Preparation of Fish Scales Hydroxyapatite (FsHAp) for Potential Use as Fillers in Polymer

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Abstract: The aims of this study were to prepare natural hydroxyapatite from fish scales (FsHAp) for potential use as a filler in polymer. The FsHAp was prepared from *Tilapia* fish scales using thermal method. The FsHAp was milled for 48 h and dried by spray method. The morphology was characterized using field emission scanning electron microscope (FESEM) which showed irregular shape of FsHAp particles with particle size around 7 μm . The analysis of FsHAp was carried out using Fourier transform infrared spectroscopy (FTIR) and X-ray diffraction (XRD) to confirm chemical structure of FsHAp.

Keywords: Hydroxyapatite, polymer, fillers, fish scales.

1. Introduction

Hydroxyapatite (HA) is a type of calcium phosphate with a chemical formula of $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ [1-4]. Many studies reported the advantages of hydroxyapatite (HA), especially in stimulating bone healing, and claimed that it has been used in orthopaedics as bone void fillers, dental surgery, orthopaedic and dental implant coating, traumatology, spine and maxillofacial [4-7]. HA is bioactive, non-toxic, non-immunogenic and osteoconductive with a crystallographic structure almost similar to that of the bone mineral [8-10]. However, HA with particle size < 10 microns is classified as common inorganic filler used to improve the mechanical properties and biocompatibility of polymer composites [11-17], because of its excellent biocompatibility properties [11, 18-22]. It is similar to the silica powder used in polymer composites [23-26].

The application of HA as a filler in epoxy was reported by Oladele et al. [13] and Alhussein et al. [11]. Meanwhile, Scalera et al. [14] prepared epoxy/HA suspensions for stereo lithography in bone tissue engineering and found that the prepared epoxy/HA exhibits good mechanical properties.

A commonly used HA for fillers in the polymer matrix composite is synthetic HA produced from the chemical reaction between ammonium phosphate $[(\text{NH}_4)_2\text{HPO}_4]$ and calcium hydroxide $[\text{Ca}(\text{OH})_2]$ [27-30]. It is widely used as a filler in thermoplastic materials, such as high-density polyethylene (HDPE) [12, 31]. However, synthetic HA is expensive to produce, and its quality is difficult to control. Biological sources of HA, such as fish scales, fish bones, bovine bones, teeth and bones of pig, are alternatives to synthetic HA [2, 32-37]. Fish waste, especially fish scales, is a good source of natural HA because it contains 50% by weight of scale. Fish scales are also abundant in valuable organic components, such as collagen [38]. The preparation of

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HA from fish scales is biologically safe, economical and biocompatible [36, 39-42]. Jaafar et al. [12] reported the use of natural HA powder (HAp) from fish scales as a filler in HDPE.

2. Materials and Methods

Fish scales from tilapia fish were collected from local market at Tanjung Malim, Perak. The fish scales were washed with water to remove dirt. The scales were soaked in hydrochloric acid (0.2 M) for 10 min to remove fat and other impurities, washed with distilled water and then dried in oven at 80°C. The fish scales (Fs) were ground into small particles using a grinding machine. The fish scales were loaded into a high-temperature furnace and then heated at 800°C for 2 h and continued at 1,000°C for 2 h to produce fish

ash. The fish ash was wet grinded using ball milling for 48 h [36].

The flow chart for the preparation of FsHAp is shown in Fig. 1. The FsHAp slurry from ball milling was dried to FsHAp powder using spray dryer. The spray dryer apparatus consisted of a high-pressure nozzle that sprays the FsHA slurry in the form of fine mist into the hot air in the chamber. The slurry was evaporated, and the fine FsHA particles were collected in the collector.

The chemical structure of the products was characterized by FT-IR, XRD and the morphology was characterized using field emission scanning electron microscope (FESEM). Meanwhile, the FsHAp particle size was determined using mastersizer 2000 particle size analyser.

Fig. 1 Process flow chart for preparation of natural hydroxyl apatite from fish scales (FsHAp).

3. Results and Discussion

HA from biological sources was prepared because of its biological safety and low cost. Several methods, such as alkaline digestion, enzymatic and direct burning, have been developed to extract HA from fish scales. Direct burning is an easy and cheap way to obtain natural HA. Fish scales are calcination to remove organic component and HA is retained as ash [35, 43, 44].

The most common method to dry the hydroxyapatite slurry is thermal drying in which the slurry was heated in an oven for 1 day at 80°C to remove water after ball milling. However, oven drying caused the HA fish scale particles to clump to each other, which made them again inseparable to be used as fillers in polymer composites. Thus, this study evaluated the properties of HA fish scale particles prepared by the spray method in which the HA fish scale slurry was not oven dried after ball milling. The spray dry apparatus consisted of a nozzle that sprays the HA fish scale slurry in the form of fine mist into the hot air. The slurry was evaporated and the fine HA fish scale particles were collected in the collector chamber.

3.1 FTIR Analysis of FsHAp

Fig. 2 shows the FTIR spectrum of FsHAp. The

hydroxyl group OH showed a sharp peak at around 3,569 cm^{-1} and a weak peak at 632 cm^{-1} , which corresponded to the stretching mode of hydroxyl group of HA. A broad peak at 1,046 cm^{-1} showed a single band for the phosphate group, and the peak at 1,091 cm^{-1} also corresponded to the phosphate group. The bending vibration of PO_4^{3-} was observed in bands located at 560-620 cm^{-1} , and a phosphate band appeared in the region of 472 cm^{-1} [45]. Typical FTIR spectra can be observed for highly crystalline HA [36, 46-49].

3.2 XRD Analysis of FsHAp

The XRD spectrum of FsHAp is shown in Fig. 3. The prominent peaks corresponded to the highly crystalline HA materials [50]. Three main peaks with high intensity were observed in FsHAp at 2θ values of 31.86°, 32.20° and 33.04° associated with the planar hkl (211), (300) and (112), respectively. This result is equivalent to the higher peak intensity reported in previous studies [2, 21, 34]. Some low-intensity peaks that appeared in the XRD pattern were associated with the planar hkl (200), (2221), (002), (102), (210), (202), (310), (222), (312), (213), (321), (410) and (004). The spectrum also resembled the standard XRD pattern of HA based on the library collection (ICDD 9-432) [51].

Fig. 2 FTIR spectrum of FsHAp.

Fig. 3 XRD pattern for FsHAp.

3.3 Particle Size of FsHAp

The filler particle size often affects the mechanical properties of composites. Hence the characterization of filler size and their distribution will help to understand the properties of composite. Fig. 4 shows the bimodal distribution of spray dried FsHAp powder used in this research. Overall, filler sizes are fairly distributed, and this contributed toward good dispersion of particles as high amount of small particulate matter is reported to have tendency to agglomerate. Table 1 summarized the particles size results based on $D_{0.1}$, $D_{0.5}$, and $D_{0.9}$. The results show that 10% of sample mass ($D_{0.1}$) comprised of particles with diameter below 0.810 μm and 90% of sample mass ($D_{0.9}$) with particle size below 23.343 μm . The median particle size ($D_{0.5}$) comprised of 50% mass particles with particle size of 5.180 μm . The median particle size 5.180 μm is taken as FsHAp particle size. This particle size is within the as fillers

in HDPE composite [12].

3.4 FESEM Analysis of FsHAp

The morphology of FsHAp was investigated by SEM, and the results are presented in Fig. 5. The results show irregular shape of FsHAp particles with some degree of agglomeration due to static force between particles. However, for synthetic HAp, the particles are more spherical or doughnut shapes as reported by Monmaturapoj et al. [32]. The SEM micrograph shows the mixture of particle size of FsHAp with particle size around 7 microns.

EDX analysis (Fig. 6) was performed to determine the Ca/P ratio of the sample. This analysis was performed to confirm the type of HA obtained after thermal heating at 1,200°C. The calculated Ca/P ratio for the FsHAp powder was 1.67, according to the chemical formula of the standard HA[30], the calcium-to-phosphoromolar ratio is approximately 1.67.

Fig. 4 Particle size distribution of FsHAp.

Table 1 FsHAp particle properties.

Particle size (μm)				
Sample	D _{0.5}	D _{0.1}	D _{0.99}	Surface area (m ² /g)
FsHAp	5.180	0.810	23.343	

D_{0.5}: median particle size; D_{0.1}and D_{0.9}: the size below which 10% and 90% of the particle diameter lie, respectively.

Fig. 5 Scanning electron micrograph for FsHAp.

Fig. 6 EDX spectrum of FsHAp.

4. Conclusion

Hydroxyapatite was successfully produced from fish scales using thermal method. The FsHAp with particle size below 10 μm is classified as fillers in polymer. The chemical structure of FsHAp was confirmed using FT-IR and XRD analysis.

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