Preparation of Low Cost Magnesium Incorporated Hydroxyapatite by Using Duck Eggshells as Calcium Source

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Abstract

This study focused on the preparation of magnesium (Mg) incorporated hydroxyapatite (HAp) samples by co-precipitation method at two different temperatures (room temperature and 40 ℃). Duck eggshells were used as a low cost calcium source. The crystal structures and phase purities of prepared samples were characterized by X-ray diffraction (XRD) and Fourier transform infrared spectroscopy (FTIR). The morphologies of prepared samples were observed by scanning electron microscope (SEM). At room temperature, hydroxyapatite (HAp) phase was observed, however, when the sample preparation temperature was increased to 40 ℃, HAp phase obviously decreased in XRD and FTIR results. Irregular shaped microstructures and microrods composed of nanoparticles were observed in SEM images of prepared samples at room temperature and 40 ℃, respectively.

1. Introduction

Calcium phosphate ceramics are applied as bone substitutes due to their compositional similarity to the mineral component of bones and dentine material in vertebrate animals. Apatite [$Ca_{10}(PO_4)_6X_2$] materials with a Ca/P ratio between 1.5 and 1.67 have unique biocompatibility feature among calcium phosphate groups, X in the [$Ca_{10}(PO_4)_6X_2$] formula represents hydroxyl (OH^-) group for hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$), fluoride (F^-) group for fluorapatite ($Ca_{10}(PO_4)_6F_2$), and chloride (Cl^-) group for chlorapatite ($Ca_{10}(PO_4)_6Cl_2$), respectively [1].

Among them, hydroxyapatite (HAp) has excellent properties such as osteoconductivity and biocompatibility. Hence, HAp can be used as implants biomaterials. Some advantages of HAp are low solubility and the ability to form a direct chemical bond with bone. However, some reports also mention the limitations of HAp, which are linked to its poor mechanical properties including low ductility and brittleness. Therefore, it is important for biomaterial researchers to focus on improving the mechanical properties of HAp [2,3].

Therefore, synthetic hydroxyapatite ceramics are incorporated or doped with small amounts of additives (e.g., fluoride (F^-), chloride (Cl^-), sodium (Na^+), potassium (K^+), magnesium (Mg^{2+}), strontium (Sr^{2+}), barium (Ba^{2+}), aluminium (Al^{3+}), manganese (Mn^{2+}), copper (Cu^{2+}), zinc (Zn^{2+}), silver (Ag^+), cerium (Ce^{3+}) and europium (Eu^{3+}) ions) to improve the mechanical and bioactive property of the implants. Among them, magnesium (Mg) has a crucial role in the calcification process, helps reducing bone fragility [4-6]. Hence, in this work, the use of Mg had considered for the development of artificial bone substitutes.

Nowadays, some researchers proposed the utilization of eggshells as applicable raw materials for various applications due to its non-expensiveness and available everywhere. The main component and the major inorganic substance in eggshells are calcium carbonate (calcite) and it makes up about 94% of chemical composition of eggshell. This makes it an essential material for HAp production. Others are organic matter which makes up 4%, magnesium carbonate (1%) and calcium phosphate (1%) as well as insoluble proteins [7,8].

Pure HAp or metals incorporated or doped HAp has been prepared by hydrothermal, solution combustion, microwave, co-precipitation and sol-gel methods. Among them, co-precipitation method is the best way to produce HAp powder and it can produce large amount of HAp with good reproducibility [9,10]. Therefore, in this research, co-precipitation method was used to prepare Mg-incorporated HAp samples.

2. Materials and Method

In this study, duck eggshells ($CaCO_3$), diammonium hydrogen phosphate ($(NH_4)_2HPO_4$) and magnesium oxide (MgO) were used as Ca, P and Mg sources. Distilled water (H_2O) was used as solvent.

2.1. Preparation of Ca source

To prepare the Ca source, duck eggs were bought from a shop at Myo Ma Market, Taunggyi and washed three times with tap water to remove dust and other impurities. The cleaned eggs were boiled to become hard-boiled egg and immersed in cold water. After cooling, the eggshells were peeled, and the obtained eggshells were sun dried for one day. The dried eggshells were crushed and ground using a motor and a pestle. The obtained eggshells powder was calcined in furnace at 900 ºC to get the calcium source.

2.2. Preparation of Mg-HAp samples

To get the Ca-Mg solution, 3 g of the calcined eggshells powder, 0.5 g of MgO and 50 mL of distilled water were stirred at room temperature for 5 min with

700 rpm. A 3.16 g of $(NH_4)_2HPO_4$ and 50 mL of distilled water were stirred at room temperature for 5 min with 700 rpm to get the P solution. Then, P solution was added dropwise to Ca-Mg solution and the mixed solution was stirred at room temperature for 30 min with 700 rpm. After that, the samples were collected by filtering with filtered paper and dried for 24 hours at room temperature. The same procedures were carried out to prepare Mg-incorporated HAp sample at 40 ºC reaction temperature.

Finally, Mg-incorporated hydroxyapatite samples were obtained by calcination the dried prepared samples at 750 ºC.

2.3. Characterization of Mg-HAp samples

X-ray powder diffraction (Shimadzu XRD-6100-S) with Cu $K\alpha_1$ radiation (λ = 0.154 nm), working current (20 mA) and voltage (40 kV) was used to study the the crystal structures and phase purities of the ground duck eggshells powder, calcined duck eggshells powder at 900 °C and prepared Mg-incorporated HAp samples. Fourier transform infrared spectroscopy (FTIR, Shimadzu IRAffinity-1S) was used to investigate the functional group of the ground duck eggshells powder, calcined duck eggshells powder at 900 °C and prepared Mg-incorporated HAp samples. The wavenumber range was set up from 400 cm^{-1} to 4000 cm^{-1} with spectral resolution of 4 cm^{-1}. The morphologies of the prepared Mg-incorporated HAp samples were observed by scanning electron microscope (SEM, JEOL JCM-6000).

3. Results and Discussion

The results of the ground duck eggshells powder, calcined duck eggshells powder at 900 °C and prepared Mg-incorporated HAp samples characterized by XRD, FTIR and SEM were discussed.

3.1. XRD Analysis

Typically, very fine calcium oxide (CaO) powder is obtained when the eggshells powder ($CaCO_3$) is calcined at 900 °C. However, fine CaO powder is sensitive to humidity. Therefore, it is transformed to calcium hydroxide ($Ca(OH)_2$) during a few hours. This phenomenon was clearly observed in this study.

Figure 1(a) shows the XRD pattern of ground duck eggshells powder (denoted as DES). All the diffraction peaks were in agreement with the standard rhombohedral calcite phase, $CaCO_3$ (ICDD-00-005-0586) which was one of the polymorphs of calcium carbonate. The result indicated that the major phase of duck eggshells powder was calcite ($CaCO_3$).

The XRD pattern of calcined duck eggshells powder at 900 °C (denoted as DES-900) is shown in Figure 1(b). All the major diffraction peaks appeared at 2θ value of 18°, 28°, 34°, 47°, 51°, and 54° corresponding to the reflection from (001), (100), (011), (012), (110) and (111) crystal planes were matched well with the hexagonal structure $Ca(OH)_2$ (calcium hydroxide) phase with ICDD card No 00-078-0135. As a minor phase, calcite ($CaCO_3$) was also found at 2θ value of 29°.

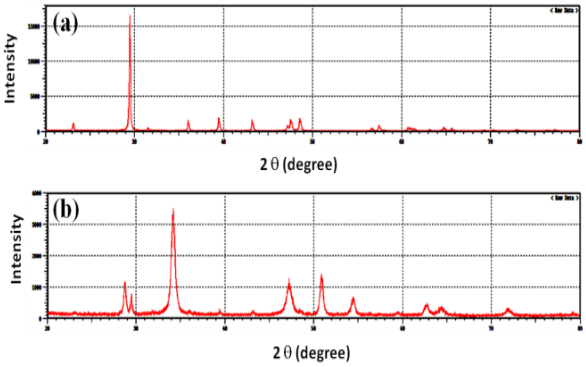

Figure 1. XRD patterns of (a) ground duck eggshells powder (DES) and (b) calcined duck eggshells powder at 900 °C (DES-900)

Figure 2(a-b) shows XRD patterns of Mg-incorporated hydroxyapatite (HAp) samples prepared at room temperature and 40 °C. According to the experimental conditions, these two samples were denoted as Mg-HAp and Mg-HAp-40, respectively. In the XRD pattern of Mg-HAp sample shown in Figure 2(a), diffraction peaks with higher intensity at 2θ values of 25°, 31°, 32° and 33° corresponding to (002), (211), (112) and (030) crystal planes were in agreement with the standard hexagonal structure hydroxyapatite (HAp) ICDD card No.00-064-0738. The result showed that hydroxyapatite (HAp) could be prepared by using duck eggshells as calcium source.

However, in the XRD pattern of Mg-HAp-40 sample shown in Figure 2(b), the intensity of diffraction peaks around 2θ values of 32° to 34° corresponding to HAp phase obviously decreased and other impurities peaks related with Mg were obviously found. The results were in agreement with the previous reports which revealed that Mg ions inhibited the HAp nucleation and crystal growth at reaction temperature 40 °C [4].

The crystallite sizes of DES, DES-900, Mg-HAp and Mg-HAp-40 samples were calculated by Debye-Scherrer's equation ($D=k\lambda/\beta\cos\theta$), where λ=0.154 is X-ray wavelength, θ is Bragg's angle, β is full width at half maxima (FWHM) and k=0.9 is a constant. The calculated results are listed in Table 1. It could be seen that the crystallite size of DES sample was 66.19 nm, which was larger than other crystallite sizes of DES-900, Mg-HAp and Mg-HAp-40 samples, respectively. This was attributed to the sharp diffraction peak of DES sample shown in Figure 1(a).

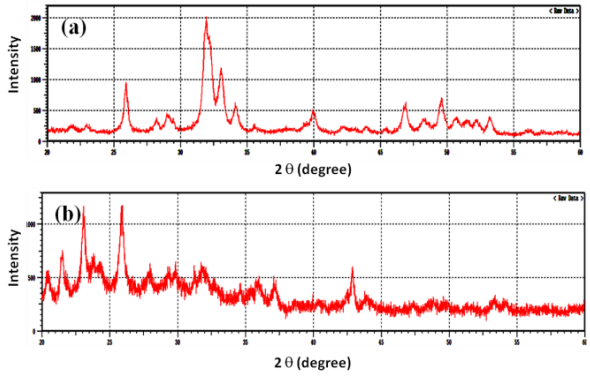

Figure 2. XRD patterns of Mg-incorporated HAp samples prepared (a) at room temperature (Mg-HAp) and (b) at 40 °C (Mg-HAp-40)

Table 1. Crystallite sizes of DES, DES-900,Mg-HAp and Mg-HAp-40 samples

Sample	2θ (degree)	FWHM (degree)	Crystallite Size (nm)
DES	29	0.0022	66.19
DES-900	34	0.0084	17.21
Mg-HAp	25	0.0086	16.63
	32	0.0138	10.48
	33	0.0106	13.58
Mg-HAp-40	25	0.0059	24.11

3.2. FTIR Analysis

Figure 3(a-b) shows the FTIR spectra of ground duck eggshells (DES) and calcined duck eggshells powder at 900℃ (DES-900). In Figure 4(a), the wide band between 1800 cm^{-1} and 1000 cm^{-1} was attributed to the stretching vibration of the C-O bond. The sharp bands at 2513 cm^{-1}, 876 cm^{-1} and 714 cm^{-1} were ascribed to the stretching and bending vibration of CO_3^{2-}. The band at 3400 cm^{-1} was assigned to the O-H stretching vibration from absorbed water. The presence of hydroxyl groups is due to the adsorbed water during sample preparation.

In Figure 3(b), the strong band at 3643 cm^{-1} was assigned to the characteristic band of calcium hydroxide ($Ca(OH)_2$) and represented the O-H stretching mode. The bands at 874 cm^{-1} and 1428 cm^{-1} showed the presence of calcite ($CaCO_3$) phase. It was found that FTIR results were in agreement with the XRD patterns shown in Figure 1(a-b).

Figure 4(a-b) shows FTIR spectra of Mg-incorporated HAp samples (Mg-HAp and Mg-HAp-40). The presence of characteristic HAp bands including phosphate groups and hydroxyl groups were observed in Figure 4(a-b). The bands between 3600 cm^{-1} and 3440 cm^{-1} were attributed to O-H stretching vibrations and around 1630 cm^{-1} were due to the H-O-H bending vibrations of hydroxyl group.

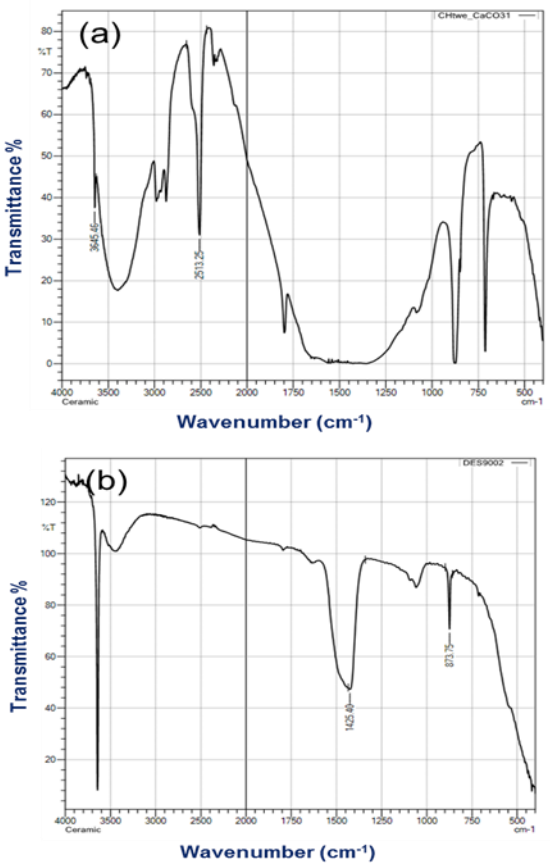

Figure 3. FTIR spectra of (a) ground duck eggshells powder (DES) and (b) calcined duck eggshells powder at 900 °C (DES-900)

The characteristic bands of (phosphate) PO_4^{3-} were observed around 1050 cm^{-1}, 962 cm^{-1}, 630 cm^{-1} and 578 cm^{-1}, respectively. The bands around 1400 cm^{-1} and 875 cm^{-1} were attributed to the C-O group. The band assigned to Mg-O-Mg vibrations was observed around 450 cm^{-1}. However, in Figure 4(b), the hydroxyl group (H-O-H) and carbonate group (C-O) were not observed. The FTIR results confirmed that HAp phase reduced when the sample preparation temperature was increased to 40 °C.

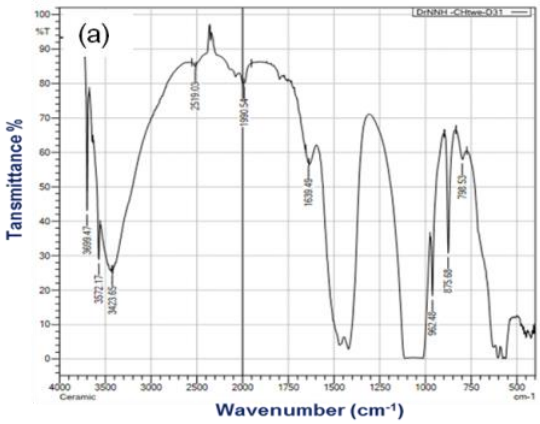

Figure 4(a). FTIR spectrum of Mg-incorporated HAp samples prepared at room temperature (Mg-HAp)

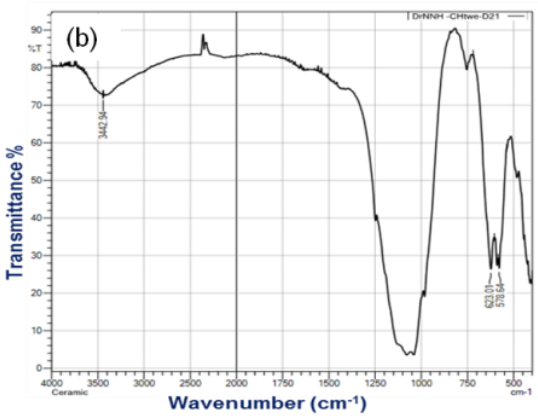

Figure 4(b). FTIR spectrum of Mg-incorporated HAp samples prepared at 40 °C (Mg-HAp-40)

3.3. SEM Analysis

Figure 5(a-b) and Figure 6(a-b) exhibit the lower and higher magnification SEM images of Mg-incorporated HAp samples (Mg-HAp and Mg-HAp-40). In the SEM images of Mg-incorporated HAp sample prepared at room temperature (Mg-HAp) shown in Figure 5(a-b), irregular-shaped microstructures were observed. Meanwhile, microrods composed of nanoparticles were found in the lower and higher magnification SEM images of Mg-incorporated HAp sample prepared at 40 °C (Mg-HAp-40) in Figure 6(a-b). The SEM images showed that reaction temperatures significantly affected on the morphologies of HAp samples.

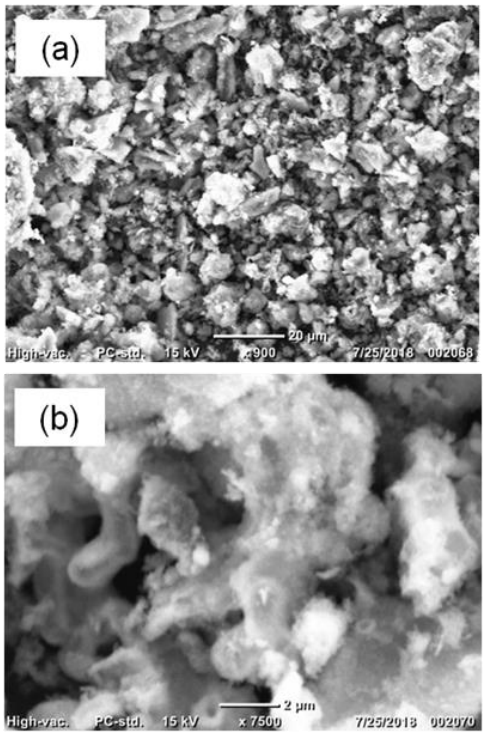

Figure 5. (a) Lower and (b) higher magnification SEM images of Mg-incorporated HAp sample prepared at room temperature (Mg-HAp)

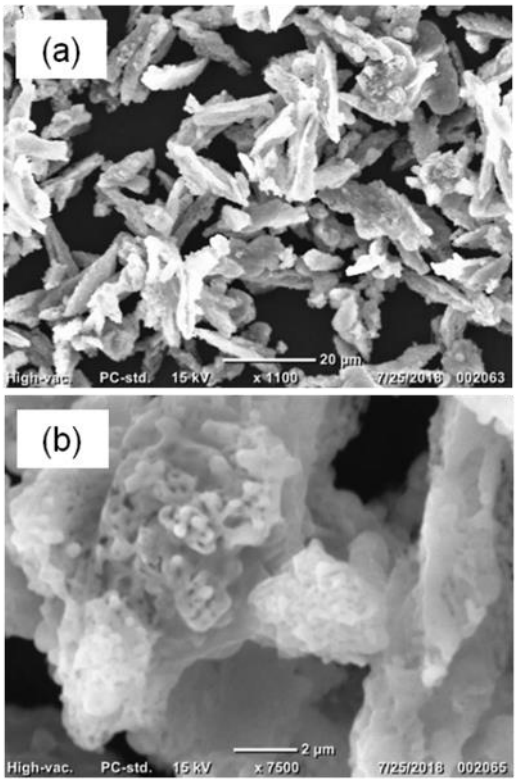

Figure 6. (a) Lower and (b) higher magnification SEM images of Mg-incorporated HAp sample prepared at 40 °C (Mg-HAp-40)

The experimental results showed that micro sized hydroxyapatite (HAp) could be obtained by using duck eggshells as calcium source. For future work, biocompatibility and mechanical properties of Mg-incorporated HAp samples will be studied.

4. Conclusion

In conclusion, Mg-incorporated HAp samples were successfully prepared by simple co-precipitation method using duck eggshells as calcium source. XRD, FTIR and SEM results showed that the sample preparation temperature was significantly influenced the crystal structures, phase purities and morphologies of Mg-incorporated HAp samples. In addition, the results revealed that duck eggshells were applicable as low cost calcium source for the preparation of hydroxyapatite (HAp).

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