Research Letter

Synthesis of Hydroxyapatite by Interfacial Reaction in a Multiple Emulsion

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Interfacial reaction in a multiple emulsion, which is one of methods for producing inorganic microspheres, was applied to synthesize hydroxyapatite. The multiple emulsion was a W/O/W emulsion, made of dipotassium hydrogen phosphate solution as an inner aqueous phase, benzene as an oil phase, and calcium nitrate solution as an outer aqueous phase. The reaction was carried out in the multiple emulsion for 24 hours at 323 K. The crystalline phase was varied with an initial pH of the inner aqueous phase, and single phase hydroxyapatite was synthesized at an initial pH of 12. The products were composed of porous microspheres of less than 3 μm in size. The microspheres were composed of nanospheres of less than 120 nm in size. By considering the mass balance, it was suggested that each nanosphere was formed in an inner aqueous phase droplet.

1. INTRODUCTION

Hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$, HAp) is a main inorganic constituent of hard tissues in the human body such as bones and teeth. Because of its intrinsic biocompatibility, applications as bioceramics have been studied since long time ago.

Actual bones are not a monolithic material made of the only HAp, but composites of HAp with collagen protein. The idea of mimicking natural state for improving the biocompatibility is reasonable. There are many reports on the preparation of composites of HAp nanoparticles with collagen or alternative polymer [1–9]. Composites with vitamins also seem to be effective for improving bone regeneration. In any case, it is easily predicted that nothing would succeed by merely mixing ingredients. For success in composite materials, it is generally required to homogeneously mix the ingredients and to prevent them separating. One of answers for such requirements is microencapsulation. Microcapsules are used for the preservation and controlled release of a core material, as well known. Therefore, microencapsulating an active component with HAp wall is thought to be meaningful.

In the most common procedure for producing microcapsules with an inorganic wall, inorganic hollow particles or porous microspheres are prepared. After that, they are impregnated with an active component, and then the wall is stuffed or covered with a suitable material. Hollow particles made of various substances have been produced by means of spray pyrolysis and interfacial reaction. Regarding HAp, many researchers adopt techniques that involve spraying raw material solutions [10–16]. On the other hand, interfacial reaction is carried out in a multiple emulsion [17–20]. This method has a lot of advantages. A common stirred tank is sufficient to be used as the reactor, and therefore any special apparatus is unnecessary. Low synthesis temperatures around room temperature are usable, whereas spraying methods need relatively high temperature. In particular, it must be emphasized that when some active material is contained in the inner aqueous phase from the first, it can be directly microencapsulated with an inorganic wall material [18]. However, the synthesis of HAp by the interfacial reaction cannot be found in the literature. The purpose of this study is to confirm whether HAp microspheres can be prepared by this method.

2. EXPERIMENTAL

An inner aqueous phase was prepared by dissolving dipotassium hydrogen phosphate (K_2HPO_4, special grade) at

0.3 mol/kg in deionized and then distilled water, and adjusting the pH in the range of 9 to 12 with potassium hydroxide (KOH, special grade). Sorbitan monooleate (Span80, practical grade) as dispersion stabilizer for the inner aqueous phase was dissolved in benzene (special grade) at 200 mol/m^3 to prepare an oil phase. Calcium nitrate tetrahydrate (Ca(NO$_3$)$_2$ 4H$_2$O, special grade) and polyoxyethylene(20) sorbitan monolaurate (Tween20, practical grade) as suspension stabilizer for the oil phase were dissolved in water at 0.5 mol/kg and 10 mol/m^3 to prepare an outer aqueous phase. All of the reagents were supplied from Wako Pure Chemical Industries Ltd. (Osaka, Japan).

The inner aqueous phase of 40.5 cm^3 and the oil phase of 94.5 cm^3 were mixed and ultrasonicated for 30 minutes to prepare a W/O emulsion. This emulsion was poured into the outer aqueous phase of 315 cm^3 and stirred at 300 rpm at 323 K and 300 rpm to prepare a W/O/W emulsion. After 24 hours, the solid product was separated from the liquid phases with centrifugation. It was washed with benzene, ethanol, and water in turn. Finally, the products were obtained by freeze drying.

The crystalline phase was identified by X-ray powder diffraction (XRD). The morphology of the emulsion droplets and the final products was examined with an optical microscope (OM), a scanning electron microscope (SEM), and a transmission electron microscope (TEM). The yield y was defined as a ratio of the total weight of the product to the theoretical weight of HAp based on the amount of K$_2$HPO$_4$ used.

3. RESULTS AND DISCUSSION

The droplets of the inner aqueous phase were so small that they could not be observed with an OM immediately after the preparation of the W/O emulsion. This suggests that the initial size of the inner aqueous phase droplets was less than 1 μm, because the droplets of larger than 1 μm were observable with the OM used. As time went on, the droplets of both inner aqueous phase and W/O emulsion grew. The multiple emulsion was broken for 24 hours into two phases. The solid products were collected from the lower aqueous phase.

Figure 1 shows the powder X-ray diffraction patterns of the products. HAp was formed under any condition. Thus, it was confirmed that HAp is produced also by the interfacial reaction method. Monetite (CaHPO$_4$) has coexisted at initial pH values of 9 to 11. In general, monetite is a dominant species for the solution syntheses at lower pH values. Riman et al. [21] have reported a phase diagram at 298 K under atmospheric pressure in the CaO$-$P$_2$O$_5-$NH$_4$NO$_3-$H$_2$O system as functions of precursor concentration and pH. Suppose that the phase equilibria in the present system are similar to Riman's diagram, the results shown in Figure 1 can be explained as follows. The chemical reaction can be described as

$$10Ca^{2+} + 6HPO_4{}^{2-} + 2OH^- \longrightarrow Ca_{10}(PO_4)_6(OH)_2 + 6H^+. \tag{1}$$
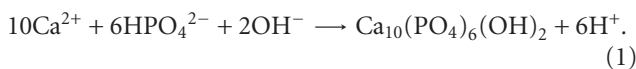
This equation means high pH values are essentially required to produce HAp. Since pH 9 is sufficiently high for the ex-

periments in which the pH is kept constant, it can be thought that the only HAp is produced at the beginning of the synthesis under any condition of this study. The above equation also means that the pH steeply decreases as the reaction proceeds. At initial pH values of 9 to 11, the pH in the inner aqueous phase may decrease to an area in which monetite is produced. Therefore, it is necessary for obtaining single-phase HAp to start at pH's high enough to keep standing in the HAp area during the synthesis process.

The yield of the product obtained at an initial pH 12 was estimated to be 48%. The exact extent of reaction should be larger than this value, because some loss at collection, separation, and drying is involved in it.

Most of the products were collected as agglomerates in irregular shape as shown in the OM micrograph of Figure 2(a). However, they seem to be composed of microspheres of less than 3 μm size. This was confirmed by SEM observation as shown in Figure 2(b). Thus, it was found that HAp microspheres can be essentially prepared by the interfacial reaction in a multiple emulsion.

Figure 2(c) indicates that such a microsphere was composed of nanospheres of less than 120 nm size. The primary particles constituting the nanosphere are still finer, less than 30 nm. These nanospheres seem to be porous. Therefore, it is expected to be usable as microcarriers or matrix-type microcapsules in the future.

The size of the microspheres will be estimated from the starting conditions to discuss the formation mechanism.

The amount of substance of HPO$_4{}^{2-}$ ion contained in an inner aqueous droplet with the diameter d is

$$\frac{\pi}{6}d^3\,C, \tag{2}$$

where C is the molarity of HPO$_4{}^{2-}$. Six moles of HPO$_4{}^{2-}$ correspond to one mole of HAp. When HPO$_4{}^{2-}$ converts to HAp at a yield of y, the volume of HAp produced is

$$\frac{\pi}{36}\frac{d^3\,C\,M_{\mathrm{HAp}}\,y}{\rho_{\mathrm{HAp}}}, \tag{3}$$

where ρ_{HAp} is the true density of HAp and M_{HAp} is the molar mass. The closest packing of spheres having the same size reveals the voidage to be 26%. The voidage varies from 40% to 60% in a common state depending on the size, size distribution, and shape. If ultrafine HAp particles are agglomerated with the voidage ε to form a nanosphere with the diameter D_{HAp}, the apparent volume of the sphere will be expressed by

$$\frac{\pi}{6}D_{\mathrm{HAp}}{}^3 = \frac{\pi}{36}\frac{d^3\,C\,M_{\mathrm{HAp}}\,y}{\rho_{\mathrm{HAp}}\,\varepsilon}. \tag{4}$$

The molarity can be converted with the molality of b and the density ρ of K$_2$HPO$_4$ solution to

$$C = \frac{b\,\rho}{b\,M_{\mathrm{HAp}} + 1}. \tag{5}$$

Hence, D_{HAp} is found to be

$$D_{\mathrm{HAp}} = \left(\frac{d^3\,M_{\mathrm{HAp}}\,y}{6\rho_{\mathrm{HAp}}\,\varepsilon} \cdot \frac{b\,\rho}{b\,M_{\mathrm{HAp}} + 1}\right)^{1/3}. \tag{6}$$
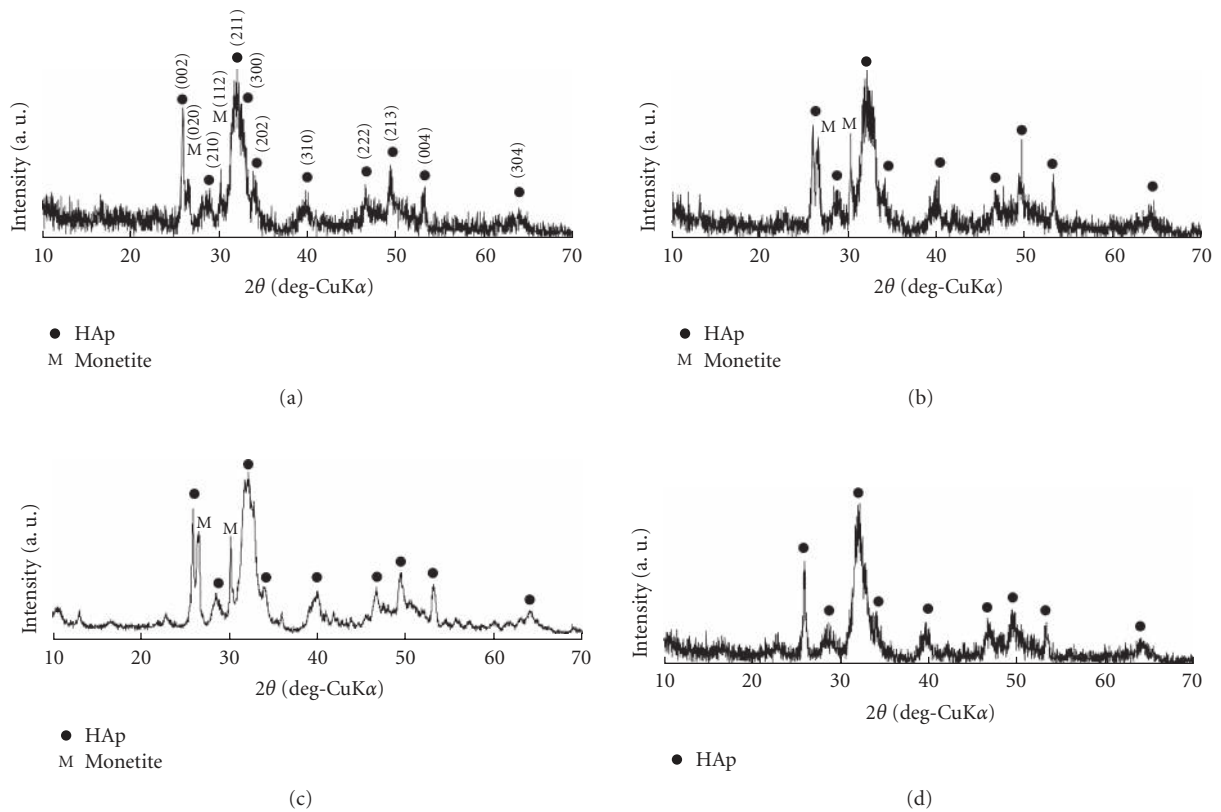
FIGURE 1: Powder X-ray diffraction patterns of the products. Initial pH of the inner aqueous phase was (a) 9.0, (b) 10.0, (c) 11, and (d) 12. HAp and monetite were indexed as compared with JCPDS no. 9–432 and no. 9–80, respectively.
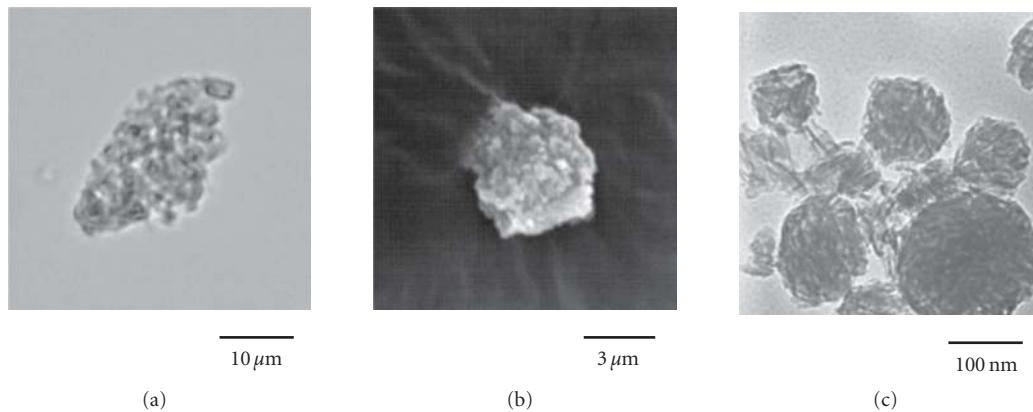

FIGURE 2: Morphologies of HAp produced at an initial pH of 12. (a) Optical micrograph, (b) SEM micrograph, and (c) TEM micrograph.

Suppose respective values as $d = 500$ nm, $y = 48\%$, and $\varepsilon = 0.5$, and assign $\rho_{\mathrm{HAp}} = 3.16\,\mathrm{g/cm^3}$, $b = 0.3\,\mathrm{mol/kg}$, $\rho = 1.05\,\mathrm{g/cm^3}$, and $M_{\mathrm{HAp}} = 1004\,\mathrm{g/mol}$, then the diameter of the HAp microsphere is estimated to be 115 nm. This value is not inconsistent with the result shown in Figure 2(c). Therefore, it is suggested that the multiple emulsion had been sustained at least until HAp microspheres were formed.

From the results shown so far, the formation mechanism of HAp microspheres can be inferred as follows. At step 1, calcium ion in the outer aqueous phase diffuses through the oil phase to the interface between the oil phase and the inner aqueous phase. At step 2, calcium ion reacts at the interface with hydrogen phosphate ion to produce primary, ultrafine HAp particles. At step 3, the ultrafine HAp particles are transported from the interface to the inside of the inner aqueous phase droplet. At step 4, they agglomerate to form nanospheres as shown in Figure 2(c). At step 5, the inner aqueous phase droplets coalesce each other and

the nanospheres coagulate to form microspheres as shown in Figure 2(b). Finally, they escape from the W/O emulsion droplet to the continuous phase.

The products in the form of discrete microspheres must be necessary for practical applications as microcarriers. For that purpose, the agglomeration at step 5 should be avoided. The multiple emulsion formed in this study was not yet recognized to be sufficiently stable. As stated above, the droplets of both phases grew as time went on. In general, the stability of droplets is altered according to the raw material species, concentration, and pH of the solutions, temperature, and co-existing species, for example, surfactants. More detailed investigation is necessary to find out the optimum condition, under which much higher stability is achieved.
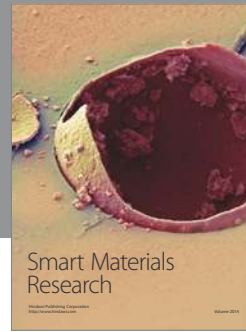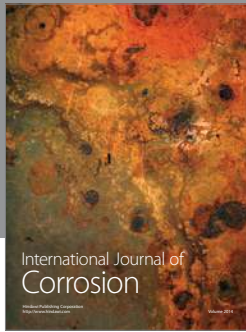
4. CONCLUSIONS

Interfacial reaction in a multiple emulsion was applied to synthesize HAp, and it was confirmed that HAp can be produced by this method. The products were HAp microspheres agglomerated. Each microsphere was constituted by a few hundred nanometer-sized porous spheres, composed of primary nanoparticles. The morphology of the microspheres lets us expect that they will be applied to microcarriers for drug delivery system and scaffolds for the regenerative medicine in the future.

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