



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

Chika Takai<sup>a,\*</sup>, Tadashi Hotta<sup>b,1</sup>, Shuji Shiozaki<sup>b,1</sup>, Shuhei Matsumoto<sup>b,1</sup>, Takehisa Fukui<sup>b,1</sup>

<sup>a</sup> Ceramics Research Laboratory, Nagoya Institute of Technology, 3-101, Hommachi, Tajimi City, Gifu 507-0033, Japan

<sup>b</sup> Kurimoto Ltd., 2-8-45, Shibatani, Suminoe-ku, Osaka 559-0021, Japan

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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/W<sub>1</sub> viscous emulsion is prepared by dropping of S/O phase in the first water phase (W<sub>1</sub>). In the second process, the S/O/W<sub>1</sub> emulsion is poured to another water phase (W<sub>2</sub>) as S/O/W<sub>1</sub>/W<sub>2</sub> emulsion. During the process, S/O/W<sub>1</sub> 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 W<sub>1</sub> phase kept inside the S/O/W<sub>1</sub> droplet. The remained W<sub>1</sub> 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 (W<sub>1</sub>/O/W<sub>2</sub>) 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

\* Corresponding author. Tel.: +81 572 24 8110; fax: +81 572 24-8109.

E-mail addresses: [c.takai@crl.nitech.ac.jp](mailto:c.takai@crl.nitech.ac.jp) (C. Takai), [t.hotta@kurimoto.co.jp](mailto:t.hotta@kurimoto.co.jp) (T. Hotta), [s.shiozaki@kurimoto.co.jp](mailto:s.shiozaki@kurimoto.co.jp) (S. Shiozaki), [shu.matsumoto@kurimoto.co.jp](mailto:shu.matsumoto@kurimoto.co.jp) (S. Matsumoto), [take\\_fukui@kurimoto.co.jp](mailto:take_fukui@kurimoto.co.jp) (T. Fukui).

<sup>1</sup> Tel.: +81 6 6686 3235; fax: +81 6 6686 3229.

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 ( $\text{SiO}_2$ , 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 ( $\text{SiO}_2$ ), 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 paled 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

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