

## Effect of Inorganic Salt on Formation of Porous PLGA Microspheres

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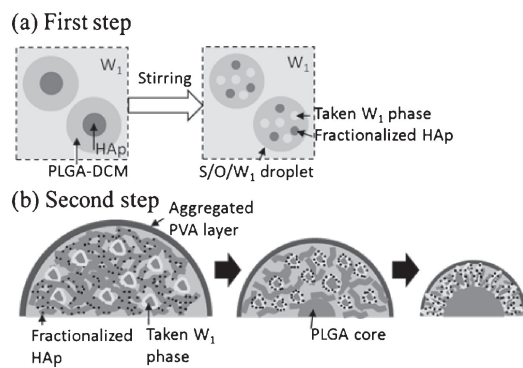
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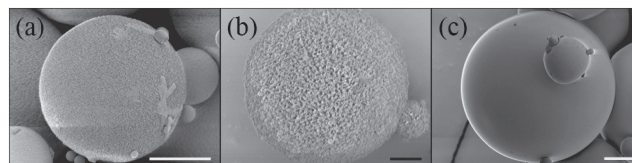
Porous microspheres were prepared by a solid-in-oil-in-water (S/O/W) emulsion. Effects of inorganic salt on pore formation were investigated. Sodium chloride (NaCl) is a proper pore inducing agent, and large uniform pores were obtained with addition of 0.5 M NaCl.

We are interested in simple chemical methods to prepare porous microspheres of biopolymer in which nanosized hydroxyapatite (HAp) particles are dispersed. These chemical conditions have been often used to fabricate porous biomaterials which in turn find applications in protein loading/release,<sup>1</sup> as bone substitutes,<sup>2</sup> and in bone tissue recovery.<sup>3</sup> Hydrophobic microspheres are prepared principally by oil-in-water (O/W) emulsions<sup>4</sup> in which oil droplets are dispersed in a continuous water phase. Some chemical additives can induce pore formation inside and/or outside the microspheres upon their removal,<sup>5</sup> by the generation of gas in oil droplets,<sup>6</sup> and by the use of water shift caused by the difference in osmotic pressures between the inner and outer phases of the oil droplets.<sup>7</sup> In the O/W emulsion systems, emulsifier molecules which are dissolved in the continuous water phase get adsorbed on the surfaces of oil droplets which consist of the polymeric substances and the organic medium, thus the two immiscible phases are stabilized. As the emulsification progresses, the organic medium in the oil droplets starts to diffuse toward the continuous water phase through the adsorbed emulsifier layer on the droplet surface. The polymeric substance solidifies and precipitates as microspheres at the same time.

In general, emulsion systems have advantages for preparation of microspheres with various morphologies because of effective combinations of emulsion type, process conditions, and additives. Previously, we have developed a novel emulsion technique with addition of inorganic salt and hydroxyapatite (HAp) using salting out and particulate emulsifier.<sup>8</sup> Unique porous microspheres can be obtained by control of process conditions.<sup>9</sup> Figure 1 illustrates the proposed technique consists of two steps.<sup>10</sup> In the first step (Figure 1a), a solid phase consisting of HAp dispersion in poly(lactic-co-glycolic acid) (PLGA)-dichloromethane (DCM) solution is added dropwise to a water phase containing PVA ( $W_1$  phase) and sodium chloride (NaCl). Vigorous stirring fractionalizes HAp granules into nanosized particles, and  $W_1$  phase is taken into the droplet to form S/O/ $W_1$  phase. In the second step (Figure 1b), the S/O/ $W_1$  emulsion is poured into a large volume of water phase containing PVA and NaCl ( $W_2$  phase) and stirred gently. The S/O/ $W_1$  droplets are stabilized by adsorption of emulsifier PVA in  $W_2$  phase. Without inorganic salt in  $W_2$  phase, DCM easily diffuses from the droplets to  $W_2$  phase and the droplets turn into dense microspheres with smooth surface. When inorganic salt is added, PVA molecules aggregate and gel by salting out. When DCM



**Figure 1.** Schematic drawings of S/O/W emulsion.



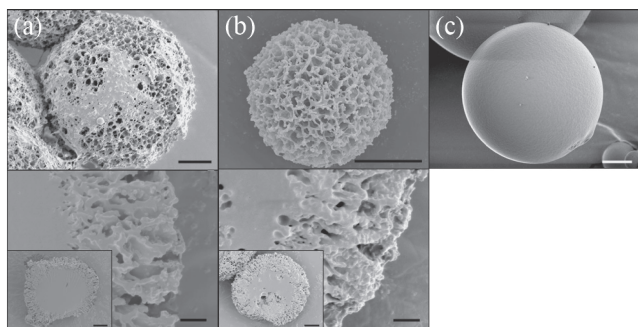
**Figure 2.** SEM images of microspheres prepared with addition of (a) KCl, (b) NaCl, and (c) CaCl<sub>2</sub>. Scale bars are 5  $\mu$ m.

diffuses from S/O/ $W_1$  droplets to  $W_2$  phase, it must pass through aggregated PVA layer. Thick aggregated PVA layer results in difficult DCM diffusion. During DCM retention in the S/O/ $W_1$  droplets, the fractionalized HAp particles work as particulate emulsifier<sup>11</sup> maintaining droplet stability between the  $W_1$  and PLGA-residual DCM phases adsorbing at the interface. The remaining  $W_1$  phase leads to conelike pores after purification.

Here, we focus on the effect of different ionic species on pore formation. Figure 2 shows scanning electron microscopic (SEM) images of microspheres prepared with addition of (a) potassium chloride (KCl), (b) sodium chloride (NaCl), and (c) calcium chloride (CaCl<sub>2</sub>). The KCl-PVA solution gave only rough surfaces without pores (Figure 2a). Pore formation on the entire microsphere surface was observed using NaCl-PVA solution (Figure 2b). Spherical smooth surfaces in CaCl<sub>2</sub>-PVA solution were obtained (Figure 2c).

According to Dai et al., PVA aggregation in salt water solution strongly depends on ionic species.<sup>12</sup> The degree of PVA aggregation can be discussed using hydration enthalpy ( $\Delta H_{\text{hyd}}$ ) of inorganic salts. Hydration is solute-solvent interaction, particularly when water is used as a solvent.  $\Delta H_{\text{hyd}}$  is an indicative factor of hydration stability among water molecules and salt ions. High  $\Delta H_{\text{hyd}}$  means that salt ions are consumed by hydration rather than complexation with PVA.

The  $\Delta H_{\text{hyd}}$  of KCl, NaCl, and CaCl<sub>2</sub> are  $-783.6$ ,  $-699.9$ , and  $-2302.6$  kJ mol<sup>-1</sup>, respectively.<sup>13</sup> CaCl<sub>2</sub> with high absolute



**Figure 3.** SEM surface (top) and cross-sectional (bottoms) images of microspheres prepared with addition of different NaCl concentrations; (a) 0.25, (b) 0.5, and (c) 1.0 M. Scale bars are 5  $\mu\text{m}$  of top images and inserted images, 1  $\mu\text{m}$  of bottom images, respectively.

value tends to form stable hydrates with water. The complexation scarcely occurs and that leads to spherical smooth surface microspheres being obtained as well as when using conventional O/W emulsions. KCl and NaCl have similar hydration stability and seem to be effective complexing agents for PVA. In particular, NaCl promotes retention of  $W_1$  phase inside the S/O/ $W_1$  droplets, and pore formation on the entire microsphere surface can be clearly observed.

The difference in gelation ability between NaCl and KCl can be explained considering their enthalpy of solution ( $\Delta H_{\text{sol}}$ ), 3.88 and 17.22  $\text{kJ mol}^{-1}$ , respectively.<sup>13</sup> Enthalpy describes balance of heat of solution when the salt dissolves in water. NaCl and KCl having positive  $\Delta H_{\text{sol}}$  show endothermic dissolution. KCl with high  $\Delta H_{\text{sol}}$  needs more heat to be dissolved. For these reasons, NaCl works as a suitable complexing agent for PVA in the emulsion system.

According to Dai and Cha, ionic concentration affects PVA gelation as well as ion species and valence.<sup>12,14</sup> Figure 3 shows SEM observations of microspheres prepared with addition of different NaCl concentrations of (a) 0.25, (b) 0.5, and (c) 1.0 M. The top and bottom photos are surface and cross-sectional images, respectively. With 0.25 M NaCl (Figure 3a), slightly deformed microspheres with porous and nonporous surfaces were obtained. With 0.5 M NaCl (Figure 3b), pores were uniformly generated on the entire microsphere surface. With 1.0 M NaCl (Figure 3c), the homogeneous pores disappeared and large pore aggregation appeared instead. From cross-sectional observations, the porous microspheres with 0.25 and 0.5 M NaCl showed surface porous layers with approximately 500-nm thickness.

With increase in NaCl concentration, PVA gelation can be promoted.<sup>12</sup> As shown in Figure 2a, 0.25 M seems to be insufficient for uniform PVA aggregation at the droplet surface. Thin and thick PVA layers give quick and slow DCM diffusion. Therefore, microspheres solidify with deformation and two surfaces as porous and nonporous. The optimum NaCl concentration to form uniform PVA aggregation is 0.5 M (Figure 2b). DCM diffuses from the entire droplet surface simultaneously passing through the PVA layer and homogeneous pores can be obtained. As shown in Figure 2c, it is hard to induce pore formation under excess NaCl. DCM retention time increases because thick hydrophilic PVA layers prevent hydrophobic DCM from passing through.

As well as DCM retention time, DCM diffusion rate from S/O/ $W_1$  droplet to  $W_2$  phase could contribute to microsphere morphology. DCM diffusion rate is affected by the differences of dissolved ionic salt species and salt concentration in  $W_2$  phase. In aqueous  $\text{CaCl}_2$  solution, solute–solvent interaction may cause slight increases in DCM water solubility, and DCM diffusion rate could be accelerated. In KCl- and NaCl- $W_2$  phases, DCM diffusion rate decreases because of hydrophilic aggregated PVA layers adsorbed on the droplet surfaces. With regards to ionic concentration in  $W_2$  phase, when less than (Figure 2a) or the optimum amount (Figure 2b) of NaCl is added, most NaCl is used in PVA aggregation and very little exists in the  $W_2$  phase. With further increase in NaCl, excess NaCl appears in the  $W_2$  phase. That causes the DCM diffusion rate increase, and spherical smooth microspheres are obtained (Figure 2c).

In summary, NaCl works as pore-inducing agent as a result of complex formation with PVA. Microspheres with large pores by addition of 0.5 M NaCl can be applied to loading large molecule drugs in therapeutics.

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- 10 PLGA (molecular weight of 10000, lactide–glycolide copolymer ratio of 50:50) was dissolved in DCM to prepare a 6.0 w/v% oil phase solution. HAp granules were dispersed in the solution as 6.5 w/v% S/O suspension. The suspension was added dropwise to 2.5 w/v% aqueous PVA (molecular weight of 88000–97000, hydrolyzation degree of 87–89%) solution ( $W_1$ ) and then emulsified with a magnetic stirrer for 20 min at room temperature. The S/O/ $W_1$  emulsion was poured into a 0.5 w/v% aqueous PVA solution with 0.5 M NaCl ( $W_2$ ) and stirred gently for 2 h. The precipitants were separated and dried under vacuum.
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