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New Chemicals and Routes for the Preparation of Gelatin/HA Composites using the Wet Precipitation Method

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Abstract

Hydroxyapatite (HA) is a material that has many uses in a wide variety of applications such as bone repair, bone implants, and bone drug delivery systems. However, the main weakness of this material is its mechanical strength, which HA is not enough to be directly applied. Gelatin addition is used to improve the mechanical properties that can support material properties for the load-bearing application. This research aimed to obtain gelatin/HA composites with high mechanical strength. This goal is achieved by finding the optimum composite composition (addition of 20, 30, and 40% w/w gelatin), CaO precursors from chicken eggshells, and gradual composite preparation. The preparation of gelatin/HA composites was carried out using the wet precipitation method. The chemical bonding, the compressive strength of HA and gelatin/HA composites, and also morphologies were analyzed by Fourier Transform Infra-Red (FTIR), Universal Testing Machine, and Scanning Electron Microscopy (SEM) respectively. The FTIR spectra show there are chemical bonds between amide and carboxyl in gelatin and Ca^{2+} in HA. The best compressive strength obtained at the composition of 20% gelatin/HA composite is 99.3 MPa (meanwhile HA is 81.5 MPa). The addition of gelatin to HA increases the particle density; this contributes to the increase in mechanical strength.

1. Introduction

Tissue engineering offers a new promising approach to the creation of biological alternatives for implants. Bone tissue consists of inorganic and organic (protein) phases [1]. Calcium phosphate is a major component of the inorganic phase. Calcium phosphate has been used for implantation due to their biological and physiochemical similarities to human hard tissue [2]. The most calcium phosphate phase that has been studied for potential biomedical applications as an implant is Hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$, HA) [3].

HA was introduced as a biomaterial in bone repair, bone implants, and bone drug delivery systems [2] due to its bioactivity, biocompatibility, osteoconductivity, osseointegration, and non-toxicity. Several studies have shown an attempt in syntheses HA using natural materials as a calcium source such as coral, seaweed, and chicken eggshells [4], shells [5], and starfish [2] by diverse methods to synthesize HA such as hydrothermal [3], sol-gel [6], precipitation [7], and microwave [8]. HA in natural bone making resistant to crushing, while protein makes it strong and elastic. Its rigidity and fragility limit the mechanical properties of pure HA. To improve the mechanical properties of HA, the addition of metal oxides such as ZrO_2 [9] and La_2O_3 [10] and a polymer such as a chitosan [11, 12], collagen [2] and gelatin [13] has been investigated.

The polymers added to HA owing to its biodegradable and non-toxic properties which contain numerous groups of hydroxyls, amines, and carboxylates. These functional groups are beneficial for enhancing the interfacial bonding between HA and polymer. A composite system which can combine HA and a polymer to resemble the structure of actual bone [2]. In recent years, gelatin/HA composites have received considerable

attention as a promising biomaterial for hard tissue regeneration due to their similar composition, likely the real bone, excellent biocompatibility, osteoconductivity, and robust-mechanical properties. Many studies have been deployed around the preparation methods, physicochemical, and biological properties, as well as in vivo studies of the gelatin/HA composites [13].

Several papers have reported the synthesis of gelatin/HA composites, and in general, the composites are prepared in situ. The resulting product deficiencies are composite particles having relatively large cavities; consequently, high mechanical strength is difficult to achieve. In this study, the synthesis of gelatin/HA composite was carried out in 2 stages, 1. HA synthesis of CaO and DHP by the hydrothermal method, and 2. The preparation of the gelatin/HA composite was carried out using the wet-precipitation method. Also, the precursors used (CaO) and DHP are different from the precursors used previously.

The preparation of gelatin/HA composite with these two stages is expected to increase the cavity density of the resulting particles. As a consequence, the mechanical strength of the composite will increase. A gradual reaction generally gives enough time for the material to rearrange the particles; consequently, the particles will be quite dense. This study will evaluate differences in the way of preparation, the source of the precursors, and the composition of the gelatin/HA composite on the change in compressive strength.

2. Methodology

2.1. Equipment/Tool/Material

Gelatin and CaO are materials used to synthesize gelatin/HA composites. HA is synthesized from diammonium hydrogen phosphate (DHP; Merck) and CaO extracted from chicken eggshells by the hydrothermal method. Bonding between functional groups between gelatin and HA was observed with Fourier Transform Infra-Red (FTIR; PerkinElmer Spectrum 100), while the surface morphology is characterized by Scanning Electron Microscopy (SEM; Jeol JSM-6360LA). Changes in the mechanical strength of composites are characterized by Universal Testing Machine (UTM, 10kN *Universal Testing Machine*).

2.2. Experiment

HA was synthesized by a hydrothermal method from chicken eggshells as the calcium source and DHP as phosphate source. CaO and DHP with the molar ratio of Ca:P= 1.67 was poured to distilled water (50 mL). The mixture then put into the autoclave 100 mL and heated at 230°C for 48 hours. The obtained HA was filtered and washed with distilled water until pH= 7 to remove the NH_4OH and then dried at 110°C for 2 hours [14]. The preparation of gelatin/HA composites was carried out by the wet precipitation method. HA was added to the gelatin solution (the gelatin concentrations are 20, 30, and 40% w/w of HA) while stirring (500 rpm) using a magnetic stirrer for 1 hour. Then the solution was allowed to stand for 24 hours. The solution was then heated in an oven at 100°C to remove water. The obtained gelatin/HA powder was then characterized by FTIR and SEM. Then samples were molded into a cylindrical shape by being compacted with 4 tons load on a 13 mm diameter mold. Subsequently, the compressive strength was tested by a 10kN universal testing machine.

3. Result and Discussion

3.1. FTIR characterization of HA and gelatin/HA composites

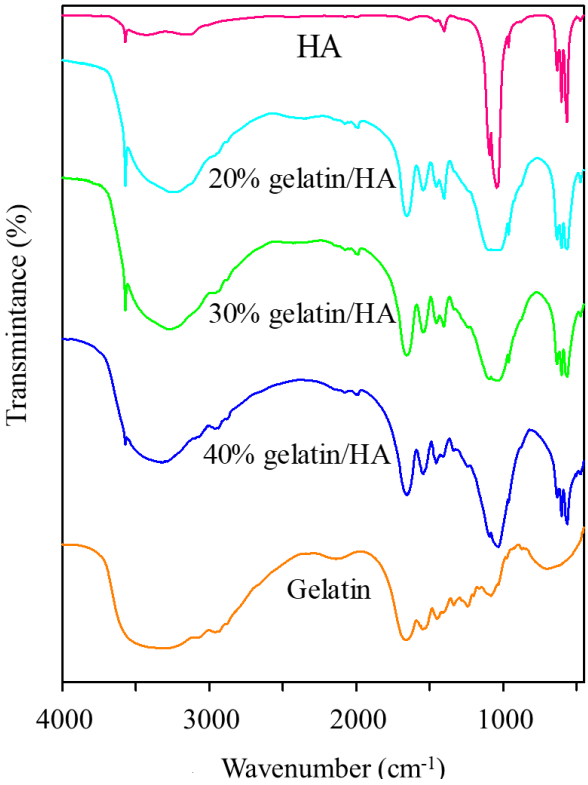

Figure 1. IR Spectra of HA, gelatin, and gelatin/HA composites.

IR spectrum revealed crucial information about the interactions between HA and gelatin. The IR spectra from HA and gelatin/HA composite shown in Figure. 1. The IR spectrum for all composites is very similar; the peaks are characteristic of groups derived from gelatin and HA. A broad peak shape for N-H in the secondary amide group (typical of gelatin) appearing at 3262.5 cm^{-1}; C=O from amide at 1655.1 cm^{-1} and N-H bending from amide at 1545.1 cm^{-1}. While the characteristics of HA are the P-O vibration in the phosphate group appears at 563.9; 602.2; 962.6; and 1041.2 cm^{-1}. At 631.8 cm^{-1} is H-O-H bending, at 3571.5 cm^{-1} is O-H stretching, and at 1402.6 cm^{-1} is a CO_3^{2-} group [1].

Changes in the spectrum in response to differences in the concentration of gelatin in the three composites cannot be adequately observed. However, qualitatively the difference in broadband intensity in the wavenumber 3500 cm^{-1} and the sharp band around 1000 cm^{-1}1 is slightly different. The functional groups detected on HA, gelatin, and gelatin/HA composites are shown in Table 1.

Table 1. Comparison of IR spectra of HA, gelatin, and gelatin/HA composites

No	Functional Groups Assignment	Wavenumber (cm^{-1})				
		HA	20% gelatin/HA composite	30% gelatin/HA composite	40% gelatin/HA composite	Gelatin
1	PO_4^{3-} stretching	565.6 and 962.9	563.9 and 962.6	564.0 and 96.,1	563.8 and 962.4	-
2	PO_4^{3-} stretching asymmetry	1043.1	1041.2	1043.5	1032.4	-
3	PO_4^{3-} bending asymmetry	602	602	602	602	-
4	H-O-H bending	632	631	631	632	-
5	O-H stretching	3571	3571	3571	3571	-
6	CO_3^{2-}	1401	1402.6	1403.5	1409.1	-
7	N-H secondary amide	-	3262.5	3278.8	3330.5	3297
8	C=O amide	-	1655.1	1657.1	1654	1659.9
9	N-H bending amide	-	1545.1	1544.1	1542.2	1547.4
10	C-O carboxyl	-	1240	1240	1240	1241.1
11	C-N amine	-	1336.3	1336.3	1336.3	1336.3

The shift of the gelatin wave number at 3297; 1659; 1547; and 1240 cm^{-1} confirms the formation of chemical bonds between amide and carboxyl in gelatin and Ca^{2+} in HA. During the gelatin-HA composite process, the interaction between Ca^{2+} ions with R-CON and R-COO from gelatin molecules may occur with mechanisms such as Lewis acids and bases [1].

3.2. SEM observation

SEM micrographs of HA and 20% gelatin/HA composite are illustrated in Figure. 2. The HA micrograph indicates HA morphology is agglomerate with spherical and porous particle shape.

Gelatin is expected shown by a red circle, while HA expected shown by a yellow square. Gelatin is present on the surface of HA that causes the density of 20% gelatin/HA composite higher than HA. The addition of gelatin to HA significantly changes the surface morphology of the composites. In general, the addition of gelatin increases the density between the grains. The density of HA composites has a significant effect on its mechanical properties (compressive strength). The compressive strength is increasing as the density of HA composites elevates [10].

The addition of gelatin to HA significantly changes the surface morphology of the composites. In general, the addition of gelatin increases the density between the grains. In line with the analysis of IR spectrum results, which predict strong electrostatic interactions between Ca^{2+} ions from HA with R-CON and R-COO from gelatin molecules, this interaction can reduce the distance between composite particles.

3.3. Mechanical Properties (Compressive Strength)

The compressive strength of HA and gelatin/HA composites were tested by a universal testing machine (data are shown in Table. 2). All the tests were conducted under a compressive load at a constant speed. The compressive strength test results showed that the addition of gelatin in the gelatin-HA composite could increase the compressive strength of HA. This confirms that the gelatin makes nature HA not fragile. The compressive strength test results also showed that the addition of 40% w/w gelatin concentration resulted in a decrease in the compressive strength of the sample because the properties of gelatin become dominant and there are groups in gelatin (amide and carboxyl) that not interact with HA [15].

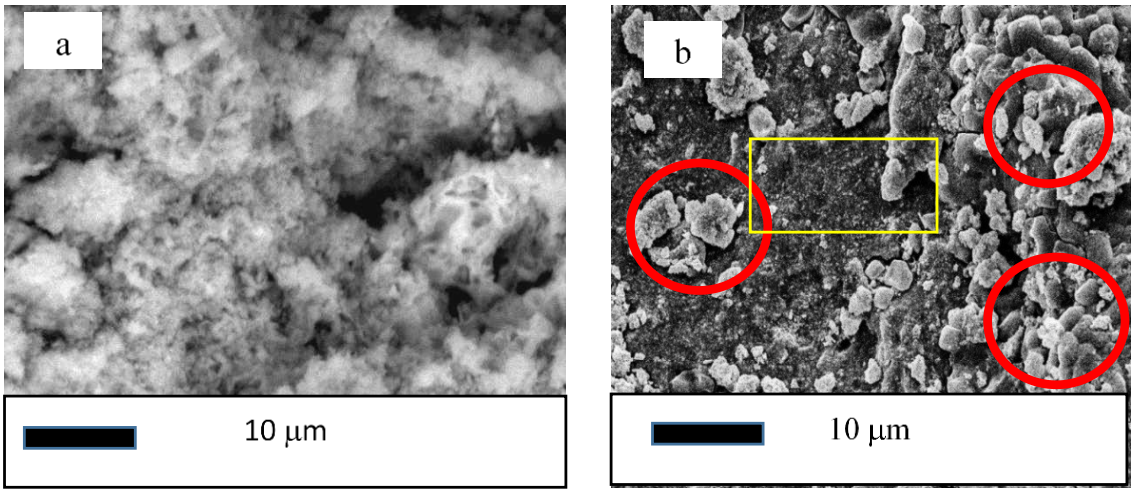

Figure 2. SEM micrograph of HA (a) and 20% gelatin/HA composites (b) surface.

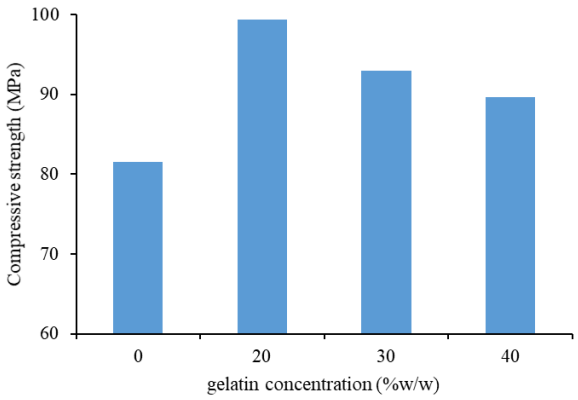

Figure 3. Compressive strength of HA and gelatin/HA composites

Mechanical properties of the gelatin/HA composites are influenced by many factors, such as particle shape, particle size, particle size distribution, the mechanical properties of the organic component (gelatin), the interfacial interactions between the HA and gelatin, and the ratio of HA and gelatin [16].

Table.1 Comparison compressive strength of HA and gelatin/HA composites

Sample	Compressive Strength/ MPa
0%	81.5
20%	99.3
30%	93
40%	89.6

The addition of gelatin to the gelatin/HA composite has been shown to increase its compressive strength. The addition of gelatin by 20, 30, and 40% gives a slightly different compressive strength. In the range of gelatin concentrations observed, the addition of 20% gelatin gives the highest compressive strength value of the composite and decreases with increasing concentration. Based on these results, it is clear that the precise concentration of gelatin against HA is needed. For further accuracy, it is necessary to increase the concentration of gelatin with a smaller concentration range, in the range x <20% <x. This assumption is reinforced by the SEM image; the addition of gelatin concentration will cover the surface more HA. In conclusion, HA has a limit on the ability to interact with gelatin, which consequently has decreased electrostatic interaction. Excess gelatin cannot interact with HA and accumulate on the composite surface. This phenomenon allows a decrease in the mechanical strength of the composite because the nature of gelatin becomes dominant, and there are groups in gelatin (amides and carboxyl) that do not interact with HA [15].

Gelatin/HA composite HA, 20, 30, and 40% have a compressive strength of 81.5; 99.3; 93; and 89.6 Mega Pascal (MPa), respectively. The compressive strength of gelatin/HA composites from this study was higher than that of similar studies, which were prepared by an in-situ method of 9.7, 25.6, and 32.1 MPA, respectively. Although researchers must determine the feasibility of this composite for biomaterial applications, for example, for cortical bone, the compressive strength needed is 100-230 MPa.

However, the results of this study can be concluded that the addition of gelatin can improve mechanical properties, especially the compressive strength approaching the compressive strength of the original bone [1].

4. Conclusion

Gelatin/HA composites have been successfully prepared using the wet-chemical method, from the precursors of CaO (from chicken egg cages) and DHP, in two stages. The IR spectrum confirms the interaction of the binding interface between HA and gelatin. The addition of gelatin increases compressive strength, and the composition of gelatin/HA 20% gives the highest compressive strength value, which is 99.3 MPa. SEM morphology shows the density of gelatin/HA composite increases with the addition of gelatin. The different stages of the composite preparation stage, the precursors used, and their composition largely determine the mechanical strength of the composite formed. Modification of the preparation and use of materials resulting from this research are expected to add a new route for preparing biomaterials that can be directly applied.

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References

[1] Mehdi Kazemzadeh Narbat, Fariba Orang, Mehran Solati Hashtjin and Azadeh Goudarzi, Fabrication of porous hydroxyapatite-gelatin composite scaffolds for bone tissue engineering, *Iranian Biomedical Journal*, 10, 4, (2006), 215-223

[2] Shao Ching Chao, Ming-Jia Wang, Nai-Su Pai and Shiow-Kang Yen, Preparation and characterization of gelatin–hydroxyapatite composite microspheres for hard tissue repair, *Materials Science and Engineering: C*, 57, (2015), 113-122 https://doi.org/10.1016/j.msec.2015.07.047

[3] V Rodríguez-Lugo, E Salinas-Rodríguez, RA Vázquez, K Alemán and AL Rivera, Hydroxyapatite synthesis from a starfish and β-tricalcium phosphate using a hydrothermal method, *RSC advances*, 7, 13, (2017), 7631-7639 https://doi.org/10.1039/C6RA26907A

[4] M. P. Ginebra, T. Traykova and J. A. Planell, Calcium phosphate cements as bone drug delivery systems: A review, *Journal of Controlled Release*, 113, 2, (2006), 102-110 https://doi.org/10.1016/j.jconrel.2006.04.007

[5] S. K. Yen and C. M. Lin, Cathodic reactions of electrolytic hydroxyapatite coating on pure titanium, *Materials Chemistry and Physics*, 77, 1, (2003), 70-76 https://doi.org/10.1016/S0254-0584(01)00562-4

[6] Wang Feng, Li Mu-sen, Lu Yu-peng and Qi Yong-xin, A simple sol–gel technique for preparing hydroxyapatite nanopowders, *Materials Letters*, 59, 8, (2005), 916-919 https://doi.org/10.1016/j.matlet.2004.08.041

[7] I. Mobasherpour, M. Soulati Heshajin, A. Kazemzadeh and M. Zakeri, Synthesis of nanocrystalline hydroxyapatite by using precipitation method, *Journal of Alloys and Compounds*, 430, 1, (2007), 330-333 https://doi.org/10.1016/j.jallcom.2006.05.018

[8] Purnendu Parhi, A. Ramanan and Alok R. Ray, A convenient route for the synthesis of hydroxyapatite through a novel microwave-mediated metathesis reaction, *Materials Letters*, 58, 27, (2004), 3610-3612 https://doi.org/10.1016/j.matlet.2004.06.056

[9] Zaw Linn Htun, Nurazreena Ahmad, Aye Aye Thant and Ahmad-Fauzi Mohd Noor, Characterization of CaO-ZrO2 Reinforced Hap Biocomposite for Strength and Toughness Improvement, *Procedia Chemistry*, 19, (2016), 510-516 https://doi.org/10.1016/j.proche.2016.03.046

[10] C. R. Gautam, Sunil Kumar, Vijay Kumar Mishra and Santoshkumar Biradar, Synthesis, structural and 3-D architecture of lanthanum oxide added hydroxyapatite composites for bone implant applications: Enhanced microstructural and mechanical properties, *Ceramics International*, 43, 16, (2017), 14114-14121 https://doi.org/10.1016/j.ceramint.2017.07.150

[11] Jayachandran Venkatesan and Se-Kwon Kim, Chitosan Composites for Bone Tissue Engineering—An Overview, *Marine Drugs*, 8, 8, (2010), 2252-2266 https://doi.org/10.3390/md8082252

[12] Naznin Sultana, Masturah Mokhtar, Mohd Izzat Hassan, Rashid Mad Jin, Fatemeh Roozbahani and Tareef Hayat Khan, Chitosan-based nanocomposite scaffolds for tissue engineering applications, *Materials and Manufacturing Processes*, 30, 3, (2015), 273-278 https://doi.org/10.1080/10426914.2014.892610

[13] Shu-Hua Teng and Peng Wang, One-pot synthesis of HA-coated gelatin microspheres by an emulsion method, *Materials Letters*, 65, 9, (2011), 1348-1350 https://doi.org/10.1016/j.matlet.2011.01.079

[14] Haile Abrham, Synthesis and characterization of zinc substituted Hydroxyapatite extracted from an egg shell by wet precipitation method, School of Mechanical, Chemical and Materials Engineering, Adama Science and Technology University,

[15] Cheng Xianmiao, Li Yubao, Zuo Yi, Zhang Li, Li Jidong and Wang Huanan, Properties and in vitro biological evaluation of nano-hydroxyapatite/chitosan membranes for bone guided regeneration, *Materials Science and Engineering: C*, 29, 1, (2009), 29-35 https://doi.org/10.1016/j.msec.2008.05.008

[16] Xuan Cai, Hua Tong, Xinyu Shen, Weixuan Chen, Juan Yan and Jiming Hu, Preparation and characterization of homogeneous chitosan–polylactic acid/hydroxyapatite nanocomposite for bone tissue engineering and evaluation of its mechanical properties, *Acta Biomaterialia*, 5, 7, (2009), 2693-2703 https://doi.org/10.1016/j.actbio.2009.03.005