Biocompatibility of Hydroxyapatite (HAp) derived from clamshell as active ingredients in sunscreen product

Saidatul Radhiah Ghazali¹, Nur Hidayah Rosli¹, Lili Shakirah Hassan¹, Mohd Zul Helmi Rozaini¹ and Habibah Hamzah²

¹Department of Chemical Engineering Technology, Faculty of Engineering, University College TATI, Terengganu, Malaysia,
²Institute of Marine and Biotechnology, University Malaysia Terengganu, Terengganu, Malaysia

E-mail: saidatulradhiah@uctati.edu.my

Abstract. Commercially, Avobenzone and Oxybenzone act as absorber of UVA and UVB while titanium dioxide (TiO₂) and zinc oxide (ZnO) are the physical “blockers” of UV radiation in sunscreen formulation. These active ingredients are known to cause photo-allergic reactions, while others are suspected as estrogen disrupters. Due to this concern, hydroxyapatite (HAp) is derived from clam shell in order to replace Avobenzone and Oxybenzone as organic component in UV absorber. The HAp has arisen as potential candidates to replace synthetic chemicals in sunscreens due to its properties that contain calcium, phosphate, chitin and protein that gives a great result in absorbing UV light. HAp is added into emulsion of sunscreen lotion and analyzed using thermal, spectroscopy and skin analysis in order to determine the potential results. The use of this biomaterial namely HAp is an alternative for the safe, organic, less chemical and good cosmetic product.

1.0 Introduction

Sunscreen is an effective protective agent against damage from the sun’s powerful rays for all ages, genders and skin types. The use of sunscreen is one of the most common and effective ways to prevent the damage associated to UVA and UVB radiations. Ideally, a sunscreen product should protect the skin in both the UVA and UVB regions to fully prevent the ascribed health problems [1]. The mechanism of sunscreen involves two parts of physical barrier and organic material. In many commercial products, the physical barrier such as TiO₂ acts as a reflector to the sun ray, while the organic material such as Oxybenzone as UV light absorber in order to prevent the UV light penetrate into the skin.

A sunscreen should be stable from time to time, and not deteriorate under irradiation. It is also important that the chemicals used in sunscreen are not toxic to human health, do not cause skin irritation and do not permeate the skin itself [2]. The excessive use of organic material (Oxybenzene) in commercial products also has been reported can cause skin cancer [3]. In addition, the use of TiO and ZnO as a physical barrier is also dangerous for humans and marine organism too [4].

The idea of this research is to determine the potential substituent for this organic material and physical barrier ingredients in sunscreen. The clamshells known as Paratapes textilis or lala were used to derive a new biomaterial namely Hydroxyapatite (HAp) which are non-toxicity sources of calcium phosphate that effectively absorb UV area. The derived HAp from clam shell are used as organic material in sunscreen.
Meanwhile, for physical barrier of Titanium Oxide and Zinc Oxide are replaced by Iron (II) Oxide. Iron oxide was already reported as a UV absorber in the material. The presence of calcium iron hydrogen phosphate increases the absorption and extends it over the whole UV range [5]. The materials showed good photostability and a UVA-To-UVB ratio greater than 0.90, which means they offer comparable protection against both UVA and UVB radiation [6]. Known as its non-toxicity, it would be very suitable material as the base for sunscreen filters.

HAp is usually used as a filler or bump in the damaged bones and implants to promote bone growth especially in orthopedic applications, dentistry, maxillofacial and biomedical and cosmetic [7-8]. Apart from that, the studies performed also proposed that HAp as a safe inorganic sunscreen agent because of its good opacity and reflectivity [6]. It also has screening capability, high dermal tolerance and a lower whitening effect than other physical sunscreen agents [9]. In addition, by using this compound, it was not only promising candidate for sunscreen, but also helping the aquaculture industry in Malaysia to manage the bone waste disposal more wisely, improve their economic status and also preserve the environment [10].

2. Methodology

2.1. Preparation of active ingredients (HAp)
The clam shells were weighed with a mass of approximately 100 g using analytical balance before the addition of alkaline solution. The clam shells were divided into two beakers which contained of water and iron chloride (98%, Merck). Each of the sample in the beaker was heated at 70°C for three hours and stirred. Then, 30 ml of 1.0 M ammonium hydroxide was added into the solution until pH meter indicating pH 8.0. The shells were dried again an overnight in oven and calcined at 900°C for three hours in a furnace. The clam shells then re-weighed to determine potential mass loss. The calcined shells were converted into powder form by grinding with high-energy ball mil for 15 min at 600 rpm. The component of synthesized clam shells in powder were identified using Fourier Transform Infrared radiation (FTIR) and X-Ray Diffraction (XRD).

2.2. Emulsification of sunscreen
The formulation was separated into two portions, the oil phase and the water phase. In the oil phase, olive oil, surfactant and petroleum jelly with 10:10:1 ratio for each, were heated to 80°C and were mixed together. While in the water phase, water was heated 80°C. After the two portions reached 80°C, the oil phase was added into the water phase solution with the 1:2 ratio for each. When the mixture reached homogeneity, the mixture was stirred slowly with stirrer until the temperature of the mixture drops to 35°C. Then, the sample was placed in cool places for storage.

2.3. UV-Spectrometry analysis and SPF calculation
The emulsions are characterized by using UV-Visible Spectrophotometer to determine the adsorption wavelength for the samples. The wavelength for this UV spectrometer is set at 200-400 nm based on the optical absorption value of TiO2 is in the range of 268-419 nm. Then, the SPF value are calculated using Mansur Equation as shown below.

\[
SPF_{spectrophotometric} = CF \sum_{\lambda = 320}^{400} EE(\lambda) x I(\lambda) x Abs(\lambda)
\] (1)
3. Result and discussion

3.1. Percentage yield
Table 1 shows the result of percentage yield of the sample for HAp, HAp-Fe and Hap-Mn. This percentage yield is obtained from the difference in the original weight of the clam shell before and after the calcination process.

| Sample     | % Yield |
|------------|---------|
| HAp        | 56.8    |
| HAp-Fe     | 99.3    |
| HAp-Mn     | 77.0    |

The different percentage yield values for the samples refer to different doping materials. HAp samples that coated with inorganic metal provide a higher value due to the new bonds formed of HAp and inorganic metal (Fe and Mn). The HAp-Fe sample gives higher percentage of yield which is 99.3%. This means the reaction is 99.3% efficient and only 0.7% of the materials was wasted either they were failed to react or their products were not captured [7-8]. While, the percentage yield for HAp is only 56.8% which shows that the reaction was not really efficient or half efficient. This result either the materials in the reaction were failed to react and become wasted or their products were not captured in the reaction [11-12]. The differences of all the results in this analysis are believed due to the moisture or liquid contained in each of the samples [10].

3.2. Fourier Transform Infrared Radiation (FTIR)
In this study, FTIR was used to determine the infrared spectrum of absorption of unmodified Hap and modified Hap-Mn and modified Hap-Fe. The FTIR spectrum of the HAp crystals hindmost at 600°C calcinations are shown at figure 1.
Figure 1. FTIR spectrum of (a)HAp, (b) HAp-Fe and (c) HAp-Mn.

There is no alkyl group emerged on the spectrum thus implying the nonappearance of organic materials residual. The residual-free water was stretched at broad band 3000-3800 cm\(^{-1}\). The band was attributed to the O-H stretching of crystallite [13]. Besides, the bending mode of the O-H group by water absorption caused the additional band at 1400-1600 cm\(^{-1}\). The phosphate group, O-P-O bending mode bands can be found in range 430-700 cm\(^{-1}\) for all the samples. These mode bands are assigned and comparable to the finding by the Vallet-Regi and Gonzalez-Calbet (2004) [14]. The symmetric P-O stretching mode are assigned at 800-990 cm\(^{-1}\) where the HAp-Fe and HAp-Mn gave weak intensity bands compared to HAp. It is due to the attraction bond of P-O with the dopant [5]. According to Wang et al., (2005), the antisymmetric P-O at 1111.0 cm\(^{-1}\) bands also gave weak intensity which is show implies a high degree of crystallinity which is persistent with the X-RD [15].
Table 2. Summary of vital functional groups from sample (a), (b) and (c).

| Functional group | Type of vibration | Wavenumber (cm\(^{-1}\)) | Intensity |
|------------------|-------------------|---------------------------|-----------|
| OH               | O-H stretch       | 3614.60 (HAp)             | br        |
|                  |                   | 3637.75 (HAp-Fe)          |           |
|                  |                   | 3626.17 (HAp-Mn)          |           |
| OH               | O-H bend          | 1440.83 (HAp)             |           |
|                  |                   | 1473.62 (HAp-Fe)          | s         |
|                  |                   | 1448.54 (HAp-Mn)          |           |
| PO\(_4^{3-}\)    | P-O (anti-sym)    | 1111.00 (HAp)             | s         |
|                  |                   | 1099.43 (HAp-Fe)          | w         |
|                  |                   | 1111.00 (HAp-Mn)          | w         |
|                  | P-O (sym stretching mode) | 875.68 (HAp) | s         |
|                  |                   | 858.32 (HAp-Fe)           | w         |
|                  |                   | 883.40 (HAp-Mn)           | w         |
| O-P-O            |                   | 553.57 (HAp)              |           |
|                  |                   | 476.42 (HAp-Fe)           | s         |
|                  |                   | - (HAp-Mn)                |           |

3.3. X-Ray Diffraction (X-RD)

The effect of dopants on the phase identification of the samples have been studied using X-ray powder diffraction (X-RD) technique.

(a)

![X-Ray Diffraction Image](image1)

(b)

![X-Ray Diffraction Image](image2)
Figure 2. X-ray diffraction pattern of (a) unmodified HAp, (b) modified HAp-Fe and (c) modified HAp-Mn doped compared to the HAp standard pattern PDF #74-566.

Figure 2 represent the XRD patterns of the HAp, HAp-Fe3 and HAp-Mn samples, which perfectly match with the JCPDS pattern #74-566 for HAp [16-17]. The hybrid system of HAp-Mn and HAp-Fe sunscreen leads to the formation of less crystalline than HAp. This will lead to the natural patterns as the dopants assumed to substitute at Ca\(^{2+}\) sites (ionic 0.99 Å) have larger charge and smaller ionic radius (Mn\(^{2+}\) 0.63 Å, Fe\(^{3+}\) 0.64 Å). For other ions usually with an ionic radius smaller than that of Ca\(^{2+}\) are known to inhibit the formation of HAp [18].

From the figure above, it can be concluded that HAp-Fe doped exhibited the highest crystalline peak at about 34.22° whereas HAp-Mn doped exhibited the lowest crystalline peak at about 34.08°. Le Geros et al., (1989) reported that incorporation of reduced amounts of Mn into HAp structure induces an evident reduction of the degree of crystallinity of the HAp phase [19]. The sizes of the crystallite grains of those materials are smaller than the pure HAp and modified HAp-Mn resulted by XRD peaks for Fe\(^{3+}\)-doped HAp [5].

3.4. UV Vis Spectroscopy

Figure 3. Ultraviolet absorption of standard (control), HAp, HAp-Fe and HAp-Mn emulsions.

Figure 3 shows the absorption of ultraviolet light from various wavelengths of HAp, HAp-Fe and HAp-Mn emulsions as sunscreen. The wavelength for this UV spectrometer is set at 200-400 nm based on the
optical absorption value of TiO₂ is in the range of 268-419 nm. TiO₂ is the most widely used inorganic material in the beauty industry for UV protection [20]. Based on figure 4, the addition of dopants (Fe and Mn) in HAp has caused some significant changes. This metal was selected based on a study conducted by Araujo et al., 2010 which proved that iron and manganese can expand the absorption range of UV compared to the chemicals found in commercial sunscreen namely TiO₂ and ZnO. The HAp-Mn emulsion produces an absorption band from UV to the visible area (200-340 nm) starting from 2.317 [5]. Meanwhile, HAp-Fe produces an absorption band from UV to visible area (267-650 nm) starting from 2.166. This value indicates that the addition of HAp as an organic matter in a sunscreen emulsion can result in a higher absorption band compared to the emulsion itself. Moreover, the addition of inorganic doping such as Fe and Mn has given a higher absorption value compared to the HAp emulsion itself [10].

3.5. SPF calculation

The increase in the SPF value indicates higher UV protection. Thus, measuring SPF value allow us to know the efficiency of formulation to protect skin from aging, cancer, sunburn and other skin damage [21]. In fact, sunscreens with sufficient SPF values are used to support the body’s natural protection mechanisms to protect against injurious UV rays through absorbing, reflecting or scattering the sun’s radiation [22]. The result from in vitro method by performing UV Visible Spectrophotometer of UV 1800 Shimadzu able to analyze the capability of sunscreen. All the calculated SPF values of control, HAp, HAp-Fe and HAp-Mn emulsions are shown in table 3.

| Emulsion      | Sun Protection Factor (SPF) |
|---------------|-----------------------------|
| Emulsion      | 10.6972                     |
| Emulsion HAp  | 15.4267                     |
| Emulsion HAp-Fe| 17.0323                    |
| Emulsion HAp-Mn| 21.1483                    |

The result shows that the SPF value for emulsion (control) was 10.6972. The control emulsion contained only base cream without added extracts. The SPF value for HAp emulsion was 15.4267. While, the SPF value for HAp-Fe emulsion and HAp-Mn emulsion are 17.0323 and 21.1483 respectively. This SPF value correlates with the results of the UV absorption value. HAp acts as an organic material in the sunscreen which provides better absorption of UV light. In the meantime, dopants (Fe and Mn) are inorganic substances that reflect UV light from penetrating into the skin. This organic-inorganic hybrid concept of sunscreen gives a great result in UV absorption and SPF value [10]. Thus, the HAp-emulsion gives a higher UV value than the plain emulsion. In addition, HAp-Fe and HAp-Mn give a higher UV absorption value than the HAp. Besides, the reason for this variation and difference in calculated SPF value might be due to the metal interaction of vehicle components and concentration of sunscreen formulations [23].

4.0 Conclusion

In conclusion, unmodified HAp, doped HAp-Fe and doped HAp-Mn emulsions were successfully synthesized from the clamshell known as Paratapes textilis or lala. The powder of HAp, HAp-Fe, and HAp-Mn were then characterized by using x-ray powder diffraction (XRD) method and it was found that all of the samples have quite high crystalline peaks. However, the peak for HAp-Mn was the lowest due to the reason that incorporation of reduced amounts of Mn into HAp structure induces an evident reduction of the degree of crystallinity of the Hap phase. In addition, all of the samples were also characterized by using FTIR and determined to have phosphate groups (PO₄³⁻) hydroxyl groups (OH⁻) carbonate group (CO₃²⁻). The samples also been characterized using UV Visible Spectrometry to determine the capability of samples to absorb UV light. The result shows the HAp-doped emulsions give
broader and higher absorbance than unmodified HAp. This UV absorbance bands correlates to the SPF value where HAp-Mn gives higher SPF value with 21.1483.

This study has developed a new hybrid of biomaterial derived from clamshell namely modified HAp. Due to its non-toxicity feature, modified HAp would be a very suitable material to replace the Oxybenzene, TiO2 and ZnO as the agent for sunscreen filter. It will reduce the risk of human health effects. Nevertheless, it is possible to generate profits if the waste is re-directed to human consumerism. Thus, efforts are being made in attempt to add value to the clam waste from seafood processing industry and convert it into viable and potential alternative to replace toxic materials used in commercial sunscreens. The extraction of HAp from clamshell will not only satisfy the Halal requirement and concerns for Halal certificate but will also increase the economic in Terengganu as business player. The proposed idea will also boost the development of Eastern Economic Region (ECER) by minimizing the environmental impact of disposed wastes.

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