Phase purity of sol–gel-derived hydroxyapatite ceramic

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Abstract

Calcium oxide was reported in the sol–gel-derived hydroxyapatite (HA) as an unavoidable major impurity. In this study phase purity of HA synthesized by sol–gel route was explored using precursors of calcium nitrate tetrahydrate and triethyl phosphate. Two different drying methods, the fast drying of as-prepared precursors and the slow drying of aged precursors were adopted as major processing variables. The dried gels were subsequently calcined up to 600°C. In the calcined powder from fast-dried gel, X-ray diffraction (XRD) patterns revealed an intense CaO peak. For the slow-dried gel, thermogravimetric analysis revealed a 2-step weight-loss behavior during heating. XRD analysis of the calcined powder, corresponding to the second weight-loss step, showed major peaks of hydroxyapatite and a very weak CaO peak. P-31 NMR analysis indicated formation of calcium phosphate complex during aging. Complete incorporation of Ca(NO₃)₂ into the complex due to proper aging therefore diminishes CaO formation. It was also found that the minor CaO derived in the slow drying method can be easily and completely washed out just by distilled water.

Keywords: Hydroxyapatite; Sol–gel; P-31 NMR; Phase purity; Aging; Drying method

1. Introduction

Ceramic materials designed to be utilized in orthopaedic and dental applications possess bio-compatible characteristics. The materials, termed bioceramics, have advantages of chemical and/or morphological similarities to the calcified tissues. With high chemical similarities of invariance, hydroxyapatite (Ca₁₀(PO₄)₆(OH)₂, HA) can also be prepared to be morphologically similar to the calcified tissues. Thus HA has been widely used to repair damaged or diseased hard tissues. Previous reports on approaches of HA synthesis include solid-state reaction [1], co-precipitation [2,3], hydrothermal method [4], and sol–gel route [5–9]. Ceramic materials synthesized by sol–gel route exhibit many advantages over the others, such as high product purity, homogeneous composition, and low synthesis temperature. Another advantage of the sol–gel route is its applicability for surface coating. A major usage of HA coatings is on titanium alloy implants for dental applications. The surface of titanium alloy is typically coated with HA to gain bioactive properties for the purpose of long-term fixation to hard tissues.

However, reports to date on the sol–gel-derived HA indicate that synthesis of HA is always accompanied by secondary phase of calcium oxide (CaO) [6–8]. Since CaO is harmful to the biocompatibility of HA, attempts to overcome this problem are thus the current research interest. A direct method to eliminate the coexisting CaO was to wash the calcined powders using dilute solution of hydrogen chloride (HCl) or other acids. Jilavenkatesa et al. pointed out that CaO, upon treatment with 0.01 M HCl, converts to calcium chloride (CaCl₂), and limited reactivity of HA with diluted HCl preserves HA. Water-soluble CaCl₂ can thus be removed by filtration [6].

The origin of formation and reduction of the secondary CaO phase can, however, be fully understood from the chemistry of sol–gel routes. One criterion for sol–gel system is to prepare stable metal-organic adducts in solution (precursors). Thermal treatment of the precursor generally plays a very important role in the formation of the adduct because stoichiometry of calcined powders is correlated with molecular arrangement in which heating provides energy to arrange the adduct, especially in the...
Pechini process [10,11]. Spectroscopic techniques, such as infrared spectroscopy [12] and nuclear magnetic resonance (NMR) [13,14] are frequently used to provide information on molecular arrangement. In this study, the
effect of thermal treatment (aging) on the molecular structure of the precursors was studied by NMR technique. Two gelation methods of precursors were investigated, the fast drying of as-prepared precursors and the slow drying of aged precursors.

2. Materials and methods

2.1. Sol–gel synthesis

Precursors with different calcium to phosphorus molar ratios (Ca/P) were prepared by mixing Ca(NO₃)₂·4H₂O (Ferak Laborate GmbH, 98%) and triethyl phosphate ((C₂H₅)₃PO, TEP, Janssen Chemicals, 99%) in 2-methoxyethanol (TEDIA chemicals, ACS grade). Compositions of each batch are given in Table 1. The flow chart of the synthesis is depicted in Fig. 1. The route includes an aging of precursors, followed by a gelation. For aging, as-prepared precursors were closely capped, and were placed in an oil bath set at 80–90°C for 16 h. The pH values of solutions before and after aging were recorded. Two gelation methods were adopted as the major processing variables: the fast drying of as-prepared precursors under reduced pressure, and the slow evaporation in the oil bath of aged precursors. The slow evaporation process was done in the same oil bath for 48 h. Table 1 also summarizes the processing variables of each precursor. The dried precursors were calcined in air from room temperature to 600°C using a ramp of 100°C/h.

2.2. Liquid phosphorus-31 NMR analysis

To elucidate bonding states of phosphate during the thermal treatment, a liquid P-31 nuclear magnetic resonance (NMR) spectrometer (BRUKER AM-400) was used in which RF pulses of 6 μs (45°) were applied. For each sample, 28 spectra were averaged to give a total measurement time of 104 s. Chemical shifts (δ) of precursors after different thermal treatments are recorded with respect to 85% H₃PO₄.

2.3. Thermogravimetric analysis (TGA) of precursors

TGA of the partial-dried precursors (dried for 10 h) was measured using a thermal analyzer (Seiko SSC 5200) from room temperature to 1200°C under an air flux of 100 ml/min ramping at 5°C/min.

2.4. X-ray analysis of calcined powder

Crystalline phases of calcined powders were investigated using X-ray powder diffraction method (Rigaku D/max-IIIB). X-ray radiation of Cu Kα (1.548 Å) is set at 30 kV and 20 mA. Identification of crystalline phases was done by comparing with JCPDS files (HA: 09-0432; CaO: 37-1497; Ca(NO₃)₂: 07-0204; β-TCP: 03-0690).

![Fig. 1. Flow chart of hydroxyapatite preparation by the sol–gel route.](image-url)
3. Results

3.1. Thermal treatment of precursors in oil bath: the aging effect of precursors

Organic phosphorus derivatives of various ester functional groups exhibit characteristics of hydrolysis upon exposure to water molecules [13,15]. The hydrolysis can be monitored by testing its pH value. In our study, pH values of aging precursors during aging were measured and the values before and after aging are summarized in Table 2. From the table, decrease in pH values due to the aging step is manifest irrespective of the Ca/P ratio, indicating hydrolysis of TEP. The hydrolysis products form a complex with calcium ions dissolved in the solution. Fig. 2 shows P-31 NMR spectra of precursors with corresponding chemical shift values summarized in Table 3. In the spectrum of a precursor aged for 16 h (Fig. 2b), chemical shift of TEP dissolved in the solvent (2-methoxy ethanol) shows a single peak ( -0.881 ppm). The acidic solution implies hydrolysis reaction, and the single peak at -0.881 ppm can thus be assigned as phosphorus atom bonded in hydrolyzed TEP. But an aged precursor with Ca/P = 1.67 explicitly shows two peaks (-2.532, -4.226 ppm) in the spectrum (Fig. 2a). These peaks indicate two different chemical environments of the phosphorus atom. For the spectra of precursors aged for different durations (Fig. 2a and c), the peak at -2.532 ppm is assigned as phosphate tetrahedron at the chain end of oligomeric phosphate. After aging, a small peak appears at -4.226 ppm, revealing further reaction of calcium ions with phosphate. Thus this small peak provides a direct evidence of phosphate complexing with calcium ion.

In terms of optical appearance of precursors, the as-prepared precursor without aging is a transparent clear solution, but the aged precursor is a transparent pale-yellow solution, indicating a homogeneous solution containing dissolved complex.

3.2. Effect of gelation methods on the phase purity of calcined powder

A set of experiments was conducted to investigate the effect of gelation methods on the phase purity. For the fast drying method, the liquid phase (mostly solvent) of the aged precursor was rapidly evaporated under reduced pressure. These precursors were calcined at a heating rate of 100°C/h from room temperature to 600°C. In Fig. 3, XRD patterns of the calcined powders show intense CaO and HA peaks. On the contrary, by slow drying the aged precursor for 48 h followed by calcining at 600°C, XRD patterns of the resultant powders (Figs. 4 and 5) show a very weak CaO peak, indicating that slow drying in an oil bath is effective to greatly reduce final CaO content.

### Table 2
pH values of precursors measured before and after aging

<table>
<thead>
<tr>
<th>Batch No.</th>
<th>Ca/P</th>
<th>pH before aging</th>
<th>pH after aging</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>1.5</td>
<td>4.15</td>
<td>1.44</td>
</tr>
<tr>
<td>8</td>
<td>1.67</td>
<td>4.24</td>
<td>1.57</td>
</tr>
</tbody>
</table>

### Table 3
Chemical shift of liquid P-31 NMR of precursors, for batch No. 5, Ca(NO$_3$)$_2$ and TEP were aged in solvent, for batch No. 6, only TEP was aged in solvent

<table>
<thead>
<tr>
<th>Batch No.</th>
<th>Ca/P ratio</th>
<th>$\delta_1$/area (ppm/cm$^2$)</th>
<th>$\delta_2$/area (ppm/cm$^2$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>1.67</td>
<td>-2.532/1.6034</td>
<td>-4.226/0.1189</td>
</tr>
<tr>
<td>6</td>
<td>0</td>
<td>-0.881</td>
<td>—</td>
</tr>
</tbody>
</table>
3.3. Thermal behavior of partial-dried precursors

From TGA curves (Figs. 6 and 7), weight-loss curves of precursors with Ca/P ratios of 1.5 and 1.67 can be divided into 2 steps. Temperature regions and corresponding weight losses of these steps are summarized in Table 4.

In step 1 (RT ~ 330°C), a weight loss of about 74–72 wt% occurs. This is the removal step of volatile substances. The hydrolysis products of TEP, hydrate and ethanol are not binding to the complex in the precursors. The evaporation of solvent mainly occurred in this step. At the end of this step, the gels were pale-yellow chunks, and were very hygroscopic when exposed to air. XRD patterns of the gels (Figs. 4 and 5) show amorphous characteristics with weak peaks of Ca(NO$_3$)$_2$ anhydride which is an undesirable precipitate representing incomplete incorporation of calcium ions into the complex. Between 330 and 540°C, a plateau of weight loss represents thermal stability of the gels. XRD patterns of the gels (Figs. 4 and 5) calcined at 450°C are mainly amorphous, the same as those of the gels calcined at 330°C.

Fig. 3. XRD patterns of powders calcined at 600°C: (a) batch No. 2, Ca/P = 1.67; (b) batch No. 1, Ca/P = 1.5; (c) batch No. 4, Ca/P = 1.67; and (d) batch No. 3, Ca/P = 1.67. (◯) CaO, other peaks: HA.

Fig. 4. XRD patterns of powders derived from precursors with Ca/P = 1.5, calcined at temperatures shown: ( ■ ) β-TCP; ( ● ) Ca(NO$_3$)$_2$; ( ◯ ) CaO; other peaks: HA.

Fig. 5. XRD patterns of powders derived from precursors with Ca/P = 1.67, calcined at temperatures shown: ( ● ) Ca(NO$_3$)$_2$; ( ◯ ) CaO; other peaks: HA.

Fig. 6. TGA curve of a precursor with Ca/P = 1.5.

Fig. 7. TGA curve of a precursor with Ca/P = 1.67.
Table 4
Temperature regions and corresponding weight loss of partial-dried precursors measured by TGA

<table>
<thead>
<tr>
<th>Step</th>
<th>Temperature range (°C)</th>
<th>Weight loss (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Batch No. 9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>RT–334</td>
<td>72.16</td>
</tr>
<tr>
<td></td>
<td>334–538</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>538–616</td>
<td>27.84</td>
</tr>
<tr>
<td>Batch No. 10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>RT–320</td>
<td>73.7</td>
</tr>
<tr>
<td></td>
<td>320–545</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>545–579</td>
<td>26.3</td>
</tr>
</tbody>
</table>

During step 2, 540–620°C, a weight loss of about 26–28 wt% occurs. The powders calcined at 600°C exhibited less hygroscopicity than those calcined at 330°C. The weight loss therefore can be attributed to the decomposition of the remaining hydrocarbons. The decomposition resulted in high content of inorganic calcium phosphates. From XRD patterns of the powder (Figs. 4 and 5), HA phase and calcium oxide phase were observed. In addition, β-tricalcium phosphate (β-TCP) phase was observed in the XRD pattern of powder derived from the precursor with a Ca/P ratio of 1.5.

3.4. Treatment to remove CaO in calcined HA powders

The chemical change of calcined powders in an aqueous environment is an interesting issue for biomedical applications, such as in vitro mineralization. In this study, calcined powders were stirred in distilled water at room temperature for 15 and 30 min, respectively. After each treatment, the solution was filtered by using a filter paper. Then, the filtrate was dried in an oven kept at 80°C. Fig. 8 shows XRD patterns of powders washed for different times. From XRD patterns (Fig. 8a–c), the CaO phase in as-calcined powders disappeared completely after the powders were washed with distilled water for 15 min. For an extended wash time of 30 min, the XRD pattern (Fig. 8c) was the same as that of 15 min. CaO readily dissolves into water and converts into dissolvable calcium hydroxide (Ca(OH)₂) [16]. This result provides an alternative approach for CaO elimination. The reason why CaO can be completely removed by washing with distilled water is simply because of the relatively minor amount present.

4. Discussion

4.1. Aging reactions

Typically, reactions involved in the sol–gel route are hydrolysis and condensation. From sol to gel, interaction between ions gradually increases. This interaction, in general, can be investigated by the NMR method. From the chemistry of sol–gel, reactants dissolved in solvent firstly undergo hydrolysis reaction. Tian et al., in the study of P₂O₅–SiO₂ sol chemistry, demonstrated that a single P-31 NMR peak is indicative of no interaction between phosphorus atom and silicate network [15]. However, the peak was broadened and chemical-shifted more negatively in the gel state. Nevertheless, the single peak might be split because of close interaction with hetero-nucleus atoms in the gel state. In this study, P-31 NMR spectra illustrated that aging can enhance the interaction of calcium ion with oligomeric phosphate to form a complex (Fig. 2). As compared with the peak at −2.532 ppm, the peak at −4.226 ppm shifts more negatively depicting the fact that calcium ion is incorporated into the oligomeric phosphate. Formation of a stable complex is helpful for subsequent drying and calcining processes, since an undesirable precipitate of calcium nitrate in precursors will accordingly result in calcium oxide formation during calcining.

4.2. Gelation reactions

According to XRD and P-31 NMR analysis, gelation at about 80–90°C is believed to play a role in the formation process of the P–O–Ca linkage. Since the hydrolysis of triethyl phosphate is much slower than alkyl phosphate [13], short-time aging, for example 5–10 h, may not be helpful for hydrolysis and the P–O–Ca linkage formation. However, prolonging aging for 20 h, for example, will again result in precipitation due to the decrease in pH value. Rapid evaporation of solvent under reduced pressure cannot provide sufficient time for intimate interaction of reactants, although concentration is rapidly increased. Therefore the undesirable precipitate,
Ca(NO₃)₂ is decomposed into CaO, as shown in Fig. 3a–c. In addition, hydrogen bonding between TEP and solvent should be considered in the drying step. Intimate interaction between reactants is also facilitated by hydrogen bonding of the solvent. Hence, reactants solvated by solvent are brought together during gelation (by slow drying). Heating history of the precursor is the key factor influencing the extent of P–O–Ca linkage formation. Slow evaporation of solvent provides not only intimate interaction but also thermal energy for reactants. After slow drying for one day, precursors exhibit viscous behavior at the drying temperature. Heat supplied therefore drives the formation of the P–O–Ca linkage in the gels.

4.3. Calcining of preceramic gel

During calcining, the following two reactions simultaneously occur:
1. the gels decompose into hydroxyapatite;
2. Ca(NO₃)₂ decomposes to CaO.

A study of the thermal decomposition of Ca(NO₃)₂ [16,17] revealed that the decomposition occurs in the temperature range 500–650°C, and it is a kind of cascade reaction comprising

\[
\begin{align*}
\text{Ca(NO}_3\text{)}_2 & \rightarrow \text{Ca(NO}_3\text{)}_2 + \frac{4}{5}\text{O}_2, \\
\text{Ca(NO}_2\text{)}_2 & \rightarrow \frac{1}{2}\text{Ca(NO}_3\text{)}_2 + \frac{3}{2}\text{CaO} + \frac{3}{5}\text{NO}, \\
\text{Ca(NO}_3\text{)}_2 & \rightarrow \text{CaO} + 2\text{NO}_2 + \frac{3}{5}\text{O}_2.
\end{align*}
\]

According to the above results, using a heating rate of 100°C/h, the temperature of HA formation was concluded to be 600°C. The CaO in the bulk of HA was originated from the decomposition of Ca(NO₃)₂.

4.4. Relationship between CaO formation and heating history of precursors

Other investigators also observed formation of CaO in sol–gel processing of HA [7,8,18,19]. For example, Chai et al. explained that starting materials do not have sufficient opportunities to react with each other [19]. Comparing TGA data of their results between aged and unaged gels shows that the unaged gel has a weight loss of 30 wt% at 650°C, whereas the aged gel has no such loss [19]. This evidence explains physical and chemical changes of precursors in preparation history, e.g. heat and chemical reactions. In this study, preparation of stable complexes in precursor solutions was found to reduce CaO content of calcined samples since the Ca(NO₃)₂ molecule has almost been incorporated into the complex.

TGA analysis demonstrated by Chai et al. [19] indeed reveals that phosphorus-containing precursors have high potential for volatilization [20]. Direct evidence of the phosphorus volatilization during preparation of calcium phosphate includes the presence of CaO peaks in XRD pattern and the positive deviation of the Ca/P molar ratio. From the phase diagram of the CaO–P₂O₅ binary system, pure HA exists only in a limited range around a Ca/P ratio of 1.67. The positive deviation is also a measure of the stability of the precursor. Before HA formation, preferential evaporation of phosphorus species leads to positive deviation in the Ca/P ratio of precursor and finally results in biphasic calcium phosphate formation.

Table 5 compares the effect of processing methods on the CaO amount in the HA powder, semi-quantitatively estimated from the XRD peak height ratio HA (211)/CaO (200) among calcined powders. The effectiveness of aging followed by slow drying to reduce CaO in calcined powders is manifest. Without aging, slow drying still provides lower CaO amount as compared with the fast-dried method. Results of Chai et al. also indicate that aging helps in the synthesis of HA by the sol–gel route [19].

<table>
<thead>
<tr>
<th>Batch No.</th>
<th>Processing method before calcining</th>
<th>Peak height ratio HA (211)/CaO (200)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>No aging, slow drying</td>
<td>0.52</td>
</tr>
<tr>
<td>4</td>
<td>No aging, rapid drying</td>
<td>0.72</td>
</tr>
<tr>
<td>1, 2</td>
<td>Aging, then rapid drying</td>
<td>0.35–0.4</td>
</tr>
<tr>
<td>5, 7–10</td>
<td>Aging, then slow drying</td>
<td>0.08–0.17</td>
</tr>
<tr>
<td></td>
<td>Water wash</td>
<td>0</td>
</tr>
<tr>
<td>Ref. [19]</td>
<td>Non-aged precursor</td>
<td>0.51</td>
</tr>
<tr>
<td>Ref. [19]</td>
<td>Aged precursor</td>
<td>0.15</td>
</tr>
</tbody>
</table>

*These ratios are measured from Fig. 3 of Ref. [19].

5. Conclusions

Pure HA can be synthesized using the sol–gel route with proper aging plus washing in distilled water. Precursors derived from calcium nitrate and triethyl phosphate were heat-treated (aging) in an oil bath, then the liquid phase was slowly evaporated from the same oil bath after aging. Simple processing procedures and comparatively low cost make the sol–gel route of producing HA superior to other methods. The key point in phase purification can be attributed to the aging step prior to the drying step. In the aging step, calcium nitrate reacts with triethyl phosphate to form a complex which is stabilized during the subsequent drying step. In P-31 NMR spectra,
a distinct peak with a chemical shift located at around −4.226 ppm was assigned to phosphorus atoms affected by complexation with calcium ion. From X-ray analysis of powders calcined at different temperatures, the formation temperature of HA was confirmed to be 600°C. The secondary phase of Ca(NO$_3$)$_2$ precipitate during calcining. To reduce CaO content in calcined HA powder, an aging step prior to liquid-phase evaporation was an important step. The minor amount of CaO remaining in calcined powder could be eliminated by mild water washing, instead of the vigorous acid washing method.

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