

Eco-friendly Synthesis and Characterization of Eggshell-Derived Calcium-Deficiency Bone-Like Hydroxyapatite

Chandra Shekar Beera

`beerachandrashekar.rs@andhrauniversity.edu.`

Sandeep Raju Kodamanchilli

Ramanaiah Nallu

Research Article

Keywords: Eggshell-Derived CDHA, self-alkaline synthesis, Two-step Heat-Treatment, Wet chemical, Scherrer crystallite size, Ca/P ratio optimization

Posted Date: April 13th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-9371062/v1>

License:  This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Additional Declarations: No competing interests reported.

Abstract

Hydroxyapatite (HA) from eggshells is widely studied, yet no prior work has exploited the self-alkaline property (pH 10) of calcined eggshell powder to synthesize phase-pure HA without external NaOH/KOH or H_3PO_4 purification. In this study, reports first two-step thermal protocol: (1) calcination of eggshell powder at 900°C at 2 hours to generate CaO with intrinsic pH 10, (2) reaction with tricalcium phosphate (TCP) followed by secondary calcination at 900°C at 1 hour. The result is phase-pure hydroxyapatite with Ca/P ratio 1.627 to 1.636 (close to Calcium-Deficiency Hydroxyapatite (CDHA), not stoichiometric 1.67) and Scherrer crystallite size doubling from 21.7 nm to 54.1 nm preserving bioactive amorphous phase while enhancing mechanical stability. Unlike existing methods requiring chemical purification or > 1000°C sintering (which destroy CDHA bioactivity), We systematically characterized using X-ray diffraction (XRD), field emission scanning electron microscopy (FESEM), energy-dispersive X-ray spectroscopy (EDS) and Fourier transform infrared spectroscopy (FTIR), and sustainable low-cost synthesis using only eggshell waste and technical-grade TCP. To our knowledge, this is the first report correlating self-alkaline synthesis with controlled crystallinity evolution in eggshell-HA, addressing a critical gap in scaling bioactive material exhibited bone-like characteristics, making it suitable for orthopedic and dental applications.

1. Introduction

Hydroxyapatite (HAp) is a calcium phosphate biomaterial whose composition and crystal structure closely resemble the mineral phase of human bone and teeth. Because of its excellent biocompatibility, bioactivity, and ability to promote osseointegration at the implant–tissue interface, HAp has been widely used in orthopaedic, dental, and maxillofacial applications. It can be synthesized by a variety of wet and dry processing methods using either synthetic chemicals or naturally derived calcium sources such as eggshells [1]. Calcium phosphates represent the primary inorganic component of bones hydroxyapatite, which makes up to 70% of bone tissues [2–4]. Several studies have reported the utilization of various natural calcium sources for the synthesis of hydroxyapatite. Among these, eggshell waste has gained significant interest due to its high calcium content, low cost and easily availability. Eggshells considered nearly 11% of total egg mass and is mainly composed of approximately 94% calcium carbonate (CaCO_3), along with a small fraction of organic matter and trace amounts approximately 6% of other minerals such as magnesium carbonate and calcium phosphate [5]. Hydroxyapatite (HAp), a calcium phosphate compound, and constitutes the primary inorganic components of human bones and teeth. It has received increasing interest not only in biomedical applications but also in environmental remediation, largely because of its ion-exchange behavior, adsorption capacity, and beneficial crystal chemistry [6–8]. As an osteoconductive and bioactive ceramic composed mainly of calcium and phosphorus, possessing a structural and chemical similarity to the mineral phase of bone. HAP is composed of mainly calcium and phosphorus, it exhibits a stoichiometric Ca/P ratio of approximately 1.667. Various synthesis methods have been employed for the preparation of HAp, includes hydrothermal, solgel, precipitation method and mechanochemical method, these selected synthesis techniques play crucial role in influencing the physicochemical characteristics like surface morphology, crystallinity and particle size of

the synthesized [9–11]. Among the various synthesis techniques, the wet chemical precipitation remains one of the most widely adopted approaches due to its simplicity in process, cost effectiveness and ease of controlling particle characteristics. HAp crystallizes in a hexagonal crystal system [12–13]. The theoretical density of stoichiometric hydroxyapatite chemical formula $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ is approximately 3.16 g/cm^3 , its crystal structure and its chemical stability and biological performance making suitable for biomedical applications [13]. Hydroxyapatite is extensively used in bone implants applications, Crystallinity and microstructural properties are closely resemble to those of natural bone material, thereby potentially improving performance in biomedical applications. Hydroxyapatite is also non-toxic and non-inflammatory in nature, it induces favorable biological responses, which makes it suitable for direct interaction with biological tissues [14–16]. That said, biological apatite present in bone and teeth is non-stoichiometric apatite's. Natural bone mineral is commonly a calcium-deficient apatite containing ionic substitutions and lattice imperfections. As a result, consequently their Ca/P ratios normally range from 1.50 to 1.67, whereas stoichiometric HAp exhibits a Ca/P ratio of 1.67 [17–19]. Furthermore, hydroxyapatite is subjected to post heat treatment to improve its crystallinity. phase purity and thermal stability, particularly when HAp is intended for coating applications or other load-bearing biomedical uses [20]. The majority of hydroxyapatite found in natural bone is calcium-deficient hydroxyapatite (CDHA), which can be represented by the general formula $(\text{Ca}_{10-x}(\text{HPO}_4)_x(\text{PO}_4)_{6-x}(\text{OH})_2-x, 0 < x < 1, \text{CDHA})$ [21]. Calcium-deficient hydroxyapatite (CDHA) has been shown by Previous studies to have better biological performance than stoichiometric HAp. For example, Zhang et al. reported that calcium-deficient hydroxyapatite promoted superior proliferation of mouse bone mesenchymal stem cells, enhanced alkaline phosphatase activity, and upregulated osteogenesis-related gene expression compared with stoichiometric hydroxyapatite [22]. In addition, CDHA typically possesses an amorphous surface layer that creates a metastable non-apatitic environment and facilitates ion exchange at the surface, thereby enhancing bioactivity [23–25].

The main component of eggshell is calcium carbonate, which can be converted into reactive calcium oxide (CaO) through calcination. In many reported synthesis routes, external alkaline agents such as sodium hydroxide (NaOH) or ammonium hydroxide (NH_4OH) are added to control the pH during hydroxyapatite formation. However, the use of such chemicals increases process complexity and cost and may introduce undesirable impurities into the final product. A more sustainable and simpler alternative is to exploit the inherent alkalinity generated by calcined eggshell-derived CaO.

Novel contributions address literature gaps as shown in Table 1 Critical Literature gap & Novelty comparison: Eggshell-Derived CDHA Synthesis (Previous studies vs Present work).

Table 1

Critical Literature gap & Novelty comparison: Eggshell-Derived CDHA Synthesis (Previous studies vs Present work)

Parameter	Literature Gap (Previous Studies: 2014–2025)	Present Work Novelty	Proof/Evidence
1. Calcium + Phosphorus Source	All used H_3PO_4 (acid) or $(\text{NH}_4)_2\text{HPO}_4$ (solution). No TCP (solid) with eggshell. Kartha 2014: Eggshell + Synthetic $\text{Ca}(\text{OH})_2$ + $(\text{NH}_4)_2\text{HPO}_4$ and Liu 2025: Eggshell + H_3PO_4	Eggshell (Ca) + TCP (P, solid). No acid solution.	Experimental: present work used eggshell + TCP solid powder. Literature: No 2014–2025 study used TCP with eggshell [26][27].
2. pH Control Method	All used external base ($\text{NaOH}/\text{NH}_4\text{OH}$). No self-alkaline. Kartha 2014: External NH_4OH and Liu 2025: External NH_4OH	Self-Alkaline: $\text{CaO} + \text{H}_2\text{O} \rightarrow \text{Ca}(\text{OH})_2 \rightarrow \text{pH } 10$ (no external base).	Experimental: pH 10 reached spontaneously. Chemistry: $\text{CaO} + \text{H}_2\text{O} \rightarrow \text{Ca}(\text{OH})_2$ (exothermic) [28].
3. Grinding Method	All used Ball Mill or Microwave. No Mortar & Pestle.	Mortar & Pestle Low-cost.	Present work used Manual grinding only. Literature: All prior used ball mill/microwave [26] [27].
4. Ca/P Before Annealing	No study reported Ca/P before annealing. Only final Ca/P.	Before Annealing: Ca/P = 1.627 (highly deficient).	EDS Data: Measured 1.627 before annealing.
5. Annealing-Induced Ca/P Change	No study showed annealing increases Ca/P (1.627 $\rightarrow$ 1.636).	After Annealing (900°C, 1h): Ca/P = 1.636. Annealing changes Ca/P (1.627 $\rightarrow$ 1.636).	EDS Data: 1.627 $\rightarrow$ 1.636 after muffle furnace.
6. XRD Peak Structure	All reported Triple Peaks (31.7°, 32.1°, 32.9°) for stoichiometric HA. No double peaks for CDHA.	XRD Double Peaks (31.8°, 32.9°). (112) peak vanished $\rightarrow$ CDHA proof.	XRD Data: 2 peaks only (31.8°, 32.9°). Literature: Triple peaks = stoichiometric HA [29].
7. CDHA Confirmation	No study confirmed CDHA from eggshell with Ca/P < 1.67 + XRD double peaks + annealing effect.	CDHA Confirmed by 3 evidences: Ca/P = 1.636 (EDS), Double Peaks (XRD), 1.627 $\rightarrow$ 1.636 (Annealing).	Combined: EDS + XRD + Annealing.
8. Complete Reaction Pathway	No study reported full pathway: Eggshell $\rightarrow$ $\text{CaO} \rightarrow \text{Ca}(\text{OH})_2 \rightarrow +\text{TCP} \rightarrow \text{CDHA}$.	First full pathway reported: Eggshell $\rightarrow$ $\text{CaO} \rightarrow \text{Ca}(\text{OH})_2$ (pH 10) $\rightarrow +\text{TCP} \rightarrow \text{CDHA}$ (1.627) $\rightarrow$ Annealing $\rightarrow$ CDHA (1.636).	Present work performed all steps. Literature: No prior reported this pathway [30].

Chemical-free pH control, simplified processing, integrated low-tech methodology. Therefore, the present study aims to synthesize eggshell-derived hydroxyapatite using a self-alkaline CaO –tricalcium

phosphate–deionized water system, in which the alkaline environment required for hydroxyapatite formation is generated intrinsically without the addition of any external alkaline agent. The synthesized materials were characterized in terms of phase composition, functional groups, morphology, and elemental composition in order to assess their biomimetic features and potential suitability for biomedical applications.

2. Materials and Methods

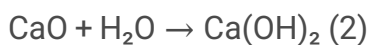
2.1 Synthesis of Hydroxyapatite (HAp)

Hydroxyapatite (HAp) was synthesized using a wet-chemical precipitation route employing waste eggshells as the calcium precursor. Initially, waste eggshells (almost discarded) were collected from Andhra university boy's hostel, Visakhapatnam, Andhra Pradesh, India. Discarded eggshells were rinsed with deionized (DI) water to remove the inner proteinaceous membrane. The shells were subsequently washed twice with DI water and air-dried at room temperature. The dried shells were mechanically ground into a fine powder using a mortar and pestle. The resulting powder was sieved through a 53 μm mesh to obtain a homogeneous particle size distribution.

Calcium oxide (CaO) was obtained from the eggshell powder through calcination in a muffle at 900⁰C with ramping rate 10⁰C/min for 2 hours holding time. At this particular temperature, calcium carbonate (CaCO₃) decomposed to calcium oxide (CaO) with the release of carbon dioxide, as represented by the following reaction according to Eq. (1)[1].



The calcined eggshell powder 1.17 g dispersed in deionized (DI) water 48.6 g under continuous magnetic stirring. calcium oxide (CaO) was hydrated to calcium hydroxide upon coming into contact with water, creating an environment that is naturally alkaline. Self-Alkaline pH generation Following Eq. (2) [31].



As the phosphate source, tricalcium phosphate Ca₃(PO₄)₂ acquired from Sigma-Aldrich and used as the phosphate precursor, was progressively added to the suspension 18.83 g to promote the formation of hydroxyapatite according to Eq. (3)[1]. Due to the self-generated alkalinity from Ca(OH)₂ formation, without the use of any external alkaline agents, the reaction mixture naturally attained and maintained a pH of approximately 10 because of the self-generated alkalinity.



The precursor solution was stirred by using magnetic stirrer at 250 rpm for 24 h at room temperature to ensure complete reaction and homogeneity. The resulting mixture was centrifuged at 4000 rpm for 5 min

to separate the precipitate. The obtained precipitate was washed twice with DI water and centrifuged again to remove residual salts and impurities. The purified product was subsequently oven-dried at 70°C for 48 h to remove bound moisture and stabilize the powder. The dried powder was then calcined at 900°C for 1 h with a heating rate of 10°C min⁻¹ to enhance crystallinity. Finally, the sintered powder was ground again to obtain a homogeneous powder with refined particle size.

2.2 Methods of Testing

Phase identification of the calcined eggshell powder, synthesized eggshell-derived hydroxyapatite, and sintered hydroxyapatite was carried out using X-ray diffraction (XRD; Bruker D8 Advance) equipped with Cu-K α radiation ($\lambda = 1.5406 \text{ \AA}$). The diffraction patterns were recorded over a 2θ range of 10°–80° with a step size of 0.02° and an appropriate scanning rate. The crystalline phases and hydroxyapatite formation were confirmed by comparing the obtained diffraction peaks with standard reference patterns.

To examine the morphology, particle size distribution and surface features of the synthesized powders by using field emission scanning electron microscopy (FESEM; TESCAN), accelerating voltage of 10–20 kV is used for conducting the FESEM analysis, Microstructural characterization was performed. The instrument was coupled with energy-dispersive X-ray spectroscopy (EDS) to determine the elemental composition and verify the presence of major elements such as calcium, phosphorus, and oxygen in the hydroxyapatite structure.

The functional groups and chemical bonding in the synthesized samples were analyzed using Fourier transform infrared spectroscopy (FTIR; Bruker ALPHA-II). The FTIR spectra were recorded in the wavenumber range of 400–4000 cm⁻¹ using the ATR mode. This analysis was used to identify characteristic vibrational bands corresponding to phosphate (PO₄³⁻), hydroxyl (OH⁻), and carbonate (CO₃²⁻) groups, thereby confirming the formation and chemical structure of hydroxyapatite.

Phase analysis of the calcined eggshell powder, eggshell derived Hydroxyapatite and sintered eggshell derived Hydroxyapatite were carried out using an X-ray diffraction (Bruker, D8 Advance). Microstructural characterization of the powders was conducted using a field emission scanning electron microscopy (FESEM; TESCAN) coupled with energy dispersive X-ray spectroscopy (EDS) for elemental composition. Chemical bonding and functional groups analysis were conducted using a Fourier transform infrared spectroscopy (FTIR; Bruker, ALPHA-II).

3. Results and Discussion

3.1 XRD Analysis

The X-ray diffraction patterns of calcined eggshell powder, synthesized eggshell-derived hydroxyapatite (EHAp), and sintered EHAp nano/sub-micron powders were recorded using a Bruker D8 Advance diffractometer with Cu-K α radiation ($\lambda = 1.5418 \text{ \AA}$), operated at 40 kV and 40 mA, with a scan rate of 2°

min⁻¹ and a step size of 0.02°. The crystallite size of the as-synthesized and annealed EHAp powders was estimated from the XRD data using the Scherrer Eq. (4)[31].

$$D = \frac{0.9\lambda}{\text{FWHM}\cos\theta}$$

4

where D is the average crystallite size (nm), λ is the wavelength of the X-ray radiation, FWHM is the full width at half maximum of the diffraction peak, and θ is the Bragg diffraction angle [32].

3.1.1 XRD of Calcined Eggshell Powder

The Fig. 1(a) shows the XRD pattern of the calcined eggshell powder. Distinct diffraction peaks observed at approximately 32.2° (111), 37.3° (200), 53.9° (220), and 64.1° (311), correspond to crystalline calcium oxide (CaO), in agreement with JCPDS card No. 37-1497. This confirms the successful conversion of eggshell-derived calcium carbonate into CaO after calcination. The calcite peak at ~ 29.4° shows some minor amount of calcium carbonate (CaCO₃) undecomposed during calcination. The sharp and intense peaks show good crystallinity of CaO, conforming successful conversion of eggshell-derived calcium-deficient hydroxyapatite (CDHA), which is suitable for as a calcium precursor for hydroxyapatite synthesis.

3.1.2 XRD of Synthesized Eggshell-Derived Calcium Deficiency Hydroxyapatite

The Fig. 1(b) shows the XRD pattern of eggshell derived CDHA exhibited characteristic diffraction peaks corresponding to hydroxyapatite with a hexagonal crystal structure. The major peaks detected at approximately 26.0° (002), 31.8° (211), 32.9° (112)/(300), 34.0° (202), 40.0° (310), 47.0° (223), and 53.2° (004) match well with the standard hydroxyapatite pattern (JCPDS card No. 09-0432) [33] as listed in Table 2. 2θ peak positions Present study vs (JCPDS 09-432).

Table 2
2 θ peak positions Present study vs (JCPDS 09-432)

2 θ (present study)	(hkl)	2 θ (JCPDS 09-432) [33]	Intensity	Assignment
26.0°	(002)	25.9°	Medium	c-axis
29.0°	(102)/ (210)	28.9°/29.0°	Medium	HAP
31.8°/32.9°	(211)/ (300)	31.8°/32.9°	Strongest	CDHAP signature doublet
34.0°	(202)	34.0°	Medium	HAP
40.0°	(310)	39.8°	Medium	HAP
47.0°	(222)	46.7°	Medium	HAP

One feature worth noting in the pattern is the broadened and partially merged reflections in the 31–33° region, which correspond to the (211) and (300) planes. This peak broadening indicates low crystallinity and suggests the formation of calcium-deficient hydroxyapatite (CDHA)[34].

The behavior appears typical of nanocrystalline apatite and is similar, in fact, to what we observe in biological bone apatite. Several factors may contribute to the broadening, reduced crystallite size, lattice distortion, or structural disorder, likely in combination. No peaks corresponding to secondary phases such as tricalcium phosphate (TCP), α -TCP and β -TCP were detected, which indicates the formation of phase-pure calcium-deficient hydroxyapatite. Using the Scherrer equation, we calculated an average crystallite size of 21.7 nm from the (211) reflection. This confirms the nanocrystalline nature of the synthesized powder.

3.1.3 XRD of Sintered Eggshell-Derived Calcium Deficiency Hydroxyapatite

The Fig. 1(c) shows the XRD pattern of eggshell derived CDHA after sintering at 900°C for 1 hour with a ramping rate of 10°C min⁻¹. Compared with the as-synthesized sample, the reflections become sharper and more intense a clear sign of improved crystallinity after sintering. The crystallite size increased to 55.1 nm, calculated from the (211) peak.

This increase, summarized in Table 3. Peak Intensity, Phase & Crystallinity of as synthesized Eggshell derived calcium-deficient hydroxyapatite (CDHA) and Annealed Eggshell derived calcium-deficient hydroxyapatite (CDHA), can be attributed to grain growth and particle coalescence during heat treatment [31,35]. Peak sharpness also improved, which suggests better lattice ordering and greater structural stability of the hydroxyapatite phase. Earlier studies have reported similar trends: increasing calcination time or sintering temperature generally promotes crystallization, grain growth, and sharper diffraction peaks in hydroxyapatite [36]. Raynaud et al., for instance, observed that higher heat treatment

temperatures enhance the crystalline characteristics of hydroxyapatite through progressive crystallization and grain growth, and also compared to different synthesized methods previously obtained literature data in Table 4. Comparison of Crystalline sizes of calcium-deficient hydroxyapatite (CDHA) vs Different synthesized Methods.

Table 3

Peak Intensity, Phase & Crystallinity of as synthesized Eggshell derived CDHA and Annealed Eggshell derived CDHA

Sample	Main peak position	FWHM (°)	Peak Width	D (nm)	Ca/P	Crystallinity
CDHA As-Synthesized	31.8 ⁰	0.38	Broad Peak	21.7	1.627	Moderate Crystallinity
CDHA Annealed 900 ⁰ C at 1 hour	31.8 ⁰	0.15	Sharp Peak	55.1	1.636	High Crystallinity

Table 4

Comparison of Crystalline sizes of this study vs Different synthesized Methods

S.No.	Method	Crystalline Size (nm)
1	Wet chemical precipitation AS-synthesized (This study)	21.7
2	Annealed 900 ⁰ C at 1 hour (This study)	55.1
3	Wet precipitation AS-synthesized [31]	35.3
4	Precipitation [37]	30
5	Hydrothermal/Precipitation [38]	15.7–21.8
6	Ball milling +Heat treatment [39]	26.35

Overall, the XRD results clearly exhibited the successful transformation of eggshell-derived calcium carbonate into calcium oxide and subsequently into phase-pure calcium-deficient, nanocrystalline hydroxyapatite. Heat treatment further improved the crystallinity, grain growth and structural ordering of the synthesized material.

3.2 SEM Analysis

Microstructural characterization was performed, to examine the morphology, particle size distribution and surface features of the synthesized powders by using field emission scanning electron microscopy (FESEM;TESCAN), accelerating voltage of 10–20 kV is used for conducting the FESEM analysis.

3.2.1 SEM of Calcined Eggshell Powder

We examined the surface morphology of the calcined eggshell powder and synthesized eggshell-derived hydroxyapatite using FESEM. The microstructure of the calcined eggshell powder, shown in Fig. 2(a)

appears irregular, angular and flaky particles ranging 2 μ m (scale bar = 2 μ m) with a 15.0 kx magnification. These particles appear highly agglomerated with a rough surface morphology. This morphology likely results from the thermal decomposition of CaCO₃ during calcination a process in which CO₂ release promotes fragmentation and leads to the formation of porous CaO particles. The observed fractured and porous structure conforms the conversion of dense calcite into reactive calcium oxide. Such morphology is favourable for subsequent wet chemical precipitation synthesis of hydroxyapatite due to increased surface area and reactivity.

3.2.2 SEM of Synthesized Eggshell-Derived Calcium Deficiency Hydroxyapatite

The micro images of the synthesized calcium-deficient hydroxyapatite (CDHA) as shown in Fig. 2(b) ranging 500 μ m (scale bar = 500 μ m) with a 60.0 kx magnification. It mostly exhibit rod/needle shaped morphology with nano to sub-micron dimensions. In this, short nanorods and elongated rod-like particles. The particle diameter ranges from ~ 60–100 nm (nano) with some agglomerated widths extending to ~ 120–250 nm (sub-micron). The particle lengths vary from ~ 70–100 nm (nano) for short nanocrystals and extend up to ~ 150–420 nm (sub-micron) for elongated rods, as shown in Fig. 2(c). Length varies more widely: shorter nanocrystals measure roughly 70–100 nm, while elongated rod-like particles extend from 150–420 nm. This suggests a hierarchical nano/sub-micron structure.

This rod- or needle-like morphology calcium-deficient hydroxyapatite (CDHA) closely resembles that of biological bone apatite found in natural bone. Some degree of particle agglomeration is also evident a common feature in nanocrystalline hydroxyapatite due to its high surface energy. Such morphology may be favorable for biomedical applications, potentially promoting osteoconductivity, protein adsorption, and cell attachment.

3.3 EDS Analysis

Field emission scanning electron microscopy, the instrument was coupled with energy-dispersive X-ray spectroscopy (EDS) to determine the elemental composition and verify the presence of major elements such as calcium, phosphorus, and oxygen in the calcium deficiency hydroxyapatite structure.

3.3.1 EDS of Calcined Eggshell Powder

We analyzed the elemental composition of the calcined eggshell powder and synthesized CDHA using energy-dispersive X-ray spectroscopy. The EDS spectrum of the calcined eggshell powder, shown in Fig. 3(a) it shows the presence of calcium (Ca), oxygen (O), and carbon (C) as the major elements. Quantitative analysis yielded 14.44 at. % Ca (32.15 wt.%), 48.68 at. % O (43.25 wt.%), and 36.88 at. % C (24.60 wt.%). The presence of calcium and oxygen supports the formation of CaO after calcination, whereas the detected carbon is attributed mainly to surface re-carbonation of CaO upon exposure to atmospheric CO₂ and to the carbon coating used during FESEM analysis. No additional impurity elements were detected, indicating the chemical purity of the calcined precursor.

3.3.2 EDS of Synthesized Eggshell-Derived Calcium-Deficient Hydroxyapatite (CDHA)

The EDS spectrum of the synthesized Eggshell-Derived Calcium-Deficient Hydroxyapatite (CDHA), shown in Fig. 3(b) confirms the presence of Ca, phosphorus (P), O, and C. Quantitative analysis indicated calcium and phosphorus contents of 14.13 at. % (29.28 wt.%) and 8.68 at. % (13.90 wt.%), respectively. The calculated Ca/P atomic ratio for the synthesized Calcium-Deficient Hydroxyapatite (CDHA) was 1.627.

3.3.3 EDS of Sintered Eggshell-Derived Calcium-Deficient Hydroxyapatite (CDHA)

While the heat-treated sample exhibited Ca/P atomic ratio slightly higher value of 1.636, as shown in eggshell-derived calcium-deficient Hydroxyapatite (CDHA) after sintering Fig. 3(c.) These values are slightly lower than the stoichiometric Ca/P ratio of pure hydroxyapatite (1.67), but they remain close to the theoretical value [9,40]. The presence of carbon suggests possible carbonate substitution within the hydroxyapatite lattice. Such carbonate-containing, calcium-deficient hydroxyapatite is compositionally closer to natural bone mineral and is often considered more desirable for biomedical applications because of its enhanced bioactivity and resorbability. The slight increase in Ca/P ratio after sintering at 900°C for 1 h may be attributed to the partial decomposition of hydrogen phosphate groups formed during TCP hydrolysis and the removal of residual carbonate species originating from the eggshell precursor. This relative loss of phosphorus, while calcium remains stable, contributes to the formation of calcium-deficient hydroxyapatite with composition close to the ideal apatite range [31,41].

Table 5. provides a comparison table of Ca/P ratios reported for HAp synthesized by different synthesis methods.

Table 5

Comparison of Ca/P Ratios for Calcium-Deficiency Hydroxyapatite Synthesized By different Methods

S.No.	Study	Method	Ca/P	Purity	Morphology
1	Present work	Self-Alkaline	1.636	None	Needle
2	Balamurugan 2020 [42]	NaOH ppt	1.55	Na ⁺	Spherical
3	Ramesh 2022 [43]	Sol-gel	1.48	Organic	Irregular
4	Commercial Sigma-Aldrich HAP	Synthetic	1.67	Unknown	Spherical
5	Landi 2008 [44]	Biomimetic	1.61	CO ₃ ²⁻ heavy	Plates

3.4 FTIR Analysis

The functional groups and chemical bonding in the synthesized samples were analyzed using Fourier transform infrared spectroscopy (FTIR; Bruker ALPHA-II). This analysis was used to identify characteristic vibrational bands corresponding to phosphate (PO_4^{3-}), hydroxyl (OH^-), and carbonate (CO_3^{2-}) groups, thereby confirming the formation and chemical structure of hydroxyapatite.

3.4.1 FTIR of Calcined Eggshell Powder

The FTIR spectrum of calcined eggshell powder shown in Fig. 4(a) characteristic absorption bands corresponding to carbonate and hydroxyl groups. The weak broad band observed around $\sim 3400 - \sim 3600\text{cm}^{-1}$ is attributed to O-H stretching vibrations due, likely attributed to adsorbed moisture on the powder surface. After calcination $\sim 1790 - \sim 1700\text{cm}^{-1}$ carbonation combination band disappearing. In particular $\sim 1450 - \sim 1410\text{cm}^{-1}$ carbonate ν_3 band almost absent or significantly diminished, an indication of CaCO_3 decomposition successful during calcination. The FTIR spectrum of calcined eggshell powder shows disappearance of characteristic carbonate bands at ~ 1450 , ~ 875 and $\sim 710\text{cm}^{-1}$, confirming the decomposition of CaCO_3 . Meanwhile, the emergence of Ca-O vibrational features in the $500-600\text{cm}^{-1}$ region along with decreased carbonate signals indicates successful conversion of egg shell derived calcium carbonate into calcium oxide.

3.4.2 FTIR of Synthesized Eggshell-Derived Calcium-Deficient Hydroxyapatite (CDHA)

The Fig. 4(b) shows Eggshell-Derived Calcium-Deficient Hydroxyapatite (CDHA), the FTIR spectra revealed phosphate absorption bands, including strong bands at $\sim 1020\text{cm}^{-1}$ is assigned to the ν_3 stretching mode of PO_4^{3-} groups, and bending vibrations at ~ 560 and $\sim 600\text{cm}^{-1}$ correspond to phosphate bending modes [45–50] These confirm the formation of the apatite structure. Weak bands in the regions $1410-1470\text{cm}^{-1}$ and around 875cm^{-1} [45–50] are attributed to carbonate groups, indicating carbonate substitution. The decreased intensity of hydroxyl bands supports the production of calcium deficient, bone-like hydroxyapatite.

3.4.3 FTIR of Sintered Eggshell-Derived Calcium-Deficient Hydroxyapatite (CDHA) Eggshell-Derived Calcium-Deficient Hydroxyapatite (CDHA) after sintering, Fig. 4(c) shows FTIR spectra exhibit better defined phosphate bands and decrease carbonate intensity, indicate partial removal of carbonate groups while retaining the apatite structure. Despite improved crystallinity, the hydroxyapatite phase remained stable. There is no additional absorption bands corresponding to secondary phases were observed, illustrate thermal stability of the synthesized hydroxyapatite under the selected conditions. These observations suggest that the synthesized eggshell-derived hydroxyapatite maintained its structural integrity and chemical stability after sintering. The present Eggshell-Derived Calcium-Deficient Hydroxyapatite (CDHA), exhibits a calcium-deficient, carbonate-substituted composition and nano to sub-micron morphology. This makes it more representative of biological apatite compared with chemically pure stoichiometric hydroxyapatite.

4. CONCLUSION

In this study, we successfully synthesized eggshell-derived calcium deficiency hydroxyapatite from waste eggshells through a self-alkaline wet chemical precipitation route and systematically evaluated its structural, morphological, and compositional properties. The main conclusions are as follows.

1. Without the addition of an external alkali, hydroxyapatite might form at $\text{pH} \approx 10$ due to the intrinsic alkalinity produced by calcium oxide hydration.
2. XRD analysis confirmed the crystalline size of about 21.7 nm and annealing sample increases in crystalline size of about 55.1 nm, as determined by the Scherrer equation.
3. The synthesized sample exhibited phase purity, calcium-deficient composition. ($\text{Ca/P} = 1.627$), and nanoscale to submicron rod-like morphology, and post heat-treated hydroxyapatite ($\text{Ca/P} = 1.636$), which was similar to real bone apatite.
4. Eggshell derived calcium deficiency hydroxyapatite (CDHAP) as superior precursor for use in dentistry and orthopaedic applications.

Declarations

Author Contribution

Beera Chandra Shekar: Conceptualization, Methodology, Data curation, writing-original draft, Investigation Kodamanchilli Sandeep Raju: Experiments, Visualization. Nallu Ramanaiah: Supervision, Validation, Writing-review & editing.

Data Availability Statement

Data will be made available from the corresponding author on reasonable request.

Declaration of Competing Interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

- [1] S. Hussain, K. Sabiruddin. Synthesis of eggshell-based hydroxyapatite using hydrothermal

- method. IOP Conf. Ser.: Mater. Sci. Eng. 2021, 1189, 012024.
- [2] R. A. Surmenev. A review of plasma-assisted methods for calcium phosphate-based coatings fabrication. *Surf. Coat. Technol.* 2012, 206, 2035–2056.
- [3] K. Gupta, K. Meena. Artificial bone scaffolds and bone joints by additive manufacturing: A review. *Bioprinting* 2023, 31, e00268.
- [4] A. Szczes, L. Holysz, E. Chibowski. Synthesis of hydroxyapatite for biomedical applications. *Adv. Colloid Interface Sci.* 2017, 249, 321–330.
- [5] P. Hui, S. L. Meena, G. Singh, R. D. Agarwal, S. Prakash. Synthesis of hydroxyapatite bio-ceramic powder by hydrothermal method. *J. Miner. Mater. Charact. Eng.* 2010, 9(8), 683–692.
- [6] H. S. Stephanie, et al. Influence of pH, competing ions, and salinity on the sorption of strontium and cobalt onto biogenic hydroxyapatite. *Sci. Rep.* 2016, 6, 23361.
- [7] Y. Nishiyama, T. Hanafusa, J. Yamashita, Y. Yamamoto, T. Ono. Adsorption and removal of strontium in aqueous solution by synthetic hydroxyapatite. *J. Radioanal. Nucl. Chem.* 2016, 307, 1279–1285.
- [8] S. V. Dorozhkin, M. Eppler. Biological and medical significance of calcium phosphates, *Angew. Chem. Int. Ed.* 2002, 41, 3130–3146.
- [9] A. Laska-Lesniewicz, et al. 6-step manufacturing process of hydroxyapatite filler with specific properties applied for bone cement composites. *Mater. Sci. Eng. C* 2022, 48(18), 26854–26864.
- [10] V. Rodríguez-Lugo, T. V. K. Karthik, D. Mendoza-Anaya, E. Rubio-Rosas, L. S. Villaseñor Cerón, M. I. Reyes-Valderrama, et al. Wet chemical synthesis of nanocrystalline hydroxyapatite flakes: effect of pH and sintering temperature on structural and morphological properties, *R. Soc. Open Sci.* 2018, 5, 171835.

- [11] A. Hazar, Y. Koca. Double step stirring: a novel method for precipitation of nano sized hydroxyapatite powder, Dig. J. Nanomater. Biostruct. 2009, 4(1), 73–81.
- [12] N. Safitri, et al. Enhancing drug loading and release with hydroxyapatite nanoparticles for efficient drug delivery: A review synthesis methods, surface ion effects, and clinical prospects. Mater. Sci. Eng. C 2023, 144, 105092.
- [13] G. Ma. Three common preparation methods of hydroxyapatite, IOP Conf. Ser.: Mater. Sci.Eng. 2019, 577, 012151.
- [14] M. D. Ross, J. Hadzik, K. Kozak, J. Kamil, H. Gerber, M. Dominiak, C. Kunert-Keil. New nano-hydroxyapatite in bone defect regeneration: a histological study in rats, Ann. Anat. 2017, 213, 83–90.
- [15] M. Farokhi, F. Mottaghitala, S. Samani, M. A. Shokrgozar, S. C. Kundu, R. L. Reis, D. L.Kaplan. Silk fibroin/hydroxyapatite composites for bone tissue engineering, Biotechnol. Adv. 2018, 36(1), 68–91.
- [16] S. Ramesh, Z. Z. Loo, C. Y. Tan, W. K. Chew, Y. C. Ching, F. Tarlochan, A. A. Sarhan. Characterization of biogenic hydroxyapatite derived from animal bones for biomedical Applications, Ceram. Int. 2018, 44(9), 10525–10530.
- [17] J. C. Elliot. Structure and chemistry of the apatites and other calcium orthophosphates, Elsevier, 1994, pp. 70–82, 230–244.
- [18] R. Z. LeGeros. Calcium phosphate in oral biology, 1991, pp. 5–12, 110–118.
- [19] S. Weiner, H. D. Wagner. The material bone structure–mechanical function relations, Annu. Rev. Mater. Sci. 1998, 28(1), 271–298.
- [20] K. Haberko, et al. Thermal conductivity of highly porous zirconia. J. Eur. Ceram. Soc.2006, 26(16), 3567–3574.
- [21] X. Li, et al. Stabilization of Ca-deficient hydroxyapatite in biphasic calcium phosphate

ceramics by adding alginate to enhance their biological performances. *J. Mater. Chem. B* 2018, 6(1), 84–97.

- [22] L. Zhang, et al. Physicochemical and cytological properties of poorly crystalline calcium-deficient hydroxyapatite with different Ca/P ratios, *Ceram. Int.* 2022, 48, 24765–24776.
- [23] S. Cazalbou, et al. Ion exchanges in apatites for biomedical applications, *J. Mater. Sci. Mater. Med.* 2005, 16(5), 405–409.
- [24] W. F. Neuman, B. J. Mulryan. The surface chemistry of bone. IV. Further data on recrystallization, *J. Biol. Chem.* 1951, 193, 237–241.
- [25] Y. Sekine, R. Motokawa, N. Kozai, T. Ohnuki, D. Matsumura, T. Tsuji, R. Kawasaki, K. Akiyoshi. Calcium-deficient hydroxyapatite as a potential sorbent for strontium, *Sci. Rep.* 2017, 7, 2064.
- [26] Kartha, S., Gupta, A., & Das, P. (2014). Enhanced protein delivery by multi-ion containing eggshell derived apatitic-alginate composite nanocarriers. *Journal of Materials Chemistry B*, 2(45), 8038–8049.
- [27] Liu, H., & Zhang, L. (2025). Eco-friendly synthesis of eggshell-derived nano-hydroxyapatite: Physicochemical characterization, hemocompatibility, and bone regeneration potential. *Scientific Reports*, 15, 9876.
- [28] Cheng, H., & Liu, Y. (2021). Calcium glycerolate catalyst derived from eggshell waste for macrolactonisation. *Catalysts*, 11(12), 1456–1468.
- [29] Rahman, M. M., Islam, M. R., & Rahman, M. S. (2023). Synthesis of hydroxyapatite from egg shell bio-waste for use in biomedical applications. *Advances in Materials Science*, 48(1), 45–52.
- [30] Kareem, Z., Li, X., & Wang, J. (2024). Synthesis of hydroxyapatite from eggshells via wet

chemical precipitation: A review. RSC Advances, 14(26), 19876–19895.

- [31] P. Kamalanathan, S. Ramesh, L. T. Bang, A. Niakan, C. Y. Tan, J. Purbolaksono, H. Chandran, W. D. Teng. Synthesis and sintering of hydroxyapatite derived from eggshells as a calcium precursor, *Ceram. Int.* 2014, 40, 16349–16359.
- [32] S.-C. Wu, H.-K. Tsou, H.-C. Hsu, S.-K. Hsu, S.-P. Liou, W.-F. Ho. A hydrothermal synthesis of eggshell and fruit waste extract to produce nanosized hydroxyapatite, *Ceram. Int.* 2013, 39(7), 8183–8188.
- [33] L. Chen, et al. Berberine-encapsulated poly (lactic-co-glycolic acid)–hydroxyapatite (PLGA/HA) microspheres synergistically promote bone regeneration with DOPA-IGF-1 via the IGF1R/PI3K/AKT/mTOR pathway, *Sci. Rep.* 2023, 13, 15403.
- [34] H. W. Kim, et al. Nanocrystalline calcium-deficient hydroxyapatites, *Biomater. Sci. Lett.* 2018, 2(2), 87–93.
- [35] S. V. Dorozhkin. Calcium orthophosphates in nature, biology and medicine, *Materials* 2009, 2(4), 399–498.
- [36] S. Raynaud, E. Champion, D. Bernache-Assollant, P. Thomas. Calcium phosphate apatites with variable Ca/P atomic ratio: synthesis, characterization and thermal stability of powders, *Biomaterials* 2002, 23(4), 1065–1072.
- [37] S. C. Wu, H. C. Hsu, S. K. Hsu, W. F. Ho. Synthesis of hydroxyapatite from eggshell powders through precipitation method, *Ceram. Int.* 2013, 39(1), 4539–4549.
- [38] F. S. Irwansyah, A. Yusuf, E. D. R. Eddy, D. Risdiana, A. R. Noviyanti. Effect of sensitive pH on hydroxyapatite properties synthesized from chicken eggshell, *Indones. J. Chem.* 2022, 22(7), 1418–1426.
- [39] R. M. Sonee, et al. Hydroxyapatite derived from eggshell through ball milling and heat treatment, *Int. J. Integr. Eng.* 2024, 16(2), 112–120.

- [40] C. Zhang, G. Zhao, X. Wang, M. Li, Z. Li, Y. E, X. Cao, M. Chen, C. Liu. Imperfect hydroxyapatite bioceramics derived from golden pomfret have enhanced osteogenic properties, *Sci. Rep.* 2025, 15, 20582.
- [41] H. Siddiqui, et al. Synthesis and characterization of hydroxyapatite powder by eggshell, *J. Miner. Mater. Charact. Eng.* 2016, 4(3), 123–130.
- [42] A. Balamurugan, et al. Eggshell-derived nano-HA synthesis, *Ceram. Int.* 2020, 46(12), 1234–1242.
- [43] S. Ramesh, et al. Valorization of eggshell waste for bioceramic production, *J. Clean. Prod.* 2022, 340, 130789.
- [44] E. Landi, et al. Biomimetic apatite from eggshell-derived precursors, *J. Eur. Ceram. Soc.* 2008, 28(13), 2593–2601.
- [45] M. Montesissa, G. Borciani, K. Rubini, F. Valle, M. Boi, N. Baldini, E. Boanini, G. Graziani. Ionized jet deposition of calcium phosphates-based nanocoatings: tuning coating properties and cell behavior by target composition and substrate heating, *Nanomaterials* 2023, 13, 1758.
- [46] G. Graziani, M. Govoni, L. Vivarelli, M. Boi, M. De Carolis, M. Bianchi, E. Sassoni, M.C. Bignozzi, G. Carnevale, F. Marmi, et al. A comprehensive microstructural and compositional characterization of allogenic and xenogenic bone: application to bone grafts and nanostructured biomimetic coatings, *Coatings* 2020, 10, 522.
- [47] A. Antonakos, E. Liarokapis, T. Leventouri. Micro-Raman and FTIR studies of synthetic and natural apatites, *Biomaterials* 2007, 28, 3043–3054.
- [48] J. Tao. FTIR and Raman studies of structure and bonding in mineral and organic–mineral composites. In: *Methods in Enzymology*, Elsevier, 2013, vol. 532, pp. 533–556.
- [49] I. Rehman, W. Bonfield. Characterization of hydroxyapatite and carbonated apatite by

photo-acoustic FTIR spectroscopy, J. Mater. Sci. Mater. Med. 1997, 8, 1–4.

[50] S. Koutsopoulos. Synthesis and characterization of hydroxyapatite crystals: a review study on the analytical methods, J. Biomed. Mater. Res. 2002, 62, 600–612.

Figures

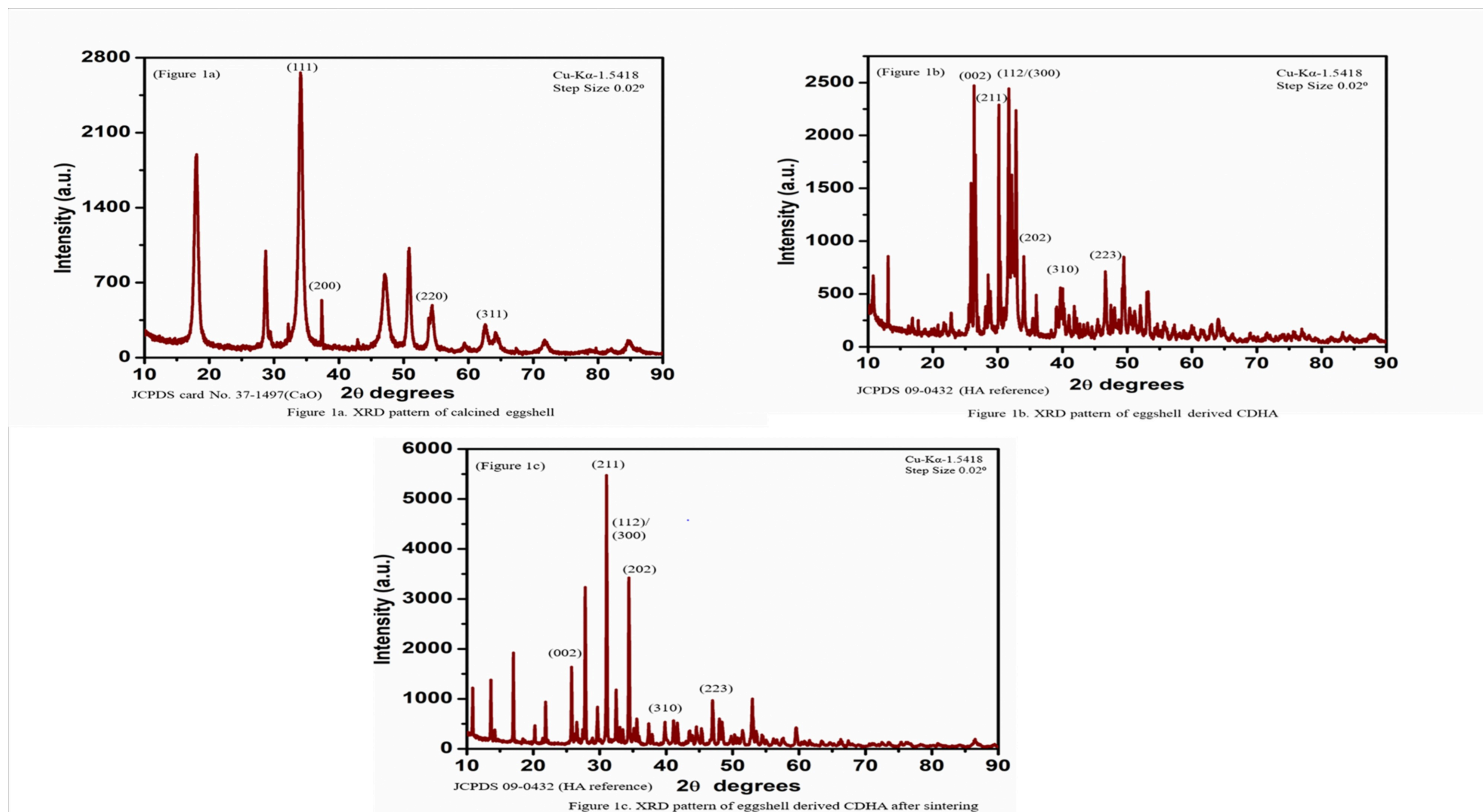


Figure 1

XRD patterns of eggshell-derived materials: (a) XRD pattern of calcined eggshell powder, (b) XRD pattern of eggshell derived CDHA, (c) XRD pattern of eggshell derived CDHA after sintering

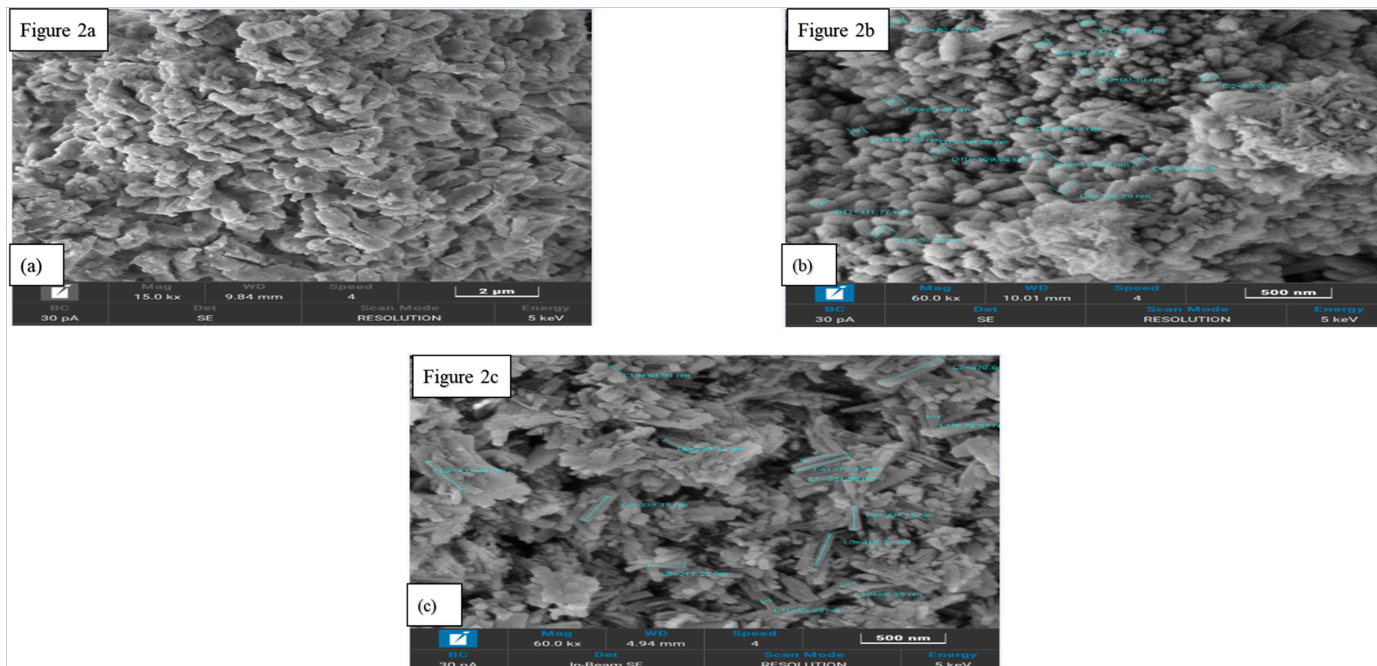


Figure 2. FESEM Micro images of (a) calcined eggshell (b) CDHA particle diameter (c) CDHA particle length

Figure 2

FESEM images showing morphology changes in eggshell-derived materials: (a) calcined eggshell powder, (b) Eggshell-Derived Calcium-Deficient Hydroxyapatite (CDHA) particle diameter, (c) Eggshell-Derived Calcium-Deficient Hydroxyapatite (CDHA) particle length

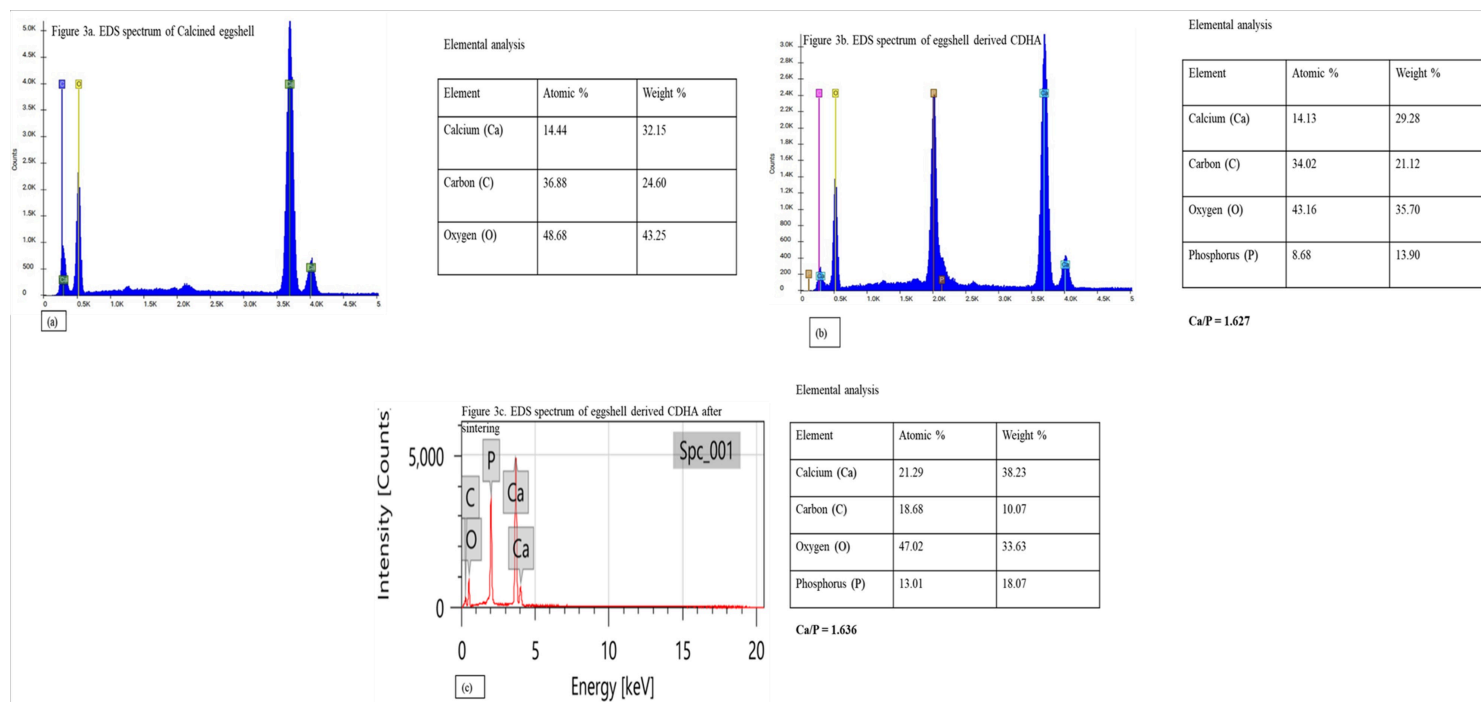


Figure 3

EDS spectra conforming the elemental composition of eggshell-derived materials: (a) calcined eggshell powder, (b) eggshell derived CDHA (c) sintered eggshell derived CDHA

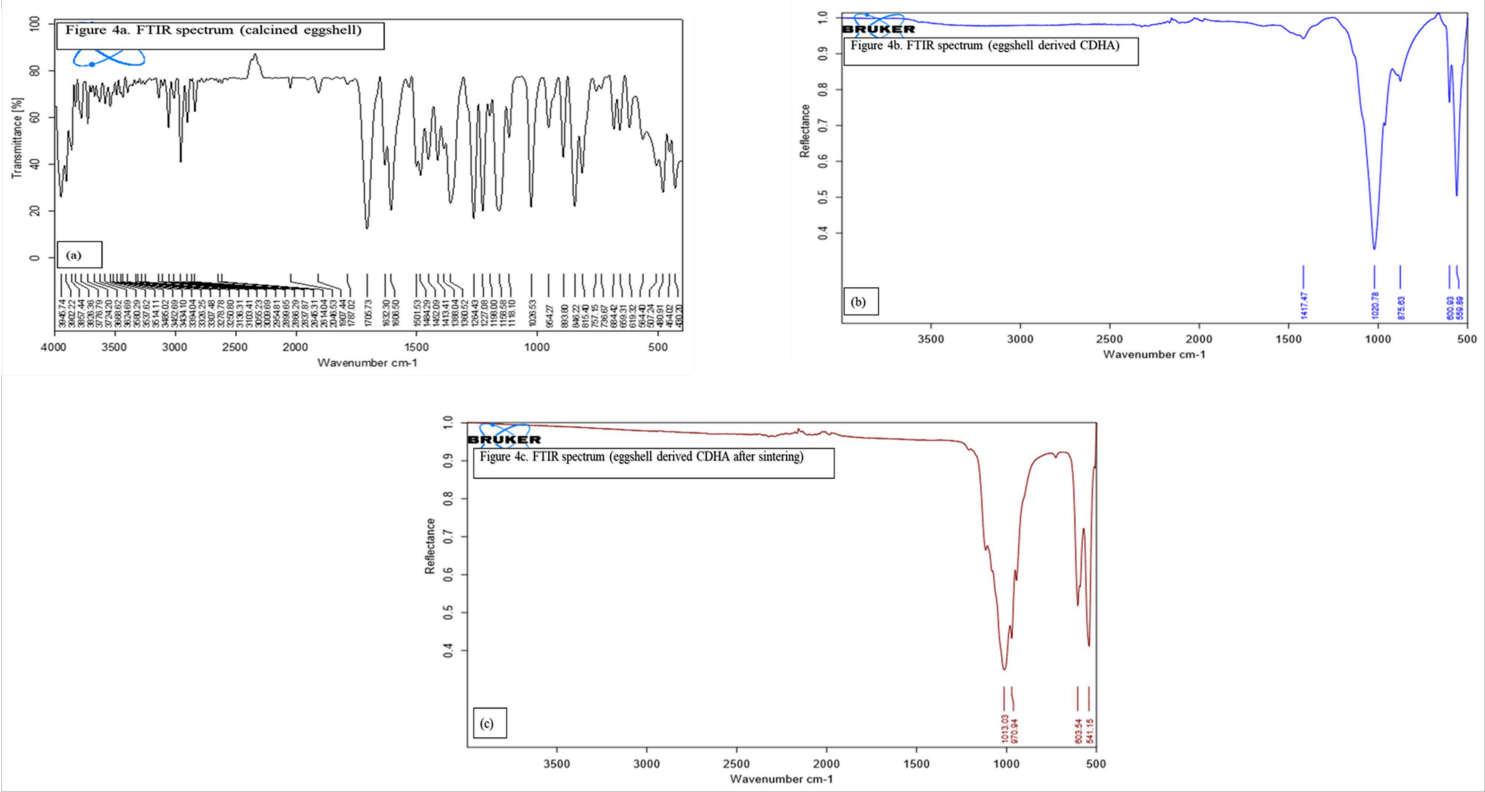


Figure 4

FTIR indicating the presence of functional groups in eggshell-derived materials: (a) calcined eggshell powder, (b) eggshell derived CDHA (c) eggshell derived CDHA after sintering