Determination of New Biomaterials of Clams as An Active Ingredient in Sunscreen

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Abstract. Sunscreen agents are widely used to protect the skin against sunburn and to prevent the degradation of cosmetics products by sunlight. The good sunscreen agents contain inorganic materials such as synthesized calcium as the active ingredients. Despite using these materials, we discovered the potential of clam shell powder as a new biomaterial product with high calcium, phosphate, chitin and protein that gives a great result in preventing UV light. The clams shell powder was treated in order to synthesis the active ingredients namely Hydroxyapatite (HAp). Hap is added into the formulation of emulsion as sunscreen lotion. The emulsion was analyzed using thermal, spectroscopy and being compared with the commercial products. The uses of this new material is an alternative for the safe, organic, less chemical and a good cosmetic products.

1. Introduction
Sunscreen is one of the most important mediums that been used to protect the skin from the harmful rays of the sun. The application of sunscreen layer on the skin can prevent the penetration of UVA and UVB from the sun that can led to the skin cancer and health problem [1]. There are two types of sunscreens which are physical and organic. For physical sunscreen, the mechanism involves the used of inorganic metals such as Titanium Dioxide (TiO₂) or Zinc Oxide (ZnO). These inorganic metals properties comes in Micro Size particles and acts as outer layer barrier of the skin and will reflect the sun ray from penetrate the skin. Meanwhile, organic sunscreen usually consists of carbon-based compounds such as Oxybenzone, Avobenzone and Octinoxite. The organic sunscreen usually comes in Nano Size particles and can be absorbed through the skin in order to diminish the impact of UV radiation [1].

A good sunscreen is lasting when applied, heat resistance and easy to degrade. Besides, the ingredients used also need to be non-allergic, environmentally friendly and not harmful to living organism [2]. Nowadays, the use of excessive Oxybenzene and ZnO in most sunscreen product gives the irritation effect to the consumer and may lead to the cancer due to its poisoning percentage composition in the ingredients [3][4]. The use of these organic and physical material also can pollute the ocean and impact the coral and marine lives [3].
This aim of this research is to minimize the harmful impact of the commercial sunscreen in the market by substituting Oxybenzene and ZnO or TiO2 with the non-toxic materials. For organic material, it will be replaced by the biomaterial that been derived from clamshell namely Hydroxyapatite (HAp). In other hand, Iron Oxide (FeO2) are used to substitute TiO2 as physical barrier due to its properties as good UV absorber. The FeO2 shows good photostability and gives a better protection on UVA UVB radiation as their ratio are 0.90 which are greater that other metals. [5].

As a biomaterial, HAp acts as an organic sunscreen protector due to its great murkiness and reflectivity [6]. In recent studies, HAp is a mineral form of calcium phosphate that had been used widely in orthopedic, dentistry, biomedical and cosmeceutical field [7][8]. The presence of HAp in sunscreen can increase the screening capability and reduce the whitening appearance of the physical sunscreen [9]. Thus, by combining the both physical and organic types in one sunscreen and substitute the material with HAp and FeO2 will increase the effectiveness of the sunscreen and decrease the harmful effect of the compound. Moreover, the effort of using clam shell waste as main resources can making a huge different to the aquaculture industry in Malaysia by transforming the waste items into valuable products [10].

2. Methodology

2.1. Preparation of pure hydroxyapatite (pure HAp) from clam shell extract.
To prepare the pure HAp, the clam shell are cleaned and boiled for 36 hours in 200°C. Then, the clam shells are dried in the furnace at 110°C for 24 hours. Next, the extraction of pure HAp will be treated with 0.8 M NaCl and follow by soaking in 0.2 M NaOH for five hours at 5°C. The extraction also will be rinsed again and treat with 0.05 M acetic acid for three hours at room temperature. Then, the extraction of HAp is left to be shaken in water bath for 12 hours at 40°C.

2.2. Preparation of hybrid hydroxyapatite (Hap)
The preparation of hybrid Hap-Mn and Hap-Fe will undergo the same procedure as the pure HAp. Then, the extraction of pure HAp is divided into three beakers containing water, iron chloride and manganese chloride. Each of the beakers is heated up and need to maintain at 70° for three hours with constant stirring. Several drops of 1.0 M of ammonium hydroxide are added into the solution of FeCl2 and MgCl2 to reduce the acidity of solution until the pH was approximately 8.0. The fish bones are dried again for overnight in oven and will be calcined at 900°C for three hours in a furnace. Lastly, the calcined bones will be grinded into powder form before storage.

2.3. Emulsification
The formulation started by preparing two different portions of oil and water phase. The oil phase consists of olive oil, petroleum jelly and surfactant been heated until 80°C. For water phase, the water was heated also to 80°C. When both phases reached 80°C, immediately pour the oil phase solution into the water phase and stir the mixture until the temperature drops to 40°C. The mixture was stored in dry and clean container to keep it homogenous and stable.
3. Result and Discussion

3.1. Appearance of sample after calcined

The determination of the appearance has been conducted after the sample was calcined in the furnace for three hours at 900°C. The samples involved was shell only known as pure sample and shell + FeCl$_3$ sample known as hybrid samples.

| Sample         | Texture  | Odour      | Colour      |
|----------------|----------|------------|-------------|
| Pure HAp       | Fine Powder | Odourless | White       |
| Hybrid HAp     | Hard Solid | Rust Iron | Slightly Brown |

Table 1 Result of appearance of sample after calcined in the furnace

Table 1 shows the result of appearance of the hybrid-HAp and Pure-HAp sample in terms of texture, odour and colour after being calcined in the furnace. For pure-HAp sample which is the shell only, the texture after calcined was a fine odorless powder and was white in colour. As for hybrid-HAp for shell + FeCl$_3$, the texture after calcined was hard with slightly brown colour solid and has an odour like rusting iron. This is because of the FeCl$_3$ itself was a metal and was brown in colour [11].

3.2. Percentage Yield

Table 2 shows the result of percentage yield of the sample for pure-HAp, hybrid HAp-Fe and hybrid HAp-Mg. The percentage yields were calculated to know the efficiency of the reaction. From the result, hybrid HAp-Fe gives highest yield with 90.8% compared to the other two. The percentage yield shows that the formation of raw material into desired product are 90.8% successful and only 9.2% failed to form the hybrid HAp product [10]. Moreover, the complexation process between Pure HAp and metals (Fe and Mg) have formed a strong covalent bond that increase the effectiveness of the formation process [10]. For Pure HAp, the percentage only achieved 51.3% which means that the formation of the products was not fully formed and half efficient. This is because of the incomplete reaction during treatment and calcination process [11].

| Sample         | % Yield |
|----------------|---------|
| Pure HAp       | 51.3    |
| Hybrid HAp-Fe  | 90.8    |
| Hybrid HAp-Mg  | 73.1    |

Table 2 Percentage yield of samples
3.3. Fourier Transform Infrared Spectroscopy

FTIR was utilized to decide the infrared range of retention of pure HAp, hybrid HAp-Fe and HAp-Mg. FTIR investigation of three distinctive sunscreen agents were performed to examine the structure of HAp particles, determining the position of top components recognized within the wavelength [12].

![FTIR Spectra](image)

**Figure 1** FTIR spectra for all HAp samples

Figure 1 shows the FTIR spectrum of pure HAp and hybrid HAp samples. The PO group was detected at the range of 560-610 cm\(^{-1}\) and 900-1100 cm\(^{-1}\). The range of wavenumber at 560-610 cm\(^{-1}\) was indicated the bending mode of PO group and 900-1100 cm\(^{-1}\) showed the stretching vibration mode of PO group. The bending mode of H\(_2\)O was noticed at the range of 1600-1700 cm\(^{-1}\) due the water of the shell was adsorbed. The carbonate ion substitution was obtained on 1400-1500 cm\(^{-1}\). The spectrum at the range of 2000-2300 cm\(^{-1}\) was due to the overtone of stretching vibration mode by PO group. The broaden peak was O-H stretching at the range of wavenumber between 3400-3900 cm\(^{-1}\). Overall, the functional groups of each spectrum were established [11].

From the above spectra, the broadening of the peak at 3300 cm\(^{-1}\) indicates the presence of hydrogen bonding [13], typically from hydroxyapatite to Fe\(^{2+}\) ion from the doping process. There were PO group bands appears at 900-1100 for pure HAp and hybrid HAp-Mg samples. However, these band did not exist in hybrid HAp-Fe sample [12]. Moreover, there was a broaden peaks appears wavenumber 800 cm\(^{-1}\) for hybrid HAp-Fe samples. This absorption peak is differed with the sharp peaks for another two samples. These differences have shown that the addition of doping metal (Fe and Mg) has broken the bond of phosphate functional in pure HAp and formed a new interaction with Ferum [14]. Thus, this proves that the complexation of new hybrid organic (HAp)-inorganic (Fe/Mg) has been fully formed.
3.4. UV-Vis Spectroscopy

Figure 2 shows the result of UV VIS analysis for both pure-HAp and hybrid-Hap. From the graph, the highest absorbance value is 3.324 at the wavelength of 280 nm for hybrid-HAp. The higher the absorbance value, the more particular wavelength is being absorbed. The wavelength for UVB is 280 nm to 315 nm [17]. This showed that the hybrid-HAp, shell + FeCl₃ is able to absorb UVB [15]. By adding this hybrid-HAp into the sunscreen, it will act as a barrier to the skin from UVB because of its potential in absorbing the UV light as well as helps to protect the skin from sunburn and skin cancer [16].

As for the pure-HAp sample, the highest absorbance value is 3.654 at the wavelength of 238 nm. The wavelength absorbed by the sample is in the range of UVC wavelength 200 nm to 270 nm [17]. It shows 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 [18]. It shows that the modification of HAp with Fe can prevents and block mostly UVA radiation [19].

3.5. Preliminary Skin Analysis

The skin analysis was evaluated by using the Dermalab® (Cortex Technology) equipment which recorded the positive outcomes in all parameter including the increment in hydration (moisture) value recorded with 160.09912 uS before applications and 186.180219 uS after the treatment as state in Table 3. Hydration (moisture) is the water content within the cells that leads them to swell and be plump and bouncy, thus reflecting light well [20][21].

| Sample        | Before       | After        |
|---------------|--------------|--------------|
| Pure HAp      | 160.10090 uS | 179.356714 uS|
| Hybrid HAp    | 160.09912 uS | 186.180219 uS|

Table 3 Hydration (moisture) value

As shown in Table 4, 22.234695 g/m²/h value of transepidermal water loss (TEWL) before and increase to 6.963493 g/m²/h after the application gives a positive result for this sunscreen lotion. Transepidermal water loss (TEWL) is the amount of water that passively evaporates through skin to
the external environment due to water vapor pressure gradient on both sides of the skin barrier and is used to characterize skin barrier function [22][23].

| Sample      | Before         | After         |
|-------------|----------------|---------------|
| Pure HAp    | 22.543672 g/m²/h | 20.845682 g/m²/h |
| Hybrid HAp  | 22.234695 g/m²/h | 6.963493 g/m²/h |

**Table 4 Transepidermal water loss (TEWL)**

Besides, in skin analysis we also need to measure the value of melanin pigments. Melanin pigment determine the skin colour of human. From the result, the reduction of melanin pigments from 41.25 to 39.10 (Table 5) shows that the application of sunscreen can prevent the absorption on sun light from penetrating into the skin [24].

| Sample      | Before | After |
|-------------|--------|-------|
| Pure HAp    | 41.10  | 40.67 |
| Hybrid HAp  | 41.25  | 39.10 |

**Table 5 Melanin pigment**

4. Conclusion
HAp can easily find on *Mytilidae* family such as clam, oyster, mussel and scallop. Not only they can be found easily, they also contain of high amount concentration of the HAp. Besides, HAp also known as non-toxic material derived from natural sources, so it is safe to be used as a material for sunscreen production. It also had a good opacity and reflectivity and also helps to decrease the whitening effect by reducing the appearance of the white cast.

The sunscreen adequacy is related to the physical and chemical properties of the active ingredients which lead to the absorption and dispersion capabilities to the UV radiation. In this study, the UV-Vis spectroscopy analysis was done to determine the absorption value for each sample.

From the FTIR analysis, it is proven that by doping the inorganic metal (Fe and Mg) in the pure HAp can form the new hybrid complexes. The positive result from UV and skin analysis has proved that the HA-Fe hybrid complex gives a great and better result in protecting the skin from UV rays compared to pure HAp itself.

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