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Low-cost Processing Technology for the Synthesis of a Biocomposite for Biomedical Applications: a Preliminary Study

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Abstract

Calcium-phosphate biomaterials, which have a composition similar to bone mineral, represent a potentially interesting synthetic bone graft substitute. In the present study, a biocomposite of calcium phosphate (CaP) and collagen (Col.) was developed; it's about a triphasic Hydroxyapatite/β-Tricalcium phosphate/Collagen (HAp/β-TCP/Col.). Both collagen and calcium phosphate were prepared, for the first time, from the same fish part; Moroccan Sardine scales (Sardina Pilchardus). Calcium phosphate was isolated by calcination at 1000°C and Collagen by extraction with an acid solution. Resulting materials were characterized using several analytical tools, including chemical analysis, Fourier transform infrared spectroscopy (FT-IR) and X-ray diffraction analysis (XRD). Due to its properties, the obtained composite appears to be potential biomaterials for rebuilding small lesionsin maxillofacial surgery, dentistry, aesthetic surgery and particularly in orthopedic surgery.

1. Introduction

It is well known that calcified tissues, bones and teeth, are mainly composed of apatitic calcium phosphate (the bone mineral) and collagen (a connective tissue protein) (Figure 1, Table 1) [1,2]. Calcium phosphate apatite gives bones their rigidity and collagen improves fracture resistance (Table 2) [3]. These calcified tissues which constitute the framework of the human body can undergo fractures or losses of substances, induced by traumas or certain pathologies, thus altering one or more functions of the bone tissue [4].To address these problems, various bone replacement biomaterials have been developed [5-13].

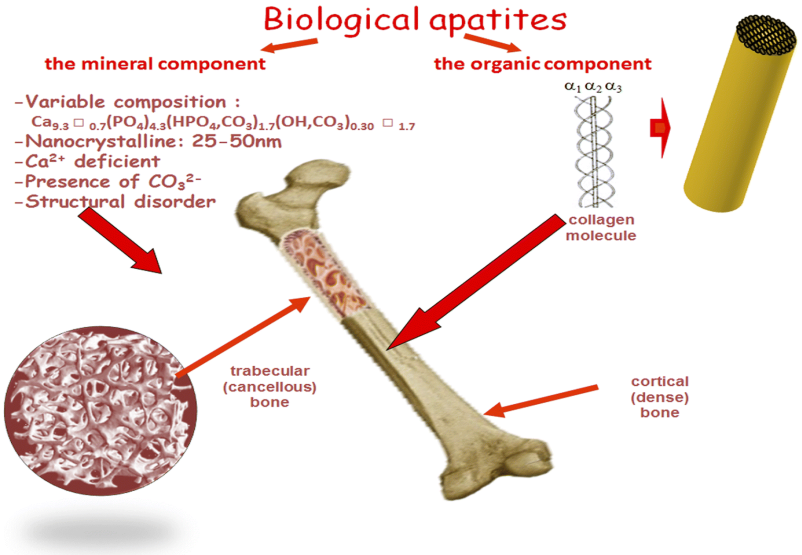

Figure 1: Inorganic–organic composite nature of trabecular and cortical bone [1].

Table 1: Biochemical composition of bones [2].

Inorganic phases	wt. %	Bioorganic phases	wt. %
calcium orthophosphates (biological apatite)	≈ 60	collagen type I	≈ 20
water	≈ 9	non-collagenous proteins: osteocalcin, osteonectin, osteopontin, thrombospondin, morphogenetic	
carbonates	≈ 4	proteins, sialoprotein, serum proteins	≈ 3
citrates	≈ 0.9	other traces: polysaccharides, lipids, cytokines	
sodium	≈ 0.7	primary bone cells: osteoblasts, osteocytes,	balance
magnesium	≈ 0.5	osteoclasts	balance
other traces: Cl^-, F^-, K^+,Sr^{2+}, Pb^{2+}, ZN^{2+}, Cu^{2+}, Fe^{2+}	balance		

The composition is varied from species to species and from bone to bone.

Table 2: General respective properties from the bioorganic and inorganic domains, to be combined in various composites and hybrid materials [3].

Inorganic	Bioorganic
hardness, brittleness	elasticity, plasticity
high density	low density
thermal stability	permeability
hydrophilicity	hydrophobicity
high refractive index	selective complexation
mixed valence slate (redoc-ox)	chemical reactivity
strength	bioactivity

Among these materials, biocomposites based on apatitic calcium phosphate and collagen possess biological compatibility properties with living tissues since they have physicochemical properties close to those of the bone [4].Amidapatitic calcium phosphates(Table 3) [14], hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$, HAp) is probably the most used in this field due to its excellent biocompatibility and bioactivity properties [15]. It is a synthetic biomaterial similar to calcium phosphate present as the inorganic components of calcified tissues. The tri-calcium phosphate ($Ca_3(PO_4)_2$,TCP) is, also, employed in this field [10,14]. It can exist in two possible forms, α and β, and normally β form gets converted into α with annealing at temperatures higher than 1250–1300°C [16]. For many biomaterial applications, a mixture of HAp and TCP is often considered; this is because TCP has better resorbability than HAp, despite being less biocompatible. It is believed to enhance the osteoconductivity and osteogenesis of the bones [17].The use of composites based on calcium phosphate apatites and collagen is one favorable approach to mimic the extracellular matrix of bone tissues [2,3,18-20]. The growing demand for these materials in biomedical and non-medical applications requires their syntheses in a reproducible manner.

Table 3: Important calcium phosphate compounds with their Ca/P ratios [14].

N°	Compound	Formula	Ellipsis	Ca/P ratio	Application
1	Monocalcium phosphate monohydrate	$Ca(H_2PO_4)_2,H_2O$	MCPM	0.5	Increase root fluoride uptake
2	Monocalcium phosphate (anhydrous)	$Ca(H_2PO_4)_2$	MCPA	0.5	Artificial bone graft
3	Dicalcium phosphate anhydrous	$CaHPO_4$	DCPA	1	Polishing agent for teeth, source of
4	Dicalcium phosphate dihydrate	$CaHPO_4,2H_2O$	DCPD	1	Ca and P in food supplements
5	α-Tricalcium phosphate	α-$Ca_3(PO_4)_2$	α-TCP	1.5	Sustained release of highly water-soluble drugs
6	β- Tricalcium phosphate	β-$Ca_3(PO_4)_2$	β-TCP	1.5	Biodegradable composite for bone repair
7	Calcium-deficient hydroxyapatite	$Ca_{10-x}(HPO_4)_x(PO_4)_{6-x}(OH)_{2-x}$	CDHA	1.5-1.6	Orthopedic surgery Bone grafting
8	Hydroxyapatite	$Ca_{10}(PO_4)_6(OH)_2$	HAp	1.67	Repairing of hard tissues
9	Fluorapatite	$Ca_{10}(PO_4)_6F_2$	FAp	1.67	Used as source of fluorine in pharmaceutical products
10	Tetracalcium phosphate	$Ca_4(PO_4)_2O$	TTCP	2	Applied as cements and coating on metallic implants

However, HAp, other calcium phosphate materials and collagen can also be prepared from natural sources and/or wastes and by-products [21-26]. The use of agri-food by-products, in particular, has attracted more and

more interest in recent years. This is due to the production of increasing amounts of waste and/or by-products, which have to be disposed of with environmental impact. Extracting compounds which have high value is, therefore, a way of addressing this problem while valorizing such wastes. In this work, biocomposite based on HAp/β-TCP/Col used in biomedicine was extracted, for the first time, from scales of Moroccan sardines (Sardina pilchardus). In fact, fish scales are mainly composed of collagen, water and the remaining 41% to 84% of other proteins [27]. Too, during evolution, scale formation process shows the same mechanism as in the formation of teeth and bone. Indeed, there are several types of calcium phosphate salts, which are present in fish scales due to their extreme biological response in physiological environment. The CaP powder was extracted by calcining the sardine scales at high-temperature and collagen solid was extracted by acid solution from the same scales.

2. Experimental details

In order to convert sardine scales into CaP apatite, these scales are washed with hot water to remove all types of proteins and other organic impurities. Then, after washing with distilled water to remove sodium chloride, and drying overnight at 40°C, scales are subject to high-temperature calcination at 1000°C overnight in air.
In order to extract collagen from washed fish scales, these scales go through extraction of collagen using acid acetic (0.5M) followed by filtration then precipitation with sodium chloride (0.2M). The obtained precipitate were washed by distilled water and dried by lyophilization to produce a solid product.
The composite material was fabricated by mixing and grinding the two obtained organic and inorganic materials using the CaP/Col ratio of 80/20 (% in weight). All obtained materials were characterized by chemical analysis, X-ray diffraction and Infrared spectroscopy (IR). Calcium and phosphorus contents were determined by wet chemical methods:
Calcium was titrated by complexometry [28]. The error on the calcium content is around 0.5%.
Phosphorus content was analyzed by colorimetry [29]. The accuracy of this dosage was determined with a relative error of 0.5%. X-ray diffraction analysis was carried out by means of a SEIFERT XRD 3000 P using CuK radiation. For infrared absorption analysis, 1 mg of the powered samples was carefully mixed with 300 mg of KBr and palletized under vacuum. The pallets were analyzed using a Perkin Elmer 1600 FTIR spectrophotometer.

3. Results and Discussion

Figure 2 shows the XRD pattern of sardine scales calcined at 1000°C. It can be seen that a second phase as well as HAp is detected in this sample, which peaks corresponding to β-TCP are marked with a β in this Figure. Thus, the scales formed a mixture of hydroxyapatite and β-tri-calcium phosphate, with a higher content of β-TCP. This bi-phasic material has a high added value, as it is employed as a bioceramic. The presence of this compound was previously reported in CaP samples of marine origin [30,31].

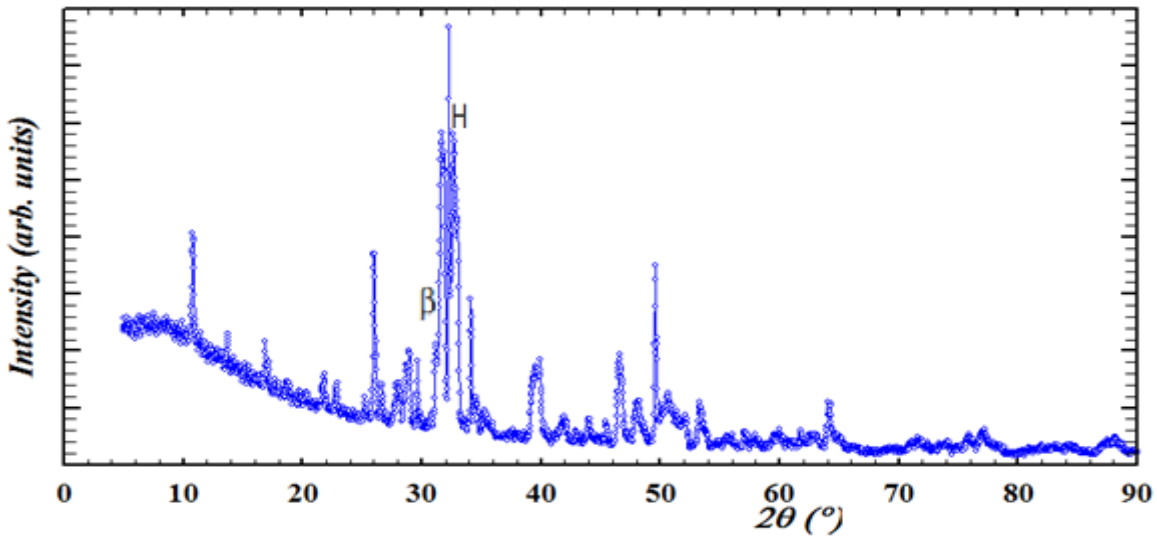

Figure 2: XRD pattern for sardine scales calcined at 1000°C.

The letter H indicates peak belonging to HAP.The letter β indicates peak belonging to β-TCP.

Table 4: Phase composition (wt%) of sardine scales calcined at 1000 °C.

HAP	β-TCP
54.2	45.8

Table 5: Concentration (wt%) on Calcium and Phosphorus for sardine scales calcined at 1000 °C.

Ca	P	Ca/P
31.8	20	1.59

Table 4 reports the percentage weight composition, calculated from the XRD patterns, with a higher content of β-TCP. Scales calcined at 1000°C were analyzed to determine their elemental composition on Ca and P, to have a more complete picture of this material presented in Table 5. It can be seen that the molar ratio between calcium and phosphorus is 1.59; this value is betweenthe stoichiometric ratio of Hap (1.67) and ratio of β-TCP (1.5).Infrared spectra of calcined scales is shown in Figure 3. Signals due to phosphate ions (PO_4^{3-}, P-O and O-P-O) from both HAp and β-TCP were detected; it is possible to see peaks in the region of (600-1100 cm^{-1}) (HAp) and at 1122 cm^{-1} (β-TCP).

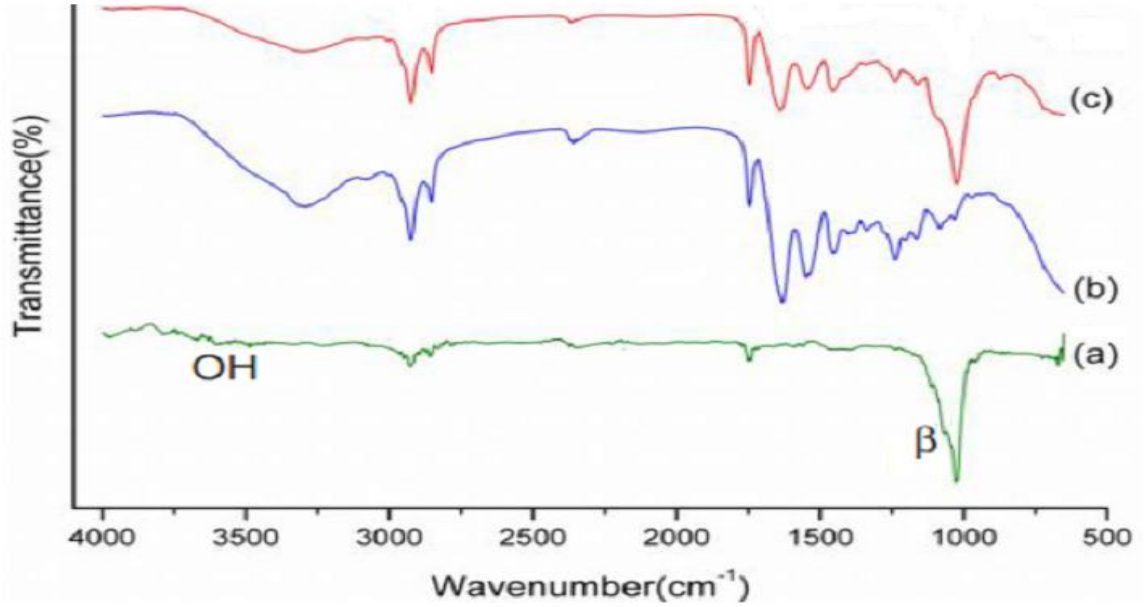

Figure 3: FTIR curves of obtained sardine scales materials:
a) HAp/β-TCP, b)Col, c) HAp/β-TCP/Col composite.

Figure 4 shows the X-ray diffraction pattern of solid product obtained by acid extraction from sardine scales that have a small end, high and sharp diffraction peak, indicating that the crystal is good in contrast to pure collagen which is amorphous [32]. This can be attributed to the presence of calcium salt residue which was not decalcified before extraction step. This biphasic material based on collagen/calcium salts has a wide range of applications especially inthe food, cosmetic, pharmaceutical and biomedical fields.
Figure 3 shows, also, Infrared spectrum of the solid product obtained by acid extraction from sardine scales. The distinctive peaks of collagen were observed, such as 1419 cm^{-1} for the COO^- group, 1640 and 1740 cm^{-1} for the C=O group, 2850 and 2930 cm^{-1} for C-H liaison, 3230 cm^{-1} for NH_2 group and 3200-3500 cm^{-1} for O-H intermolecular bonding. Changes to peaks are due to interaction between calcium salts and amino nitrogen group in the collagen.
Finally,the molecular structure of fabricated tri-phasic composite, HAp/β-TCP/Col, was analyzed by FTIR (Figure 3) and the absorption bands could be characterized as corresponding to bi-phasic HAp/β-TCP and collagen.

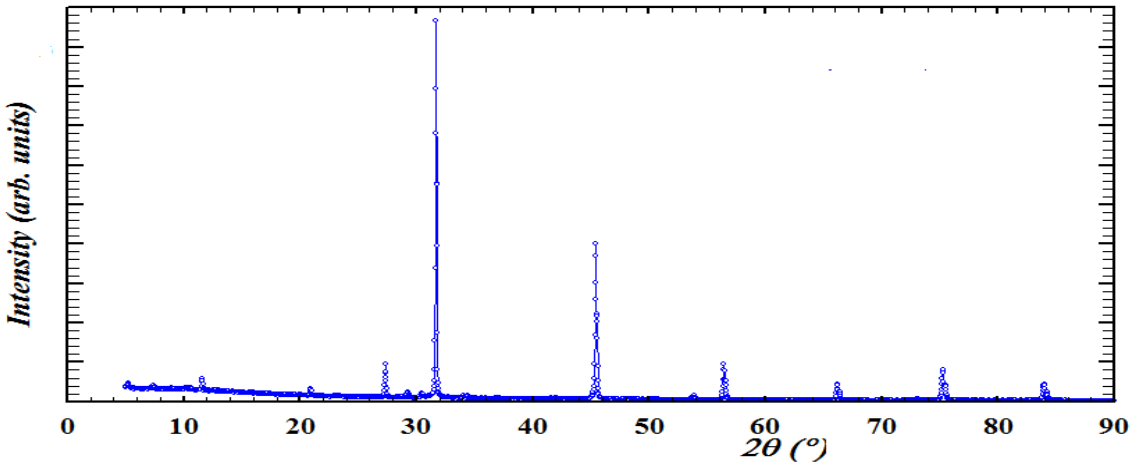

Figure 4: XRD pattern for extracted Collagen from sardine scales.

Conclusion

Sardine scales were successfully used to extract a tri-phasic apatitic calcium phosphate (Hap/βTCP) and collagen/calcium salts; thus one part of the sardine was used to extract a biocomposite presenting high potential as biomaterial. This study shows how it is possible to valorize by-products of the food industries, obtaining high added value products which can be used in biomedicine and other potential applications.

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