

PREPARATION OF SILICON DOPED HYDROXYAPATITE

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Abstract

The disadvantage of using hydroxyapatite implant is its low reactivity with human bone. An interesting way to enhance the bioactivity is addition of silicon to hydroxyapatite structure. Silicon plays important role on bone formation and calcification of implant. In this study Si-hydroxyapatite is prepared from cuttlefish bone (*Seppia Officinalis* L, from Adriatic Sea) by hydrothermal method. The phase composition of the converted bone samples depend on the conditions of hydrothermal treatment, on Si content in Si substituted hydroxyapatite and additional heat treatment. X-ray diffraction and Fourier transform ATR infrared spectroscopy were used to study the transformation processes during hydrothermal conversion of cuttlefish bone.

Key words: bioceramics, hydroxyapatite, hydrothermal method, silicon

1. INTRODUCTION

The requirement for biomaterials to assist in many medical applications replacing original musculoskeletal tissues and improve quality of life is rapidly increasing. Hydroxyapatite, $[\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2]$, has achieved significant application as a bone graft material in a range of medical and dental applications. However, a disadvantage of using pure Hydroxyapatite (HA) implants is that its reactivity with existing bone is low [1] and therefore it integrates relatively slowly with bone [2]. These properties could have implications for the time required for patient rehabilitation [3]. A way to enhance the bioactive behavior of HA ceramic is to obtain substituted HA, which resemble the chemical composition and structure of the mineral phase in bones [4,5] An interesting way to improve the bioactivity of HA is the addition of silicon to the apatite structure [6]. The earliest studies on the role of silicon in bone dates back to the early 1970s, when a series of experiments were published by Carlisle [7], using electron probe microanalysis. He found the presence of silicon (0.5 wt%) in vivo within the mineralizing osteoid regions, i.e. the active calcification sites, of normal tibiae from young mice and rats. This study suggested that silicon plays a critical role in the bone calcification process. Tanizawa and Suzuki [8] and Sugiyama *et al.* [9] tried hydrothermal methods obtaining materials with a Ca/(P+Si) ratio higher than that of pure calcium hydroxyapatite, but they did not mention about stability of Si substituted hydroxyapatite above 145°C. Kim *et al* [10] reported preparation of silicon-incorporated hydroxyapatite by hydrothermal treatment of natural corals. To the best of our knowledge hydrothermal treatment of Si-substituted hydroxyapatite obtained by hydrothermal technique of cuttlefish bones has not been reported in literature till now. The aim of the present study is to prepare Si-substituted hydroxyapatite by hydrothermal treatment of cuttlefish bones, *Seppia Officinalis* L, from Adriatic Sea. Additionally, structural characterization of the Si-substituted hydroxyapatite has been carried out.

2. MATERIALS AND METHODS

As the starting material peaces of native cuttlefish bones, *Seppia Officinalis* L, from Adriatic Sea were used. For hydrothermal treatment only the peaces, cut from internal cuttlebone matrix (lamellae spacing) were used, since by the pre-treatment at 350°C the

aragonite in external wall (dorsal shield) partially transforms into calcite. The content of aragonite (CaCO_3) in cuttlebones was determined by DTA-TG analysis. Small peaces of bones (about 2 cm^3) were put with the required volume of an aqueous solution of $0.6 \text{ M NH}_4\text{H}_2\text{PO}_4$ ($\text{Ca/P} = 1.67$) in teflon lined stainless steel pressure vessel and sealed at 200°C for 24 hours in the electric furnace. The converted HA was washed with boiling water and dried at 110°C . Only completely converted cuttlefish bone into hydroxyapatite were used for further preparation of Si substituted hydroxyapatite. The Si substitution was performed hydrothermal (at 200°C for 24 hours) adding about 15 cm^3 solutions of $\text{Si}(\text{CH}_3\text{COO})_4$ saturated with acetone, or aqueous solution of $\text{Na}_2\text{Si}_3\text{O}_7$, or tetraetoxysilane ($\text{Ca}_6\text{H}_2\text{O}_4\text{Si}$).

The conversion of hydrothermal transformation of the cuttlefish bone and the phase composition of the Si-converted HA samples were studied by: X-ray diffraction analysis (Philips PW 1820 counter diffractometer with $\text{Cu K}\alpha$ radiation) and Fourier transform infrared spectroscopy (FTIR- Bruker Vertex 70). The FTIR analysis was performed by attenuated total reflectance (ATR) spectroscopy for solids with a diamante crystal. Samples were scanned on resolution 4cm^{-1} with 16 scans.

3. RESULTS and DISCUSSION

3.1. X-Ray diffraction analysis

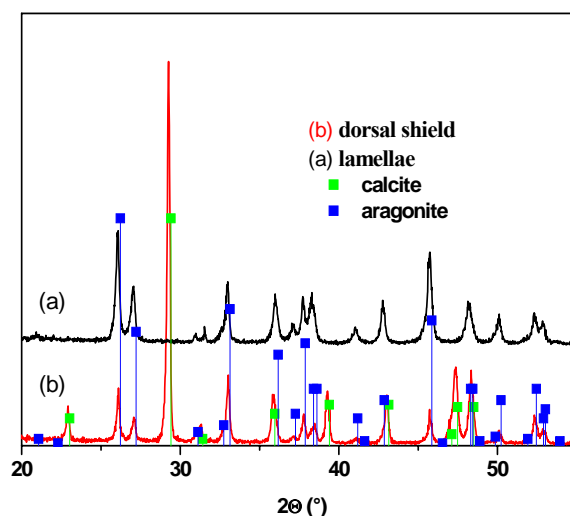


Fig 1. XRD patterns of (a) lamellae and (b) dorsal shield of cuttlefish bone heat-treated at 350°C for 3 hours.

Powder X-ray diffraction analysis (PXRD) of the internal lamellae part of bones have aragonitic structure even after heat treatment at 350°C for 3 hours, as seen on Fig 1, while the external wall (dorsal shield) partially transforms into calcite. Therefore for hydrothermal treatment only peaces of internal cuttlefish bone matrix (lamellae spacing) were used. As can be seen on Fig. 2 aragonite transforms rapidly into HA, and for complete transformation into HA at 200°C 24 hours were needed.

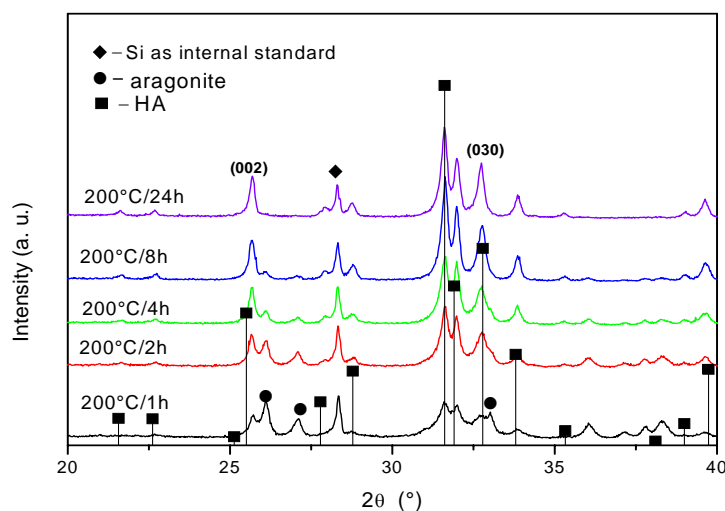


Fig. 2. XRD patterns of hydrothermal treated cuttlefish bone at 200°C at different times. For purpose of clarity the HA lines are not marked

HA with addition of TEOS; or calcium acetate, or sodium silicate were then hydrothermally treated at 200°C during 24 h washed with water and then analyzed by XRD. Si-substituted hydroxyapatite is compared with pure HA in Fig. 3.

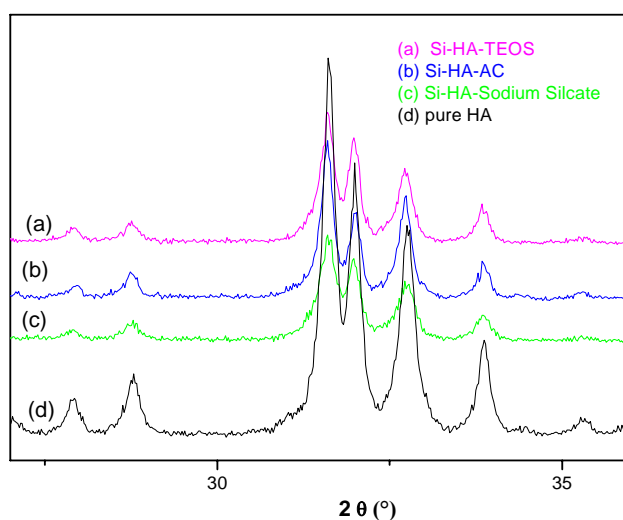


Fig. 3. XRD patterns of hydrothermal transformed Si-substituted HA (a) Si-HA-TEOS, (b) Si-HA-AC, (c) Si-HA-Sodium Silicate and (d) pure HA

The intensity of XRD pattern is the smallest by treatment of pure HA with TEOS as shown in Fig. 3. Unit cell parameters of Si-substituted HA are determined in the sample treated with TEOS using Si as internal standard. Silicon substitution results in a decrease of *a*-axis, and increase in the *c*-axis of the unit cell of hydroxyapatite as seen in Table 1.

Table 1. Lattice parameters of HA and Si-HA treated with TEOS

Sample	<i>a</i> /nm	<i>c</i> /nm
5 wt% Si (added as TEOS)	0.94182(3)	0.68937(3)
Pure HA	0.94259(3)	0.68898(2)

The evidence of incorporation of Si into the HA is obvious by measuring the unit cell parameters and is proposed by Gibson *et al.* [11]:



XRD analysis of patterns calcined at 1200°C (Fig. 4) shows the presence of tricalcium-phosphate as the result of system instability caused by Si-incorporation into hydroxyapatite and changing of Ca/P molar ratio. The samples with different wt% content of silicon were prepared in order to determine the effect of silicon ratio on stability of Si-HA. There is clearly seen that intensity of HA peaks reduce with silicon increase incorporated into Si-HA, and simultaneously increase the amount of TCP formed in the samples. It has to be mentioned that with Si increase also other phosphates occur in the system, most probable monetite, CaHPO_4 ; JCPDF 75-1520, and brushite $\text{CaPO}_3(\text{OH}) \cdot 2\text{H}_2\text{O}$; JCPDF 11-0293.

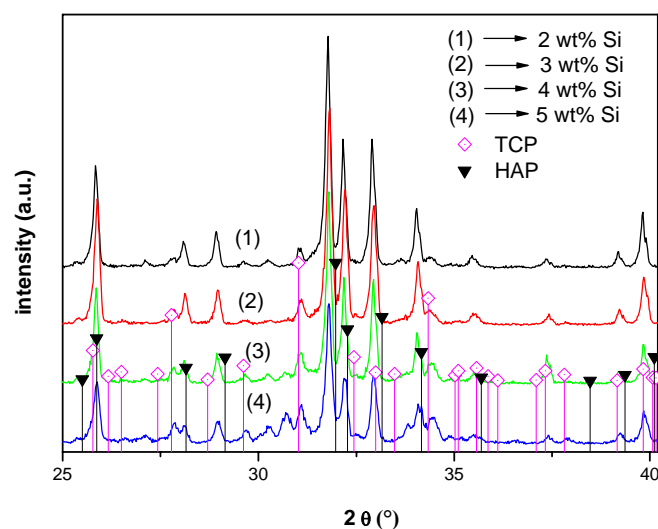


Fig. 4. XRD patterns of Si-HA samples with different wt% of silicon calcined at 1200°C

3.2. FT-IR spectroscopy

FTIR spectra were used to quantify the effect of the silicon substitution on the different functional groups, such as hydroxyl and phosphate groups of hydroxyapatite. Fig. 5. shows that the bands at 3571 and 631 cm^{-1} correspond to the stretching and vibrations modes, of hydroxyl groups, respectively. The doublet at 1471-1413 cm^{-1} is assigned to CO_3^{2-} groups. In the HA spectrum (spectrum (a) in Fig. 5) the intense bands at 1085, 1014 and 960 cm^{-1} correspond to P-O stretching vibration modes, whereas the doublet at 593-572 cm^{-1} corresponds to O-P-O bending mode, whose wavelengths are in accordance with the literature [12]. The additional band at about 872 cm^{-1} can be assigned to the SiO_4^{4-} groups [13]. The decrease in intensity of the peak in the FTIR spectra that corresponds to OH- groups on Si-HA at 630 cm^{-1} was expected since the substitution of phosphate groups for the silicate group leads to some loss of OH^- in order to maintain the charge balance.

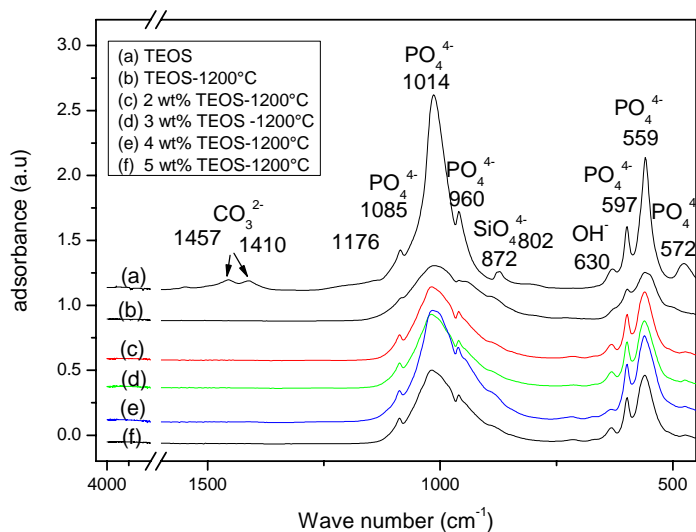


Fig. 5. FTIR spectra of (a) HA, (b) HA-1200°C, (c) 2 wt% TEOS-1200°C (d) 3 wt% TEOS-1200°C, (e) 4 wt% TEOS-1200 and (f) 5 wt% TEOS-1200 °C .

4. CONCLUSION

Hydroxyapatite and silicon substituted hydroxyapatite can be prepared by hydrothermal conversion of aragonite from cuttlefish bone. Incorporation of silicon into hydroxyapatite structure is successful and TEOS appears as the most suitable for in situ reaction of preparation of Si-HA. XRD diffraction and FTIR analysis confirmed substitution of PO_4^{3-} with SiO_4^{4-} groups. Incorporation of silicon results in removal of OH^- groups in order to maintain the charge balance, which causes the changes and defects in structure of HA. The heat-treatment of Si-substituted HA at about 1200°C partially transforms into tricalcium phosphate, however the greater amount of Si incorporation causes the appearance of other calcium phosphates, preferable monetite, CaHPO_4 ; JCPDF 75-1520, and brushite $\text{CaPO}_3(\text{OH}) \cdot 2\text{H}_2\text{O}$; JCPDF 11-0293.

5. ACKNOWLEDGMENTS

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