

BIOMATERIALS DERIVED FROM TAMBAN, *Sardinella fimbriata* BONES AS PROMISING ANODYNE SUNSCREEN

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Abstract: Development in the fish processing industry has led to a large amount of waste including, fish head, scales, bones and guts, every year around the world. Certain fish bones have an attractive added value which contains hydroxyapatite (HAP), $(Ca_{10}(PO_4)_6(OH)_2)$ that can potentially be used as UV absorbance (unmodified bones). *Sardinella fimbriata* bone was synthesized and utilized as sunscreen in cosmeceuticals with the addition of manganese and ferum, new hybrid sunscreen materials have been initiated via hydroxyapatite-Fe and hydroxyapatite-Mn doped (modified bones). The modified bones have been characterized using Nuclear Magnetic Resonance (NMR) spectroscopy and X-Ray Diffraction (XRD) and UV-Visible spectroscopy. The results clearly indicated that the HAP existing in the waste of *S. fimbriata* bone exhibits high potential as sunscreen with addition of $FeCl_2$ compared to manganese and unmodified Tamban bones. Thus, the utilization of waste from the fish bone not only produce value-added products from low-cost resources, but also help in reducing pollution to the environment and preserve the global sustainability.

Keywords: Crystallite, fish-waste, hybrid sunscreen, hydroxyapatite, *Sardinella fimbriata*, utilization

Introduction

Sunscreen has become a necessary products among human to protect from hazardous ultraviolet (UV) due to the hot and extremely weather. It is known that longer UV exposure can cause sunburns and severe effects (Piccirillo *et al.*, 2014). Sunscreen can be defined as substance that protect the skin from the deleterious UV-light and avoiding or minimizing the damage that radiation may cause from radiation on human health (Salvador & Chisvert, 2005). The UV light composed of three regions which are UVA (320-400 nm) UVB (290-320 nm) and UVC (200-290 nm).

However, UVA and UVB can pose a threat to human according to Piccirillo *et al.* (2014). If the skin repeated exposed, skin aging and skin cancer could caused damage to human DNA and RNA (Chisvert & Salvador, 2007).

The use of sunscreens as protective barriers against DNA damage and illness by absorbing harmful UV ray is becoming important. However, there have been huge concerns on commercial sunscreen regarding potential toxicity of the materials ingredients and adverse environmental disturbances of marine organisms such as coral reefs. Studies have found that the damage of exquisite

coral bleaching was because of oxybenzene substances that are used in commercial sunscreen (Welch, 2015). Nano-sized titanium dioxide (TiO_2) and zinc oxide (ZnO) are widely used as inorganic sunscreen pigments due to their greater shielding (Lewicka & Colvin, 2012) to absorb ultraviolet UV radiation. Concerns have emerged on the safety and efficacy of synthetic sunscreen that they can react with UV rays from the sun, resulting in free radical production. Free radicals have the potentiality to damage the DNA found in cells and contribute to the development of skin cancer (Burnett & Wang, 2011).

High fish demand from worldwide has contributed uncontrolled development on fish industry. Large amounts of fish skin, scales, and bones produced every year led to a huge waste around the world (Wang *et al.*, 2013). Fascinatingly, fish skin, scales and bones can be recycled because they are enriched with collagen and other possible nutrients. Fish waste after filleting can be as much as 75% of the raw materials, with the amount of fish by-products produced contributes almost 36% of the total weight of the fish. This waste is usually processed into low value products such as fish meal and natural fertilizers but more commonly being discarded into the sea. Such problems may affect the areas of environmental protection and its sustainability (Russ & Pittroff, 2004). Fish skins and bones are a major by-product of the fisheries and aquaculture industries (Mohtar *et al.*, 2010). The use of fishbone as a material in bone therapy and development of artificial bones is very reassuring (Tutoru, 2012). Furthermore, fish-bones can also be utilized as a bio-sorbent where it was efficiently absorbed heavy metals in the water (Huang, 2007).

Sunscreen agents for instance TiO_2 and ZnO can potentially be replaced with HAP non toxicity calcium phosphate sources from human and animal bones that effectively absorb the UV region (Araujo *et al.*, 2010; Piccirillo *et*

al., 2014). HAP is commonly used as a filler or coating on damaged bone and implants in order to promote bone in-growth bone especially in orthopedic, dental, maxillofacial and biomedical applications (Ferraro *et al.*, 2013) and cosmetics (Wang *et al.*, 2013). Bones known as white colour, brittle properties and cheaper source that provided abundance of HAP that can turn into good carriers in sunscreen (Springsteen *et al.*, 1999). It also provides a promising candidate for advanced sunscreen. Furthermore, the UV absorption limit can be tuned to absorb in the desired range from UVB to UVA by simply introducing a doping (Piccirillo *et al.*, 2014).

Iron (Fe) and manganese (Mn) can widen UV range absorption in comparison to TiO_2 and ZnO (Araujo *et al.*, 2010). Therefore, by doping fish bone with proper element can initiate a huge potential of anodyne sunscreen agent as novel hybrid biomaterials. It was not only promising candidate for sunscreen, but also helping the local fishing industry especially in Terengganu (East Coast Peninsular Malaysia) to manage bone waste disposal more wisely, improve their economic status and also preserve the environment.

In this paper, the synthesis and characterization of modified HAP *Sardinella fimbriata* with Fe and Mn as anodyne UV filter were discussed.

Materials and Methods

Sample Collection and Storage

Fresh Tamban fish, *Sardinella fimbriata* were collected from Kampung Mangkuk, Setiu, Terengganu, Peninsular Malaysia, Malaysia. The samples were boiled for 8 hours before drained and dried. The fish was cut in half vertically, separated and dry bones were collected. Bones were washed and kept under -20°C in chiller defrosted and dried overnight at 50°C in oven before synthesized (unmodified bones).

Synthesization of Bones

S. fimbriata bones were synthesized with (FeCl₂) (98%, Sigma-Aldrich) and manganese chloride (MnCl₂) (98%, Sigma-Aldrich) at 70°C respectively. 1.0 M of Ammonium hydroxide (NH₄OH) (98%, Sigma-Aldrich) was dropped into solution contained of FeCl₂-bones and MnCl₂-bones to reduce the acidity of solution which initially at pH 2.5 until 3. The bones were dried overnight at 50°C in oven and then were calcined at 900°C for three hours in a furnace. The calcined bones were grinded with high-energy ball mill for 15 minutes at 450 rpm to yield powder form (unmodified bones). For the Mn²⁺ and Fe³⁺ -doped samples, a 0.1 M solution managnous nitrate, Mn(NO₃)₂.6H₂O and ferrous nitrate, Fe(NO₃)₂.9H₂O, respectively, were added unmodified bones.

Characterization of Bones

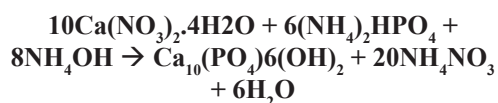
¹H NMR spectrum was recorded on a 400 MHz spectrometer with CDCl₃ as a solvent. To signify the phase composition in compound, X-Ray Diffraction (XRD) was performed using Rigaku MiniFlex II diffractometer with Cu Kα to signify the phase composition in the compound. Samples were analyzed with a step of 0.02°, over 2θ and between the range of 10° and 80°. By comparing the experimental XRD patterns to standard compiled by the Joint Committee on Powder Diffraction and Standards (JCPDS), crystallographic identification of the phase was achieved with PDF number 74-566 (HAP), 45-553 (Ca₉FeH(PO₄)₇), 46-905 (Ca₉HPO₄(PO₄)₅OH), 79-7 (Fe₂O₃), 84-309 (Fe₂O₃), 23-402 (Mn₅(PO₄)₃Cl), 24-214 (Ca₅(PO₄)₃Cl), and 73-1728 (Ca₅(PO₄)₃Cl).

The precipitated HAP powders were physically and chemically characterized using powder X-ray diffraction (XRD) and UV-Vis diffuse reflectance. The powders were selected between 75 mm/μm and 150 mm/μm and drizzled for characterization of the samples.

Powder XRD measurements were performed using CuKα radiation at 40 kV/40 mA with a monochromator detector and spinning sample holder at room temperature. The UV-Vis diffuse reflectance spectrum was measured using UV-Vis recording spectrophotometer (UV-2401, Shimadzu). Barium sulphate was utilized as reference to calibrate the spectrophotometer.

Results and Discussion

HAP was produced using a precipitation route of calcium nitrate, Ca(NO₃)₂.4H₂O from fish bone of *S. fimbriata* with ammonium phosphate, (NH₄)₂HPO₄. The HAP reaction formation is expressed as follows:



The formation of single proton in HAP (δ_H = 1.54) obtained from unmodified *S. fimbriata* was confirmed by ¹H spectroscopy (Figure 1). The effect of dopants on the phase identification of the modified bones, were characterized. Figure 2 represent the typical XRD patterns of the pure HAP and Fe³⁺-doped and Mn²⁺ -doped HAP samples, which perfectly match with the JCPDS pattern #74-566 for HAP. The incorporation of Mn²⁺ and Fe³⁺ into the material apparently leads to the formation of less crystalline HAP. This effect seems natural as the dopants assumed to substitute at Ca²⁺ sites (ionic radius 0.99 Å) have larger charge and smaller ionic radius (Mn²⁺ 0.63 Å, Fe³⁺ 0.64 Å). Several ions, usually with an ionic radius smaller than that of Ca²⁺ are known to inhibit the formation of HAP (Miyaji et al., 2005).

Figure 2 (a) showed the patterns and phase composition of fish bone treated at 900°C. The HAP pictograph showed that 54% of bone contained Hydroxylapatite, Ca₁₀(PO₄)₆(OH)₂ as the main component.

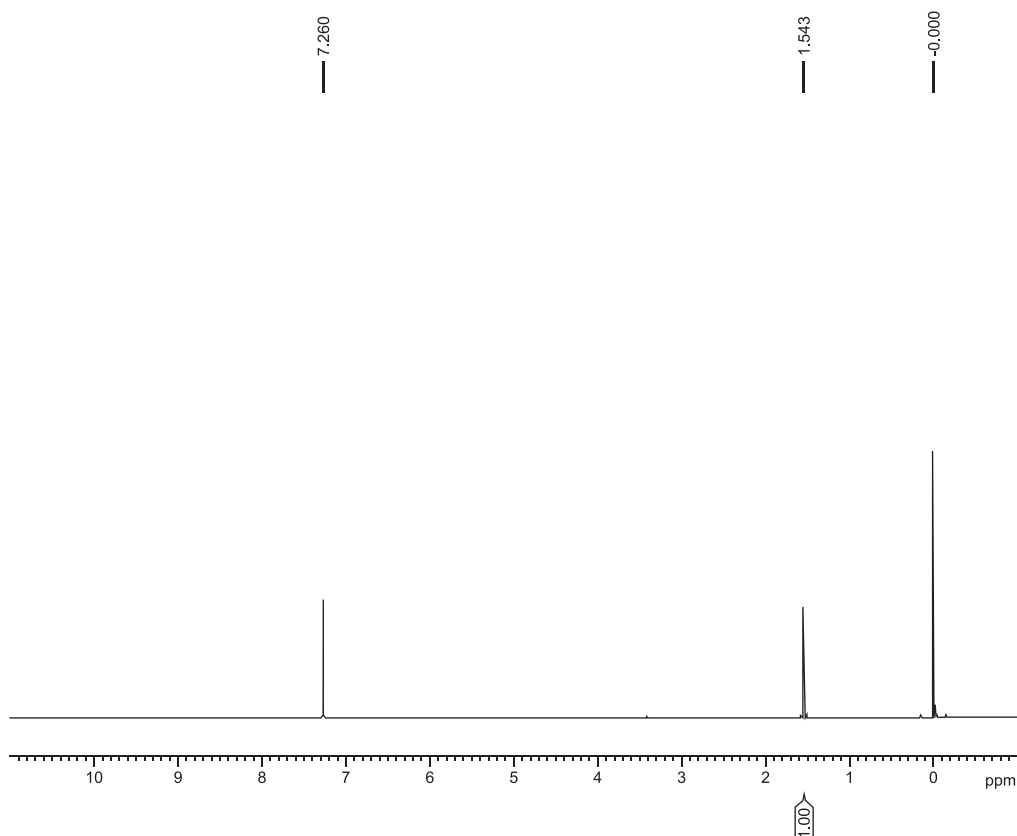


Figure 1: ^1H NMR (400 MHz, CDCl_3) of unmodified HAP derived from *Sardinella fimbriata* fish bone

Figure 2 (b) showed the calcium hydrogen phosphate hydroxide, $\text{Ca}_9\text{HPO}_4(\text{PO}_4)_5\text{OH}$ as the main phase which contained about 30 % but there is different phosphate-based compound, which contains both iron and calcium - $\text{Ca}_9\text{FeH}(\text{PO}_4)_7$, 21%.

A small amount of alpha hematite, $\alpha\text{-Fe}_2\text{O}_3$ and Fe_2O_3 also detected with 28% and 25% respectively. This indicates that iron is present with two oxidation states as Fe (II) and Fe (III) in the mixed of phosphate and hematite (Piccirillo *et al.*, 2014). It can be clearly seen that the colour of bones changed from white into maroon colour during calcination process. When Mn doped with unmodified bone by mixing

with $\text{Mn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$, two different formula; Chloroapatite, $\text{Ca}_5\text{Cl}(\text{PO}_4)_3$ and $\text{Ca}_{10}\text{Cl}_2(\text{PO}_4)_6$ were obtained as new modified materials. Figure 2(c) showed that, Mn is still presents but has been combined with phosphate producing approximately 21% of manganese chloride phosphate, $\text{Mn}_5(\text{PO}_4)_3\text{Cl}$. Both modified bones resulting in the formation of Fe(III) and Mn (II) in the form of hematite and chloroapatite which help to prevent from UV rays.

Mn^{2+} -doped HAP can be obtained without producing significant alterations in the structure of HAP. On the other hand, Le Geros *et al.* (1989) reported that incorporation of reduced amounts of Mn^{2+} into HAP structure induces an

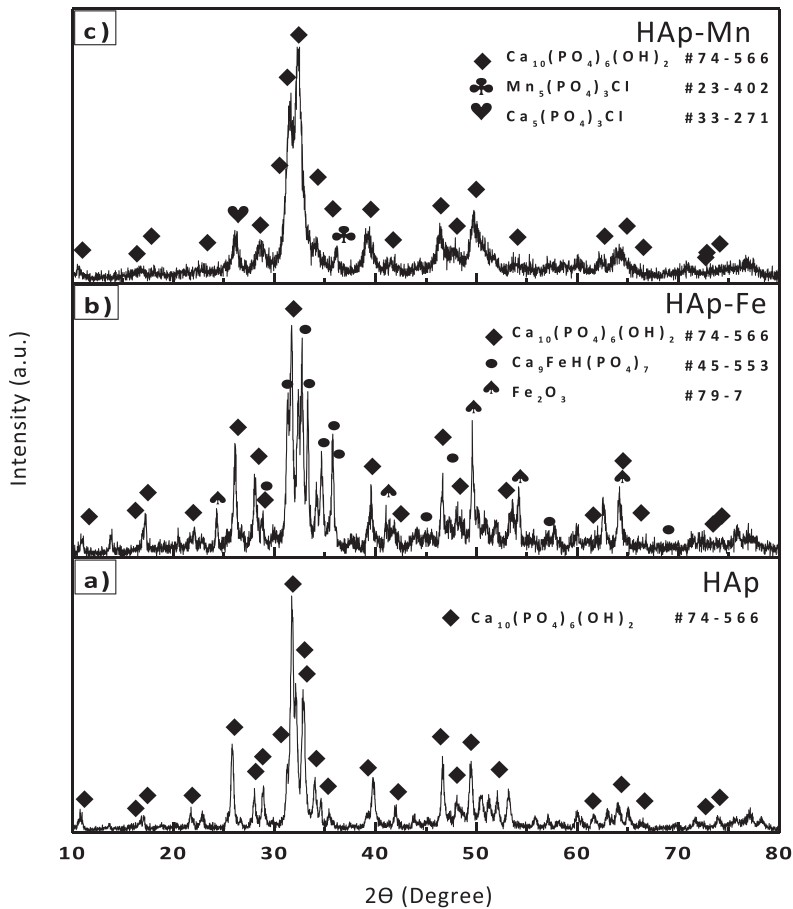


Figure 2: X-ray diffraction pattern of (a) Pure HAP, (b) Fe³⁺-doped HAP and (c) Mn²⁺-doped HAP compared to the HAP standard pattern PDF #74-566

evident reduction of the degree of crystallinity of the HAP phase. HAP-Fe³⁺ exhibited high crystalline peak of reduced amounts of Fe³⁺ into HAP structure induces an evident reduction of the degree of crystallinity of the HAP phase (Walters *et al.*, 1997).

In this work, HAP-Fe³⁺-doped exhibited high crystalline peak at about 33.11°; almost identical to the patterns that were recorded for pure and doped powders. The relatively broadening of the XRD peaks for Fe³⁺-doped HAP indicates that the sizes of the crystallite grains of those materials are smaller than the pure HAP and Mn²⁺-doped HAP (Table 1).

Table 1: Medium size crystallite obtained via XRD measurements using Scherer’s equation to HAP prepared with different dopant at room temperature

Sample	Crystallite size ± 1 (nm)
Pure 2	7
HAP–Mn ²⁺ - doped	23
HAP -Fe ³⁺ - doped	5

Through Scherrer's equation, the medium crystallite sizes were estimated. The Scherrer equation, in X-ray diffraction and crystallography, is a formula that relates the size of sub-micrometre particles, or crystallites, in a solid to the broadening of a peak in a diffraction pattern. It is named after. It is used in the determination of size of particles of crystals in the form of powder. The Scherrer equation can be written as:

$$\tau = K\lambda / \beta \cos \Theta$$

Where τ is the mean size of the ordered (crystalline) domains, which may be smaller or equal to the grain size. K is a dimensionless shape factor, with a value close to unity. The shape factor has a typical value of about 0.9, but varies with the actual shape of the crystallite. λ is the X-ray wavelength. β is the line broadening at half the maximum intensity (FWHM), after subtracting the instrumental line broadening, in radians. This quantity is also sometimes denoted as $\Delta(2\theta)$ and θ is the Bragg angle (in degrees). Note that the effect of Mn^{2+} on the growth of HAP particles is more remarkable

than Fe^{3+} , although the reason for this difference remains unclear at the present. The particle size of a powder for application as sunscreen should obey, in beginning, the size of the passage of a sieve 200 mesh (Harry, 2003).

The attachment with HAP in nano size has been modified, exploring its absorption capability and its intense dispersing of the solar radiation in the UV region. The sunscreen or photoprotection action of that material the method of diffuse reflectance was applied. Titanium dioxide (TiO_2) was performed as measurements of reflectance which commonly used in sunscreens formulations especially in cosmetics industry. Furthermore, TiO_2 is an inorganic UV filter in sunscreen products to protect the skin from harmful UV rays when exposed to sunlight in Figure 3, TiO_2 showed one large absorption around 268-419 nm can be seen. Basically compound with this kind of band gap can be promoted by radiation at wavelengths below than 380 nm. Thus, TiO_2 may be vulnerable to excitation of electron by UVB and UVA in sunlight. TiO_2 also could promote a single electron from the valence

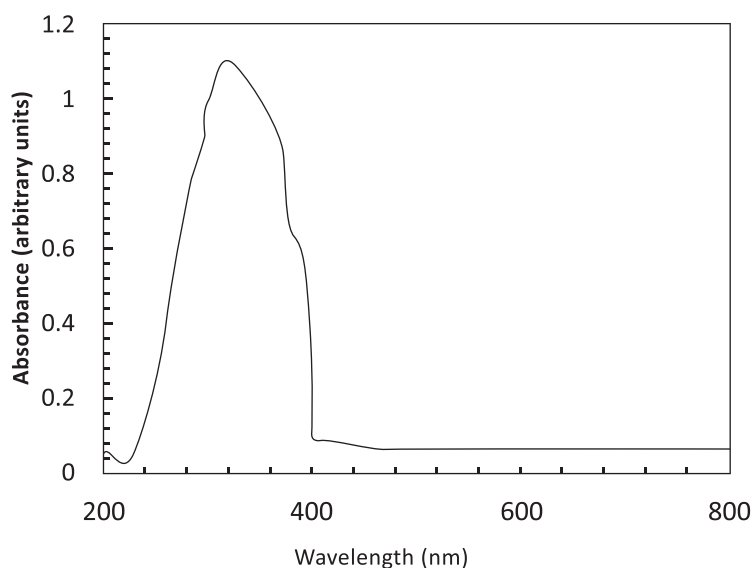


Figure 3: UV-Vis absorption spectrum of TiO_2

band to the conduction band by photoexcitation and leaving a positively charged space that also known as a hole behind. Normally, the electron reunites with the hole, but sometimes the hole migrates to the particle surface, where it can react with absorbed species. It can react with water or hydroxyl ions and forming hydroxyl radicals in an aqueous environment (Brezova & Stasko, 1994). For aqueous preparations of TiO_2 is widely known such reactions, exposed to either artificial UV light or natural sunlight. At this stage, the photocatalytic potential of TiO_2 has been utilized to break down suspensions of organic materials and purify drinking water experimentally.

Zinc oxide was found that prevents and block mostly UVA radiation and has an

attenuation maximum around 380 nm, but this can differ slightly with particle size (Pinell *et al.*, 2008). It also has been clearly shown to be similar to that of TiO_2 based on photocatalytic mechanism (Dindar & Icli, 2001; Rozaini & Brimblecombe 2009). Food and Drug Administration (FDA) in 1993 has clarified that a sunscreen or photoprotective should maintain wavelength of UVA around 360 nm to declare to be anti-UVA and the material used in the formulation should be able to present absorption in this spectrum region.

The spectra of pure, Mn^{2+} -HAP and Fe^{3+} -HAP produced by UV-Vis absorption is shown in Figure 4 and the table 2 is presented the maximum absorption wavelengths for TiO_2 , pure and -doped attached with HAP.

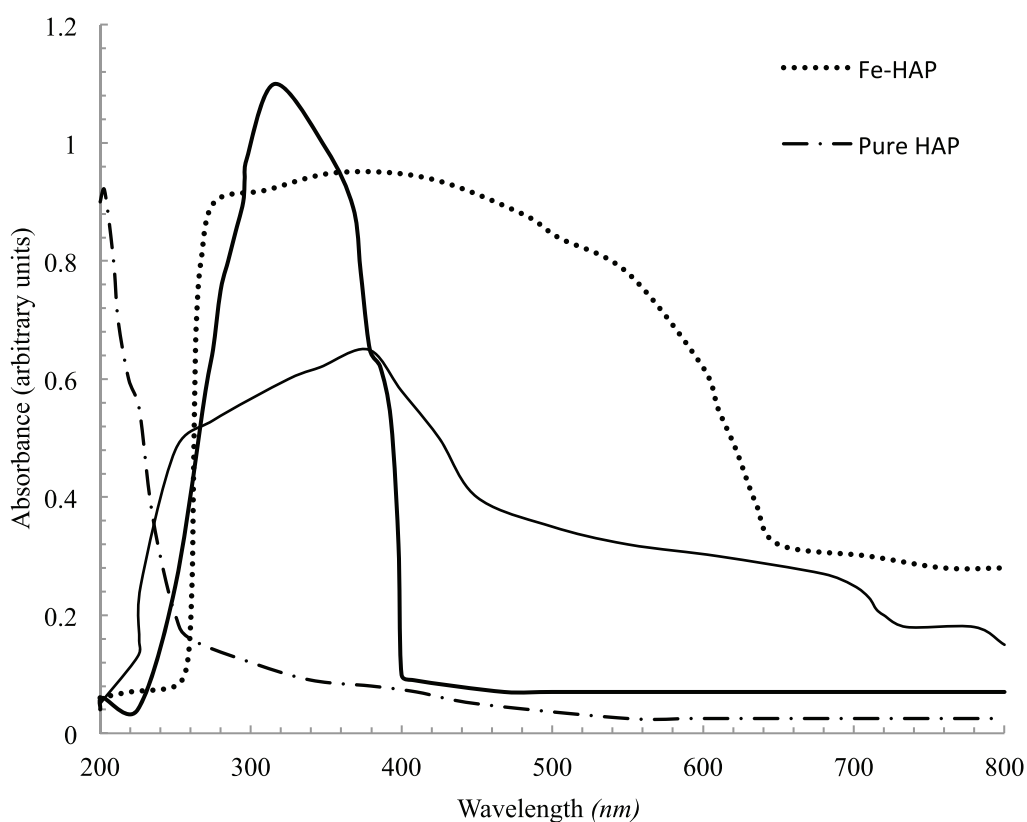


Figure 4: UV-Vis absorption spectra of Mn^{2+} , Fe^{3+} -doped HAP

UV region around 200–340 nm with a strong band below 247 nm shows optical absorption in pure HAP. Results for producing pure HAP by an aqueous precipitation procedure from $\text{Ca}(\text{OH})_2$ and H_3PO_4 at temperature 900°C similarly were found by Nishikawa (2001). The optical absorption of HAP had undergone remarkable changes with the introduction of Fe^{3+} and Mn^{2+} as can be seen in Figure 4. Mn^{2+} -doped HAP generates absorption bands from UV to the visible (250–380, 450–700, 730–800 nm). Fe^{3+} -doped HAP showed a broadened absorption from UV to the visible (267–650 nm) regions. The possibility of an unacceptable visual color effect in sunscreens can be produced by absorption bands in the visible region. To prevent the agglomeration of particles, care has to be taken during sunscreen production, because the final effect of the product will decrease and the bad dispersion will yield a colored film. Thus, this problem can be worked out if there is a better dispersion of the sunscreen on the skin and a colored sunscreen will have transparent appearance when correctly applied on the skin (Flor & Davolos, 2007). The Fe^{3+} -doped HAP is the best sample because it showed absorption features in the wider spectral interval of 213–650 nm, which is similar to that obtained for TiO_2 even if the Mn^{2+} -doped HAP had good results in their absorption spectrum. Moreover, it is known that a highlighted sunscreen should have maximum absorption capacity in relationship to the UV radiations in wavelengths between 296.7 and 700 nm. Substances with higher absorbance embraced the highly UV absorbance potential (Harry, 2003). Undoubtedly, the most promising sample produced in this work is the Fe^{3+} -doped HAP with this aim.

Conclusion

Valuable compounds such as HAP, alpha hematite and chlorapatite were successfully obtained from modified *S. fimbriata* bones. ^1H NMR, XRD and UV-Vis results showed that the fish bone heated at 900°C produced a

major HAP phase with a similar composition to that of a biomaterial commonly used for bone implants. Hence, the material showed good potential absorption properties in the UV range.

Sunscreen effectiveness is directly related to the physical and chemical properties of the ingredients, in specific their absorption and/or dispersion capabilities with respect to solar radiation. The present study provides information on the hybrid production (organic–inorganic) and optical absorption of Fe^{3+} or Mn^{3+} -doped (inorganic) HAP (organic) as possible active components of sunscreen agent.

Similar to TiO_2 powders which are regularly used in sunscreen formulations, the doped HAP samples show absorption bands in the UV region as needed. The Fe^{3+} doped HAP has a promising potential to be applied as a good sunscreen due to the extremely biocompatibility of HAP and low toxicity of the Fe^{3+} ions. In spite of that, to assure the protection against the undesirable effects of the ultraviolet radiation, the long way from the sunscreen's elaboration to its commercialization demands several further studies by experts from various areas.

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