare stable S/O/ W_1 / W_2 in the second process. During the second process, organic medium starts to diffuse from the S/O/ W_1 droplet to W_2 phase and polymeric substance solidifies. With progress in the diffusion, emulsion stability of S/O/ W_1 droplet decreases because of increase in hydrophobic polymer and hydrophilic W_1 phase in the droplet. Therefore, solid phase with nanoscale can be emulsifier adsorbing at the interface between W_1 phase and oil phase to stabilize them. When organic medium completely diffuses, cone-like pores can be formed on the microsphere surface after W_1 phase removal.

Scanning electron microscopic (SEM) observations of the obtained microspheres in (a) HAp-PLGA-CF, (b) HAp-PLGA-DCM, (c) HAp-PLGA-AC, (d) HAp-PMMA-DCM, and (e) HAp-PSt-DCM systems were shown in Fig. 2. Compared with difference in organic medium (Fig. 2(a)–(c)), pore formed on the entire microsphere surface with slightly deformed shape in CF (Fig. 2(a)) and totally spherical in DCM (Fig. 2(b)) while spherical microspheres with aggregated particulates on the surface in AC (Fig. 2(c)) were observed. It seems that microsphere morphology can be fixed by medium diffusion property. CF takes more time to diffuse to W_2 phase than DCM because of its higher boiling point and lower polarity. The hard-to-diffuse property prevents uniform diffusion from the entire droplet surface. Then HAp-PLGA-CF system gives deformed microspheres. It needs diffusion control by process conditions such as reaction temperature to prepare spherical ones.

In HAp-PLGA-AC system (Fig. 2(c)), HAp particulates were observed on the microsphere surface and pore formation was not

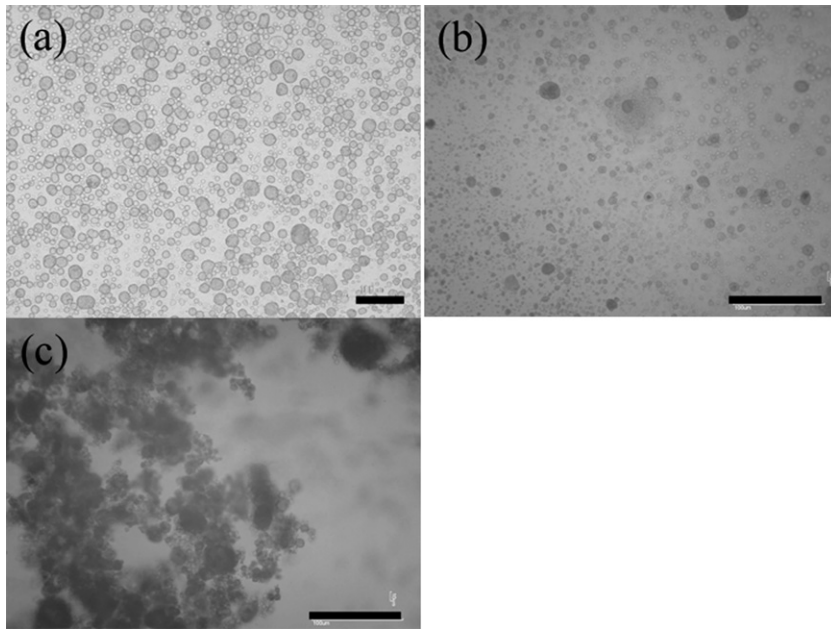


Fig. 1. HAp dispersibilities in (a) PLGA-DCM, (b) PMMA-DCM, and (c) PSt-DCM observed by an optical microscope. Scale bars are 100 μm .

confirmed though HAp dispersability was high in PLGA-AC. It is no need PLGA dispersant for HAp in PLGA-AC system because HAp and AC have already good affinity. AC diffusion flow could take HAp outside the droplet and PLGA dense microspheres with adsorbed of HAp on the surface were obtained.

With regards to difference in polymer (Fig. 2(b)–(e)), porous microspheres were obtained in HAp-PLGA-DCM (Fig. 2(b)) and no porous formed in HAp-PMMA-DCM (Fig. 2(d)) and HAp-PSt-DCM (Fig. 2(e)) systems. Similar to PLGA, PMMA showed good dispersing ability for HAp in S/O phase. Nonetheless, no pores formed on the obtained microsphere surface (Fig. 2(d)). It can be thought that the chemical interaction between PMMA and HAp is weak because of the contribution of methoxy (OCH_3) group. In HAp-PSt-DCM (Fig. 2(e)), HAp could be taken outside the droplet as “foreign sub-

stance” during medium diffusion because PSt scarcely interacts with HAp surface.

When SiO_2 was used for HAp as solid phase, even though it shows hydrophilic, these solids were observed outside the droplet. As the solid phase, it needs not only hydrophilic but also the specific property such as interaction with polymer.

From these results, high affinity needs between solid and polymer so that polymer can adsorb on the solid surface. In order to achieve this, organic medium must be good solvent for polymer and not be good dispersing medium for solid. In this study, HAp-PLGA-DCM system was selected as S/O phase and morphology control of porous microspheres has been investigated.

In this experimental scale, stirring rate below 195 rpm made S/O/W₁ emulsion separated as S/O and W₁ phase, otherwise no

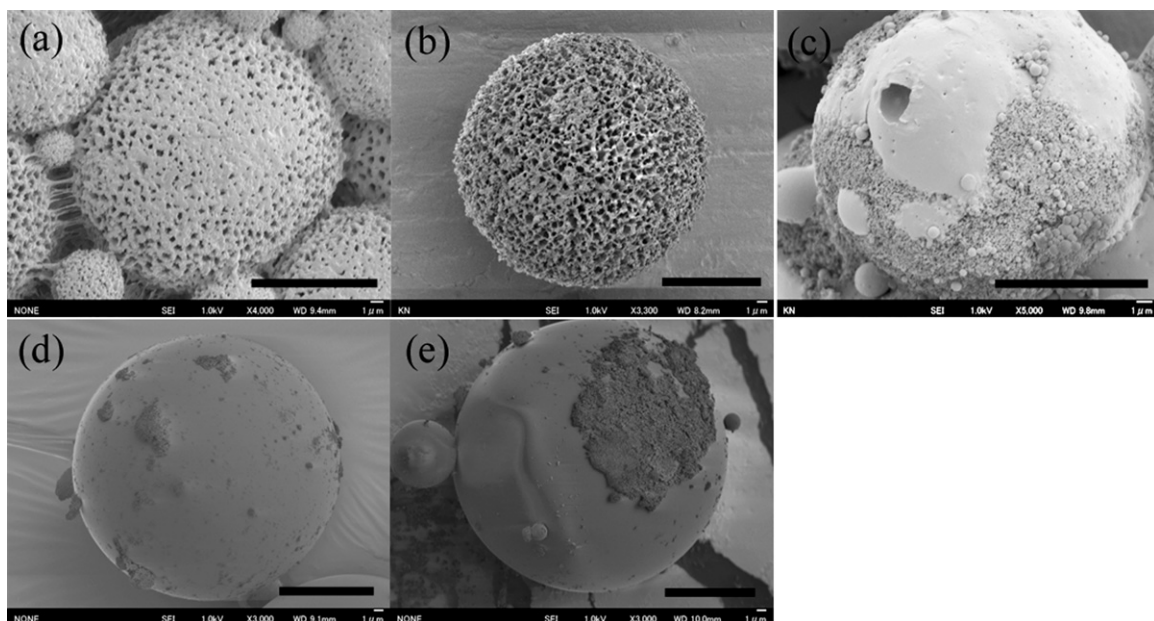


Fig. 2. Surface morphology observations of microspheres prepared in (a) HAp-PLGA-CF, (b) HAp-PLGA-DCM, (c) HAp-PLGA-Ace, (d) HAp-PMMA-DCM and (e) HAp-PSt-DCM systems. Scale bars are 10 μm .

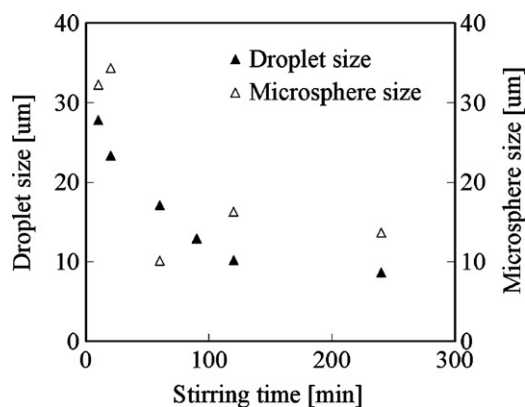


Fig. 3. Changes in the droplet and obtained microsphere sizes as a function of stirring time in the first process.

pores formed on the obtained microsphere surface though the surface was rough.

First, stirring time of the first process was changed with range of 10–240 min at fixed stirring rate of 195 rpm. Fig. 3 shows time-dependent size changes of S/O/W₁ droplets and microspheres which were determined by ImageJ software from corresponding optical and SEM photographs. Fig. 4 shows surface observations of the obtained microspheres which were prepared for various stirring times of (a) 10 min, (b) 20 min, (c) 60 min, (d) 120 min, and (e) 240 min in the first process.

As shown in Fig. 3, size change of the obtained microspheres showed similar behavior to that of the droplets such as rapid decrease and almost constant at the boundary of 60 min. It seems that microsphere size can be determined by stirring condition.

Optimum stirring time which gives porous microspheres exists in the range of 20–120 min. For 10 min stirring (Fig. 4(a)), totally spherical microspheres with smooth surface were obtained. For 20 min (Fig. 4(b)), approximately 500 nm sized pores generated on the entire microsphere surface. With increase in the stirring time of 60 (Fig. 4(c)) to 120 min (Fig. 4(d)), pore size decreased. For 240 min (Fig. 4(e)), the small pores completely disappeared.

In the first process, the stability of S/O/W₁ emulsion increases with increase in the stirring time. The 10 min stirring (Fig. 4(a))

seems to be insufficient to obtain the stability. Keeping W₁ phase inside the S/O/W₁ droplet is not promoted in the second process and no pores are obtained. The 20 and 60 min stirring times (Fig. 4(b) and (c)) are suitable for stable S/O/W₁ phase and pore forms on the microsphere surface. The stirring time more than 60 min seems to be excess even though they are enough time for stable S/O/W₁ emulsion. Because long time stirring gives DCM diffusion, it decreases the emulsion stability as well.

As the other key factor of porous microsphere preparation, stirring rate of the first emulsification process was changed with the range of 195–375 rpm for fixed stirring time of 20 min. Fig. 5 shows surface (top views) and cross-sectional (bottom views) images of microspheres prepared at (a) 195, (b) 270, (c) 300, and (d) 375 rpm. All of the S/O/W₁ phases can be formed as viscous white emulsions without phase separation and the entire microsphere surfaces were covered with homogeneous pores.

As shown in surface observations (top of Fig. 5(a)–(d)), surface pore size increased with increase in the stirring rate. Lots of particular shaped protrusions appeared on the wall surface which divides pores. The cross-sectional images (bottom of Fig. 5(a)–(d)) indicated that microspheres have core–shell structure such as dense core and surface porous layer which consists of assembled of cone-like pores. The thickness of the porous layer increased with increase in the stirring rate. Besides, particulate protrusions increased wall surface roughness.

In the first process, strong shear force caused by fast stirring fractionalizes lots of W₁ phase to be stable existed as S/O/W₁ emulsion in 20 min. In the second process, when DCM diffusion and PLGA solidification start, the solidification flow occurs from the S/O/W₁ droplet core toward surface. The droplet stability decreases and HAP works as particulate emulsifier adsorbing at the interface between W₁ and PLGA-residual DCM to stabilize them. The solidification flow takes W₁ phase with HAP toward droplet surface. When the droplet surface is filled with W₁–HAP phase, PLGA solidifies involving them. This is the first foundation of porous layer. In the similar way, as the solidification progresses, W₁ phase assembles to become cone-like shaped porous layer which is divided by PLGA wall. After W₁ phase removal by purification and drying, PLGA wall remains with HAP.

With increase in the stirring time, effective W₁ phase amount increases in S/O/W₁ droplet. Inside the droplet is filled with lots

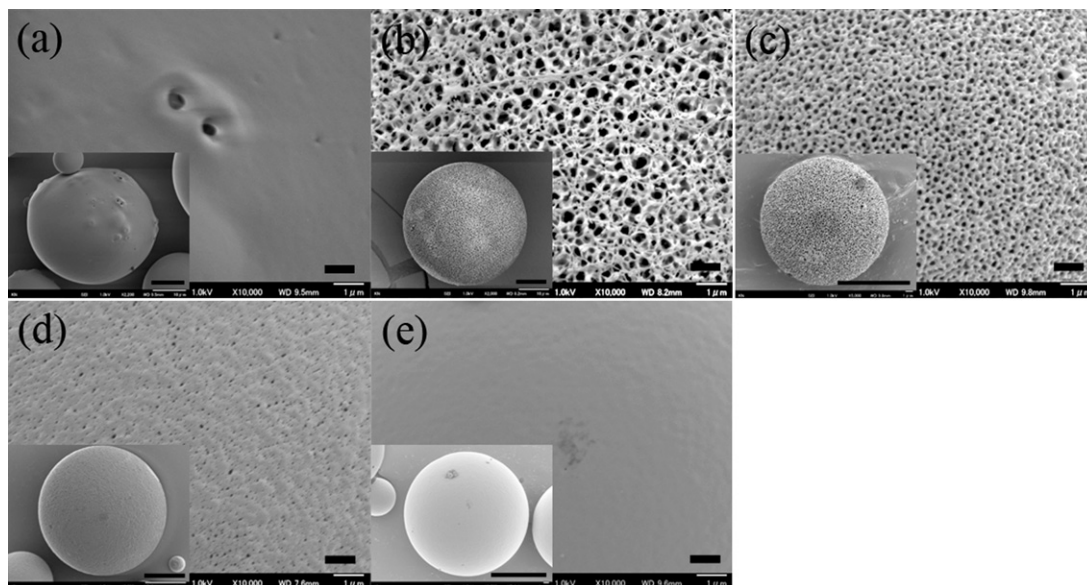


Fig. 4. SEM images of microspheres which were prepared for different stirring times; (a) 10, (b) 20, (c) 60, (d) 120, and (e) 240 min. Scale bars are 10 μm in the inserted figures and 1 μm in the magnified figures.

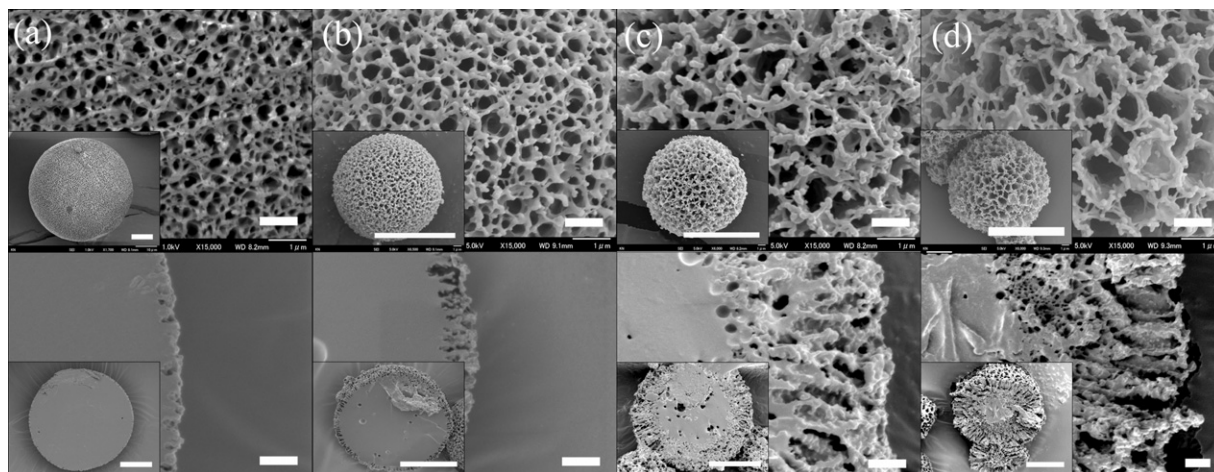


Fig. 5. Surface (tops views) and cross-sectional (bottom views) images of microspheres prepared at stirring rate of (a) 195, (b) 270, (c) 300, and (d) 375 rpm. Scale bars are 10 μm in the inserted figures and 1 μm in the high magnified figures.

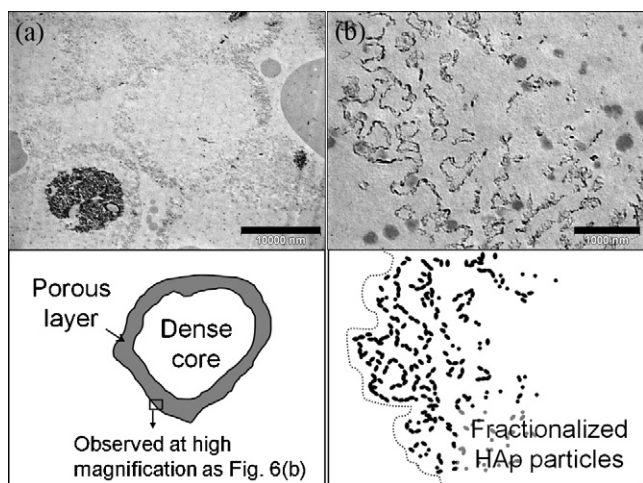


Fig. 6. TEM images of sliced porous microspheres observed at (a) low and (b) high magnifications and their schematic illustrations. Scale bars are (a) 10 μm and (b) 1 μm .

of W_1 phase. When diffusion starts, the first foundation of porous layer easily forms. Consequently, fast stirring induces deep porous layer and large pore size. The porous microsphere morphology can be controlled by stirring rate.

In order to investigate porous layer in detail, thin sections of porous microspheres which are embedded in resin were observed by transmission electron microscope (TEM). Fig. 6 shows TEM images of sliced microspheres corresponding to Fig. 5(c) observed at (a) low and (b) high magnifications and their schematic illustrations. The gray colored circles in Fig. 6(a) are porous layer. Detailed observation (Fig. 6(b)) indicates that the porous layer consists of black colored ellipsoidal particulates with the size of several tens nanometer. These particulates were detected as Ca by an energy dispersive spectroscopy (EDS). The results show that black colored particulates are HAp. The particulates protrusions on PLGA wall surface are caused by HAp stabilizer.

4. Conclusions

With the aim of design porous microsphere morphology, the proposed S/O/W emulsion system has been optimized considering affinities between phases. It is clear that high solid dispersion in polymer solution in S/O phase is the best combination and properly

stirring time exists to form stable S/O/W₁ emulsion. In addition, pore size and porous layer thickness can be controlled by stirring rate easily.

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