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Development and Characterization of Calcium Based Biocomposites Using Waste Material (Calcite Stones) for Biomedical Applications

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Abstract

Calcium-based biocomposite materials have a pivotal role in the biomedical field with their diverse properties and applications in combating challenging medical problems. The study states the development and characterization of Calcium-based biocomposites: Hydroxyapatite (HAP), and PVA-Gelatin-HAP films. For the preparation of Calcium-based biocomposites, an unconventional source, the waste material calcite stone, was used as calcium raw material, and by the process of calcination, calcium oxide was synthesized. From calcium oxide, HAP was prepared by chemical precipitation method, which was later added in different proportions to PVA-Gelatin solution and finally dried to form biocomposite films. Then the different properties of PVA/Gelatin/HAP composite, for instance, chemical, mechanical, thermal, and swelling properties due to the incorporation of various proportions of HAP in PVA-Gelatin solution, were investigated. The characterization of the HAP was conducted by X-ray Diffraction Analysis, and the characterization of HAP-PVA-Gelatin composites was done by Fourier Transform Infrared Spectroscopy, Thermomechanical Analysis, Tensile test, Thermogravimetric Differential Thermal Analysis, and Swelling Test. The produced biocomposite films might have applications in orthopedic implants, drug delivery, bone tissue engineering, and wound healing.

Keywords

Hydroxyapatite, Calcium-Based Biocomposites, PVA-Gelatin Films, Drug Delivery, Bone Tissue Engineering

1. Introduction

The development of biocompatible composites has drawn a growing interest in recent years due to the demand for novel and sustainable materials for biomedical applications. These composites are crucial for meeting the ever-changing requirements of regenerative medicine and modern healthcare because of their wide range of traits and applications. A component or mixture of substances that makes up an object is known as a "material." They can be pure or impure materials, organic or inorganic. Additionally, materials can be classed according to their physical and chemical qualities, as well as their geological origin and biological function [1]. Materials are categorized into three broad categories based on their chemical and atomic structure: Metals, ceramics, and polymers. Furthermore, composites consist of at least two distinct material types. Nanomaterials, biomaterials, and energy materials are just a few of the new and sophisticated materials being produced [2].

A biomaterial is typically defined as any natural or manufactured biocompatible material that is utilized to replace or assist a portion of an organ or tissue while maintaining close touch with living tissue. They can be made from a variety of materials, including solids, liquids, and gels (metallic components, polymers, ceramics, or composite materials). Biomaterials, an intriguing and highly interdisciplinary field, have evolved into a critical component of modern advancements in human health and quality of life. It is performed by treating a variety of health-related challenges originating from a variety of sources. Biomaterials have expanded their applications over the last few decades, from diagnostics (gene arrays and biosensors) and medical equipment (blood bags and surgical tools) to therapeutic medications (medical implants and devices) and emerging regenerative therapies (tissue-engineered skin and cartilage) [3].

Numerous biomaterials, both natural and synthetic, have been studied as scaffolds for bone repair. Among these, calcium phosphate-based materials, particularly bioactive hydroxyapatite, are commonly used due to their chemical and structural similarities to bone's mineral component. Natural bone is a three-dimensional structure composed of organic and mineral phases, with collagen serving as the primary organic constituent and HAP serving as the primary mineral constituent. To create a scaffold that closely resembles the natural structure of bone, composite systems have been designed that combine the desired features of organic and mineral components into a single material system. Polymer/ceramic systems such as HAP/collagen, HAP/chitosan, HAP/collagen/poly (lactic acid), HAP/alginate/collagen, and HAP/gelatin are the most extensively researched composites for creating tissue engineering scaffolds. Because of multiple physiologic functional groups that stimulate osteoblast adhesion, migration, and mineralization, gelatin (GEL), a collagen derivative, is an intriguing component for extracellular matrix replacement. Polyvinyl alcohol possesses outstanding film-forming, emulsifying, and adhesive characteristics. Additionally, it is impervious to oil, grease, and solvents. It possesses exceptional tensile strength and flexibility, as well as excellent oxygen and odor barrier qualities [4].

Hydroxyapatite (HAP) is a calcium phosphate with form and content identical to human hard tissues. One of the most important aspects of hydroxyapatite compared to other calcium phosphates is its stability. Hydroxyapatite is the most thermodynamically stable calcium phosphate molecule under physiological conditions, for instance, temperature, pH, and body fluid composition. The advancement of nanotechnology has had a significant impact on materials science. Nanomaterials have gotten much attention lately for adsorption, catalysis, and optical applications, especially when biomaterials are involved. A mineral is found in great amounts in the enamel of bones and teeth. Due to its outstanding properties: Biocompatibility, Bioactivity, Osteoconductivity, and Non toxicity and non-inflammatory nature. Metal items are frequently coated with hydroxyapatite (HA) to increase their biocompatibility. Under atmospheric conditions, crystalline HA particles are disseminated inside an amorphous calcium phosphate matrix to form HA coatings. Even with abundant HA, the early breakdown of the coating's amorphous phase during bone remodeling leads to the release of calcium phosphate particles with a wide range of characteristics and compositions.

Extensive research has been conducted on the synthesis of hydroxyapatite (HAP) using natural and renewable resources. Natural hydroxyapatite is commonly obtained from various biological sources and waste materials, including mammalian bone (such as bovine, camel, and horse), marine or aquatic sources (such as fish bone and fish scale), shell sources (such as cockle, clam, eggshell, and seashell), as well as plants, algae, and mineral sources (such as limestone) [5].

Londono-Restrepo *et al.* employed a hybrid approach involving hydrothermal and calcination methods to extract hydroxyapatite (HAp) from bovine bone. The hydrothermal method was used to eliminate the excess fat and protein from the bone. The bone was subsequently subjected to calcination at temperatures ranging from 700˚C to 1100˚C. The morphology of Hap underwent alterations in response to changes in calcination temperatures, such as being irregular at 700˚C and becoming semi-spherical at 800˚C. The production of hydroxyapatite (HApdehydroxylate) was observed at temperatures over 800˚C [6]. Sunil & Jagannatham documented the production of highly crystalline pure hydroxyapatite (HAp) from fish bones through calcination at temperatures ranging from 600 to 1000 degrees Celsius. The sample subjected to calcination at a temperature of 600˚C exhibited a Ca/P ratio of 1.63, which is marginally below the stoichiometric HAp ratio. Furthermore, the EDS analysis of the sample subjected to calcination at a temperature of 600˚C detected the existence of magnesium ions in the sample. These magnesium ions serve as micronutrients that support metabolic processes in tissues [7]. The cockle shell was subjected to varying calcination temperatures (450˚C and 800˚C) by Mohamad Razali *et al.*, who subsequently employed a hydrothermal technique to generate hydroxyapatite (HAp). The cockle shell that underwent calcination consisted of calcim carbonate ($CaCO_3$). This calcium carbonate was subsequently subjected to treatment with di-ammonium

hydrogen phosphate ($(NH_4)_2HPO_4$) and water in order to convert $CaCO_3$ to hydroxyapatite (HAp) at a molar ratio of 5:3:1 of $CaCO_3$:$(NH_4)_2HPO_4$:H_2O. The pH of the mixture was adjusted to 10.5 by adding the ammonia solution. Subsequently, the mixture underwent a hydrothermal reaction at a temperature of 100˚C for a duration of 1 hour, while being continuously stirred. The solution was subsequently washed and filtered prior to desiccation in order to acquire HAp powder. The X-ray diffraction (XRD) analysis revealed that the sample subjected to calcination at 450˚C yielded a mixture of hydroxyapatite (HAp) and calcite, whereas calcination at 800˚C resulted in the production of pure HAp. The observed calcite peak can be attributed to the incomplete conversion of calcite to hydroxyapatite (HAp) at a temperature of 450˚C. The used technique yielded rod-shaped structures measuring 207 nm in length and 27 nm in diameter [8].

In contrast, the primary objective of this study is to investigate and analyze calcium-based biocomposite: hydroxyapatite (HAP) generated from an unconventional source, namely waste material-derived calcite stone. The calcite stone, which had been thoroughly cleansed, was pulverized into a fine powder through the utilization of a mortar and pestle. The calcite powder underwent calcination at a temperature of 1000 degrees Celsius in order to produce calcium oxide, which is an essential constituent for the formation of hydroxyapatite (HAP). The HAP material was synthesized by the precipitation process using CaO as a precursor. Calcite, an inherent manifestation of calcium carbonate, has been well acknowledged for its ample presence, economic value, and compatibility with biological systems. The incorporation of waste material calcite stone as a primary constituent in the production of biocomposites is in accordance with the concepts of sustainability and resource efficiency, hence facilitating waste reduction and promoting advancements in the realm of biomedical materials.

Furthermore, much research has been conducted on the advancement of thin films through the utilization of Gelatin-PVA, Gelatin-HAP, and PVA-Gelatin-HAP combinations. In their study, Al-Mamun *et al.* (2020) fabricated gelatin-PVA films through the combination of PVA and gelatin solutions at a 1:1 ratio. The blending process was conducted at a pH of 2, and the resulting mixture was cast onto silicon cloths. Subsequently, the films were dried under ambient air conditions until they reached their final form. The film underwent crosslinking using gamma-irradiation at various dosages for a duration of one hour [9]. In a study conducted by Md. JakirHossan *et al.*, in 2014, composites consisting of Gelatin and Hydroxyapatite (HAP) were synthesized using the freeze-drying process. The thermal stability, mechanical stability, and morphological potentiality of gelatin composites with varying concentrations of hydroxyapatite (HAP) were then investigated in relation to their suitability for bone tissue engineering [10]. In their study, Basak *et al.* (2018) fabricated a film composed of Hydroxyapatite (HAP), Gelatin, and Polyvinyl Alcohol (PVA). This was achieved by combining solutions of PVA and gelatin in a ratio of 1:2 and subsequently incorporating 200 mg of HAP into 10 mL of acetic acid. The

solution underwent crosslinking with the addition of 2% glutaraldehyde in a dropwise manner, followed by casting at a temperature of 40˚C for 24 hours. The biocompatibility of the produced film was assessed in order to determine its potential suitability for various medical applications. This evaluation involved conducting various physical characterizations [4].

In this study, we prepared the PVA-gelatin composites with different concentrations of HAP (100 mg, 200 mg, 400 mg, and 500 mg) by solvent casting method. The HAP used for the development of the composite was synthesized from an unconventional source: waste material derived from calcite stone, as mentioned earlier. Then, we conducted characterization for different chemical, mechanical, thermal, and swelling properties for each type of composite to observe whether they have the potential to be used in biomedical applications. The Thermomechanical Analysis and Thermogravimetric Differential Thermal Analysis for the thermal property, the tensile test for the mechanical property, the swelling test for the water absorption property and Fourier Transform Infrared Spectroscopy for the chemical analysis of PVA-gelatin composites were conducted.

2. Material and Methodology

2.1. Chemicals

Calcite stone was collected from the waste material gathered at the construction site in Bangladesh Council of Scientific and Industrial Research (BCSIR), Dhaka, Bangladesh, PVA[$(-C_2H_4O)_n$](Merck Specialities Private Limited, Worli, Mumbai, India), citric acid monohydrate crystal extra pure ($C_6H_8O_7{\cdot}H_2O$) (Merck Specialities Private Limited, Worli, Mumbai, India), Disodium hydrogen phosphate anhydrous, ExpertQ®, ACS, Reag. PhEur (Scharlau, Istanbul, Turkey), Sodium dihydrogen phosphate dihydrate, extra pure, Pharmpur®, PhEur, BP, USP ($NaH_2PO_4{\cdot}2H_2O$) (Scharlau, Istanbul, Turkey), Sodium hydroxide pellets for analysis (NaOH) (Merck KGaA, Darmstadt, Germany), Gelatin purified (Merck Specialities Private Limited, Worli, Mumbai, India), Glutaraldehyde solution 25% ($[CH_2(CH_2CHO)_2]$ (anhydrous) (Merck Specialities Private Limited, Worli, Mumbai, India)) and acetic acid glacial (CH_3COOH) (BDH Chemicals Ltd Poole England).

2.2. Preparation of Calcium Carbonate ($CaCO_3$)

A block of calcite stone was acquired from the waste material collected during construction work. The calcite stone was washed and cleaned properly. It was then broken down into smaller pieces using a steel mortar and pestle. The pieces were crushed into a fine powder of calcium carbonate using an agate mortar and pestle.

2.3. Preparation of Calcium Oxide (CaO)

Calcium oxide was prepared from calcium carbonate by calcination. Three por-

celain basins were half-filled with finer granules of calcium carbonate (micro size) and placed into the furnace (AWF 13/42, Lenton, UK) at 800 degrees Celsius. Here the samples were heated from the room temperature (25 degrees Celsius) upto 800 degrees Celsius at the heating rate of 10 deg/ min and was hold at that temperature for 24 hours inside the furnace. Then the furnace was allowed to cool down.

2.4. Preparation of Hydroxyapatite (HAP)

Hydroxyapatite is produced by reacting disodium hydrogen phosphate and calcium oxide. First, 1 gm of CaO was taken in a beaker containing 100 ml of water and was stirred in a magnetic stirrer for 30 minutes. Next, 19.2125 gm of citric acid (it maintains the crystal size of the nanoparticles) was added to the beaker and stirred until the citric acid was dissolved and a clear solution was produced. The beaker was then placed in the ultra-sonicator. When the temperature reached 45˚C, 1.9656 gm of disodium hydrogen phosphate was added in small quantities. During this period, the pH of the solution was measured, and sodium hydroxide (2M solution) was added to make the pH 11. For this whole procedure, the temperature was maintained at around 65°C. The beaker was removed from the ultrasonicator and placed in the magnetic stirrer at approximately 50 degrees Celsius for about 3 hours to complete the reaction. Then, the heating is stopped and the beaker was left undisturbed. A white precipitate was formed at the bottom of the beaker after it was left undisturbed overnight. Then, the solution was filtered with distilled water. Then, the filter papers with the white ppt. were air dried. Finally, the air-dried HAP was crushed into a finer powder.

2.5. HAP/Gelatin/ PVA Film Synthesis

10 mL of acetic acid solution was taken in a beaker, and 0.4%Hydroxyapatite, 5 mL of 5% PVA solution, and 25 ml 5% of gelatin solution were added to the acetic acid solution. The solution was placed in a magnetic stirrer and stirred for 30 minutes at 40 - 60 degrees Celsius, as shown in **Figure 1(a)**. Then 1 mL of 2% Glutaraldehyde solution was added dropwise to the solution, and the solution was again stirred for 30 minutes at about 600 rpm and 40 degrees Celsius (**Figure 1(b)**).

Then, the solution was solvent cast in a mold of thermal paper in a petri dish and let dry at room temperature as shown in **Figure 1(c)**. After drying, a film-type material was formed, as shown in **Figure 1(d)**. The above-described process was followed to prepare three more films by only varying the weight of Hydroxyapatite to 0.2% (**Figure 1(e)**), 0.8% (**Figure 1(f)**), and 1% (**Figure 1(g)**). A Gelatin-PVA film was also prepared similarly without the Hydroxyapatite, as shown in **Figure 1(h)**.

2.6. Preparation of PVA Film

10% PVA was measured and taken in a beaker with 100 ml water. Then, the beaker was placed on a magnetic stirrer at 90 degrees Celsius for 2 hrs to dissolve

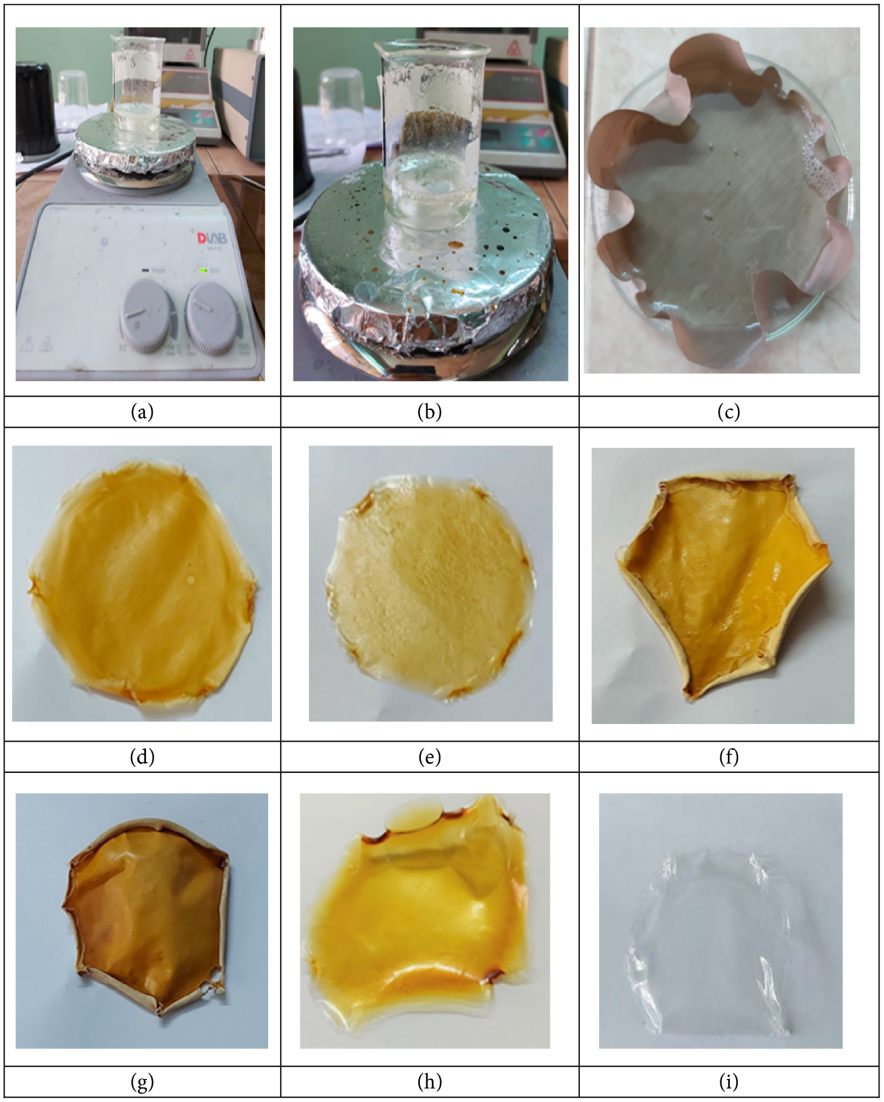


Figure 1. (a) The solution with HAP, PVA, Gelatin and acetic acid being stirred at 40 - 60 degrees on the magnetic stirrer, (b) The final solution after adding the crosslinking agent Glutaraldehyde being stirred at 40 degrees, (c) The solution being solvent casted in a thermal paper mold in a petri dish, (d) The HAP-PVA-Gelation film with 0.4% HAP concentration, (e) The HAP-PVA-Gelatin film with 0.2% HAP concentration, (f) The HAP-PVA-Gelatin film with 0.8% HAP concentration, (g) The HAP-PVA-Gelatin film 1% HAP concentration, (h) The PVA-Gelatin film without HAP, (i) PVA film.

all the PVA beads. Then, the hot PVA solution was solvent cast in a mold of thermal paper in a petri dish and let dry at room temperature. After drying, a film-type material was formed, as shown in **Figure 1(i)**.

2.7. Characterization

2.7.1. X-Ray Diffraction Analysis (XRD)

The phase analysis of the HAP powder and composite samples was done by XRD (Model X'Pert Pro, Manufacturer: Panalytical, Netherland) using 40 mA and 40 kV current. The mean crystallite size was calculated using Scherer's equa-

tion, that is, $D = 0.9/\beta\cos\theta$, where D is the average crystallite size in A˚, β is the peak broadening of the diffraction line measured at half of its maximum intensity in "radian", λ is the wavelength of X-rays, and θ is the Bragg's diffraction angle [4].

2.7.2. Fourier-Transform Infrared Spectroscopy (FTIR)

The goal of FTIR is to find different functional groups in the present sample [10]. The identification of functional groups in the 0.2%, 0.4%, 0.8%, and 1% HAP-Gelatin-PVA composites was analyzed by FTIR analysis on Nicolet iS5 (4.16A, 12V DC) [4].

2.7.3. Thermomechanical Analysis (TMA)

Thermomechanical analysis was performed by TMA/SS 6300, SII NanoTechnology, Japan, system controlled by an EXSTAR 6300. TMA experiments were conducted on Gelatin-Hap composite samples of varying weights after calibrating the machine. Simultaneous TMA analysis was used to heat the sample at a rate of 5˚C/min in the temperature range of 25 to 100˚C in a nitrogen atmosphere. The Thermal Mechanical Analyzer (TMA) uses the linear coefficient of thermal expansion as a means to identify changes in the characteristics and structure of materials. On a quartz platform, a little sample of polymeric material was heated, and a rod applied a small amount of pressure. The substance expanded as the samples warmed up, and the rod moved around. Data was given as a function of the material's dimensional change over time, and significant milestones were noted [11]. The TMA curves for 0.2%, 0.4%, 0.8%, and 1%HAP-Gelatin-PVA and PVA/gelatin films were obtained.

2.7.4. Thermogravimetry Differential Thermal Analysis (TG/DTA)

Thermogravimetry Differential Thermal Analysis was performed by TG/DTA 6300, SII Seiko Instrument Inc, Japan, a system controlled by an EXSTAR 6000 controller. A thermogravimetric/differential thermal analysis (TG/DTA) is a technique used to quantify the change in weight of a sample in relation to temperature (and/or time) while maintaining a controlled gas environment and temperature conditions. Using a graph to depict the percentage change in weight throughout a predetermined range of temperatures facilitates the analysis of physical or chemical phenomena resulting in the sample experiencing weight loss or growth [12].

TG/DTA experiments were conducted on Gelatin-Hap composite samples of varying weights after calibrating the machine. Here, a small sample weight of 3.211 mg was taken in an aluminum crucible and placed in the crucible chamber. The analysis was conducted by comparing the composites with the aluminum metal starting at around 25 degrees Celsius up to 600 degrees Celsius, where the temperature was increased by about 20 degrees per minute in a nitrogen atmosphere. For the HAP 0.2%, 0.4%, 0.8%, and 1%/Gelatin/PVA and PVA/gelatin films, the TG curves and The DTA curves were obtained, respectively.

2.7.5. Swelling Test

The swelling test was performed to observe the water absorption and sustainability in water with the samples of dimension 1 cm × 1 cm. Pre-weighed air-dried composite films with various HAP-PVA-Gelatin film loading were taken and soaked in 100 mL distilled water for 5 minutes, 15 minutes, and 45 minutes. The weight of the HAP-PVA-Gelatin film with different concentrations of HAP was measured after soaking for 5 minutes, 15 minutes, and 45 minutes. Then, the water absorption was calculated using the following equation.

$$DS\ (\%) = [(Ws - Wo)/Wo] \times 100\% \quad (1)$$

where DS (%) was the degree of swelling, Wo was the weight of films before immersing, and Ws was the weight of swollen films [13].

2.7.6. Tensile Test

Tensile strength (TS), force at break, and percent elongation at break (Eb) of the composites were measured by a Universal Testing Machine (Model H10K, Hounsfield, UK) following ASTM D3039 having efficiency within ±1%. The machine speed was 100 mm·min^{-1} with gauze length and a load of 8 cm and 500 N, respectively. 4 different composites with different concentrations of HAP (HAP-0.2%, 0.4%, 0.8%, and 1%) in PVA-Gelatin and 10% PVA films were analyzed. The films were cut into rectangular sizes with the dimensions of 0.1 mm, width 5 mm, and 8 mm and the cut sample was placed into the machine along the length.

3. Result and Discussion

3.1. Characterization Study

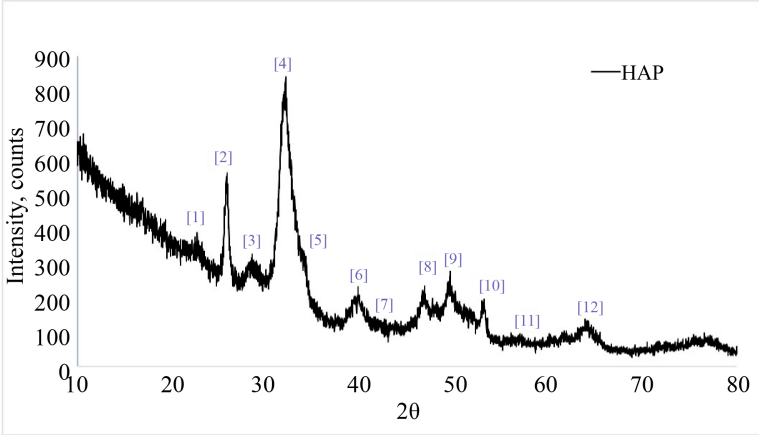
3.1.1. XRD Study

The diffractogram resulting from XRD analysis of the synthesized powder is shown in **Figure 2(a)**. The straight baseline and the sharp peaks crystal of this diffractogram marked as [1]-[12] in the 20 to 70 degrees range, which confirms that the product is well crystallized. The peaks are obtained at 22.8˚, 25.812˚, 28.5˚, 31.975˚, 33.49˚, 41.4˚, 46.78˚, 49.5˚, 53.172˚, 56.91˚, and 63.94˚ [4]. The pattern perfectly matched with JCPDS 00-001-1008 (**Figure 2(b)**).

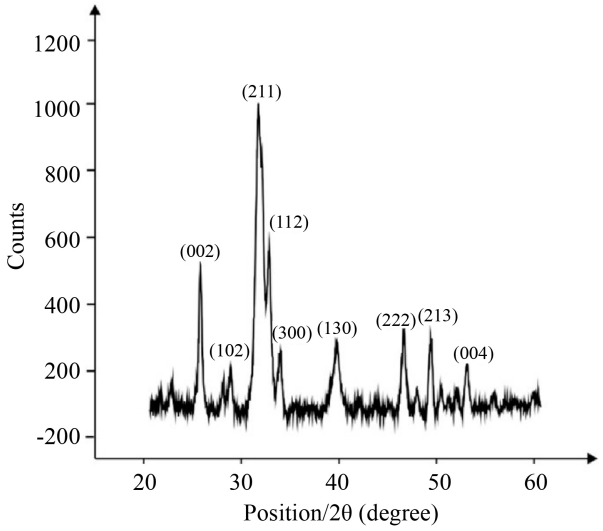
3.1.2. FTIR Study

In **Figure 3(a)**, the FTIR study clearly states the presence of the OH, C-H, and C-O-C functional groups in 100% PVA. The peak observed at 3248.5 cm^{-1} is due to the presence of the OH group. This corresponds to the stretching vibration of the hydroxyl group. The peaks at 2936 cm^{-1} and 2906 cm^{-1} are due to the stretching vibrations of C-H bonds. The peak at 1138 cm^{-1} is associated with the stretching vibrations of the C-O-C linkage.

FT-IR spectra confirmed the interfacial interaction between PVA-Gelatin/HAP composites in **Figure 3(b)**. In **Figure 3(b)**, for the composite with 1% HAP, the peaks around 3350 cm^{-1} are associated with the OH stretching due to the presence of PVA in the composite. Similarly, for the composite with 0.8%,

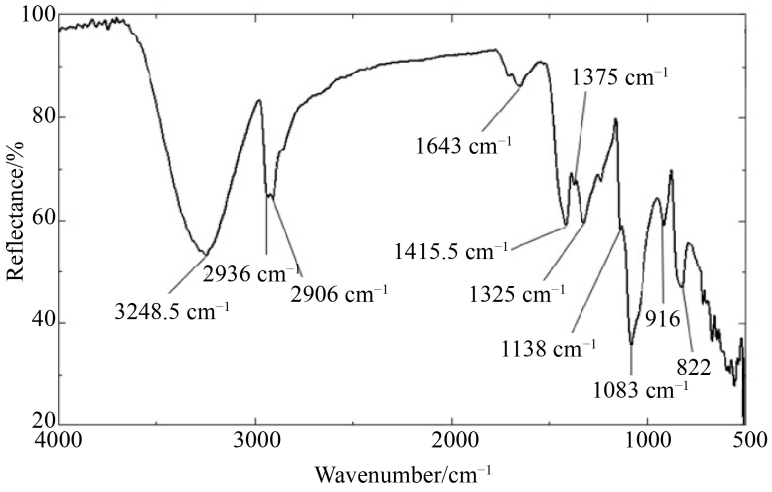


(a)

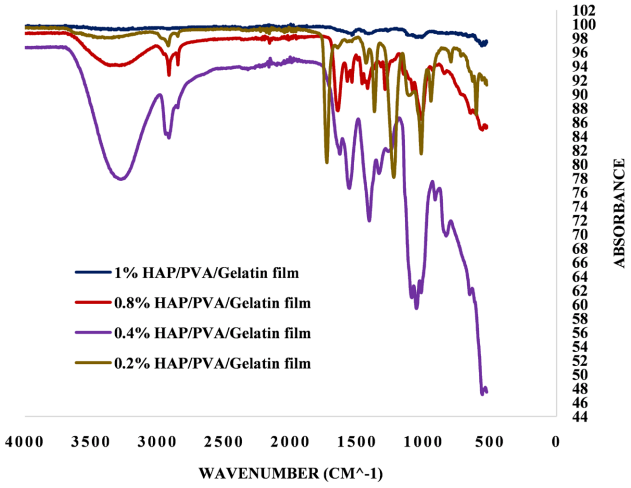


(b)

Figure 2. XRD of (a) HAP prepared from waste material calcites, (b) Standard HAP pattern—JCPDS (Joint Committee on Powder Diffraction Standards) 01-072-1243 [16].



(a)



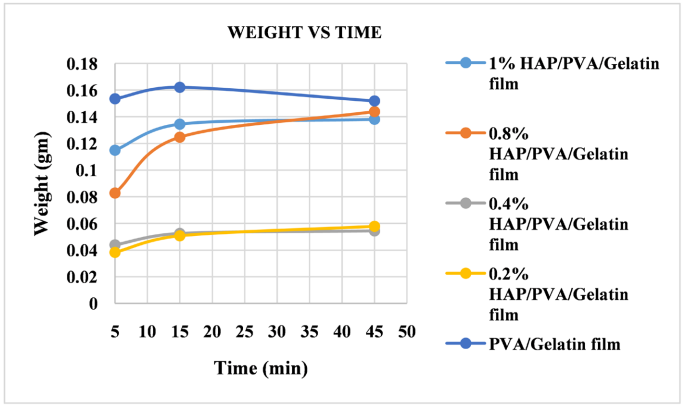
(b)

Figure 3. FTIR study of (a) 100%PVA [14], (b) PVA-Gelatin/1% HAP composite, PVA-Gelatin/0.8% HAP composite, PVA-Gelatin/0.4% HAP composite, PVA-Gelatin/0.2% HAP composite.

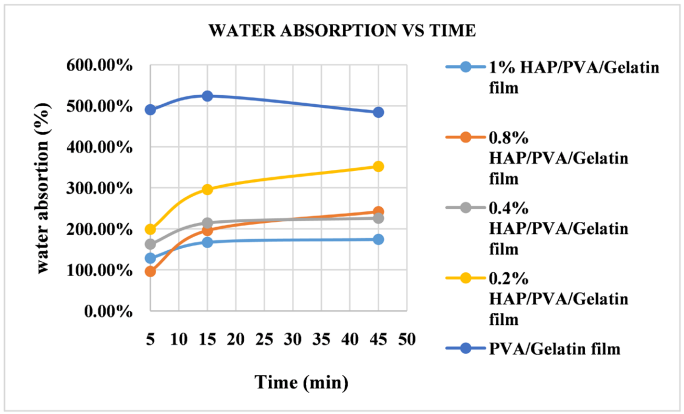
0.4%, and 0.2% HAP, the peaks due to the O-H stretching changes to 3300 cm^{-1}, 3400 cm^{-1}, and 3390 cm^{-1}, respectively. For the composite with 1% HAP, the band of 1050 cm^{-1} is due to stretching vibrations of P-O bonds. Now, for the composite with 0.8%, 0.4%, and 0.2% HAP, the band due to stretching vibrations of P-O bonds changes to 1010 cm^{-1}, 1040 cm^{-1}, and 1005 cm^{-1}, respectively. The peaks at 1500 cm^{-1} and 1600 cm^{-1} could be attributed to the presence of aromatic C=C bonds stretching vibrations for almost all the types of composites with different concentrations of HAP. The change in concentration of HAP in the composite did not drastically shift the peaks for the O-H stretching, P-O stretching vibrations, and the presence of aromatic C=C bonds [4] [10].

3.1.3. Swelling Test

Within the context of the multiple biopolymer, each individual biopolymer not only independently influences the characteristics of the film but also participates in interactions with other biopolymers, thereby influencing the overall properties of the system. At times, these relationships hold greater significance compared to individual actions [15]. Chemical structure, the ratio of HAP in the biocomposite, and the synergistic effect between PVA, gelatin, and HAP influence the permeability of water of the PVA/Gelatin/ HAP biocomposite films. In **Figure 4(a)**, the weight of PVA/gelatin is the highest. It can happen due to the hydrophilic nature of PVA and gelatin that allows them to absorb water through hydrogen bonding and other interactions readily. In the early stages of immersion, PVA and Gelatin films can rapidly absorb water. The hydrophilic groups within the polymer structures facilitate this initial uptake of water molecules. When PVA and Gelatin come into contact with water, they can form a hydrogel matrix. This hydrogel structure can retain a significant amount of water, contributing to high water absorption. Although HAP (Hydroxyapatite) exhibits a



(a)



(b)

Figure 4. (a) The weight values of the PVA/ Gelatin biocomposite with different concentrations of HAP after immersing in distilled water for 5 min, 15 min and 45 min, (b) Water uptake-time values obtained for PVA/ Gelatin biocomposite with different concentrations of HAP.

certain degree of hydrophilicity, it is possible that its hydrophilic properties are somewhat less pronounced when compared to those of PVA and Gelatin. Thus, the addition of HAP counterbalances the comparatively greater hydrophilicity exhibited by PVA and gelatin in the composite material, resulting in a modification of the equilibrium of water absorption. Films with high initial water absorption may be preferred in applications where rapid hydration is essential, such as wound dressings or drug delivery systems. However, when different concentrations of HAP are incorporated, this weight is changed significantly, where composite with 0.2% HAP has the least weight and with 1% has the maximum weight. This weight change occurs because the use of hydroxyapatite, a ceramic material with inherent porosity, can introduce supplementary microstructures inside the composite. An increase in the concentration of HAP might lead to an increase in the overall porosity of the composite. These holes have the potential to function as reservoirs for the absorption of water. The hydrophilic nature of gelatin is characterized by its propensity to readily absorb water. With a rise in HAP concentration, Gelatin may continue to be present in substantial quantities, hence adding to the composite's overall hydrophilicity. Both hydroxyapatite

(HAP) and gelatin exhibit the ability to engage in interactions with water molecules via hydrogen bonding and other attractive forces. These interactions have the potential to enhance the process of water absorption, leading to the gradual uptake of water molecules by the composite material. Capillary action can be induced within the composite through the incorporation of Hydroxyapatite (HAP) and Gelatin, resulting in the formation of capillary-like structures. These structures have the capacity to attract and retain water, resulting in a gradual augmentation in mass.

In Figure 4(b), owing to the hydrophilicity of PVA and gelatin, the PVA/Gelatin shows a high water absorption. However, as different concentrations of HAP are added to the PVA/gelatin biocomposite, there is a significant decrease in the water absorption of the biocomposite. The PVA/Gelatin film with 0.2% has the highest water absorption among the four biocomposite films. Then, increasing the HAP concentration to 0.4%, 0.8%, and 1%, the water absorption of the biocomposites decreases and becomes the least for the PVA/gelatin film with 1% HAP. In this system, irrespective of the concentration of HAP in the PVA/gelatin, the water absorption after 45 min slows down and reaches a plateau, corresponding to the water uptake equilibrium. Nevertheless, the PVA/Gelatin composite, before reaching its plateau state, starts to dissolve in water in very small quantities. In general, with the increase of concentration of HAP in the composite, the water absorption for the composite also increases. But sometimes, the opposite may happen due to various factors. One of the reasons might be that the PVA dissolves in the water as the time passes. Thus, the composite loses the capability to absorb water resulting in the decrease of % water absorption. Besides, the absorption behavior of water can be influenced by several environmental factors, including temperature and humidity. Variations in water absorption may occur as a result of alterations in such parameters.

It is imperative to consider the water absorption properties of the PVA/Gelatin/HAP system while considering its prospective uses. In the field of tissue engineering, the regulation of water absorption plays a pivotal role in creating an optimal cellular environment for growth. Similarly, in drug delivery systems, the extent of water absorption can significantly influence the kinetics of drug release.

3.1.4. Thermomechanical Analysis (TMA)

TMA analysis, the change of dimension of the samples is observed with respect to the change of temperature. In Figure 5(a), the PVA-Gelatin/ 1% HAP composite initially contracts as the temperature increases, and this contraction remains almost constant and occurs approximately between the temperatures 29.98 degrees to 31.99 degrees Celsius. Then the sample starts softening around 32.01 degrees Celsius and continues up to 80.11 degrees Celsius finally and melts down completely.

Figure 5(b), the PVA-Gelatin/0.8% HAP composite initially contracts as the temperature increases, and this expansion occurs approximately between the temperatures 33.84 degrees to 56.02 degrees Celsius. Thus, the sample takes

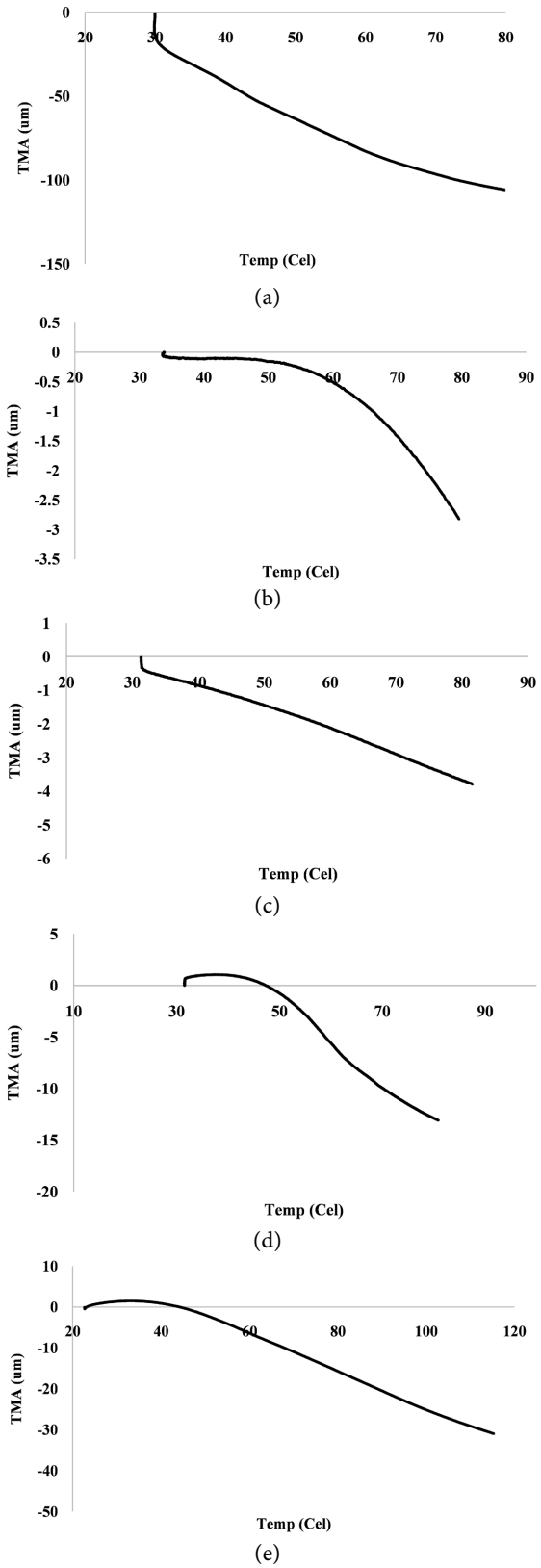


Figure 5. TMA curves of (a) PVA-Gelatin/1% HAP composite, (b) PVA-Gelatin/0.8% HAP composite, (c) PVA-Gelatin/0.4% HAP composite, (d) PVA-Gelati/0.2% HAP composite, (e) PVA/Gelatin composite.

quite some time to reach the softening point. Then the sample starts softening around 56.04 degrees Celsius and continues up to 79.58 degrees Celsius finally and melts down completely.

Figure 5(c), the PVA-Gelatin/0.4% HAP composite initially contracts as the temperature increases, and this contraction remains almost constant and occurs approximately between the temperatures 31.32 degrees to 33.26 degrees Celsius. Then the sample starts softening around 33.27554 degrees Celsius and continues up to 81.55 degrees Celsius finally and melts down completely.

In Figure 5(d), the PVA-Gelatin/0.2% HAP composite initially expands as the temperature increases, and this expansion occurs approximately between the temperatures 31.55 degrees to 39.69 degrees Celsius. Then the sample starts softening around 39.7 degrees Celsius and continues up to 80.9 degrees Celsius finally and melts down completely.

In Figure 5(e), the PVA/Gelatin composite initially expands as the temperature increases, and this expansion occurs approximately between 23.17 degrees to 44.14 degrees Celsius. Then the sample starts softening around 44.15 degrees Celsius and continues up to 115.26 degrees Celsius finally and melts down completely.

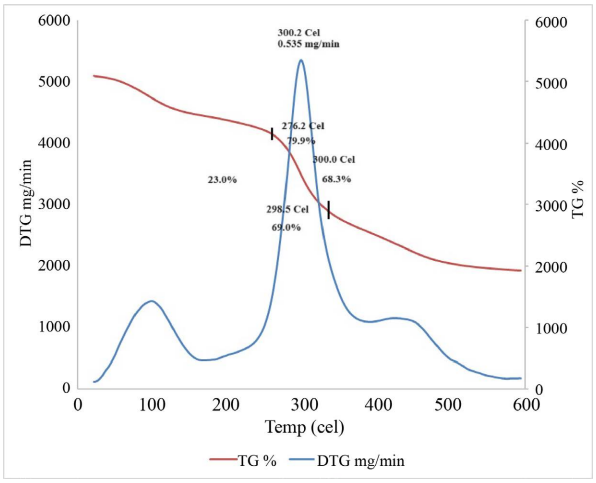
From the TMA analysis of the samples, we observe that the PVA/gelatin composite shows the expansion of its dimension with the increase in temperature. As the concentration of HAP increases in the PVA/Gelatin composite, 0.4%, 0.8%, and 1% HAP-containing composites show contraction in their dimensions. Such contractions occur because HAP has a low thermal expansion from the PVA and Gelatin, leading to the contraction of the composite. Besides, cross-linking or strong interactions between HAP and the polymer matrix can reduce thermal expansion and result in contraction in the TMA curve. Again, the PVA-Gelatin/0.8% HAP composite shows more contraction than the 1% HAP-containing composite. It might happen due to the distribution and dispersion of HAP within the composite. At lower concentrations, HAP particles may be less uniformly dispersed, potentially causing localized and pronounced thermal expansion for the lower-concentration composite. As concentration increases, dispersion decreases, leading to less expansion and potential contraction. However, only the 0.2% HAP-containing composite shows expansion. Because HAP can undergo phase transitions or sintering at specific temperatures at lower concentrations, these effects may not be significant, and the composite may exhibit expansion. However, as HAP concentration increases, these phase transitions may become more pronounced, leading to contraction. The specific processing conditions used to prepare the composite can affect the distribution of HAP particles and their interactions, which, in turn, can influence thermal expansion properties.

3.1.5. Thermogravimetry Differential Thermal Analysis (TG/DTA)

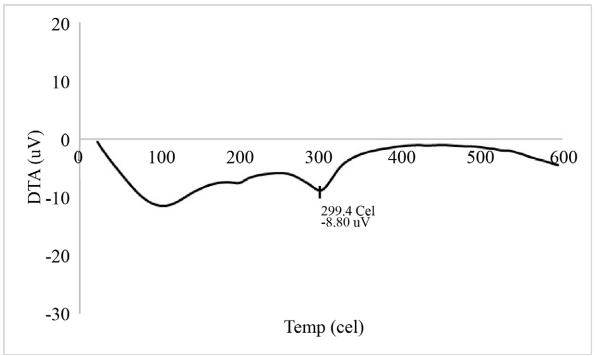
The physical characteristics of the polymer used to prepare the biocomposite can be seen by TGA. TGA demonstrates how mass changes as the temperature rises. Figure 6(a) shows the TG curve for PVA-Gelatin/1% HAP composite where the

onset temperature, first 50% degradation temperature, and maximum slope are 276.2 degrees Celsius, 300 degrees Celsius, and 298.5 degrees Celsius, respectively. The total degradation loss is 23%. The DTG curve shows one significant peak at 300.2 degrees Celsius. There is one step of crystallization or phase transition due to the crystallization of the composite. Figure 6(b) shows the DTA curve shows the exothermic peak at 299.4 degrees Celsius due to heat release in crystallization or phase transition.

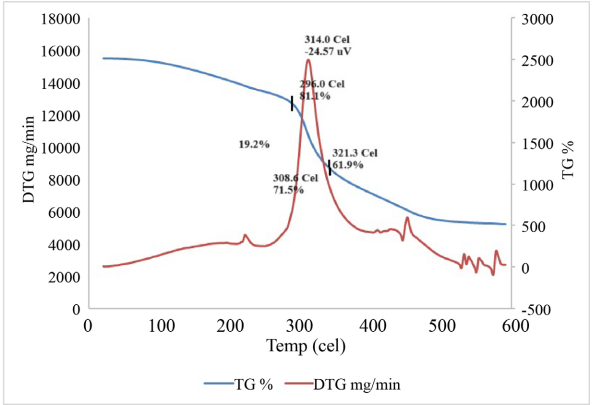
Figure 6(c) shows the TG curve for PVA-Gelatin/0.8% HAP composite where



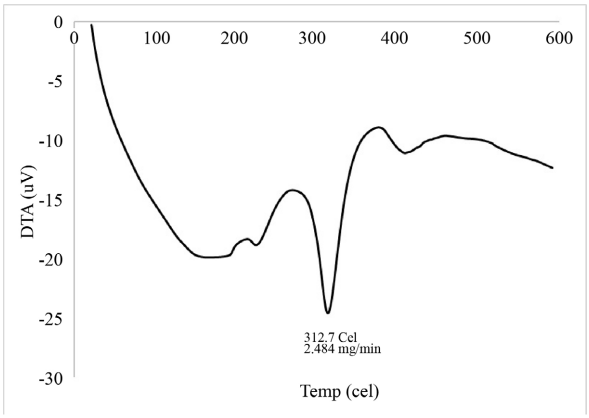
(a)



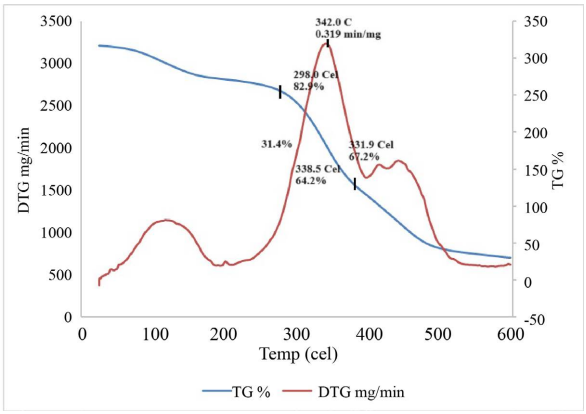
(b)



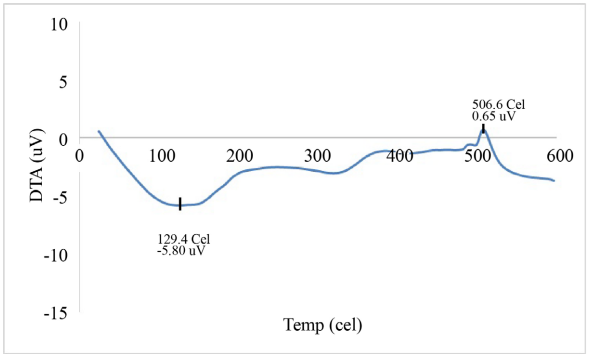
(c)


DTA (uV)
Temp (cel)
312.7 Cel
2.484 mg/min

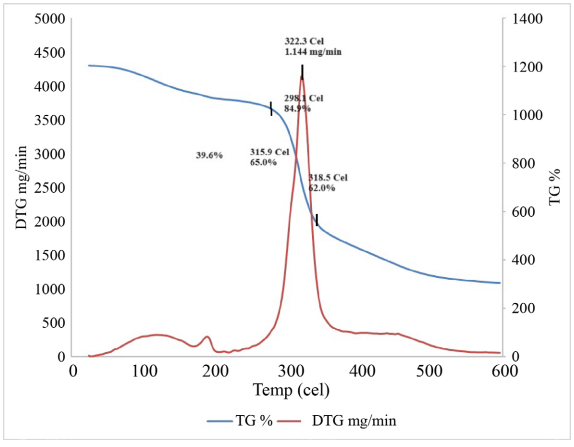
(d)


DTG mg/min
TG %
Temp (cel)
342.0 C
0.319 min/mg
298.0 Cel
82.9%
31.4%
331.9 Cel
67.2%
338.5 Cel
64.2%
TG %
DTG mg/min

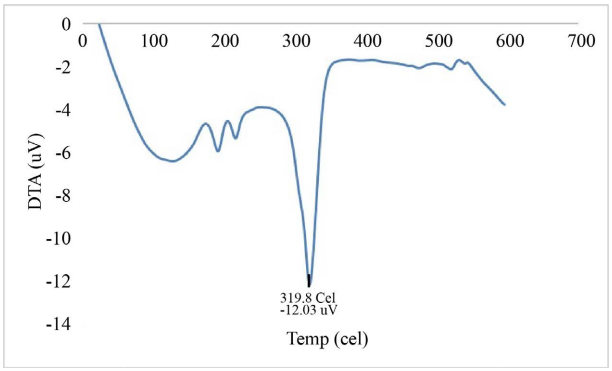
(e)


DTA (uV)
Temp (cel)
506.6 Cel
0.65 uV
129.4 Cel
-5.80 uV

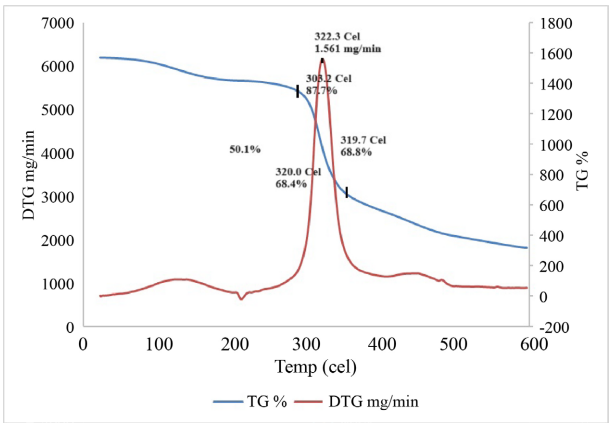
(f)


DTG mg/min
TG %
Temp (cel)
322.3 Cel
1.144 mg/min
298.1 Cel
84.9%
39.6%
315.9 Cel
65.0%
318.5 Cel
62.0%
TG %
DTG mg/min

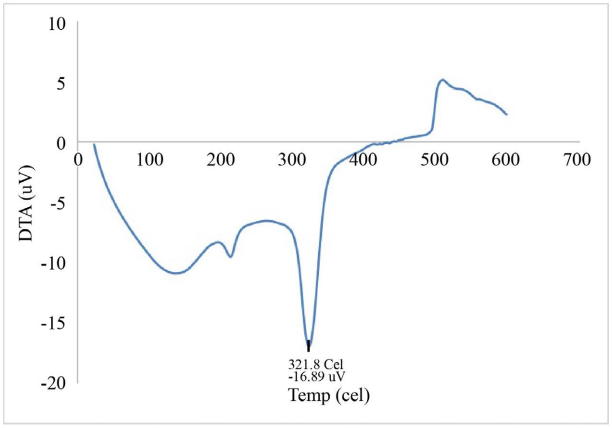
(g)



(h)



(i)



(j)

Figure 6. (a) TG and DTG curves of PVA-Gelatin/1% HAP composite (b) DTA curve of PVA-Gelatin/1% HAP composite, (c) TG and DTG curves of PVA-Gelatin/0.8% HAP composite, (d) DTA curve of PVA-Gelatin/0.8% HAP composite, (e) TG and DTG curves of PVA-Gelatin/0.4% HAP composite (f) DTA curve of PVA-Gelatin/0.4% HAP composite, (g) TG and DTG curves of PVA-Gelatin/0.2% HAP composite, (h) DTA curve of PVA-Gelatin/0.2% HAP, (i) TG and DTG curves of PVA-Gelatin composite, (j) DTA curve of PVA-Gelatin composite.

the onset temperature, first 50% degradation temperature, and maximum slope are 296 degrees Celsius, 321.3 degrees Celsius, and 308.6 degrees Celsius, respectively. The total degradation loss is 19.2%. The DTG curve shows one significant

peak at 314 degrees Celsius. There is one step of crystallization or phase transition due to the crystallization of the composite. Figure 6(d) shows the DTA curve shows the exothermic peak at 312.7 degrees Celsius due to heat release during crystallization or phase transition.

Figure 6(e) shows the TG curve for PVA-Gelatin/0.4% HAP composite where the onset temperature, first 50% degradation temperature, and maximum slope are 298 degrees Celsius, 331.9 degrees Celsius, and 338.5 degrees Celsius, respectively. The total degradation loss is 31.4%. The DTG curve shows one significant peak at 342 degrees Celsius. There is one step of crystallization or phase transition due to the crystallization of the composite. Figure 6(f) shows the DTA curve shows the exothermic and endothermic peaks at 129.4 and 506.6 degrees Celsius. The exothermic and endothermic peaks simultaneously can indicate the presence of multiple components with distinct thermal behaviors leading to concurrent endothermic and exothermic processes, each associated with different materials or reactions. Both peaks can also indicate phase transitions occurring simultaneously in different parts of the composite. Variations in processing conditions, such as temperature gradients within the sample or the speed of heating, can lead to overlapping endothermic and exothermic peaks as well.

Figure 6(g) shows the TG curve for PVA-Gelatin/0.2% HAP composite where the onset temperature, first 50% degradation temperature, and maximum slope are 298.1 degrees Celsius, 318.5 degrees Celsius, 315.9 degrees Celsius, respectively. The total degradation loss is 39.6%. The DTG curve shows one significant peak at 322.3 degrees Celsius. There is one step of crystallization or phase transition due to the crystallization of the composite. Figure 6(h) shows the DTA curve shows the exothermic peak at 319.8 degrees Celsius due to heat release in crystallization or phase transition.

Figure 6(i) shows the TG curve for the PVA-Gelatin composite where the onset temperature, first 50% degradation temperature, and maximum slope are 303.2 degrees Celsius, 320 degrees Celsius, and 319.7 degrees Celsius, respectively. The total degradation loss is 50.1%. The DTG curve shows one significant peak at 322.3 degrees Celsius. There is one step of crystallization or phase transition due to the crystallization of the composite. Figure 6(j) shows the DTA curve shows the exothermic peak at 321.8 degrees Celsius due to heat release in crystallization or phase transition.

TGA and DTGA show that all the samples exhibited two distinct weight loss phases at 100˚C - 500˚C (decomposition of the main chain of gelatin and PVA). However, significant weight losses are observed at about 50 wt % in the range of 150˚C - 500˚C for all the samples, corresponding to the structural decomposition of gelatin and PVA.

The first-order derivative of TGA curves reveals the temperature at which the maximum decrease of mass occurs. The temperature at the maximum loss rate is 322.3˚C for the PVA-Gelatin composite, 322.3˚C for the PVA-Gelatin/100 mg HAP composite, 342˚C for the PVA-Gelatin/200 mg HAP composite, 314˚C for the PVA-Gelatin/400 mg HAP composite and 300.2˚C for the PVA-Gelatin/ 500

mg HAP composite. DTGA data clearly show that endothermic peaks are due to thermal degradation [10].

From the TG/DTA, it is seen that as the concentration of HAP increases in the PVA/Gelatin/HAP composite, the thermal stability remains constant from 0% HAP to 0.4%, then the stability increases for 0.4% and lastly decreases for 0.8% and 1%. The pattern where the stability increases with an increase in HAP concentration and then decreases can occur as higher concentrations of HAP in the composite occur because HAP may act as a thermal insulator, reducing the heat transfer within the composite. This phenomenon can lead to localized heating and degradation of the organic components, potentially causing a decrease in thermal stability. Moreover, at very high concentrations of HAP, the composite may become less homogeneous, and the organic components may be diluted to a point where their influence on the overall thermal stability is reduced. Lastly, the processing conditions, such as temperature and pressure during composite preparation, can impact the distribution and interactions of HAP within the matrix, affecting thermal stability.

3.1.6. Tensile Test

In **Figure 7**, we see the comparison of the stress-strain study of 1% HAP/PVA/Gelatin, 0.8% HAP/PVA/Gelatin, and PVA film. The PVA-Gelatin films with 0.2% and 0.4% did not produce consistent data in the graph. For PVA film, the curve follows a typical elastomeric behavior, with an initial linear region representing elastic deformation, followed by a yield point at a strain 5.5 with stress 8.03 MPa and plastic deformation.

The ultimate tensile strength (UTS) is relatively lower than biocomposite materials. The inclusion of HAP significantly impacts the stress-strain curve. HAP is a rigid and stiff material, and its addition increases the overall stiffness of the composite. The curve shows a higher Young's Modulus, indicating increased

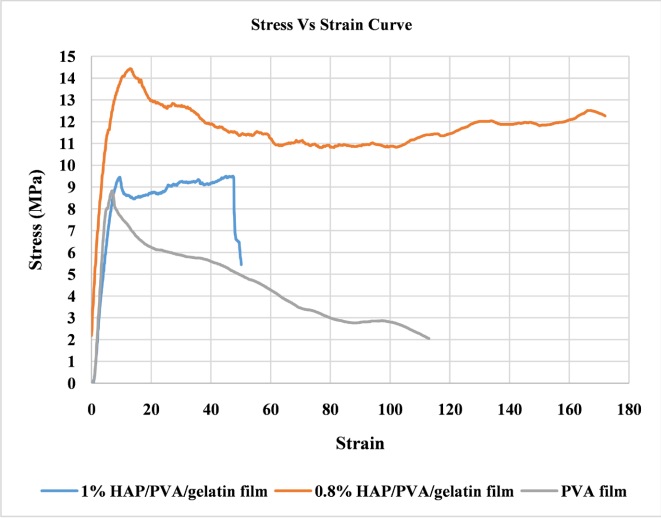


Figure 7. Stress Vs Strain curves of 1% HAP/PVA/Gelatin, 0.8% HAP/PVA/Gelatin and PVA films superimposed.

Table 1. The table mentioning tensile strength, stress at break, E-modulus and energy between for different concentrations (1%, 0.8%) of HAP in PVA/Gelatin film and PVA film.

Sample	Tensile Strength (MPa)	Stress at break (MPa)	E-modulus (MPa)	Energy between (J)
1% HAP	9.08	3.208	364.5	0.0049
0.8% HAP	16.15	16.15	425.6	0.1408
PVA	1.698	0.3942	43.76	0.0251

stiffness, especially in composites with higher HAP concentrations. The tensile strength increases due to the reinforcing effect of HAP, resulting in higher UTS. Table 1 summarizes the tensile strength, stress at break, E-modulus, and energy between the concentration of 0.8% and 1% HAP in PVA/Gelatin film and PVA film. The 0.8% HAP/PVA/Gelatin film exhibits higher elastic modulus, tensile strength, and stress at break compared to the 1% HAP/PVA/Gelatin film. The reason for such higher elastic modulus, tensile strength, and stress at break for the 0.8% is because the film with lower HAP concentrations may have matrix-enhancing agents or crosslinking agents that strengthen the PVA and gelatin matrix, resulting in higher stiffness for the composite at a lower HAP concentration HAP/PVA/Gelatin film comparing to the film with higher HAP concentration. A well-structured, highly aligned distribution of HAP in the film with lower HAP concentration may contribute to better load-bearing characteristics and, thus, a higher E-modulus.

The poor strength properties (elastic modulus, tensile, stress at break) of PVA film are significant drawbacks to their use in biomedical applications such as bone tissue engineering. Improvements in the mechanical properties of film are therefore mandatory. With the incorporation of HAP, we can significantly improve the tensile strength, elastic modulus, and stress at break, as seen in Figure 7 and Table 1.

4. Conclusions

From the study, we can observe the following characteristics of Calcium based biocomposites (Hydroxyapatite and PVA-Gelatin-HAP films):

- The XRD analysis confirms that the synthesized powder prepared from the waste material calcite is pure Hydroxyapatite (HAP) powder.
- The FTIR analysis of all the PVA/Gelatin composites with 0.2%, 0.4%, 0.8%, and 1% HAP reveals chemical bond formation with PVA, Gelatin, and Hydroxyapatite (HAP).
- The swelling test indicates that the water absorption by the PVA/Gelatin composites decreases with the increasing concentration of HAP and the test is also indicative of the biocompatibility of the composites.
- The TMA reveals the mechanical stability with respect to the temperature of the PVA/Gelatin composites with the increasing concentration of HAP in the

composite.

- The TG/DTA analysis depicts that PVA-Gelatin-HAP films are highly stable. The degradation temperature of the composites is approximately 300 degrees Celsius.
- The tensile test reveals the mechanical stability of the PVA-Gelatin-HAP films with a higher concentration of HAP in the composite.

Based on the above results, we can reasonably conclude that the PVA/Gelatin composites with 0.8% and 1% have the potential to be used in diverse biomedical applications with their high thermal stability, high mechanical stability, biocompatibility, bioactivity, osteoconductivity and water absorption properties. Thus, the developed biocomposite can be used in bone tissue engineering, orthopedic implants, and wound healing.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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