

Hydroxyapatite from Natural Resources

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Abstract: The improve of well-being and longevity of the population in Europe results in an increasing demand for the development of biocompatible materials with new properties which can be applied as implants for several decades. The one of most used biomaterial worldwide is a hydroxyapatite (HAP). This chemically similar material to the mineral component of bone and hard tissues is one of the bioactive materials, which can react with the tissue and generates good chemical bonding. The different preparation routes of HAP are known. In this study, the environmental friendly and cheap process from natural resources is shown. The examination of biogenic HAP confirmed the submicron sized structure with ~ 100 – 150 nm globular grains. The measurements confirmed the high structural stability of HAP grains.

Keywords: Eggshell, hydroxyapatite, attritor milling, submicron size.

1. INTRODUCTION

Biomaterials used for implant should possess some important properties in order to long-term usage in the body without rejection [1-2]. One of most important properties is a biocompatibility [3]. The biomaterial is „any substance, synthetic or natural in origin, which can be used for any period of time, as a whole a part of a system which treats, augments or replaces any tissue, organ or function of the body” [4]. Biomaterials are used in different parts of the human body as artificial valves in the heart, stenosis in blood vessels, replacement implant in shoulders, knees, hips and orodental structures [5-7]. Bioinert or bioactive materials used as different biomaterials should be made with certain properties. Bioinert refers to a material that retains its structure in the body after implantation and does not include any immunologic host reaction. Bioactive materials could be used for modification of the surface that occurs upon implantation. Bioactive refers to material that direct chemical bonds with bone or even with soft tissue of a living organism.

One of most used bioactive materials is a hydroxyapatite. It is one of the bioactive materials which can react with the tissue and generates good chemical bonding. HAP can be employed in different forms, such as powder, porous blocks or beads to fill bone defect. The HAP thin films presents widespread applications as bioactive surface layers of bone replacements. In literature work synthetic and natural raw materials are also used for HAP preparation.

Environmental issues are taken into consideration when natural materials are employed for HAP production. Australian researchers converted corals to monophasic HAP by the use of hydrothermal method [8]. It was found that the pore structure of coralline apatite is similar to human cancellous bone, making it suitable materials for bone-graft applications. In the other hand, there are well-known preparations of HAP derived from extracted deproteinized human teeth or bovine bones [9]. Eggshells that are rich in calcium and are available in large quantities in nature are gaining more attention and are becoming increasingly important for HAP preparation. The eggshell represents the 11% of the total weight of the egg and is composed by calcium carbonate (94%), calcium phosphate (1%), organic matter (4%) and magnesium carbonate (1%). As can be calculated from the above, thousands tons of eggshell are produced as by-products in poultry-farms or food industry each year. A considerable amount of this is classified as dangerous waste, because of organic contents, and only a small part is reused as fertilizer or nutrients considering the calcium and nitrogen content. Therefore the usage of eggshell as a raw material for biocompatible ceramic is significantly important from environmental and scientific point of views.

2. GENERAL INFORMATION ABOUT HYDROXYAPATITE

HAP has close similarities with inorganic mineral component of bone and teeth [10]. Hydroxyapatite (HAP) is chemically similar to the mineral component of bone and hard tissues. This similarity provides HAP based materials excellent bioactivities like bone bonding capability, osteoconductivity, and biocompatibility. To date, calcium phosphate biomaterials have

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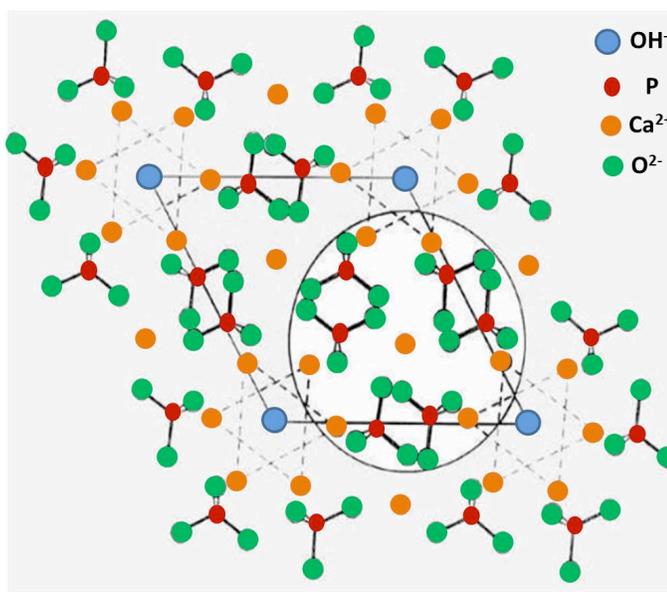


Figure 1: Schematic view of hydroxyapatite (HAp) ($a = b = 9.432 \text{ \AA}$, $c = 6.881 \text{ \AA}$).

been widely used clinically in the form of powders, granules, dense and porous blocks and various composites. Calcium phosphate materials form the main mineral part of calcified tissues. Naturally occurring HAp is hexagonal in structure (Figure 1) with the chemical formula of one unit cell being $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ [11]. The hydroxyl ion (OH^-) or it can be replaced by F^- , Cl^- , CO_3^{2-} , etc in the collagen fiber matrix.

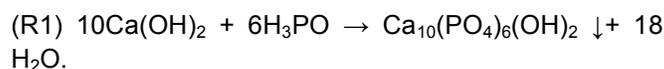
The arrangement of phosphate ions in the crystal structure is such to provide the channel structure, in which calcium atoms are located within two different surroundings [12], Ca (I) and Ca (II). The Ca (I) one is parallel to the c -axis bounding nine oxygen atoms, i.e., 3 atoms in each of the positions O (1), O (2) and O (3). The Ca (II) surroundings contains one O (1), one O (2), four O (3) and one OH^- ion. Ca (II) has a larger atomic radius than Ca (I). The substitution of the carbonate group occurs either in the PO_4^{3-} position (for the B type of apatite), or in the OH^- position (for the A type of apatite) [13, 14].

3. PREPARATION TECHNIQUES

Several methods have been utilized for the synthesis of HAP include precipitation technique [15], sol-gel approach [16], multiple emulsion technique [17], biomimetic deposition technique [18], electrodeposition technique [19].

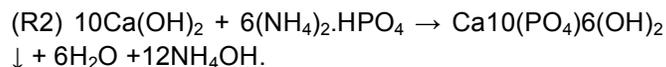
Santos et al. prepared the synthetic HAP by precipitation technique used 3 different routes [15].

Route 1:



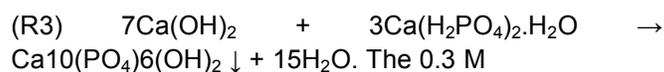
The 0.5 M calcium hydroxide - $\text{Ca}(\text{OH})_2$ - suspension was prepared using $\text{Ca}(\text{OH})_2$ powder. The suspension was degassed, vigorously stirred and heated for one hour at $40 \pm 2 \text{ }^\circ\text{C}$ temperature. The 0.3 M orthophosphoric acid - H_3PO_4 - solution was dropped into the $\text{Ca}(\text{OH})_2$ suspension at same temperature for approximately one hour. The pH was adjusted by addition of 1 M ammonium hydroxide - NH_4OH - solution at the end of the precipitation process.

Route 2:



The 0.3 M ammonium phosphate - $(\text{NH}_4)_2.\text{HPO}_4$ - suspension was dropped into the 0.5 M $\text{Ca}(\text{OH})_2$ suspension prepared as R1 temperature at $40 \pm 2 \text{ }^\circ\text{C}$ for approximately one hour.

Route 3:



$\text{Ca}(\text{OH})_2$ suspension and 0.12 M calcium hydrogen phosphate hydrate - $\text{Ca}(\text{H}_2\text{PO}_4)_2.\text{H}_2\text{O}$ - solution were prepared at room temperature and vigorously stirred for ten min. The $\text{Ca}(\text{H}_2\text{PO}_4)_2.\text{H}_2\text{O}$ solution was added slowly to the $\text{Ca}(\text{OH})_2$ suspension and the mixture was stirred at room temperature for one hour. Secondary

phase CaO was observed on R1 and R2, and HAP + tricalcium phosphate (TCP) was verified on R3. Process parameters during the HA synthesis, such as pH and thermal treatment have indicated strong influence on the Ca/P phases obtained. The crystallinity of the material was largely increased by thermal treatment and directly influenced the appearing of CaO and TCP secondary phases. The amorphous phase remained in lower amount after thermal treatment.

Chai et al. presented a new method of producing hydroxyapatite and mixed calcium phosphate *via* the sol-gel alkoxide route in two meetings [16]. The sol-gel approach is an effective method for the synthesis of nanophasic HAp, due to the possibility of a strict control of process parameters. The sol-gel methodology used in this investigation is similar to the conventional alkoxide method used to produce various oxides and mixed powders. This method involves the dissolution of metal alkoxides in organic solvents and the subsequent combination of these solutions. The coatings were crack free and consisted of two distinct regions: the surface was covered with small grains, approximately 200 nm in size. These smaller grains exhibited a "cauliflower-like" surface, which was broken up by larger grains, approximately 800 nm in diameter. These were observed at random separations across the coating surface and can be identified as CaO. Structural examination revealed the presence of two distinct regions consisting of grains 200 and 800 nm in size, respectively, after being sintered at 1000°C.

Kimura et al. have been used the multiple emulsion to synthesize HAp [17]. An inner aqueous phase was prepared by dissolving dipotassium hydrogen phosphate (K_2HPO_4 , special grade) at 0.3mol/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/m³ to prepare an oil phase. Calcium nitrate tetrahydrate ($Ca(NO_3)_2 \cdot 4H_2O$, special grade) and polyoxyethylene (20) sorbitan monolaurate as suspension stabilizer for the oil phase were dissolved in water at 0.5 mol/kg and 10mol/m³ to prepare an outer aqueous phase. The inner aqueous phase of 40.5 cm³ and the oil phase of 94.5 cm³ were mixed and ultrasonicated for 30 minutes to prepare a W/O emulsion. This emulsion was poured into the outer aqueous phase of 315 cm³ 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 products were HAP microspheres agglomerated. Each microsphere was constituted by a few hundred nanometer-sized porous spheres, composed of primary nanoparticles.

Tas et al. prepared the HAp by biomimetic deposition technique [18]. Metastable synthetic body fluids (modified SBF), with an inorganic salt composition similar to that of human blood plasma, incubate and facilitate the spontaneous nucleation and growth of a nanosized, carbonated and "bone-like" calcium HA at physiological pH and temperature. The procedural steps for the preparation of hydroxyapatite in SBF involve dissolution of $Ca(NO_3)_2 \cdot 4H_2O$ and $(NH_4)_2HPO_4$ in SBF solution contained in separate beakers at the start of precipitation experiments. As soon as both reagents get dissolved in SBF, immediate formation of fine precipitates took place as indicated by the slight turbidity of the solution. The HAP samples were uniaxially compacted and calcined for 2 hours at different temperatures (560, 750 and 850°C). The powder calcined at 750°C was then sintered for 2 hours at different temperatures (1000, 1100 and 1200°C). The as prepared HAP powder contains broad peaks of HAP with crystallite size of 25.49 nm.

Shikhanzadeh et al. used the electrodeposition technique for HAP preparation [19]. The electrolytes used for electrodeposition of calcium phosphate coatings were prepared by dissolving reagent-grade $Ca(NO_3)_2$ and $NH_4 \cdot H_2PO_4$ in deionized water. $NaNO_3$ was also added to improve the ionic strength of the electrolytes. Electrodeposition of calcium phosphates was carried out for 2h at 85°C in a conventional cell fitted with saturated calomel electrode. Pure titanium plates pre-etched in hydrofluoric acid were used as the substrate for deposition of calcium phosphates. CO_2 -free nitrogen was continuously sparged into the electrolyte during electrodeposition process to minimize the risk of contamination of the deposits with carbonates. Ultrafine-grained, nanophase coatings of hydroxyapatite were synthesized by electrocrystallization from dilute electrolytes ($[Ca]=6.1 \times 10^{-4}$ M, $[phosphate]=3.6 \times 10^{-4}$ M) at pH values comparable with the biological pH. At these comparatively low supersaturations, hydroxyapatite is shown to be precipitated without the formation of a precursor phase. A description of the sequence of events occurring at the electrode-electrolyte interface is given to explain the mechanism involved in the direct formation of nanophase hydroxyapatite on polarized electrodes.

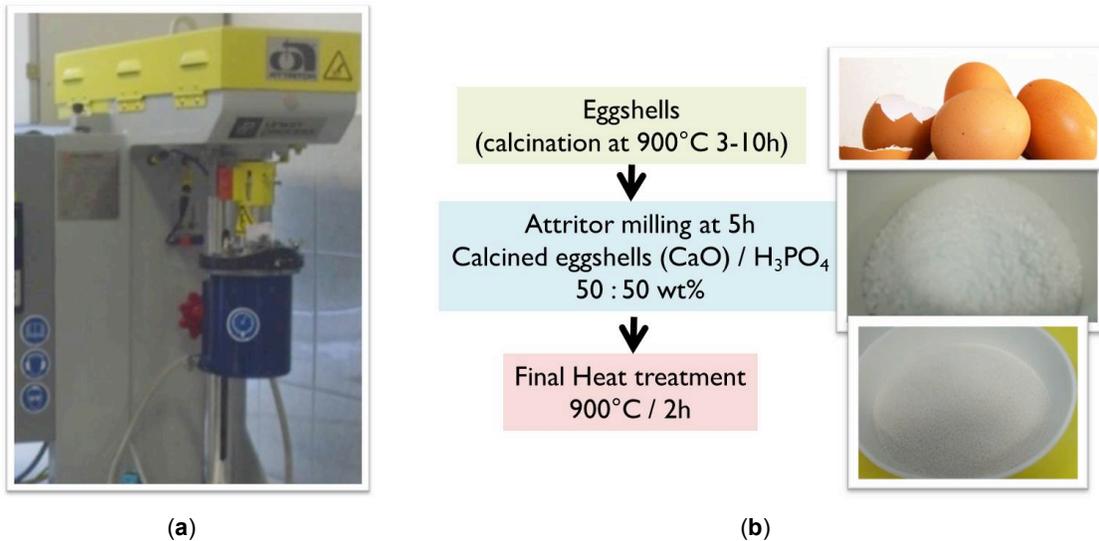


Figure 2: Preparation process, (a) attritor mill, (b) preparation steps.

In this work, the HAP powder was prepared by attritor milling using an eggshell and phosphoric acid [20].

4. POWDER METALLURGY PROGRESS (PMP) AS HIGH EFFICIENT METHOD FOR HAP PREPARATION

The principle of attritor grind processing is achieved by an expanded moving bed of media. This condition is described as kinematic porosity. The particles are subjected to various forces such as impact, rotational, tumbling, and shear; therefore, micron/nano range fine powders can be easily achieved. Additionally, combinations of these forces create a more spherical particle than other impact-type milling equipment [21].

Eggshells were collected and washed with detergent, then calcined in air at 900°C for 10 h.

Calcined shells were crushed, reacted with phosphoric acid (H₃PO₄) in an exothermic reaction and milled in an attritor mill (Union Process, Figure 2) with zirconia tanks and Ø2 mm zirconia balls during 5 h at 4000 rpm to achieve homogenous mixtures and to prevent agglomeration. From our previous tests, 50: 50 wt% ratio of shell: H₃PO₄ was found as optimal [20, 22]. Milled HAP powder was heated at 900°C for 2 h in air.

Structural investigations of powder were studied by transmission electron microscope (TEM, Philips CM-20). The elemental composition was measured by JSM-25 S-III SEM using Bruker Si(Li) EDS detector and Quantax system using area scan mode for averaging.

Bimodal submicron structure was observed after 5h intensive attrition milling (Figure 3). The existence of two main phases was shown; hydroxyapatite

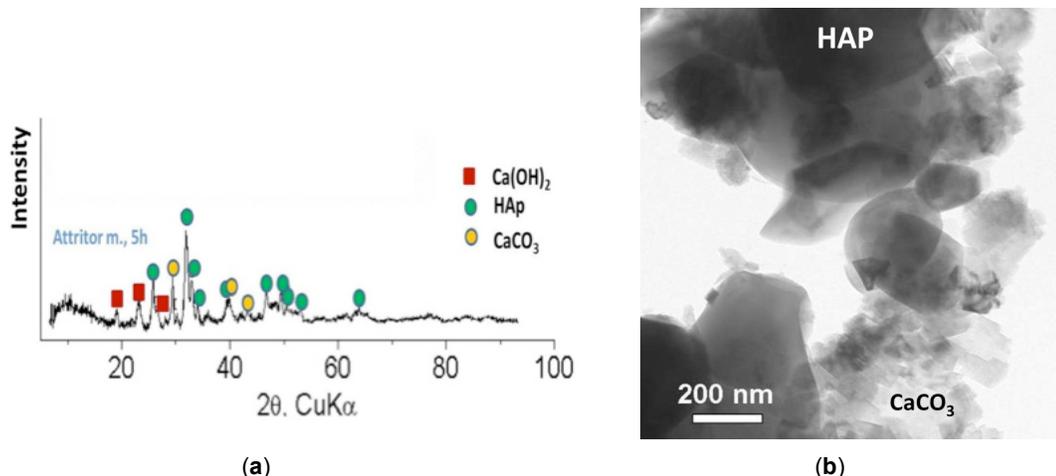


Figure 3: Structural investigation of HAP after attritor milling. (a) XRD measurements, (b) TEM investigations.

(JCPDFSPDF 74-0565) and calcite (CaCO_3 , JCPDFSPDF 05-0586). Milled sample consisted of HAP grains with average size 400–500 nm and few nanometer sized CaCO_3 phase.

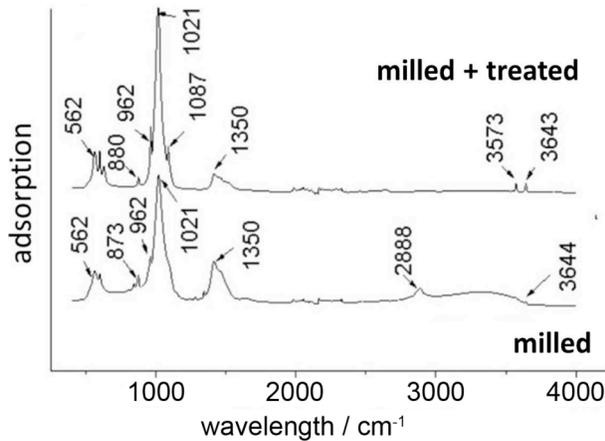


Figure 4: FITR investigation of HAP after milling and treatment.

The Fourier Transformed Infrared Spectroscopy (FITR) spectrum (Figure 4) indicated mainly the typical PO_4 bands of poorly crystalline apatite (triply degenerate ν_3 PO_4 asymmetric stretch at 1021 and 1087 cm^{-1} (shoulder), non-degenerate symmetric stretch of ν_1 PO_4 at 962 cm^{-1} , and components of the ν_4 PO_4 bending triplet at 599 and 562 cm^{-1} [16,19]. Carbonate bands are observed at 1550-1350 cm^{-1} (ν_3), 873 cm^{-1} (ν_2) and 712 cm^{-1} (ν_4). By analogy with bone mineral. 1456, 1415 and 872 cm^{-1} showed the presence of carbonated apatite with B-type substitution. The broad, weak 3000-3400 cm^{-1} band can be attributed to traces of water, confirmed by the very weak, broad H-O-H bend around 1640 cm^{-1} . νOH and surface OH at 3644 cm^{-1} appear in the OH stretching vibration region both hydroxyapatite. The surface OH band also increases with increasing temperature. Hydroxyapatite ν OH and surface OH, probably connected to CaO, are present which is good agreement with other structural investigations.

The elemental composition of HAP was measured by Spectro Genesis ICP-OES spectrometer (Table 1). It is a fast, automatic and simultaneous optical

emission spectrometer equipped CCD detector system. Due to the technical configuration of the instrument 20-30 or more elements (metals and some non- metals such as phosphorus and sulphur) can be measured at the same time in minutes from 3-4 ml fluid samples in concentration of mg/l – $\mu\text{g/l}$. Very low detection limits can be achieved by using together of axial plasma viewing and a special optical-plasma-interface (OPI) system, besides matrix effects is reduced dramatically. The plasma itself is extremely stable due to its broad bandwidth and special operating frequency. These features can compensate fluctuations caused by volatile organic compounds.

The main elements are O, P, Ca and Mg (Table 1). The minor elements are 0.05-0.08 wt% Na, 0.06-0.1 wt% Si, 0.1 wt% S, 0.01-0.03 wt% Cl, 0.06 wt% Zn and 0.14-0.16 wt% Zr. These elements are the remains of preparation process. Mg is one of the main substitutes for calcium in biological apatites and has been known as one of the cationic substitutes for calcium in the HAP lattice [23,24]. Enamel, dentin and bone contain, respectively, 0.44, 1.23, and 0.72wt% of Mg [12]. Biomaterials with high Mg concentration showed excellent biocompatibility and biological properties [25]. The high concentration of Mg in HAP has an effect on increasing of HPO_4^{2-} incorporation, decreasing the crystallinity of particles [24].

The important data is a magnesium trace element in milled and treated HAP powders (0.45-0.47 wt%). The incorporation of Mg in synthetic HAp is limited (maximum of about 0.4wt% of Mg) unless other ions, such as carbonate or fluoride are simultaneously incorporated together with magnesium as paired substitutions [24]. The Ca / P ratio is higher after heat treatment at 900°C during 2.06 compared with only milled HAP powder 1.98.

CONCLUSION

Biomaterials used for implant should possess some important properties in order to long-term usage in the body without rejection. The creation of nanocomposites of ceramic materials with particle size few ten

Table 1: ICP Measurements of HAP after Milling and after Heat Treatment

Sample	O	Na	Mg	Si	P	S	Cl	Ca	Zn	Zr	Ca/P
	wt %										
Attritor milling (5h)	42.05	0.08	0.45	0.06	16.01	0.1	0.03	41.01	0.06	0.16	1.98
Attritor milling (5h) and heat treatment	31.76	0.05	0.47	0.1	18.34	0.1	0.01	48.98	0.06	0.14	2.06

nanometers can significantly improve the bioactivity of the implant and enhance the osteoblast adhesion.

The detailed study of the submicron sized hydroxyapatite prepared by high efficient attritor milling from biogenic raw materials – eggshells – was showed. The intensive milling of eggshells in phosphoric acid and next heat treatment were sufficient for hydroxyapatite powder preparation. It is a cheap and environmental friendly technology for biomaterial preparation with compare to other synthetic technology.

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