Block-copolymer-assisted synthesis of hydroxyapatite nanoparticles with high surface area and uniform size

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Abstract

We report the synthesis of hydroxyapatite nanoparticles (HANPs) by the coprecipitation method using calcium D-gluconate and potassium hydrogen phosphate as the sources of calcium and phosphate ions, respectively, and the triblock copolymer F127 as a stabilizer. The HANPs were characterized using scanning electron microscopy, x-ray diffraction, and nitrogen adsorption/desorption isotherms. Removal of F127 by solvent extraction or calcination alters the structure of HANPs. The solvent-extracted HANPs were single crystals with their ⟨001⟩ axis oriented along the rod axis of the HANP, whereas the calcined HANPs contained two crystal phases that resulted in a spherical morphology. The calcined HANPs had much higher surface area (127 m² g⁻¹) than the solvent-extracted HANPs (44 m² g⁻¹).

Keywords: triblock copolymer F127, hydroxyapatite, nanoparticles, formation, coprecipitation method, calcium D-gluconate, potassium hydrogen phosphate

1. Introduction

Because its chemical and morphological properties are similar to those of bone and teeth, hydroxyapatite (HA, Ca₁₀(PO₄)₆(OH)₂) with a Ca : P ratio of 1.67 : 1 has attracted much attention as a new biomaterial for dental and orthopedic applications [1–7]. The mechanical strength of synthetic HA is one of the most important properties when using HA in load-bearing bone-grafting materials. HA synthesized by wet chemical methods without a structural stabilizer has a bulk morphology with low toughness and poor reliability. HA particles smaller than 1 µm should perform better owing to their high surface area and improved sintering density.
shows the nitrogen adsorption
properties of synthetic HA nanoparticles (HANPs) depend on
their size and morphology [18, 19]. For example, the smaller
HA crystals can be more easily dissolved, and this property
is important in the case of osteoporosis and other metabolic
diseases [20]. Therefore, understanding the factors that affect
the crystal size and growth of HA should be very helpful for
studying the dissolution mechanism and microscopic behavior
of HA as it undergoes osteoclast-mediated dissolution in
acidic medium.

To synthesize HANPs with uniform size and large
surface area, we adopted a coprecipitation approach, using
calcium D-glucurate and potassium hydrogen phosphate as
the sources of calcium and phosphate ions, respectively, and
poly(ethylene glycol)-poly(propylene glycol)-poly(ethylene
glycol) triblock copolymer F127 (EO106PO70EO106) as a
dispersing agent. Although block copolymers such as P123
and F127 have been widely used as structure-directing agents
for mesoporous materials, in our synthesis, F127 acts as a
stabilizer rather than a structure-directing agent. Recently,
nanoparticles synthesized using nonionic surfactants as a
stabilizer have attracted much attention because the stabilizer
can inhibit the aggregation of nanoparticles, providing an
easy and reproducible route for generating a uniform particle
size [21–24]. In addition, the high external surface area of
nanoparticles is beneficial for biomedical applications such as
intracellular drug delivery [25–27], cell bioimaging [28–30],
and purification and adsorption of biological molecules [31].
Various inorganic nanoparticles of silica [32, 33], titania
[34, 35], carbon [36, 37], etc, have been reported. However,
the uncertainty in the safety of these inorganic nanoparticles
is a concern for biomedical applications. Therefore, the
production of HP nanoparticles exhibiting not only high
biocompatibility but also biodegradability has been highly
demanded.

2. Experimental details

2.1. Chemicals

Calcium D-glucurate ([HOCH2(CH2OH)4CO2]2−Ca),
potassium hydrogen phosphate (K2HPO4), sodium
hydroxide (NaOH), ethanol (C2H5OH, 95%) and
Pluronic F127 (poly(ethylene glycol)106-poly(propylene
glycol)90-poly(ethylene glycol)106) were purchased from
Sigma-Aldrich and used without further purification.

2.2. Synthesis of hydroxyapatite nanoparticles

Typically, 3 g of F127 and 0.04 mol of calcium D-glucurate
were dissolved in 100 ml of distilled water, and the mixture
was stirred vigorously until a clear sol was obtained. Then,
another solution containing potassium hydrogen phosphate
(K2HPO4, 0.024 mol) dissolved in 60 ml of distilled water
was prepared and its pH was adjusted to 12.0 by adding NaOH
(2 N). The PO43− solution was then slowly added into the
Ca2+/F127 solution under stirring. The pH of the final mixture
was 8.6, which favors the formation of hydroxyapatite. After
this mixture was kept at 90 °C for 24 h, a white precipitate was
formed. It was washed several times with boiling water and
ethanol to remove the excess calcium D-glucurate. The final
product (i.e. as-synthesized HANP) was collected by filtration
and dried at 100 °C for 24 h. F127 was removed by solvent
extraction and calcination. For solvent extraction, 0.5 g of the
as-synthesized sample was added in 100 ml of ethanol and
the mixture was heated to 60 °C for 2 h (i.e. solvent-extracted
HANP). For calcination, the as-synthesized HANPs were
calcined at 550 °C for 6h in air (i.e. calcined HANP). The
as-synthesized and solvent-extracted HANP samples were
white powders, but the calcined HANPs were light brown. The
complete removal of F127 was confirmed by carbon, nitrogen
and hydrogen (CHN) analysis.

2.3. Characterization of HANPs

The sample crystallinity was analyzed by powder X-ray
diffraction (XRD) on a Rigaku Ultima IV instrument using
cu Kα radiation (40 kV, 40 mA). The sample morphology
was observed by scanning electron microscopy (SEM,
Nova TM Nano SEM). The powders were dispersed in
ethanol and dropped onto copper grids for transmission
electron microscopy (TEM) observations, which were
carried out with a JEOL JEM-3010 microscope operated
at 300 kV. The surface areas of the products were
analyzed via N2 adsorption/desorption isotherms recorded with
a Micromeritics ASAP 2000 instrument at −196 °C.
The samples were degassed at 100 °C overnight before the
measurements. The surface areas were evaluated by the
Brunauer–Emmett–Teller (BET) method.

3. Results and discussion

The morphology and particle size of the HANPs were
examined by SEM. The SEM images in figure 1(a) show
that the as-synthesized sample consists of small rod-like
nanoparticles. Their smaller dimension was about 10–20 nm
for all the nanoparticles and the larger one varied between
30 and 90 nm. The nanoparticles were not connected to
each other but well dispersed owing to the use of the
F127-stabilized coprecipitation method. After the removal
of F127 by solvent extraction, the morphology and average
particle size of the resulting HANPs remained very similar
to those of the as-synthesized HANPs (figures 1(b) and 2).
F127 was also removed from the as-synthesized HANPs by
another method, i.e. calcination. After the calcination, the
particles shrank to 30–70 nm in the larger dimension and grew
to 20–30 nm in the smaller dimension. Some calcined HANPs
had a spherical shape, as shown in figures 1(c) and 2.

Figure 3 shows the nitrogen adsorption/desorption
isotherms, which have typical shapes for macroporous solids
with multilayered adsorption, both for the solvent-extracted
and calcined HANPs [38]. The hysteresis loops at high
relative pressures can be assigned to the spaces between
the HANPs. The BET specific surface areas were calculated as 44 and 127 m$^2$ g$^{-1}$ for solvent-extracted and calcined HANPs, respectively, revealing that calcination increased the surface area. We could not confirm the presence of uniform mesopores either from Barret–Joyner–Halenda (BJH) pore size distributions calculated from the adsorption isotherms or by SEM and TEM observations (figures 2 and figure 4). The role of F127 in the present synthesis was a stabilizer rather than a structure-directing agent, although block copolymers such as P123 and F127 have been widely used as structure-directing agents for mesoporous materials.

As a control experiment, we synthesized HA without using F127 as the structural stabilizer, and the HANPs were highly aggregated. Therefore, we conclude that F127 is essential for the synthesis of HANPs with a uniform, nanometer-scale size. The OH groups on the gluconate anion would form hydrogen bonds with the ethylene oxide groups of F127, which could stabilize the HA structure formed in the reaction between the Ca$^{2+}$ ions of calcium D-gluconate and PO$\text{3}^-\text{4}$ ions of potassium hydrogen phosphate.

The crystallinities of the solvent-extracted and calcined HANPs were characterized by TEM (figure 4). Lattice fringes

**Figure 1.** SEM images of (a) as-synthesized, (b) solvent-extracted and (c) calcined HANPs.
Figure 2. Magnified SEM images of solvent-extracted and calcined HANPs.

Figure 3. Nitrogen adsorption–desorption isotherms of solvent-extracted and calcined HANPs.

cohereently extended over the whole particles, indicating a single-crystalline structure. In the solvent-extracted HANPs, the (001) direction (c-axis) was aligned to the long axis of the rod-shaped HANPs (figure 4(a)). The rod shape was distorted by the calcination. The TEM image shown in figure 4(b) was viewed along the (-11-1) direction. The corresponding electron diffraction pattern (figure 4(b), inset) reveals that two different crystal orientations overlap in this area and that the (001) direction (c-axis) is not oriented along the long axis of the HANP. Thus, further crystallization occurred during the calcination, although the crystal phases did not change.

Many researchers have studied the effects of organic ligands on the crystal growth of HA. Puvvada et al reported that the presence of citric acid, tartaric acid and acetic acid inhibits the growth along the (001) and (100) directions of the HA crystal, thereby promoting the formation of needle-shaped HANPs [39]. Pan et al reported that the amino acid glycine (Gly) preferentially adsorbs on the HA (001) face rather than (100) face, resulting in the formation of plate-like HANPs. However, another amino acid, glutamic acid (Glu), did not show such preferential adsorption [40]. In our present system, we suggested that F127 helps the growth of rod-shaped HA crystals with their (001) direction aligned to the long axis of the HA. The as-prepared rod-shaped morphology remained unchanged after the solvent extraction. However, the thermal energy provided during calcination promoted a further HA crystal growth without any preferential direction, resulting in a spherical morphology.

The XRD patterns in figure 5 show the crystal phases for the as-synthesized, calcined and solvent extracted HANPs. Even for the as-synthesized sample, several XRD peaks agree with the reference data of a standard hexagonal HA crystal (space group P6̅3 m) [39]. Both the intensities and positions of the peaks are well consistent with the reference JCPDS profile for HA (Card No. 09-0432), confirming the absence of intermediates such as tricalcium phosphate. The hexagonal cell parameters a and c can be calculated using

\[
d = \frac{1}{\sqrt{\frac{4 h^2 + h k + k^2}{3 a^2} + \frac{l^2}{c^2}}},
\]

where \(d\) is the spacing between the adjacent planes with the indexes \((hkl)\). The cell parameters are listed in table 1.

The XRD peaks for the as-synthesized HANPs were broad, which can be attributed to the lattice strain and low crystalline size of the formed hydroxyapatite. The degree of crystallinity \(X_c\) can be evaluated as

\[
X_c = \left(\frac{k}{\beta_{002}}\right)^3,
\]

where \(\beta_{002}\) is the full-width at half-maximum of the 002 reflection and the value of constant \(k\) is 0.24 for most HA materials. \(X_c\) for the as-synthesized HANP sample was calculated as 0.15. This value is similar to that of the solvent-extracted HANPs (0.17) but smaller than that of the calcined HANPs (0.23), indicating that the degree of HANP crystallinity could be improved by the calcination.

The crystallite sizes of the three HANPs were estimated using the Scherrer equation

\[
D = \frac{0.9 \lambda}{\beta \cos \theta},
\]

where \(D\) is the crystallite size, \(\lambda\) is the x-ray wavelength (0.15406 nm), \(\beta\) is the full-width at half-maximum (in
Figure 4. TEM images of (a) solvent-extracted HANPs and (b) calcined HANPs. The insets show selected-area electron diffraction patterns taken from one HANP.

Figure 5. XRD patterns of as-synthesized, solvent-extracted and calcined HANPs.

Table 1. Specific surface areas, crystallinity degrees and lattice parameters of the studied HANPs.

| HANPs       | Surface area (m² g⁻¹) | Degree of crystallinity | Lattice parameters (nm) |
|-------------|-----------------------|-------------------------|-------------------------|
| As-prepared | 35.2                  | 0.15                    | \(a = 0.9418\)          |
|             |                       |                         | \(c = 0.6859\)          |
| Solvent-extracted | 43.6                  | 0.17                    | \(a = 0.9417\)          |
|             |                       |                         | \(c = 0.6858\)          |
| Calcined    | 127                   | 0.23                    | \(a = 0.9415\)          |
|             |                       |                         | \(c = 0.6871\)          |

Ikawa et al reported the synthesis of amino acid-containing amorphous calcium phosphates and further converted these amorphous calcium phosphates to crystalline apatite by immersion in a simulated body fluid [41]. In this method, glutamic acid (Glu), aspartic acid (Asp) and lysine (Lys) were added to the synthesis reaction of calcium phosphate, and the pHs of the amino acid-containing systems were all different. In contrast, although an amino acid-containing calcium source (i.e. calcium D-gluconate) was used in our study, we adjusted the pH to 8.6 after adding calcium and phosphate ion sources. This treatment favors the formation of crystalline HA directly, without the transformation from amorphous calcium phosphate, as shown in figure 5 where the as-prepared HANPs exhibit several characteristic HA peaks.

4. Conclusions

We demonstrated the synthesis of hydroxyapatite nanoparticles by coprecipitation in the presence of the
triblock copolymer F127 as a stabilizer. Post-treatments, namely solvent extraction or calcination, resulted in HANPs with different particle sizes, specific surface areas and degrees of crystallinity. The design developed here of biocompatible and biodegradable HANPs with controllable structural properties should be useful for biomedical applications.

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