Synthesis of β-Calcium Pyrophosphate by sol-gel method

T Windarti1, Taslimah1, A Haris1, Y Astuti1 and A Darmawan1

1Research group of Material and Process, Chemistry Department, Diponegoro University, Indonesia

Email: tri.windarti@live.undip.ac.id

Abstract. Beta calcium pyrophosphate [β-CPP, β-Ca2P2O7] can be used as bone graft extender in posterolateral lumbar fusion. In this research, β-CPP was synthesized by sol-gel method using phosphorus pentaoxide [P2O5] and calcium nitrate tetrahydrate [Ca(NO3)2.4H2O] as phosphorus and calcium precursors. The reaction was carried out in ethanol medium with Ca/P ratio of 1.67. After 21 hours of reaction and 20 hours of drying at 80°C, white powder of amorphous calcium phosphate (ACP) was produced. Transformation of ACP to β-CPP was undertaken by firring at 400-800°C for 8 hours. Transformations of amorphous to microcrystalline, semicrystalline and crystalline structures occur at 400, 600 and 800°C, respectively. The β-CPP with the crystallite size of 61.71 nm, Ca/P ratio of 0.89 and Ca/O ratio of 0.21 was achieved by firing at 800°C. Morphology changes due to firing in which irregular shape of β-CPP at 400°C changed to regular cuboid at 600 °C and above.

1. Introduction
Calcium phosphate compounds such as hydroxyapatite [HA, Ca10(PO4)6(OH)2] and β-tricalcium phosphate [β-TCP, Ca3(PO4)2] are known as the biomaterial for bone filling due to their biocompatibility and osteoinductivity properties [1]. To achieve successful implantation in a difficult environment such as posterolateral lumbar fusion, osteoconductivity property must be combined with the porous structure of the biomaterial [2]. HA has a low resorption rate so that HA will remain in the body for a long time and hinders the constructive and reformative process as in a living bone tissue. While β-TCP even has good resorbability property, it has high biodegradability and might not persistent until the new bone formed [3]. β-calcium pyrophosphate [β-CPP, β-Ca2P2O7] has biodegradability in between HA and β-TCP. Moreover β-CPP has all properties needed as biomaterial such as the same osteoinductivity degree with HA, bioactive and nontoxic. β-CPP was reported has an excellent fusion rate compared to other calcium phosphate ceramics. Many researchers suggested using β-CPP as an alternative material for bone filling [4,5,6]. On the other hands, only a few report of β-CPP synthesis method available.

The sol-gel method can be used in the synthesis of calcium phosphate compounds. The sol-gel method is known as a simple method to produce high homogeneity, nanosized, and semi-crystalline-phase product, also can be done at a relatively low temperature [7]. A study about the synthesis of HA with Ca/P molar ratio 1.5 – 1.67 showed that for Ca/P = 1.5 a secondary product as β-TCP was found. The amounts of β-TCP were decreased as the increase of Ca/P and HA was found as the only product for synthesis with Ca/P= 1.67 [8]. Synthesis of anhydrous dicalcium phosphate [DCPA, CaHPO4] with
Ca/P= 1, ageing time of 24 hours and firing process at 600°C also produced β-TCP as a secondary product [9]. Liu et al [10] studied structure evolution of HA on sol-gel method and found that amorphous gel transformed into crystalline by firing at 400°C and above. So it can be concluded that production of calcium phosphate by the sol-gel method is determined by several factors such as Ca/P molar ratio, ageing time, and firing temperature.

In this paper, β-CCP was synthesized by sol-gel method. As calcium and phosphorus precursors were used calcium nitrate tetrahydrate [Ca(NO\textsubscript{3})\textsubscript{2}. 4H\textsubscript{2}O, Merck\textregistered] and phosphorus pentoxide [P\textsubscript{2}O\textsubscript{5}, Merck\textregistered], respectively. The reactions were carried out in ethanol medium with Ca/P ratio of 1.67. The reactions were conducted for 21 hours and continued by drying at 80°C for 20 hours to produce a white powder of amorphous calcium phosphate (ACP). Transformation of ACP to β-CPP was undertaken by firing at 400-800°C for 8 hours. The products were characterized by FTIR, XRD, and SEM-EDS to analyze calcium phosphate compound type, crystallite size and surface morphology.

2. Experimental procedures

Calcium nitrate tetrahydrate [Ca(NO\textsubscript{3})\textsubscript{2}. 4H\textsubscript{2}O, Merck\textregistered] and phosphorus pentoxide [P\textsubscript{2}O\textsubscript{5}, Merck\textregistered] were dissolved in ethanol [C\textsubscript{2}H\textsubscript{5}OH, Merck\textregistered] to form 1.67 M of Ca(NO\textsubscript{3})\textsubscript{2}. 4H\textsubscript{2}O and 0.5 M of P\textsubscript{2}O\textsubscript{5} solution.

One hundred ml of 0.5 M P\textsubscript{2}O\textsubscript{5} solution was added slowly to 100 ml of 1.67 M Ca(NO\textsubscript{3})\textsubscript{2}. 4H\textsubscript{2}O solution. A gently mixing was conducted for 21 hours to form a stable gel phase. The Gel was washed with aquabidest and continued by drying process to produce amorphous calcium phosphate (ACP). The drying process of gel phase was proceed at 80°C for 20 hours. The dry powder of ACP was transformed to crystalline phase by firing process for 8 hours at various temperature of 400, 600 and 800°C.

Products characterization were done by FTIR (Shimadzu) and XRD (Shimadzu XRD-7000). The mean crystallite size (d) of the particle base on XRD data was calculated by Scherer equation [11].

$$d_{\text{scherer}} = \frac{0.95\lambda}{\beta \cos\theta}$$  

(1)

Where λ is the wavelength, β is the full wide on the half-maximum (FWHM) of β-CPP line and θ is the diffraction angle. To confirm the surface and calcium to phosphorus molar ratio (Ca/P) of products, SEM-EDS (JSM-6510LA) was used.

3. Results and discussion

There are several stages in the synthesis of β-CPP by sol-gel method. At first, calcium and phosphorus precursors were dissolved in ethanol separately. Dissolving precursors in ethanol would produce sol, a nano-sized colloidal suspensions of solid in liquid. Calcium nitrate tetrahydrate [Ca(NO\textsubscript{3})\textsubscript{2}. 4H\textsubscript{2}O] would be ionized in ethanol and formed Ca\textsuperscript{2+}, NO\textsubscript{3}\textsuperscript{−} and H\textsubscript{2}O. When colloidal suspension of phosphorus pentoxide [P\textsubscript{2}O\textsubscript{5}] was slowly mixed with calcium precursor solution, P\textsubscript{2}O\textsubscript{5} would react with H\textsubscript{2}O and produce H\textsubscript{3}PO\textsubscript{4}. Further ionization of H\textsubscript{3}PO\textsubscript{4} would produce H\textsuperscript{+}, H\textsubscript{2}PO\textsubscript{4}\textsuperscript{−}, HPO\textsubscript{2}\textsuperscript{−} and PO\textsubscript{3}\textsuperscript{3−} ions that depend on pH of solution. The slow reaction of those ions and Ca\textsuperscript{2+} in ethanol media would produce calcium phosphate particles. Then the particles would agglomerate each other and produce particulate in the form of a gel, a colloidal suspensions of liquid in solid. This stage was continued with drying process to slowly remove solvent from the gel and produced amorphous calcium phosphate (ACP). The last stage was firing the ACP to transform amorphous phase of calcium phosphate into crystalline structure.

FTIR spectra of ACP can be seen in figure 1. The spectra show specific peaks of calcium phosphate compounds. A peak at 956.69 cm\textsuperscript{-1} is the vibration adsorption ν\textsubscript{1} of PO\textsubscript{4}. Vibration ν\textsubscript{3} and ν\textsubscript{4} appear at wave number of 1041.56 and 570.93 cm\textsuperscript{-1}, respectively. A peak at 1041.56 cm\textsuperscript{-1} corresponds to asymmetric stretching of P-O. Meanwhile, the band at 956.69 cm\textsuperscript{-1} corresponds to symmetric P-O stretching of PO\textsubscript{4}\textsuperscript{3−} ion. A broadband at 3448.72 cm\textsuperscript{-1} and a band at 1635.64 cm\textsuperscript{-1} indicate the presence of water. The spectra show the appearance of CO\textsubscript{3} at wave number of 1519.91 cm\textsuperscript{-1}.
Figure 1. FTIR spectra of ACP

The reactions involved in the formation of β-CPP during the sol-gel procedures can be estimated as follows:

I. Solvation

\[
\begin{align*}
Ca(NO_3)_2 \cdot 4H_2O & \rightarrow Ca^{2+} + 2 NO_3^- + 4 H_2O \\
P_2O_5 + 3 H_2O & \rightarrow 2 H_2PO_4^- \\
H_3PO_4 & \leftrightarrow H^+ + H_2PO_4^- \\
H_2PO_4^- & \leftrightarrow H^+ + HPO_4^{2-}
\end{align*}
\]

II. Gelation

\[
\begin{align*}
Ca^{2+} + 2 H_2PO_4^- & \rightarrow Ca(H_2PO_4)_2 \\
Ca^{2+} + HPO_4^{2-} & \rightarrow CaHPO_4
\end{align*}
\]

III. Drying

Gel \rightarrow ACP

IV. Firing (600 and 800°)

\[
2 CaHPO_4 \rightarrow Ca_2P_2O_7 + H_2O
\]

The X-rays diffraction analysis was performed using the CuKα source operated at 30 kV and 30 mA. The diffractograms were recorded at 2θ = 5 – 90° with a scan speed of 2°/min and a step angle of 0.02°. XRD diffractogram of ACP with and without washing with aquabidest indicated that washing process was razed ACP crystal structure (figure 2).

Figure 2. Diffractogram ACP with (a) and without (b) washing with aquabidest.
Figure 3. Diffractogram of products after firing process at 400°C (a), 600°C (b) and 800°C (c) Inset JCPDS card no 09-0346

Figure 3 showed the diffractogram of firing product at 400, 600 and 800 °C for 8 hours. The high peaks were occurred at 2θ = 25 - 35°. Transformations of amorphous to microcrystalline, semicrystalline and crystalline structures occur at 400, 600 and 800°C, respectively. The pattern of firing at 600 and 800 °C was similar. This signifies that the β-CPP crystal structure starts to form at 600°C but only diffractogram of firing at 800°C can be compared to JCPDS data. The strong peaks at 2θ = 27.84, 29.07, 29.75 and 33.59° indicated that product was β-calcium pyrophosphate [β-CPP, β-Ca$_2$P$_2$O$_7$] base on JCPDS card no. 09-0346. Those angles derived from X-ray diffraction of (202), (203), (008) and (205) β-CPP crystal planes. The β-CPP crystallite size was 61.71 nm.

Figure 4. Morphology of (a) ACP and firing product at (b) 400 (c) 600 and (d) 800°C

Figure 4. Morphology of (a) ACP and firing product at (b) 400 (c) 600 and (d) 800°C
Morphology changed due to firing, in which irregular shape of β-CPP at 400° changed to regular cuboid at 600 °C and above (figure 4). The Ca/P and Ca/O ratio of products decreased as firing temperature increases, from 1.06 to 0.88 for Ca/P ratio and 0.23 to 1.21 for Ca/O ratio (table 1). Those numbers are closed to Ca/P ratio and Ca/O ratio of β-CPP that are Ca/P= 1 and Ca/O=0.29.

| Sample                  | Ca/P | Ca/O |
|-------------------------|------|------|
| ACP                     | 1.06 | 0.23 |
| After Firing 400°C      | 0.99 | 0.22 |
| After Firing 600°C      | 0.89 | 0.21 |
| After Firing 800°C      | 0.89 | 0.21 |

4. Conclusions
Synthesis of β-CPP can be done by sol-gel method with Ca/P ratio precursors of 1.67, reaction time of 21 hours, drying process at 80°C for 20 hours and firing temperature at 800°C for 8 hours. The β-CPP has crystallite size of 61.71 nm, Ca/P ratio of 0.89 and Ca/O ratio of 0.21. Morphology changes due to firing in which irregular shape of β-CPP at 400° changed to regular cuboid at 600 °C and above.

Acknowledgement
This work was supported by The Indonesia Ministry of Education and Culture (DIPA: 023.04.2.673453/2015).

References
[1] Zhang J, Liu W, Schnitzler V, Tancret F and Bouler J-M 2014 Calcium Phosphate Cement For Bone Substitution: Chemistry, Handling and Mechanical Properties Acta Biomaterialia 10 1035-49
[2] Lee J H, Chang B-S, Jeung U-O, Park K-W, Kim M-S and Lee C-K 2011 The First Clinical Trial of Beta-Calcium Pyrophosphate as A Novel Bone Graft Extender In Instrumented Posteriorlateral Lumbar Fusion Clinics in Orthopedic Surgery 3 238-44
[3] Arcos D, Boccaccini A R, Bohner M, Diez-Perez A, Eppe M, Gomez-Barrena E, Herrera A, Planell J A, Rodriguez-Manaz L and Vallet-Regi M 2014 The Relevance of Biomaterials to The Prevention and Treatment of Osteoporosis Acta Biomaterialia 10 1793-05
[4] Tadic D and Epple M 2004 A Though Physicochemical Characterisation of 14 Calcium Phosphate-Based Bone Substitution Materials In Comparison to Natural Bone Biomaterials 25 987-94
[5] Ryu H-S, Youn H-J, Hong K S, Chang B-S, Lee C-K and Chung S-S 2002 An Improvement in Sintering Property of β-Tricalcium Phosphate by Addition of Calcium Pyrophosphate Biomaterials 23 909-14
[6] Lin F-H, Lin C-C, Lu C-M, Liu H-W, Sun J-S and Wang C-Y 1995 Mechanical Properties and Histological Evaluation of Sintered β-Ca3P2O7 with Na4P2O7.10H2O Addition Biomaterials 16 793-02
[7] Brinker C J and Scherer G W 1990 Sol-Gel Science: The Physics and Chemistry of Sol-Gel Processing (Academic Press Inc. San Diego)
[8] Chen J, Wang Y 2011 A Simple Sol-Gel Technique for Synthesis of Nanostructured Hydroxyapatite, Tricalcium Phosphate and Biphasic Powders Materials Letters 65 1923-26
[9] Eshtiagh-Hosseini H, Houssaindokht M R 2008 Preparation of Anhydrous Dicalcium Phosphate, DCPA, Through Sol–Gel Process, Identification and Phase Transformation Evaluation Journal of Non-Crystalline Solids 354 3854-57

[10] Liu D, Yang M Q 2002 Structural Evolution of Sol–Gel-Derived Hydroxyapatite Biomaterials, 23 1679-87

[11] Volkmer T M, Lengler F, Barreiro O, Sausa V C and Dos Santos L A 2013 Novel Method for The Obtainment of Nanostructured Calcium Phosphate Cements: Synthesis, Mechanical Strength and Cytotoxicity Powder Technology 235 599-05