

**EFFECT OF REDUCED GRAPHENE OXIDE CONTENT ON THE
MORPHOLOGY AND STRUCTURE OF EGGSHELL DERIVED
MAGNESIUM DOPED HYDROXYAPATITE COMPOSITE
USING MICROWAVE PROCESSING**

BY

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Requirements for the Degree of

MASTER OF SCIENCE

In

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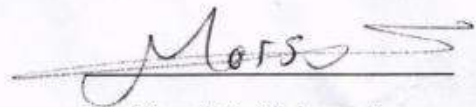
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[Dedicated to my beloved parents and family members, who always stood by my side and wholeheartedly supported me throughout the work with the unparalleled inspiration and motivation.]

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LIST OF ABBREVIATIONS

Table 1 - List of Abbreviations

HAp	Hydroxyapatite
GO	Graphene oxide
rGO	Reduced graphene oxide
Mg	Magnesium
rGO-Mg-HAp	Magnesium - doped hydroxyapatite reduced graphene oxide composite
CaP	Calcium phosphate
MW	Microwave
EM	Electromagnetic
XRD	X-ray diffraction
SEM	Scanning electron microscopy
EDS	Energy-Dispersive Spectroscopy
FTIR	Fourier transform infrared spectroscopy
Wt. %	Weight percent
hr	Hour
min	Minutes
μm	Micrometer
nm	Nanometer

ABSTRACT

Full Name : Ismaeel Alshnkiti

Thesis Title : Effect of Reduced Graphene Oxide Content on the Morphology and Structure of Eggshell Derived Magnesium Doped Hydroxyapatite Composite using Microwave Processing

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In this study, a one-step microwave (MW) processing is used to prepare magnesium doped hydroxyapatite with reduced graphene oxide (rGO-Mg-HAp) composite utilizing eggshell waste. All three constituents of the composite have gained a lot of attention in the biomedical field due to their special characteristics for bone tissue engineering. Microwave processing is expected to provide a fast and promising economic processing method for the proposed composite. Eggshell waste was used as the main calcium source and starting material to prepare the Mg-doped HAp which offered an excellent way to manage such waste in a better way and have a value-added product. The reduced graphene oxide was prepared via the well-known Hummel's method. The effect of different contents of rGO on the microwave prepared eggshell based Mg-doped HAp composite structure and morphology was investigated and analyzed. The prepared samples were characterized using X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR), and Scanning electron microscopy (SEM). The microwaved samples showed high levels of crystallinity and the XRD profiles of HAp and Mg-HAp did not reveal any phase changes. The rGO addition caused an increase of the crystal size and overcame the Mg tendency to

lower the crystal size. FTIR results showed the presence of all HAp bands and the intensity changed to phosphate bond confirmed the incorporation of Mg ions into HAp lattice. All samples showed an irregular shape morphology with tendency to agglomerate; the homogenous distribution of different elements proved that the composite was successfully synthesized.

ملخص الرسالة

الاسم الكامل: إسماعيل الشنقيطي

عنوان الرسالة: تأثير محتوى أكسيد الجرافين المخفض على تشكل وبنية مركب هيدروكسيباتيت المشتق من قشر البيض والمطعم بالمغنيسيوم باستخدام المعالجة بالميكروويف

التخصص: هندسة المواد

تاريخ الدرجة العلمية: شوال 1446 هـ

في هذه الدراسة، تم استخدام معالجة بالموجات الدقيقة (المايكرويف) بخطوة واحدة لتحضير مركبات هيدروكسيباتيت (HAp) المشتقة من مخلفات قشر البيض والمطعمة بالمغنيسيوم (Mg) وأكسيد الجرافين المخفض (rGO). اكتسبت المواد المركبة rGO-Mg-HAp الكثير من الاهتمام وتستخدم في العديد من التطبيقات مثل مجال الطب الحيوي و هندسة الأنسجة العظمية. كما أنه من المتوقع أن توفر المعالجة بالموجات الدقيقة طريقة معالجة اقتصادية سريعة وواحدة للمركب المقترح. تم استخدام مخلفات قشر البيض كمصدر رئيسي للكالسيوم والمواد الأولية لتحضير الهيدروكسيباتيت المطعم بالمغنيسيوم (HAp-doped Mg) الذي قدم طريقة ممتازة لإدارة هذه المخلفات بطريقة أفضل والحصول على منتج ذي قيمة مضافة.. تمت دراسة وتحليل تأثير محتويات مختلفة من أكسيد الجرافين المخفض (rGO) والمغنيسيوم (Mg) على البنية المركبة وتشكل الهيدروكسيباتيت المعتمد على قشر البيض. تم تشخيص العينات المحضرة باستخدام حيود الأشعة السينية (XRD)، ومطيافية تحويل فورييه للأشعة تحت الحمراء (FTIR)، والمجهر الإلكتروني الماسح (SEM). أظهرت العينات مستوى عالٍ من التبلور ولم تكشف صور الأشعة السينية عن أي تغيرات في الطور. تسببت إضافة أكسيد الجرافين المخفض في زيادة حجم البلورة وتغلبت على ميل المغنيسيوم لخفض حجم البلورة. أظهرت نتائج مطيافية فورييه للأشعة تحت الحمراء عن وجود جميع نطاقات الهيدروكسيباتيت وتغير الشدة إلى رابطة الفوسفات مما أكد دمج أيونات المغنيسيوم في شبكة الهيدروكسيباتيت. أظهرت جميع العينات مورفولوجيا سطحية تشبه اللوحة ثنائية الأبعاد مع ميل إلى التكتل؛ أثبت التوزيع المتجانس للعناصر المختلفة أن المركب قد تم تصنيعه بنجاح.

CHAPTER 1

INTRODUCTION

Composite materials have accumulated expanding interest recently because of their wide applications across different industries. The unification of distinct components into a single material considers the synergistic enhancement of qualities, leading to further enhanced execution and new functionality. Among the plenty of composite materials, graphene oxide (rGO) and hydroxyapatite (HAp) stand apart for their uncommon properties and applications in the biomedical field. Because of its remarkable mechanical, electrical, and thermal characteristics, graphene oxide, a graphene derivative, has gained prominence. Its two-layered construction and high surface region make it an amazing contender for supporting materials, adding to upgraded strength and conductivity. Graphene oxide has found applications in fields like gadgets, energy capacity, and biomedical designing, attributable to its capacity to give prevalent mechanical strength, electrical conductivity, and biocompatibility to composite materials [1].

Hydroxyapatite, then again, is a calcium phosphate compound with an organization like that of normal bone mineral. Its biocompatibility, bioactivity, and osteoconductive make it an essential part in bone tissue designing and dental applications. Hydroxyapatite-based composites are generally used for their capacity to emulate the normal bone construction, advancing cell grip and bone recovery [2]. The reconciliation of hydroxyapatite into

composites upgrades their bioactivity, making them important in biomedical and dental applications [3].

Organic and inorganic components make up bone tissues, which have a high capacity for self-healing and mechanical stress. Fixing bone fractures and non-unions, correcting spinal abnormalities, and replacing worn-out joints are vital clinical requirements that have yet to be addressed [2]. For remolding or regenerating major orthopedic defects, historically bone restoration has been based on bone grafts. However, bioactive Calcium phosphate ceramics (CP) have recently emerged as potential osteoinductive and osteoconductive substitute materials [1,4].

Bones and teeth consist of two major parts, organic and inorganic. The latter part is chemically and crystallographically similar to the mineral hydroxyapatite (HAp) and exhibits excellent osteoconductivity [1]. A bioactive coating of this type has been used to enhance the biointegration of bone tissues and implants in a clinical setting. This allows osteoblast cells to connect with and develop on the surface of the prosthetic implant [2]

The fundamental problem is that when placed under severe loads, it has weak mechanical qualities. Carbon nanotubes, polyethylene, Al_2O_3 , and titania are commonly used in HAp composites to improve mechanical qualities [5]. These polymers, on the other hand, are not as bioactive as pure HAp. Graphene, which is an atom thick carbon sheet in a hexagonal lattice, is a better biomedical material and quickly becoming a viable medical substance because of its significant surface area, high conductivity, and remarkable mechanical qualities. Any composite doped with graphene has the potential to trigger osteogenic and chondrogenic differentiation of stem cells, which opens new possibilities for bone

regeneration and repair [6]. Graphene and its oxides are good mechanical fillers for biomaterials because of their remarkable elasticity and flexibility.

The blend of composite materials assumes a crucial part in deciding their properties and, thus, their pertinence. One-step microwave handling has arisen as an effective and financially suitable strategy for the blend of composite materials. This method includes the utilization of microwave irradiation to prompt quick and uniform heating from molecular level to increase the kinetics of the chemical reaction and nucleation of the composite. The benefits of one-step microwave handling incorporate decreased response times, energy proficiency, and the capacity to get homogenous composite. Advanced materials with individualized properties can be developed using this approach with great potential [7].

With respect to composite materials, the joining of graphene oxide and hydroxyapatite through one-step microwave handling addresses a state-of-the-art approach with huge ramifications. The blend of these two materials tends to the requirement for multifunctional composites that display a synergistic exchange of mechanical, electrical, and organic properties. The one-of-a-kind qualities of graphene oxide, for example, its high conductivity and mechanical strength, supplement the bioactivity and osteoconductive of hydroxyapatite, bringing about a composite material with improved by and large execution [8].

This study centers around tackling the upsides of one-step microwave handling to combine a composite material involved reduced graphene oxide and magnesium-doped hydroxyapatite (rGO-Mg-HAp). The incorporation of magnesium further improves the composite's qualities that make it particularly attractive for use in medical fields. The use

of eggshell waste as a calcium source adds a naturally feasible aspect to the composite. Also, providing a great method of administration of waste materials.

Magnesium (Mg) is an important element for the human body. About 60 % of total Mg in the human body is in bones. Enamel, dentin, and bone have Mg concentrations of 0.44 wt.%, 1.23 wt.%, and 0.72 wt.%, respectively of their total weight [9]. Magnesium ions influence ion channel regulation, DNA integrity, enzyme activation, and cell growth and proliferation. Lack of Mg or Mg deficiency causes a halt in bone production, loss, and fragility, as well as decreased osteoblast and delayed osteoclast activity [10]. Because it encourages osteoblast growth, Mg-HAp is a better bone replacement than pure HAp. The biological activity of HAp can be increased by doping with Mg.

As the interest in composite materials keeps on developing, especially in fields such as biomedicine and materials science, the synthesis of a novel material by different routes became important since it brings materials with distinctive qualities to the industry and serve the required demand. This study plans to address this interest by investigating the one-step microwave handling way to deal with create a composite material with customized properties, opening new roads for applications in different modern areas.

Figure 1 below shows the raising numbers of publication on magnesium doped hydroxyapatite with reduced graphene oxide composites [11]. This rapid increase of research articles indicates the topic importance and novelty.

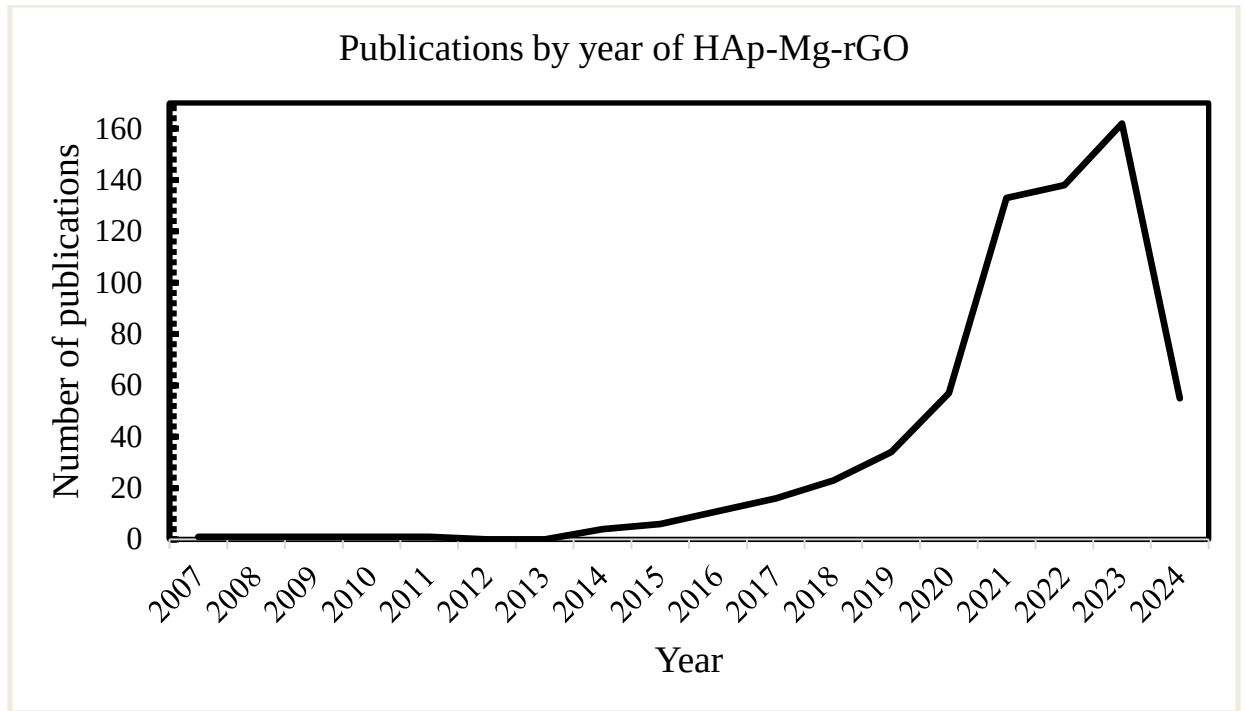


Figure 1. Number of publications of HA-Mg-rGO research articles between 2007 and 2024 [11]

1.1 Importance and application of rGO-Mg-HAp

This section discusses the significance of the composite material composed of magnesium (Mg), hydroxyapatite (HAp), and reduced graphene oxide (rGO). This material is very useful in tissue engineering, biomaterials, and biomedicine:

- **Importance of the composite**

1- Enhanced Mechanical Properties:

Strength and toughness are two mechanical qualities of HAp that can be improved by adding rGO and Mg. Graphene-based materials, including rGO, are known for their exceptional mechanical strength, which can reinforce the HAp matrix [12].

2- Improved Biocompatibility:

HAp is a biocompatible substance that is frequently utilized in biomedical settings since it resembles the mineral found in real bone. The biocompatibility of the composite can be further improved by adding Mg and rGO, which qualifies it for use in tissue engineering scaffolds and medical implants [13].

3- Electrical Conductivity:

Graphene-based materials like rGO offer excellent electrical conductivity. Introducing rGO into the composite can impart electrical conductivity to HAp, enabling applications in electrical stimulation therapies, biosensors, and regenerative medicine [14].

4- Biodegradability and Bioactivity:

Because of its superior biodegradability and bioactivity, HAp is advantageous for the regeneration of bone tissue. These characteristics can be improved even further by the addition of Mg and rGO, which promotes osteogenic differentiation, cell adhesion, and proliferation [15].

5- Tailorable Properties:

One can modify the ratios and processing parameters to customize the properties of rGO-Mg-HAp composites. Because of its adaptability, researchers can tailor the composite for use in load-bearing implants, medication delivery systems, or bioactive coatings [16].

- **Applications of the composite**

1- Bone Tissue Engineering:

Bone tissue engineering applications may be developed with the rGO-Mg-HAp composite. Because of its mechanical attributes, bioactivity, and biocompatibility, it can be utilized to make bone scaffolds for mending and regeneration [17].

2- Orthopedic Implants:

Implants made from rGO-Mg-HAp can provide mechanical support and promote bone ingrowth. The composite's bioactivity and ability to mimic the natural bone structure make it ideal for orthopedic implants, such as screws, plates, and joint replacements [17].

3- Drug Delivery Systems:

Both the large surface area and the porous structure of rGO-Mg-HAp composites qualify them for use in drug delivery systems applications. Drugs or bioactive molecules can be loaded onto the composite matrix and released in a controlled manner, making it useful for localized drug delivery in bone regeneration therapies [18].

4- Biosensors and Bioelectronics:

The electrical conductivity of rGO-Mg-HAp composites enables their use in biosensors and bioelectronics. They can be incorporated into devices for detecting biomolecules, monitoring cellular activities, and interfacing with biological systems [19].

5- Dental Applications:

Dental implants, fillings, and coatings can be made with rGO-Mg-HAp composites because of their mechanical qualities and biocompatibility. These materials can promote osseointegration and prevent bacterial colonization, enhancing dental health outcomes [20].

In summary, the rGO-Mg-HAp composite offers a versatile platform with tunable properties for various biomedical applications. Its exceptional mechanical strength, electrical conductivity, bioactivity, and biocompatibility make it a potential material for the development of implantology, regenerative medicine, and biosensing. Sustained investigation and advancement in this field have the capacity to tackle significant healthcare predicaments and enhance patient results.

1.2 Hydroxyapatite (HAp)

Calcium cations (Ca^{2+}) and metaphosphates (PO_3^{3-}), orthophosphates (PO_4^{3-}) or pyrophosphates ($\text{P}_2\text{O}_7^{4-}$) anions, and hydroxyl (OH^-) or hydrogen (H^+) ions are all referred to as "CaPs." CaP is the main element of enamel (90 %) and bone (60%), which is why it is being researched for bone repair and regeneration [1], [21]. They have a similar crystal structure and chemistry to bone apatite and are extremely biocompatible with living cells. CaP, in any form, is known to have bone conduction properties that facilitate bone cell adhesion, proliferation, and migration (such as coating, powder, bulk, or porous scaffold) [2]. CaPs have also been shown to have bone-inducing characteristics, implying that they promote new bone development and regeneration.

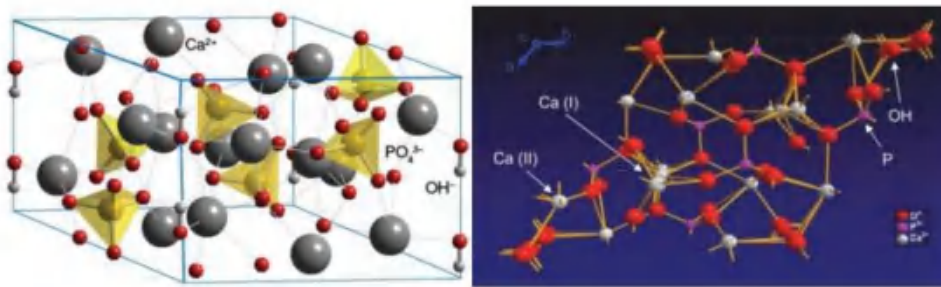


Figure 2. The crystalline structure of HAp [22].

The following ions, along with their distribution of total weight, make up the two formula units in the crystallographic unit cell that illustrate HAp's complicated crystalline structure (Figure 2):

- phosphate ions (PO_4^{3-}) make up 56.77 %.
- hydroxyl ions (OH^-) make up 3.39%.
- calcium ions (Ca^{2+}) make up 39.84 %.

The crystal development occurs along the c-axis, and the unit cell's cell characteristics are $a = b = 9.4225$ and $c = 6.8850$. Its structure is hexagonal in nature, and it belongs to space group P63/m. With a pH range of 4.2 to 8, HAp is more stable in aqueous environments than other CaP ceramics [22].

Each unit cell has two I and II sites that can be occupied by calcium ions, and there are two distinct planes for PO_4^{3-} ions, each of which has a crystal height of 14 or 34. Along the c-axis, there are two different kinds of channels: channel A and channel B. At the corners of two equilateral triangles that rotate 60 degrees apart, there is Channel A, which contains the oxygen atom of the phosphate group and the type II calcium (Ca (II)) ion. Only Type I calcium ions (Ca (I)) are present in the Type B channel, which has a diameter of 2 Å [22].

The number of cation and anion in each HAp unit cell is as follows:

- Ca^{2+} : the total number in a unit cell is 10 with 6 enclosed by the unit cell and 8 shared with neighboring cells and contribute with 1/2 to the cell.
- PO_4^{3-} : the total number in a unit cell is 6 with 2 enclosed in the unit cell and 8 shared with neighboring cells and contribute with 1/2 to the cell.

- OH^- : It only located in the corners and the total number is 2 contributing by 1/4 to the cell.

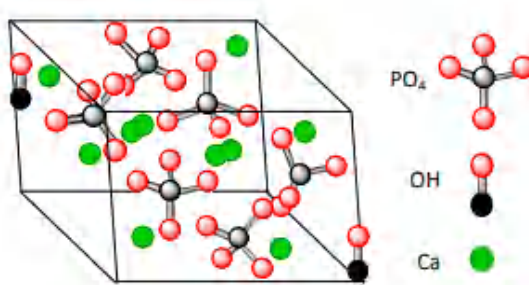


Figure 3. The structure of hydroxyapatite crystals [22].

Because HAp resembles biological apatite found in human bones and teeth, it is highly suited for application in orthopedics, dentistry, and maxillofacial surgery. Its Ca/P atomic ratio is 1.67. As the Ca/P ratio rises, the HAp in the human body becomes more stable. Additionally, it has been discovered that as the Ca/P ratio rises, the mechanical strength of CaP increases, peaking at 1.67 and then declining. The main mechanical properties of HAp are listed in table 2 below.

Table 2. properties of HAp [22].

Property	Value
Density	3.16 g/cm ³
Tensile strength	38–300 MPa
Young's elastic modulus	35–120 GPa
Poisson's ratio	0.27

1.3 Reduced graphene oxide

Carbon atoms are organized in two-dimensional sheets in a honeycomb lattice structure to form graphene, a carbon allotrope that is usually one atom thick [17]. Graphene exhibits exceptional mechanical, thermal, and electrical capabilities. Recently, there has been a lot of interest in graphene and its hybrids as possible microwave absorber material (MAM) due to their exceptionally high specific surface area, light weight, and stable mechanical and thermal resilience. Furthermore, the introduction of novel and simple fabrication procedures for those materials has made them easier to procure and use in microwave absorption material applications. Because of its unique band structure and electron conduction mechanism, graphene has impedance matching properties that allow independent hole and electron migration. In contrast, a kind of graphene called reduced graphene oxide (rGO) can be produced by reducing graphene oxide where the oxidized functional groups are removed. The energy transition and improved impedance matching features are caused by residual groups and faults in rGO. rGO's microwave absorption properties are improved by residual groups' dipole relaxation and defects' polarization. The graphene sheet works as a 2D planar platform in graphene hybrids, supporting and anchoring other nanomaterials [17].

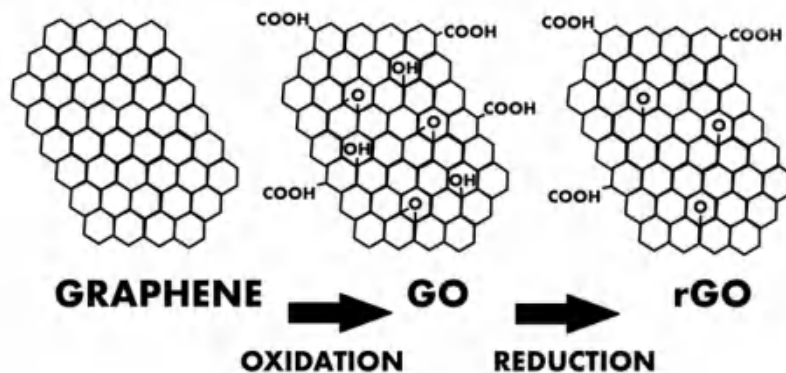


Figure 4. Molecular structure of graphene, GO and rGO [14].

The functional groups carbonyl, carboxyl, and hydroxyl are among the several oxygen-containing ones that are prevalent in graphite monomolecular layers [23]. This increases the atomic structure's inter-layer distance and makes the layers hydrophilic. Graphene oxide (GO) is typically produced using a modified Hummers method [23] from natural flake graphite powder. Thermal, chemical, microwave, photo-chemical, photo-thermal, and biological treatments can all be used to convert graphene oxide to reduced graphene oxide (rGO). The GO to rGO conversion does not produce a complete graphene sheet because not all sp^3 bonds are converted to the sp^2 configuration, leaving residual oxygen and oxygen-containing functional groups as shown in figure 4 above [23].

rGO appears to be a viable material for many biological applications where it is critical to use materials that are harmless to human cells. Many publications in the literature [24] have validated rGO's biocompatibility. Because of its enormous active surface area, rGO can be used to safely introduce biological molecules (both organic and inorganic) into the organism without the risk of uncontrolled and undesired dissemination into the surrounding tissues.

1.4 Magnesium

Magnesium is one of the most vital ions in the body and is primarily found in bone. Mg^{2+} ions promote the production of stem cell osteoblasts, osteoblast/osteoblast activity, cell adhesion and proliferation, and bone metabolism [10]. Mg^{2+} is a growth agent that promotes bone production in the early stages, although deficiency can cause bone loss and fragility [25].

According to Matsunaga, ion size differences cause the neighboring O_2 ions to move ahead to the replacement cations when Ca^{2+} ions are replaced by smaller cations. Consequently, O_2 ions get closer to one another and repel one another electrostatically [26]. Some O_2 ions are isolated from adjacent cations in order to reduce electrostatic repulsion, and the coordination number is decreased [27]. The c-axis decreased while the a-axis grew slightly. Compared to Ca^{2+} , Mg^{2+} has lesser radius of ions (0.072 nm), which means that its unit cell volume is less [27].

In the synthesis of Mg-HAp, a fundamental challenge is the lack of completely substituting Ca^{2+} . When substituted components are added to the HAp phase, it can become unstable, leading to the development of undesired secondary phases that might injure cells. Farzadi et al. [28] explained the synthesis of $Mg(OH)_2$ in Mg-substituted HAp. Secondary phase formation in the HAp lattice shows a limited degree of Mg^{2+} ion replacement. Secondary CaP phases, on the other hand, should not be harmful to cells, and They might enhance the multiphase systems' biological performance and resorption characteristics [28].

1.5 Microwave processing.

A pure form of energy that travels at the speed of light is called electromagnetic radiation. When the magnetic and electric fields fluctuate at right angles to one another and in the same propagation direction, an electromagnetic wave is produced (Figure 5).

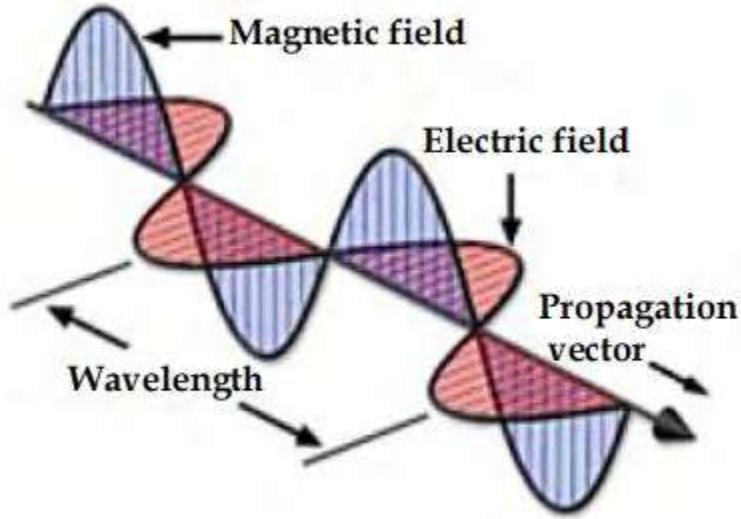


Figure 5. Schematic representation of an electromagnetic wave [29]

The electric and magnetic fields carry the energy of an electromagnetic wave. The energy per unit volume in an alternating electric field (U_{elec}) propagating in vacuum is generally given by [30]

$$U_{elec} = \frac{1}{2} \cdot e_o \cdot E^2 \quad (1)$$

where E represents the alternating electric field's amplitude and e_o denotes the vacuum's dielectric permittivity.

Now, the energy per unit volume in an alternating magnetic field (U_{magn}) propagating in vacuum is:

$$U_{magn} = \frac{1}{2 \cdot \mu_o} \cdot B^2 \quad (2)$$

B is the alternating magnetic field's amplitude, and the magnetic permittivity in vacuum is μ_o . The energy density of an electromagnetic wave can be expressed in terms of either the magnetic or electric fields because the energy associated with each is equal [30].

$$U_{elec} = \frac{1}{2} \cdot e_o \cdot E^2 = \frac{1}{2 \cdot \mu_o} \cdot B^2 \quad (3)$$

The proportionality of the E and B fields is also implied by this. By rearranging, equation 4 can be obtained.

$$E = \frac{1}{\sqrt{e_o \mu_o}} \cdot B \quad (4)$$

Hence, this factor $\frac{1}{\sqrt{e_o \mu_o}}$ is a constant and matches to the vacuum light speed (c).

$$E = C \cdot B \quad (5)$$

Examining the amount of energy that the wave transports from one location to another is a more popular method of handling energy. An effective measure of this is the wave's intensity (S), which is computed by dividing the power by its size. There exists a factor of c between the intensity, S, and the energy density.

$$S = C \cdot U = C \cdot \left(\frac{1}{2} \cdot e_o \cdot E^2 + \frac{1}{2 \cdot \mu_o} \cdot B^2 \right) \quad (6)$$

The microwave sector of the electromagnetic spectrum lies between infrared and radio wave radiation (Figure 6). Microwaves have wavelengths between one millimeter and one meter, or between zero and three gigahertz, in relation to wave frequencies. There are different frequency allocations for microwave equipment, but the standard wavelength for home and commercial microwave equipment is 12.25 cm (2.45 GHz) [31].

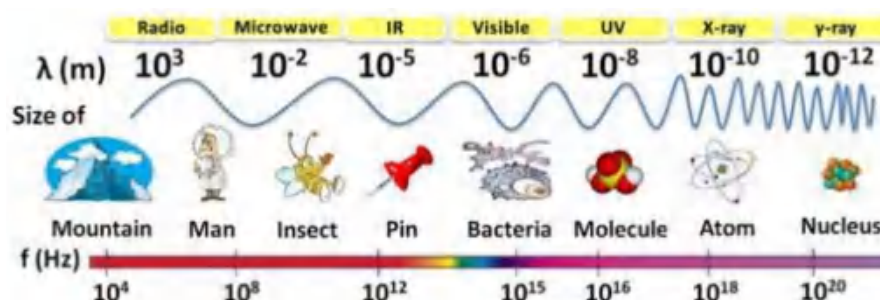


Figure 6. The electromagnetic spectrum [32]

Several needs influence the choice of this frequency. First things first, any interference with cellular phones, wireless networks, or telecommunications must be avoided. The wavelength of 2.45 GHz, which is smaller than the cooking chamber at 12.25 cm, is the second factor that makes it appropriate for use in kitchen microwaves. However, the resonance frequency of liquid water is higher, at 18 GHz, therefore this frequency is not the best for heating water. As a result, in water containing food products, microwave energy would be most efficiently converted into thermal energy at this frequency [32].

The distinction between microwave energy and other radiation types, like X-rays or γ-rays, lies in its non-ionizing nature. Unlike other radiation types, microwave energy does not fully knock out an electron from its atom or molecule; instead, it just increases thermal energy (eat) due to molecular motion. Because microwave radiation comes from

the low-energy part of the electromagnetic spectrum, its initial heating effects are a little surprising. Table 3 demonstrates that the energy of a weak bond, like the hydrogen bonding, is 0.21 eV, which is significantly less than the microwave frequency at 2.45 GHz, which is comparable to 0.00001 eV (Table 4). The material's dipoles react to the microwave's alternative electromagnetic field by producing a significant amount of heat. Heat is produced by the resistance to the 2.4×10^9 times per second electric field change caused by the rotation of dipolar molecules like water. This might be the primary reason for heating in microwaves [32].

Table 3. Energy of popular radiations [32]

	Typical frequency (MHz)	Quantum energy (eV)
γ -rays	3×10^{14}	1.24×10^6
x-rays	3×10^{13}	1.24×10^6
UV	1×10^9	4.1
Visible	6×10^8	2.5
IR	3×10^6	1.2×10^{-2}
Microwaves	2450	1×10^{-5}
Radio	1	4×10^{-9}

Table 4. Energy of few chemical bonds [33]

	Chemical bond energy (eV)
H-OH	5.2
H-CH ₃	4.5
H-NHCH ₃	4
H ₃ C-CH ₃	3.8
phCH ₂ -COOH	2.4
Hydrogen bonding	0.21

To grasp the various mechanisms of interaction between microwaves and matter, one must have a solid understanding of the mechanism underlying microwave heating.

1.6 Condensed matter interacting with microwaves.

The heating effect resulting from interactions between microwaves and matter can have at least five distinct contributions [30], [31]:

- Dielectric Polarization
- Dielectric losses (conduction currents)
- Magnetic heating
- Magnetic losses (induction currents)
- Second Order effects

The high effectiveness of microwave heating can be explained by the existence of five possible contributions, even if numerous phenomena may contribute to the heating simultaneously. It also makes regulated reactions more difficult, though, as it is usually challenging to ascertain the relative contributions of each process to the heating.

1.7 Dielectric polarization mechanism

Dielectric permittivity and dielectric losses play key roles in microwave heating of polar materials. The amount to which a material's electric dipoles may rotate in the presence of an electric field is known as its "polarizability," or dielectric permittivity. Equation 7, describes the many accumulative components that make up the overall polarization, denoted as (α_1), which include electronic polarization (α_e), interfacial polarization (α_{if}), dipolar polarization (α_p), ionic polarization (α_{ion}), and atomic polarization (α_a) [34].

$$\alpha_1 = \alpha_e + \alpha_a + \alpha_p + \alpha_{ion} + \alpha_{if} \quad (7)$$

- Electronic polarization α_e : An atom's internal charges redistribute in response to an external electric field, producing an induced dipole moment. As a result, the atomic electron cloud moves away from the positive nucleus' equilibrium position. This phenomenon can be seen in any solid substance where the electrons are scattered locally [31,32].
- Atomic polarization α_a : Similar to electronic polarization, this takes into consideration the change that the applied electric field causes in positively charged atomic cores. Typically, it's little in comparison to α_e [31,32].
- Dipolar polarization α_p : When the material reaches thermal equilibrium, it will have randomly oriented electric dipoles that do not indicate any net polarization. Inducing a net polarization in the material, an external field can partially align these dipoles. It can only be found in polar materials with strong ionic chemical bonding, like dielectrics or polar liquids [31,32].
- Ionic polarization (or ionic displacement) α_{ion} : Positive and negative ions are relative to one another and move from their equilibrium lattice positions, which causes this effect. Ions with positive and negative charges are naturally dispersed, and in the crystals, the centers of positive and negative charge within a single unit cell do not line up. Applying strong electric fields can flip this net dipole moment. This effect is limited to ferroelectric and piezoelectric devices [31,32].
- Interfacial polarization α_{if} : There is a possibility of polarization on surfaces, grain boundaries, and interphase boundaries (which includes precipitate surfaces). E. since they have dipoles that could partially align with an outside field, adding to

the material's overall polarization. Any type of condensed matter can experience these effects, but because bulk material interface volume fractions are typically small, Usually, they provide a very small quantitative contribution to the total polarization [31,32].

Figure 7 outlines how the frequency of the connected electric field has an imperative impact on the polarization of dielectric materials. All dipoles can take after the connected field at low frequencies. Be that as it may, as the frequency increments, the different inputs that make up the entire polarization “slow down, " demonstrating that the substituting connected electric field can now not be taken after or polarized by comparing dipoles. subsequently, the dielectric permittivity signified as ϵ' continuously diminishes. A double-peak structure for the dielectric permittivity is observed in both the electronic and nuclear commitments. This structure emerges when the connected field frequency and the exchanging dipoles' resonance frequency overlap [31].

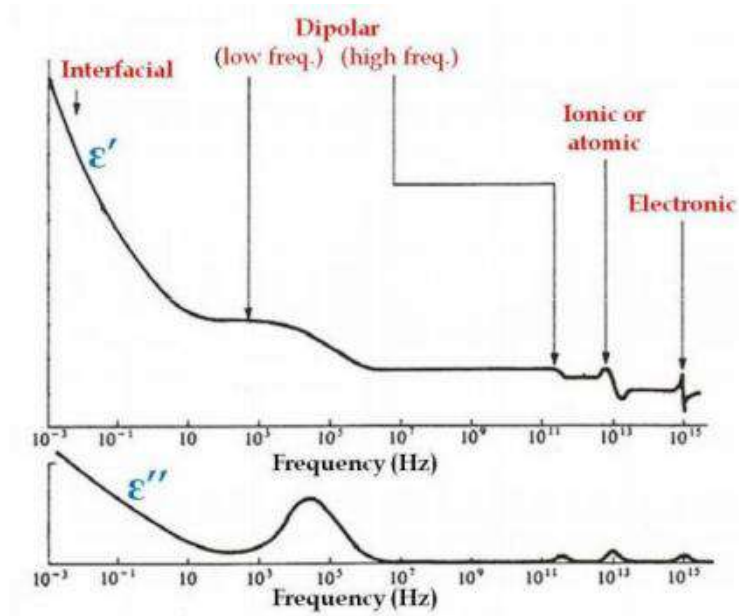


Figure 7. Polarization mechanisms dependence on the electric field frequency for dielectrics at room temperature [32].

A material can produce heat when exposed to microwave radiation if it possesses a dipole moment, as the water molecule does. If a molecule is exposed to low frequency radiation, it will rotate precisely with the oscillating electric field. This rotation does give some energy to the molecule, but because of the low frequency, the overall heating effect is minimal. Conversely, high frequency prevents the dipoles from rotating because they lack sufficient time to respond to the oscillating field, which inhibits the heating [33].

When the oscillating field falls within the microwave radiation zone, a scenario that lies in the middle of these two extremes occurs. The dipoles can rotate in the alternating electric field's direction because the frequency is low enough. Furthermore, the frequency is high enough at 2.45 GHz to produce very rapid dipole spins, which produce a lot of heat. Remember that the dipole's rotations may not always line up precisely with the field, and there may occasionally be a phase discrepancy between the dipole's and the

field's orientation (Figure 8). This phase difference accounts for the energy wasted in dipole molecule friction and collisions [34].

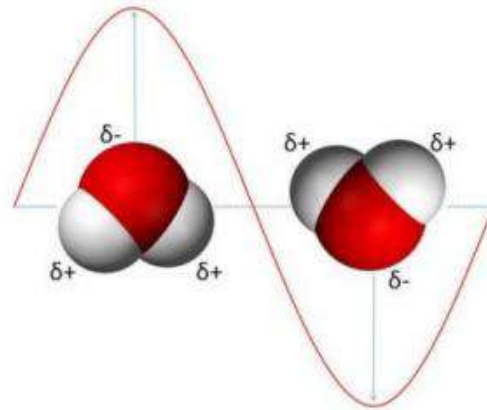


Figure 8. The oscillation of water molecule under electrical field effect [35].

1.8 Dielectric losses: conduction mechanism

In response to an applied electric field, a process known as conduction causes free charge carriers to move over a long distance. Figure 9 shows how the charge carriers oscillate when an AC electric field is applied. The process known as "ion conduction" describes the ability of ions to travel with electrons, the typical charge carriers [35].

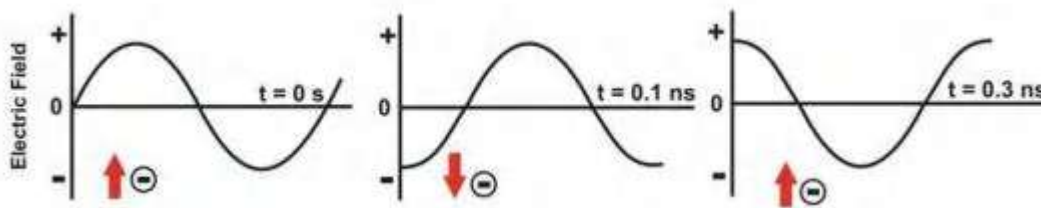


Figure 9. mechanism of conduction, when a sample is exposed to microwave radiation, the charge carriers in the electric field oscillate [36].

The term "dielectric loss" (ϵ'') is frequently used to characterize the behavior of free charge carriers when an alternating electric field is applied. Heat dissipation resulting from Joule heating of the loss currents is known as the dielectric loss heating effect, and it is strongly reliant on the material's electrical resistance. Only at lower frequencies at room temperature does conduction-based heat dissipation have a significant impact. In semiconducting materials, heat conduction has a faster increasing effect with temperature and eventually gains equal importance to dielectric polarization. Elevating a beaker filled with distilled water and another containing a sodium chloride water solution in a home microwave oven allows one to effortlessly illustrate the concept of conduction heating. The main source of heating in distilled water is dipolar polarization; noticeably, the rate of heating is substantially slower than in a diluted salt solution heated to the same volume. In the latter case, the heating effort is enhanced by the salt solution's Na^+ and Cl^- ion conduction [35].

Due to dielectric losses, a sample's power density per unit volume (P) of absorbed microwaves can be expressed in W/m^3 using equation 8. P , however, only describes the microwave energy that the material absorbs; this energy is connected to the thermal energy that the material creates, though it is not precisely the same. Due to dielectric losses, the average power (P) is determined by:

$$P = \sigma \cdot E_{rms}^2 = \omega \epsilon_o \epsilon'' E_{rms}^2 = 2\pi f \epsilon_o \epsilon'' E_{rms}^2 \quad (9)$$

Where σ is the material's conductivity, E_{rms} is the microwave's internal electrical field, $\omega = 2\pi f$ is its angular frequency, ϵ_o is the vacuum's dielectric permittivity, and ϵ'' is the material's relative dielectric losses.

It is highly challenging to heat polar materials with losses $\epsilon'' < 10^{-2}$; for those with $\epsilon'' > 5$, the surface is heated instead of the bulk. The best materials for microwave heating are those with intermediate values, $10^{-2} < \epsilon'' < 5$.

The dielectric permittivity's real and imaginary components (ϵ' and ϵ''), as shown in equation 10, determine how deeply the microwaves will permeate the material. The penetration depth (D) of microwave radiation in a material is the depth at which, for a given incoming wavelength (λ_0), the power density of the radiation is reduced to 37% of its initial value at the surface. The following formula provides the penetration depth for materials whose loss tangent ($\tan\delta = \tan(\epsilon''/\epsilon')$) is smaller than unit [29].

$$D = \frac{\lambda_0}{2\pi} \frac{\sqrt{\epsilon'}}{\epsilon''} \quad (10)$$

The penetration depth values are low for materials with relatively high values of ϵ'' . Since it affects how evenly the material is heated throughout, the depth of penetration is significant. There will be surface heating for small values of ϵ' and large values of ϵ'' , and greater volumetric heating but with lower efficiency for large values of ϵ' and small values of ϵ'' . A constraint on the controlled application of microwave energy for material processing is the absence of temperature-dependent dielectric data for relevant materials in the microwave frequency range [29].

1.9 Classification of material

Condensed matter can absorb, transmit, or reflect electromagnetic microwaves primarily depending on what kind of material it is. According to Figure 10, three distinct classes of materials are typically identified for non-magnetic condensed matter interacting with

microwaves. According to equation 10, this classification has to do with the microwave's penetration depth, as well as to absorption brought on by losses and dielectric polarization [29]:

- Much of the microwave's intensity is reflected, and reflecting materials prevent the electromagnetic wave from penetrating deeper and have shallower penetration depths. This is how materials with free electrons, like some metals, behave. Because of their high electrical conductivity, metals have high losses.
- Materials that are transparent, like Teflon and silica glass, allow the wave to travel through them completely with little attenuation. In this context, insulators or materials with low losses and low dielectric permittivity are of interest. The dielectric polarization results in a high penetration depth and minimal microwave energy absorption.
- Materials that absorb electromagnetic energy effectively convert it to heat. This is true for polar materials like ferroelectrics with high dielectric permittivity and dipolar liquids like water. The material is more likely to absorb microwave energy when the dielectric losses are in the middle range. Due to dielectric polarization, there is a high absorption of microwave energy and an intermediate penetration depth.

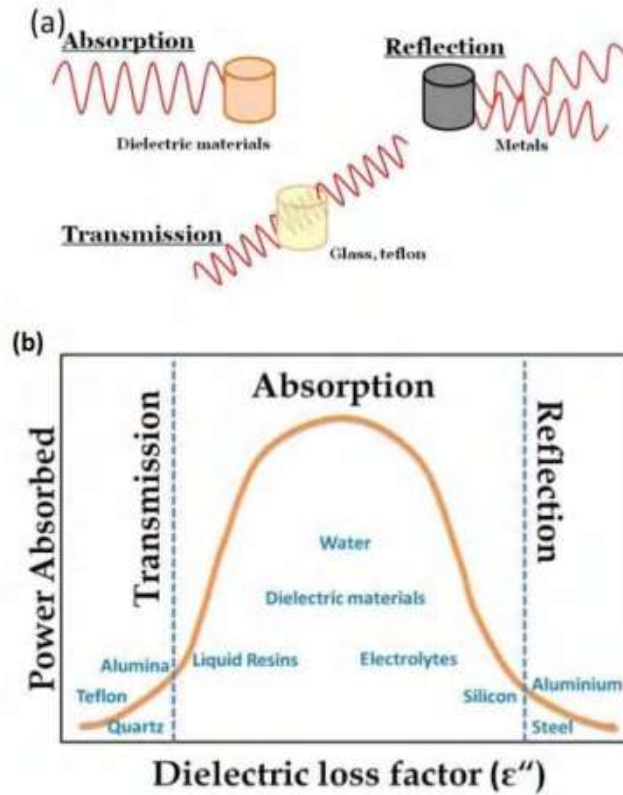


Figure 10. a) Electromagnetic field's interaction with various materials. b) Relationship for certain common solid and liquid substances between the dielectric loss factor and microwave power absorption capacity. Reproduced from Thostenson et al. [29].

Materials with low dielectric loss have a very deep penetration rate. As a result, the material is transparent to microwave waves and absorbs relatively little energy. This tendency means that the materials most suited to benefit from the energy transfer effects of microwaves are those whose dielectric losses are in the middle of the conductivity range (Figure 10-b). Conversely, traditional heating is the most efficient way to absorb heat from materials with high thermal conductivity.

1.10 Microwave heating.

Three key characteristics set microwave heating apart from traditional methods: material selection, rapid heating, and a faster rate of energy transfer. When heated in a microwave furnace, this causes the material's temperature distribution to change.

Energy transfer

During conventional heat treatments, energy is transmitted to the material by convection and heat conduction, which results in thermal gradients. Through molecular interactions between the substance and the electromagnetic wave, energy is immediately transmitted to a material when it is heated using microwaves (Figure 11).

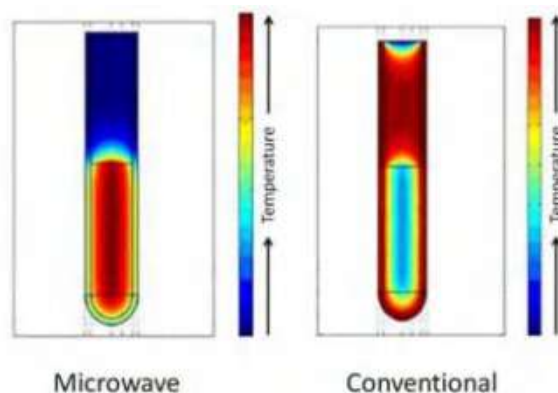


Figure 11. Differences in the temperature distribution in microwave and conventional processes [29].

Energy transfer processes are said to be essential to the synthesis of materials. Microwaves can heat materials quickly and efficiently without overheating them because they can penetrate materials and heat them from the inside out. Bulky materials may be heated more evenly with microwaves since they penetrate deeper and heat up faster, improving the quality and uniformity of the final product [29].

Fast heating

Microwaves significantly shorten the processing time for chemical synthesis when compared to traditional methods, but they typically do not result in appreciable changes to the material's physical and crystallographic characteristics. According to some authors, chemical reactions can be accelerated 10–1000 times faster by using microwave assistance than by using traditional techniques [29].

1.11 Microwave-enhanced reaction rates.

This formula represents the simplest basic chemical reaction. $A + B \rightarrow AB$. Activating reactants A and B to a state of greater energy, represented as $[A-B]^{\ddagger}$, is the initial step. What separates these energy levels from one another is known as the activation energy (E_a), and it is the energy needed for the system to react. The reaction begins when enough activation energy has been absorbed, and product AB (Figure 12) is the result, which releases either more energy than E_a (exothermic) or some of E_a (endothermic) [29].

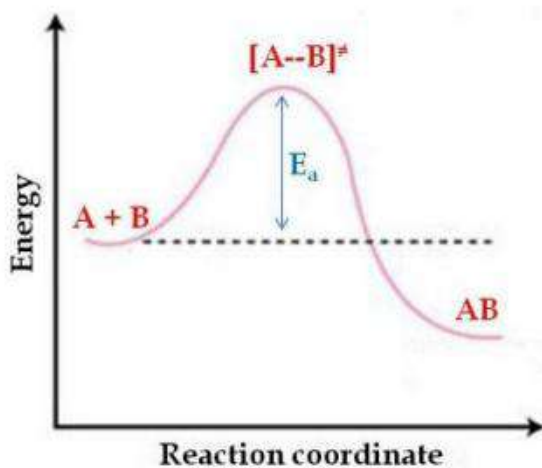


Figure 12. Reaction coordinates of a typical exothermic chemical reaction [29].

The widely recognized Arrhenius rate equation (11) can be used to characterize the reaction's rate:

$$K = Ae^{-Ea/RT} \quad (11)$$

The temperature is represented by T, the gas constant by R, the temperature-dependent reaction rate constant by K, and the characteristic of molecular mobility, which is dependent on the frequency of vibrations of the molecules at the reaction interface, is represented by A, a pre-exponential factor constant [37].

CHAPTER 2

LITERATURE REVIEW

2.1 Producing hydroxyapatite by coprecipitate method and microwave irradiation for the first time

In the biomedical industry, hydroxyapatite (HAP) and calcium phosphates are important inorganic biomaterials with a range of applications. Recently, there has been a lot of interest in the use of microwaves to synthesize nanostructured biomaterials. Lerner and colleagues first described the process of creating HAp particles by microwave irradiation from a precursor solution in 1991 [38]. The calcium (Ca^{2+}) source utilized by the authors was calcium chloride (CaCl_2), the phosphate (PO_4^{3-}) sources were sodium dihydrogen phosphate (NaH_2PO_4) and diammonium hydrogen phosphate ($(\text{NH}_4)_2\text{HPO}_4$). Following the initial five minutes of HAp production, a second microwave irradiation step was carried out to improve the precipitates' crystallinity [38].

The study explored a novel method for manufacturing pure HAp through precipitation from an aqueous medium using microwave irradiation. The authors managed to produce HAp within less than an hour by utilizing microwave energy. Traditional methods involved slow precipitation and maturing processes. The study provided physicochemical explanation for enhanced HAp maturation. The main finding was that microwave energy interact with water molecules, leading to efficient heat transfer and rapid crystallization of HAp [38].

2.2 Synthesis of hydroxyapatite from eggshells precursor

The EC regulations state that industrial eggshells are hazardous waste that needs to be properly managed to prevent risk pathogens (like Salmonella) from spreading into the environment and endangering human health [39]. Waste made of eggshells from bakeries, kitchens, poultries, restaurants, and the food processing sector is contaminating the environment. The majority of eggshell is made up of calcium carbonate (CaCO_3), with minor amounts of organic compounds (~4%), calcium phosphate (~1%), and magnesium carbonate (~1%) [40]. Additionally, it has trace amounts of Na, Fe, Mn, K, Cu, and Sr. Because eggshell waste is abundant and sustainable and has a calcium-rich composition, it is a useful source that can be utilized to synthesize HAp [40].

Generally, chicken eggshells are made of ceramic and have three layers: the innermost shell membrane, calcite's middle layer (which is spongy), and the outermost cuticle layer (Fig. 13(a)) [41]. The matrix that is in both spongy and mammillary layers form is made up by protein fiber attached to calcite crystals in a porous microstructure. In the meantime, most of the organic materials that make up the eggshell membrane are protein fibers and other substances including chondroitin, hyaluronic acid, and glucosamine. The inner protein membrane that is attached to the eggshell will be thrown away in order to synthesize HAp, after which the leftover eggshell will be washed, powdered, and used as a precursor [41].

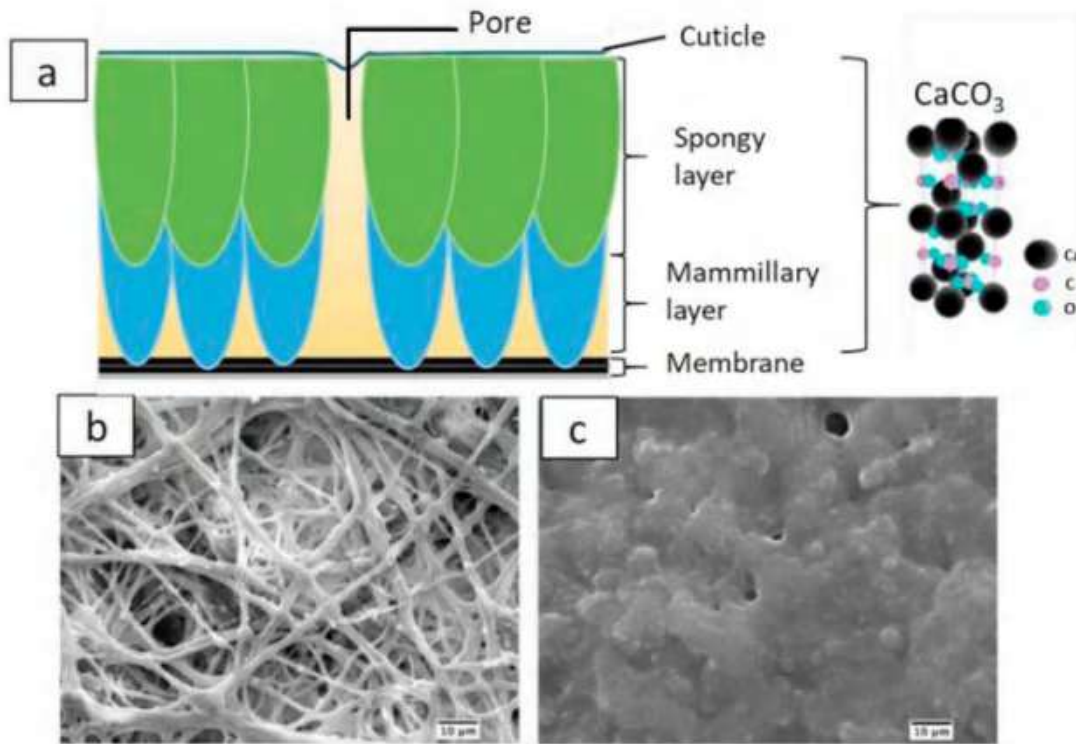


Figure 13. (a) Eggshell structure schematic representation; (b) SEM of eggshells inner surface with a morphology resembling fibers, and (c) SEM of eggshells outermost surface with intrinsic pores [41].

Eggshell wastes have generally been used to create HAp nanostructures with a variety of morphologies, including spherical [42], needle [44], prolate spheroidal [42], rod [43], and flower-like [45] forms. The synthesized HAp's diverse microstructure is largely influenced by the processing technique employed.

2.3 Solid-state method

The comparatively simple solid-state reaction preparation method has been broadly utilized to create HAp on a comparably large scale from eggshell waste [46]. To create HAp, a solid-state process ordinarily involves processing both calcium and phosphorus precursors into a homogeneous blend by milling and after that sintering the blend at a high temperature ($\geq 900\text{ }^{\circ}\text{C}$) [47]. HAp crystallization occurs at elevated high temperature in this process. A

significant problem frequently linked to HAp produced using this method is heterogeneous phase composition, which is brought on by the emergence of secondary phases such β -TCP. Crucially, the processing variables, such as the kind of reagents employed, the ratio of chemicals to water, the length of milling time, the rotating speed, and the temperature at which the calcination occurs have a great determining impact on the phase purity of the finished composite. Thus, having perfect control over the previous outline processing parameter will lead to the formation of HAp with high degree of phase purity [47].

Lee et al. used eggshell squander and H_3PO_4 as precursors using a solid-state method to successfully synthesis single phase HAp and β -TCP ceramics [48]. In this study, cleaned eggshells were first heated to 900 °C to facilitate the transformation of CaCO_3 into CaO. Following a ball milling operation with H_3PO_4 at several mixing ratios (weight percentage of eggshell: H_3PO_4), the CaO powder was calcined for one hour at 900 °C. It was demonstrated that the final products' phase composition depends on CaO: H_3PO_4 mixing proportion: for mixing ratios of (1: 1.1), HAp was generated; increasing phosphorus precursors to this ratio (1: 1.3 to 1: 1.5) will produce β -TCP. The synthesis of HAp follows these chemical reactions [48]:



2.4 Hydrothermal method

Another popular method for synthesizing HAp from natural resources is the hydrothermal approach [49]. Using an aqueous solution at high pressure and temperature, phosphorus and calcium precursors can combine chemically to create HAp nanoparticles using the

hydrothermal technique. The hydrothermal method can impact condensation and utilize both high temperature and pressure to enhance the chemical reaction, which can effectively raise the products' crystallinity and favorably contribute to the synthesis of HAp as the main phase [49]. However, this method has relatively limited control over particle size distribution and shape. Alternative pathways, referred to as the template-mediated hydrothermal approach, have been extensively utilized in recent times to synthesize HAp with regulated shape. This method uses "soft" templates, such as cetyltrimethylammonium bromide (CTAB), and sodium dodecyl sulfate (SDS) surfactants to control the nucleation and crystal propagation of HAp. Most of the time, 1-D nanostructured HAp, which resembles nanorods, are synthesized using this technique [49].

Recently, Nga et al. employed Na_2HPO_4 and eggshell as starting materials along with CTAB as a regulator to produce HAp with rod like form, the synthesized HAp diameter and length was 52 nm and 161 nm respectively [50]. Using this approach, CaCO_3 was transformed into CaCl_2 by dissolving eggshell powder in HCl solution to remove CO_2 . Subsequently, the produced CaCl_2 solution was mixed with a solution comprising 0.1 M Na_2HPO_4 and 0.64 g of CTAB. Then, the produced solution was treated for 12 hours at 180 °C, and then calcined for 1 hour at 500 °C to yield HAp. In this investigation, carbonated HAp with both A and B type CO_3^{2-} replacements was produced, with a CO_3^{2-} content of 2.14 wt.%. Furthermore, the synthesized HAp contained the elements Mg, Na, and K [50].

2.5 Wet chemical precipitation method

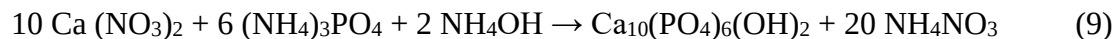
Wet chemical precipitation is one of the most practical and commonly used to synthesize HAp since it is an easy-to-understand synthetic process [51]. A phosphorus-based reagent

and an alkaline calcium supply react to produce precipitation, with the molar ratio of Ca to P being locked at a stoichiometry that corresponds to its ratio in HAp (1.67). The process undergoes these phases: precipitation, aging, filtration, drying, and ultimately calcination. This method doesn't require elevated temperature for Reaction, temperatures ranging from room temperature to 100 °C are sufficient, and pH values between 3 and 12 are frequently used [51]. Properties of the synthesized HAp are determined by controlling several processing parameters, including but not limited to type and concentration of the reactants (precursors), the reaction temperature, the rate at which the reactants are added, the pH value, the aging length, and the calcination temperature. To regulate the shape of synthesized HAp, the wet chemical precipitation approach may occasionally be used in the presence of templates [51].

According to Kamalanathan et al., eggshell and H_3PO_4 were used as the precursors to successfully generate HAp with a needle-like shape using wet chemical precipitation [52]. Calcination of eggshells produced CaO, which, after being hydrated, produced $\text{Ca}(\text{OH})_2$. The $\text{Ca}(\text{OH})_2$ solution was then added gradually with a rate of 10–20 drops/min to H_3PO_4 , then NH_4OH was used to maintain the suspension's pH above 10.5. At last, the reaction was given a full day to develop before producing HAp. It was discovered that this approach produced HAp with B-type carbonated substitution. The HAp synthesized by this method showed phase stability up to 1250 °C based on its sinterability; any temperature above this will produce secondary phases like TTCP and α -TCP [52].

Nevertheless, when $\text{Ca}(\text{NO}_3)_2$ and $(\text{NH}_4)_3\text{PO}_4$ solutions interact at pH level between 11 and 12, HAp is produced in the form of nanorods [53]. It was found that the product of

calcined eggshell (CaO) and HNO₃ solution is Ca (NO₃)₂. The investigation indicates that the overall process is represented by Eqs. (8) and (9) [53]:



2.6 Sol-gel method

The sol-gel method involves preparing precursors solutions containing the necessary elements required to produce HAp i.e. calcium, phosphate., then the solutions are mixed to form a homogeneous mixture that undergo hydrolysis reactions that breaks down the precursor's molecules into smaller units. Condensation reactions are then followed leading to the formation of gel-like product. Further aging is required for the gel-like product. Then, drying to remove the solvent and water content. Finally, calcination (heat treatment) is applied at elevated temperatures (600-800 °C). During calcination the gel transform into crystalline HAp [54].

A study reported a sol-gel reaction involving eggshell-derived Ca (NO₃)₂ and KH₂PO₄ produced single-phase nanosized HAp. The reaction mixture matured at room temperature for 24 hrs. before being thermally calcined for 30 minutes at 700°C. The pH level was kept at 10. According to the findings, the produced HAp belonged to the B-type carbonated substitution and had an average particle size of 35–50 nm and a prolate spheroidal morphology [54].

2.7 Microwave irradiation method.

Conversely, HAp can also be produced via the microwave irradiation technique [55]. This method often involves quickly heating the initial ingredients to a high temperature using a high-frequency microwave. The dipole polarization and ionic conduction that result from

microwave irradiation within the starting materials can be used to unravel the fundamental heating mechanism of microwave processing [55]. The advantages of microwave processing over conventional techniques include great homogeneity, rapid heating rates, energy savings, high repeatability, non-polluting, and shorter processing times[55].

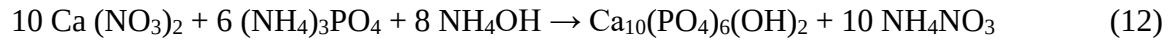
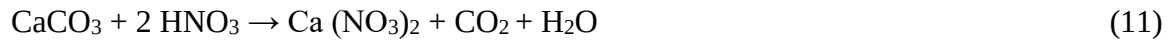
The microwave irradiation method is frequently used in conjunction with other traditional procedures to provide synergistic results from various approaches and quickly reach higher yields [56].

Krishna et al.'s study emphasized the advantages of employing eggshells as starting materials and microwave processing for the manufacturing of HAp [56], microwave irradiation approach was used to produce HAp from calcined eggshell waste and $(\text{NH}_4)_2\text{HPO}_4$, then, the product was compared to synthetic HAp made from commercial calcium precursor to investigate difference of products qualities. The outcomes demonstrated that, when compared to commercially available HAp, HAp synthesized by microwave irradiation had a higher SSA (specific surface area). Additionally, eggshell-derived HAp exhibits remarkable sinterability, surpassing its synthetic counterparts in both hardness and density. The HAp that was produced from eggshell waste was classified as B-type carbonated HAp, and it primarily displayed platelets structure with agglomeration with lengths of 33–50 nm and widths of 8–14 nm [56].

To synthesize HAp nanostructures, microwave power and exposure time are important factors [56]. Sajahan et al., revealed that when synthesizing HAp from eggshells and $(\text{NH}_4)_2\text{HPO}_4$, longer microwave irradiation times significantly affected the size and morphology of synthesized product [56]. For short irradiation time (less than 5 minutes), the HAp produced will have irregular circular nanosized. Extending irradiation time to

between 15 to 45 minutes, the HAp crystal size will further grow and more uniformity in shape is noticed. Further irradiation didn't change the phase of produced HAp [56].

Rahman et al. employed eggshell waste and $(\text{NH}_4)_2\text{HPO}_4$ as the precursors in a wet precipitation process with microwave irradiation to create HAp [57]. In summary, the $\text{Ca}(\text{NO}_3)_2$ solution (produced by treating eggshells with HNO_3) was combined with aqueous $(\text{NH}_4)_2\text{HPO}_4$ dropwise. The final pH was then brought to 11 using NH_4OH , and then the mixture was microwave irradiated for 15 minutes at 80 °C. According to the findings, this method promoted HAp crystal development along the c-axis, leading to an uneven morphology of needle-like form and an SSA of 100.6 m²/g. Below, the chemical process involved in the synthesis of HAp is depicted in Equations (11) and (12) [57]:



2.8 Ion doped hydroxyapatite.

Biomaterials development and application for bone tissue engineering has experienced a notable upsurge in interest over the last several years. Because bone regeneration is thought to be the most intricate and well-coordinated physiological process, creating biocompatible materials with exact physicochemical and biomechanical properties that can encourage bone production and regeneration has been the focus of scientific research (Figure 14). Moreover, the objective of these biomaterials is to trigger cellular reactions at the molecular level, encompassing everything from extracellular matrix synthesis to cell attachment, differentiation, proliferation, and interaction.

Because HAp is naturally occurring in the human body and exhibits osteogenic qualities as well as being biocompatible and able to create a strong link with osseous tissues, it has been the subject of substantial investigation in biomedical applications [58].

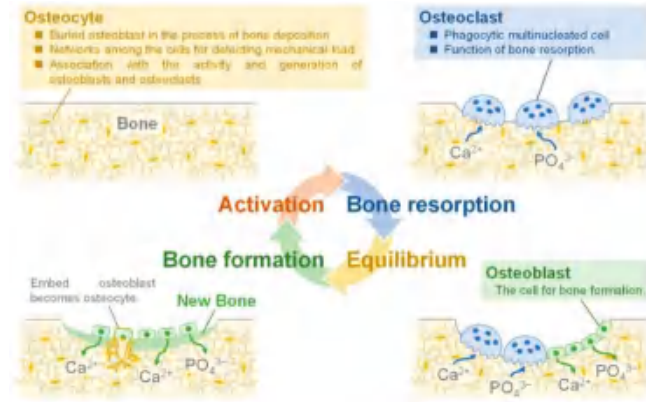


Figure 14. Representation of bone formation process [58].

The simplest way to also address the primary shortcomings of implants based on HAp, primarily the mechanical qualities, is to dope or substitute atoms for HAp (Figure 15). All previously listed problems can be resolved by executing various substitution types, particularly cationic (Ca^{2+}) and anionic (PO_4^{3-}) substitutions.

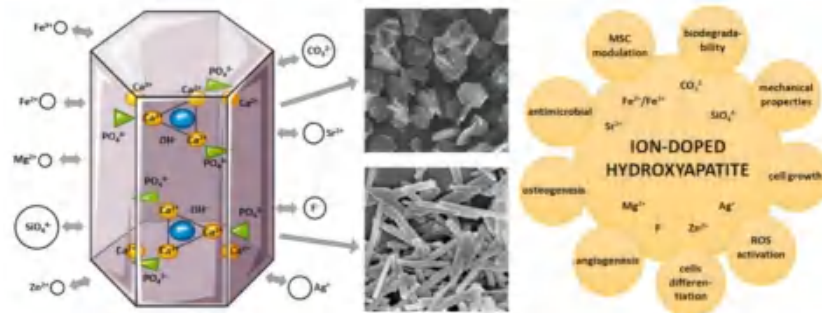


Figure 15. Representation of HAp structure and applications of each doped nanoparticles [58].

In addition, Bassyouni et al. reported that the material's bioavailability would be enhanced by HA's increased degree of crystallinity and decreased particle size. As a result of the

increased disintegration rate, suitable structural modifications may also be considered.

HA's structure can be changed by [59]:

- substituting Zn^{2+} , Sr^{2+} , Mg^{2+} , and Na^{+} ions for the Ca^{2+} sites.
- substituting the PO_4^{3-} sites with CO_3^{2-} , or SiO_3^{2-} ions.
- substituting the OH^{-} group with Cl^{-} , F^{-} , and CO_3^{2-} ions

The disruption of crystallinity, lattice parameters, morphology, and surface charge, caused by these examples of ionic exchanges can dictate changes in the mechanical characteristics, thermal stability, solubility, and bioactivity of HAp as shown below.

Table 5. Doping ion incorporated into HA structures and their enhanced properties [59].

	Doping Ion	Biologic Activity
Cation	Na	Enhanced osteoconductivity and improved cell proliferation
	K	Enhanced thermal stability
	Mg	Improved crystal growth, crystallization, thermal stability, and dissolution rate
	Sr	Enhanced bone regeneration and formation
	Mn	Enhanced cell adhesion
	Zn	Enhanced bone regeneration, increased antimicrobial activity
Anion	CO	reduced crystallite size, enhanced osteoconductive properties,
	SiO	Enhanced bioactivity, improved dissolution rate
	F	reduced solubility, and increased remineralization
	Cl	Enhanced osteoconductive properties, and increased solubility

2.9 Magnesium substituted hydroxyapatite.

Among replacement cations, magnesium (Mg), the fourth most common ion in the human body, has been thoroughly investigated. Mg^{2+} ions are present in various parts of the human skeletal. Mg^{2+} accounts for 0.73 wt.% of bones, 1.23 wt.% of dentin, and 0.44 wt.% in natural enamel. At the beginning of the osteogenesis process, it is roughly 2 wt.% in the bone tissue and cartilage, but it tends to decrease in the mature tissues. All phases of skeletal metabolism are negatively impacted by magnesium deficiency, which results in osteopenia, bone fragility, a reduction in osteoblastic and osteoclastic activity, and a halt to bone formation [58].

Mg^{2+} addition considerably distorts the crystallographic properties of the HAp lattice. The c-axis dimension of the apatite lattice is reduced when magnesium is incorporated because the radius of Mg^{2+} (0.069 nm) is less than that of Ca^{2+} (0.099 nm). Ren et al., however, note that the a and c reticular characteristics also drop with an increase in magnesium content. It is observed that this substitution can only lead to a little increase in the lattice parameter. These differences result from the preparation methods' significant influence on the lattice properties [58].

Precipitation and hydrolysis techniques are frequently used to create magnesium-substituted HA (Mg-HA). Previous research reports have varied about the maximum amount of Mg^{2+} that can replace Ca^{2+} . Apatite can contain up to 0.3 wt.% and less than 1 wt.% of magnesium substitution, according to certain research [58]. Mg substitution was shown to be greater in other experiments. For instance, without concurrent carbonate substitution, Mayer et al. precipitated HA containing up to 1.5 wt.% of magnesium. On the other hand, Bigi et al. reported synthesizing Mg-HA with up to 7.5 wt.% (~30 mol.%) of

Mg under hydrothermal conditions at 120 °C. Golden and Ming were able to synthesize Mg-HA with up to 2 wt.% of Mg. Using the mechanochemical–hydrothermal approach, Riman et al. even reported a stable, phase-pure crystalline HAp that was magnesium-substituted and contained between 2.0 and 29 wt.% Mg. At least 75 wt.% of the Mg replaced the calcium ions in the HAp lattice structure. It should be noted that complete substitution of Mg^{2+} for Ca^{2+} in apatite produced in a high pH solution was also observed [59].

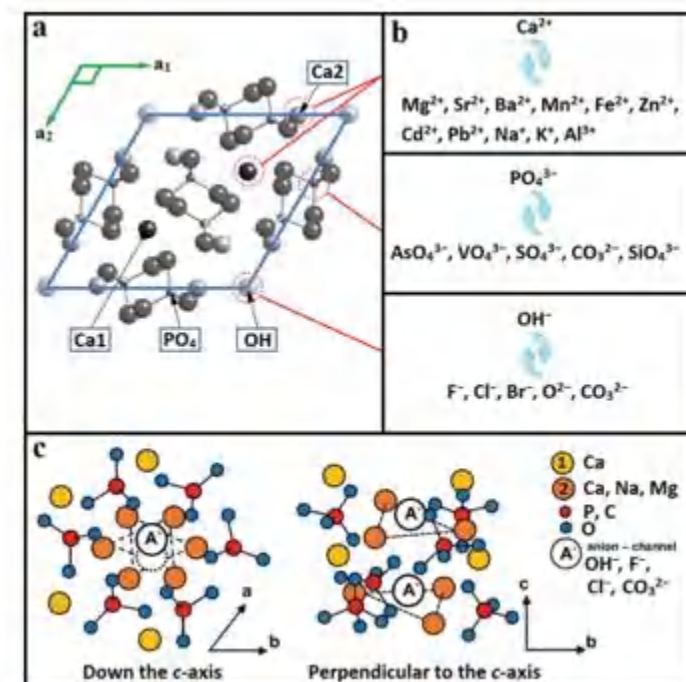


Figure 16. (a) The c-axis projection of the atomic arrangement of Ca (I), Ca (II), PO₄³⁻, and OH⁻ groups in the hexagonal crystal structure of HA; (b) Its modification by ionic substitutions; and (c) Atomic structure of HA [59].

Using Rietveld structure improvements, Bigi et al. found that magnesium was effectively integrated into the HA structure in the Ca (II) site [60]. This preference was attributed to the various geometries of the two separate metal sites in the apatite structure as well as the unique metal-oxygen interactions. When Mg^{2+} is partially substituted for Ca^{2+} , the

dimension of the c-axis is lowered because Mg^{2+} radius (0.069 nm) is substantially smaller than Ca^{2+} (0.099 nm) [60]. The HA lattice becomes substantially deformed because of this disparity, which reduces crystallinity. However, Ren et al. used ab initio generalized gradient approximation density functional theory to do a computer analysis to find variations in the lattice parameters. They found that both lattice constants, a and c, decreased with increasing Mg substitution and that the Ca (I) site in the HA lattices afforded energetically preferred sites for Mg substitution [61].

In addition, it was shown by Tampieri and colleagues that Mg substitution was preferred at the Ca (I) position, while Farzadi and colleagues found that the (a) lattice parameter increased slightly [61]. These variations can be explained by pointing out that the preparation method utilized to produce the HA-like materials has a significant influence on the lattice characteristics. Different preparation methods would probably result in different levels of hydration and possibly other defects in the materials [62].

In an investigation by Zyman et al. focused on the impact of magnesium percentage on HAp, he found that up to 1.8 wt.% causes noticeable decrease in compressive strength and microhardness values for Mg-HA ceramics. However, these values they found are still higher than the values of bones [63]. However, there was a trend for the fracture toughness (a force suggestive of destruction) to rise with increasing magnesia concentration up to 0.6 wt.% of magnesium, which is like what naturally occurring in bones. This is due to a weak intergranular boundary, loss of texture, increased microporosity, and decreased average grain size. Researchers Fadeev and associates showed the similar pattern [63].

2.10 Synthesis of reduced graphene-hydroxyapatite composite

Between rGO and HAp, there is an electrical contact. Because of the numerous oxygen functional groups that are present on its surface and edges, including phenolic hydroxyls and carboxylic acids, rGO has a significant negative charge [64]. Electrostatic interactions between rGO and HAp occur when the positively charged calcium atoms (C sites) in the (100) crystal plane of HAp can be absorbed by the negatively charged rGO. The positively charged calcium cations would electrostatically adsorb the negatively charged rGO following compositing, as seen in figure 17 [64].

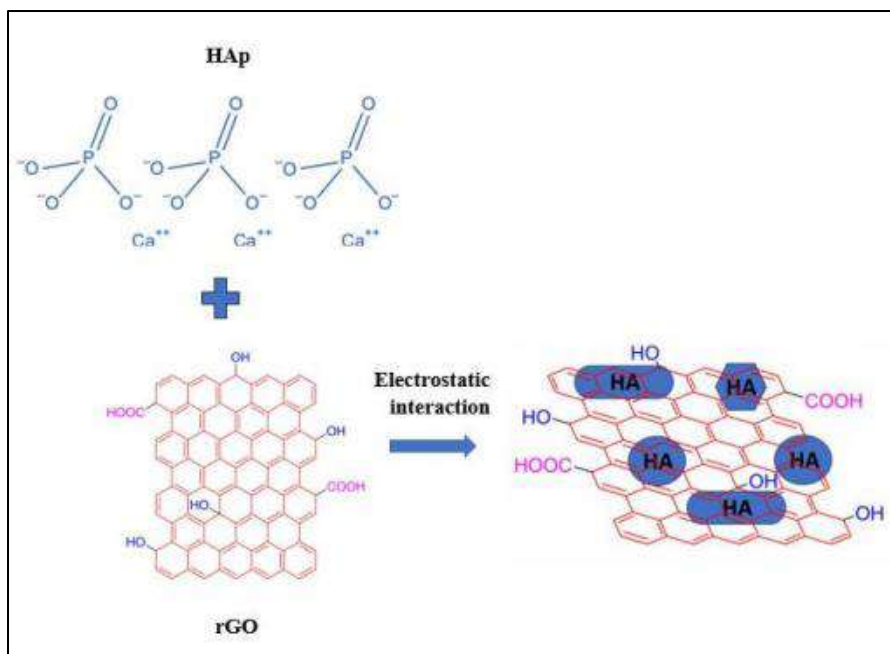


Figure 17. interaction of rGO–HA composite.

Like graphene, rGO has a layered structure, but because oxygen groups are present in its carbon atom planes, the interlayer distance expands, and the hydrophilic atomic layer thickens. Functional oxygen groups such as epoxide groups (C-O-O), carbonyl group (C=O), and hydroxyl groups (-OH) are known to be present in the rGO sheet structure and operate as supports on the surface of a graphene monolayer (Figure 18) [64].

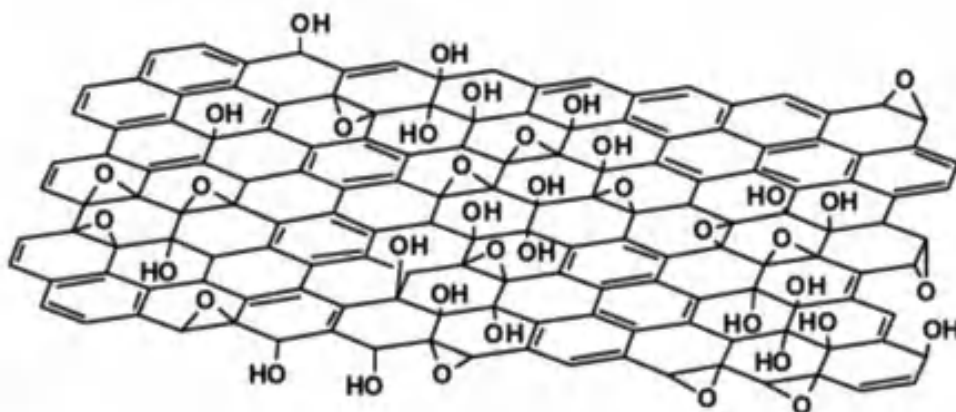


Figure 18. rGO sheet structure [64].

The presence of oxygen-containing groups gives rGO its enhanced hydrophilic characteristics and permits dispersion in various solvents, including water [64]. Stated differently, water molecules can readily intercalate between the graphite layers due to its hydrophilic nature. With hydrophobic epoxy, hydroxyl, and graphitic domains on its basal plane and hydrophilic carboxyl groups at the edges, rGO functions as a 2D amphiphile [65]. Because of their partially hydrophobic and hydrophilic regions as well as their negatively charged carboxylate groups at the margins or defect sites, rGO surfaces can form hydrogen bonds and complex metal ions. rGO has an abundance of carbonyl, epoxide, and hydroxyl groups, which produce a lot of chemically reactive sites. rGO is also a viable nanoscale reinforcement filler in bio composites that can improve interfacial bonding between the components due to its favorable mechanical properties, high hydrophilicity, and strong biocompatibility [65].

The main function for rGO hybrid is to enhance the composite mechanically. rGO is frequently used in HAp biomimetic synthesis due to its lack of toxicity to cells and the high mechanical strength of graphene-based composites [65].

A variety of methods, including spark plasma sintering (SPS), bioinspired mineralization, biomimetic mineralization, in situ chemical precipitation reaction, simple precipitation, hydrothermal method [66], and radio frequency chemical vapor deposition (RF-CVD) technique, can be used to prepare HAp nanoparticles with graphene nanosheets. While all these technologies showed promise for in-vitro bioactivity, they are more difficult and time-consuming to produce than in-situ synthesis [65], [66].

While in-situ synthesis produced rGO-based HAp nanocomposite more easily, cheaply, and effectively, it also resulted in superior HAp distribution and robust interfacial interaction with rGO nanosheets [66].

Better dispersion and robust interfacial bonding of HAp towards rGO nanosheets were achieved by in-situ synthesis, which used a simpler, more affordable, and more efficient method to create rGO-based HAp nanocomposite [66].

The mechanical approach uses a magnetic stirrer and electrospinning devices to physically mix HAp that has been spread onto a rGO surface while applying mechanical forces. To improve HAp dispersion on rGO, chemical mixing can be added to mechanical mixing, which takes a lengthy time. HAp can be distributed across the matrix via a variety of techniques, such as physical blending and mixing. The wet chemical precipitation approach was used to prepare rGO-HAp. With good survivability, composite rGO-HAp may function as an osteoconductive composite to promote osteoblast cell adhesion [67].

Comparing the rGO-HAp composite to uncoated rGO nanoparticles, it performs well and has a high osteoblast cell viability. To improve bone formation, HAp surface functionalizes rGO, increasing cell attachment and proliferation [67].

Table 6. Properties of rGO for biomaterial applications [67].

Biocompatibility	rGO is generally biocompatible and does not induce significant cytotoxicity
Large Surface Area	rGO possesses a large surface area due to its 2D planar structure
Easy Functionalization	The oxygen-containing functional groups on rGO (such as hydroxyl, carboxyl, and epoxide) allow straightforward chemical modification.
Mechanical Strength	rGO exhibits remarkable mechanical strength, making it suitable for tissue engineering scaffolds and implants.
Electrical Conductivity	rGO has excellent electrical conductivity
Drug Delivery and Therapeutics	rGO-based nanocarriers can efficiently deliver drugs, nucleotides, and biomolecules.
Photothermal Properties	rGO can absorb near-infrared light, leading to localized heating.
Bioimaging and Biosensing	rGO's strong near-infrared light absorption makes it useful for bioimaging.
Antibacterial Activity	rGO exhibits antibacterial properties due to its interaction with bacterial cell membranes.
Tissue Regeneration	rGO-based scaffolds promote cell adhesion, proliferation, and differentiation.

CHAPTER 3

EXPERIMENTAL PROCEDURE

In this research, magnesium doped hydroxyapatite with reduced graphene composite was prepared by co-precipitation method and microwave irradiation technique, closely following the research paper by K. Ijaz, M. Zahid, A.S. Khan, and A.A. Chaudhry, published on May 9, 2015, in the journal "Materials Science & Engineering" [63]. The samples were differentiated based on variance in wt.% of rGO, and Mg content. Samples were prepared with 0%, 0.5%, 1%, 1.5%, and 3% rGO and 0%, 0.3%, 0.5%, and 0.7% Mg to hydroxyapatite weight ratio for co-precipitation method with microwave irradiation. All samples were microwaved for 30 min at 800 W and at 100 °C. For further ease in identification and classification of samples, they were divided into five groups based on rGO content. Hydroxyapatite (first sample) was used as a standard sample to compare its XRD-spectra and other properties with other samples to make sure that the samples are not just mixed, but a chemical reaction has taken place. The preparation of eggshell derived magnesium doped hydroxyapatite with reduced graphene has not been reported before as to our best knowledge and this is first time this particular composite has been prepared using these precursors.

Table 7 . Classification of various samples

	Sample No	Sample name	Magnesium Concentration (%wt.)	rGO Concentration (%wt.)
Group 1	1	HAp	0	0
	2	0.3Mg-HAp	0.3	0
	3	0.5Mg-HAp	0.5	0
	4	0.7Mg-HAp	0.7	0
Group 2	5	0.5rGO-HAp	0	0.5
	6	0.5rGO-HAp-0.3Mg	0.3	0.5
	7	0.5rGO-HAp-0.5Mg	0.5	0.5
	8	0.5rGO-HAp-0.7Mg	0.7	0.5
Group 3	9	1rGO-HAp	0	1
	10	1rGO-HAp-0.3Mg	0.3	1
	11	1rGO-HAp-0.5Mg	0.5	1
	12	1rGO-HAp-0.7Mg	0.7	1
Group 4	13	1.5rGO-HAp	0	1.5
	14	1.5rGO-HAp-0.3Mg	0.3	1.5
	15	1.5rGO-HAp-0.5Mg	0.5	1.5
	16	1.5rGO-HAp-0.7Mg	0.7	1.5
Group 5	17	3rGO-HAp	0	3
	18	3rGO-HAp-0.3Mg	0.3	3
	19	3rGO-HAp-0.5Mg	0.5	3
	20	3rGO-HAp-0.7Mg	0.7	3

3.1 Raw materials

Using the microwave irradiation technique, these raw ingredients are mixed in a specified order as outlined in the regulating reaction for the experiment to create the rGO-Mg-HAp composite. To create the appropriate composite material, magnesium nitrate, calcium carbonate (derived from eggshells), and ammonium dihydrogen phosphate react in a controlled process. The experimental approach entails adjusting the magnesium and rGO concentrations to generate twenty distinct samples for comparison and characterization.

The molecular weights and molar ratios produced by the controlling reaction provide the basis for calculating the quantities of each input ingredient.

Table 8. Raw materials of the experiment

Eggshells (CaCO_3):	Gathered from KFUPM restaurant as eggshells waste.
Hydrochloric acid (HCl)	Hydrochloric acid from HONEYWELL FLUKA with 30% purity for laboratory.
Reduced Graphene Oxide (rGO)	Single layer graphene oxide from Nanografi with 99.5% purity for laboratory.
Magnesium-nitrate hexahydrate ($\text{Mg}(\text{NO}_3)_2(6\text{H}_2\text{O})$)	Provided from Sigma-Aldrich with 99% purity for laboratory.
Ammonium-dihydrogen phosphate ($\text{NH}_4\text{H}_2\text{PO}_4$)	Ammonium phosphate monobasic from DAEJUNG with 98% purity for laboratory.
Sodium hydroxide (NaOH)	Sodium hydroxide pellets from CARLO ERBA

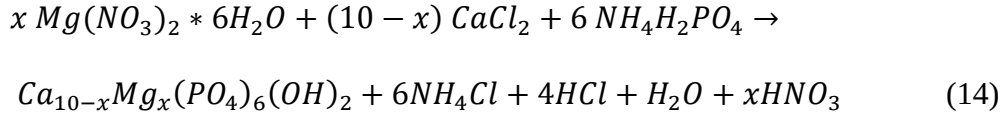
3.2 procedure of experimental work (design of experiment)

In this experiment, the microwave irradiation method will be used to produce the rGO-Mg-HAp composite from an aqueous solution. By varying the percentage of Mg and rGO, we will get 20 samples to characterize and compare:

- The percentage of magnesium from 0.3 to 0.7 wt.% was chosen based on previous studies in literature. Previous research reports have varied about the maximum amount of Mg^{2+} that can replace Ca^{2+} . Apatite can contain up to 0.3 wt.% and less than 1 wt.% of magnesium substitution, according to certain research [58]. Mg substitution was shown to be greater in other experiments. For instance, without

concurrent carbonate substitution, Mayer et al. precipitated HAp containing up to 1.5 wt.% of magnesium [58]. Therefore, to study the effect of Mg to decrease the crystal size of HAp without causing any phase changes, these three different values (0.3, 0.5, 0.7) were selected for the experiment.

- rGO percentage was determined based on literature. Composite of rGO (1.5%) and HAp has been investigated for mechanical behavior that showed increased elastic modulus [65]. The rGO (0.25, 0.5, 1, and 2% wt.) has been reinforced in HA and investigated for mechanical and biological behavior using MTT analysis. It has been concluded that the rGO-HA with 1% and 0.5% rGO showed biocompatibility on NIH-3T3 fibroblast cells and enhanced mechanical strength [66]. It was reported that for high rGO ratios cytotoxicity was effect and in order to produce a biocompatible composite these percentages were selected (0.5, 1, 1.5, 3 wt.%).
- Alkaline medium was chosen for the precipitation process because there is a higher concentration of hydroxide ions (OH^-). These ions are essential for the reaction involved in the precipitation of magnesium-doped hydroxyapatite. Also, alkaline pH helps prevent the dissolution of metal ions such as magnesium and calcium from starting solution. Therefore, the process of the experiment start with high acidity and all precursors ions are present in the solution, metal ions tend to dissolve more readily due to increased protonation of surface site and the formation of metal-hydrogen complexes. Then when sodium hydroxide is added and pH reaches 10, metal ions (Mg, Ca) are less soluble because the hydroxide ions (OH^-) compete with metal ions for binding sites on the HAp surface.
- The controlling reaction for the experiment takes this sequence:



The amount of Calcium, Phosphorus, and Magnesium must follow the controlling reaction to achieve correct synthesis of the composite. Waste eggshells ($CaCO_3$) were collected and cleaned with distilled water, and then they were exposed to the sun for a day to get them dried. After that, they were crushed into fine powder. Then they were dissolved in diluted Hydrochloric acid (HCl) to remove CO_2 gas from carbonate (CO_3). Then, rGO and magnesium nitrate hexahydrate were added to the solution dropwise. Then, ammonium dihydrogen phosphate ($NH_4H_2PO_4$) was added dropwise to the solution. Finally, the solution was placed in a microwave for 30 minutes at 800W power at 100 °C to get the rGO-Mg-HAp powder. Figure 19 below shows the sequence of the experiment procedure with details.

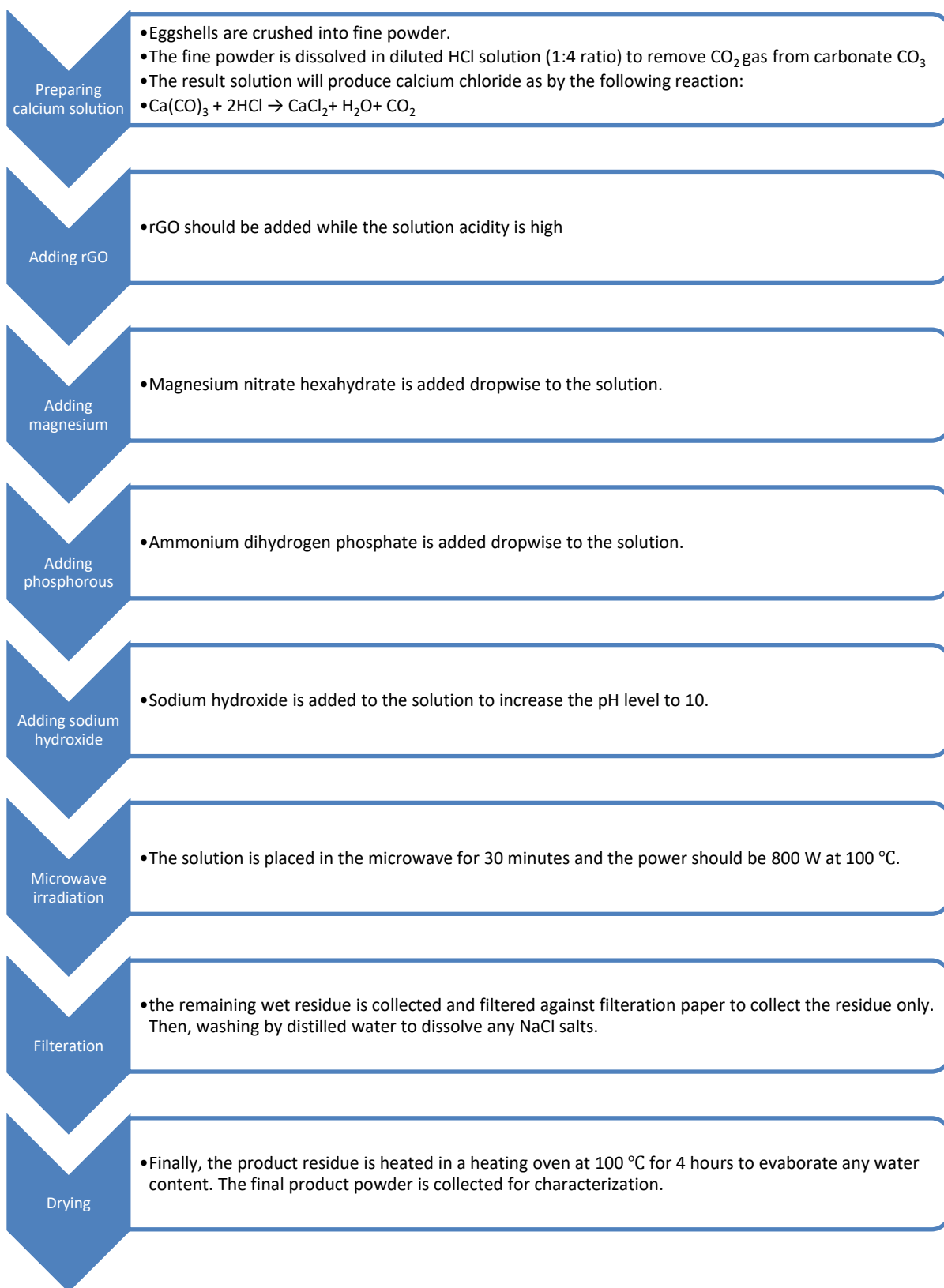
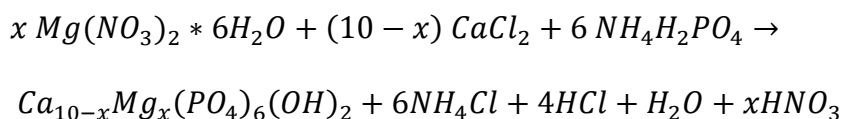


Figure 19. sequence of experiment procedure

3.3 Experiment calculations.

1. To get the experiment calculation, we must first follow the general/ruling equation of our experiment, this equation controls the reactions and decides all quantities needed for all elements. This equation represents the reaction between magnesium nitrate hexahydrate ($Mg(NO_3)_2(6H_2O)$), calcium carbonate ($CaCO_3$), and ammonium dihydrogen phosphate ($NH_4H_2PO_4$). The products include magnesium doped hydroxyapatite ($Ca_{10-x}Mg_x(PO_4)_6(OH)_2$), water (H_2O), carbon dioxide (CO_2), and nitric acid (HNO_3).



2. Secondly, all molar mass for chemical elements in the equation must be obtained for the calculations:

- $NH_4H_2PO_4 = 115.03 \text{ g/mol}$
- $Mg(NO_3)_2(6H_2O) = 256.41 \text{ g/mol}$
- $CaCl_2 = 110.98 \text{ g/mol}$

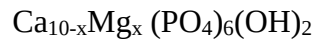
Now, since the experiment includes 20 samples with variation of Mg and rGO we can categorize all samples into four distinctive categories according to magnesium content: HAp, 0.3Mg-HAp, 0.5Mg-HAp and 0.7Mg-HAp. The content of rGO is fixed in each sample and it is not included in our equation or calculations. Thus, we will only focus on magnesium content while rGO will be incorporated according to its percentage without calculation. Nevertheless, rGO has four categories 0.5wt.%, 1wt.%, 1.5wt.%, and 3 wt.% representing 0.005, 0.01, 0.015, and 0.03 grams. To calculate the number

of grams needed from each element, we first must find the molecular weight for each sample according to the magnesium content needed and then apply it to the equation to get the weight in grams. The table below uses each element's molecular weight and multiply it by the fraction of Ca and Mg needed to get the final molecular weight (molar mass) which direct us to find the number of moles of each element which leads to the number of grams.

Table 9. Molecular weight calculations

	Molecular weight	Mg wt. %	molar ratio	10-x (Ca)	X (Mg)
HAp	1004.6	0	0	10	0
0.3Mg-HAp	1002.70712	0.3	0.012	9.88	0.12
0.5Mg-HAp	1001.45	0.5	0.02	9.8	0.2
0.7Mg-HAp	1000.18328	0.7	0.028	9.72	0.28

Let us calculate for 0.3Mg-HAp sample, we have the product formula,



and each element molecular weight is as follows:

Mg: 24.304 Ca: 40.078 P: 30.97 O: 16 H:1

Now, by plugging each molecular weight in the equation with the correct fraction of Ca and Mg for 0.3Mg-HAp sample according to the table, we get:

$$(0.12 \times 24.304) + (9.88 \times 40.078) + (6 \times (30.97 + (4 \times 16))) + ((16 + 1) \times 2) = 1002.707$$

3. Thirdly, the following rule calculates the number of moles and grams required for each element in the reaction.

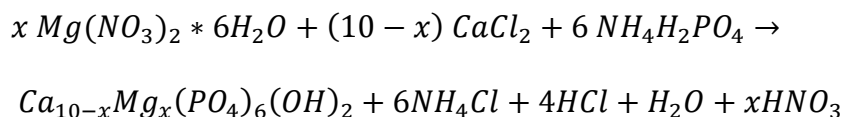
$$\text{Weight in grams} = \text{no. of moles} \times \text{molar mass}$$

We start by calculating the number of moles for our product which is Mg-HAp and from there, each element mole fraction must coincide with the product to balance the reaction and get the correct weight in grams required. We will follow the same example for 0.3Mg-HAp, First, we decide the number of grams we want from the product, let's say 10 grams. Then, we get the molecular weight which was calculated earlier, and plug both numbers into the equation.

$$10 = \text{no. of moles} \times 1002.707$$

$$\text{No. of moles} = 0.009973$$

Then, we calculate each element mole required for our experiment by checking the number it shares in the controlling equation.



It can be noticed that the number beside the reactants (underlined) is essential in this step. So, we can notice that the Calcium carbonate contributes by 10 because number 10 is next to it, number 6 for the phosphate element, and number 1 for the magnesium element. Now, those element numbers must be multiplied by the calculated number of moles of the product to get the element moles required in the reaction:

$$\text{Mg}(\text{NO}_3)_2 (6\text{H}_2\text{O}) = 0.12 * 0.009973 = 0.001197$$

$$CaCl_2 = 9.88 * 0.009973 = 0.098533$$

$$NH_4H_2PO_4 = 6 * 0.009973 = 0.059838$$

Now to get the grams needed, each element no. of moles must be multiplied by its molecular weight to get the grams needed.

$$Mg(NO_3)_2(6H_2O) = 0.001197 * 256.41 = 0.30692 \text{ grams}$$

$$CaCl_2 = 0.098533 * 110.98 = 10.9352 \text{ grams}$$

$$NH_4H_2PO_4 = 0.059838 * 115.03 = 6.883166 \text{ grams}$$

4. All remaining samples follow the same procedure.

Table 10. Summary of all samples molecular weight and elemental weights in grams

	X (Mg)	10-x (Ca)	Molecular weight (g/mol)	Calcium chloride (g)	Magnesium nitrate hexahydrate (g)	Nitrate dihydrogen phosphate (g)	Reduced graphene oxide (g)
HAp	0	10	1004.6	9.962	0	6.870197093	0
0.3Mg-HAp	0.12	9.88	1002.70712	10.935	0.307	6.883166442	0
0.5Mg-HAp	0.2	9.8	1001.45	10.854	0.5118	6.891839913	0
0.7Mg-HAp	0.28	9.72	1000.18328	10.785	0.718	6.90053527	0
0.5rGO-HAp	0	10	1004.6	9.962	0	6.870197093	0.005
0.5rGO-HAp-0.3Mg	0.12	9.88	1002.70712	10.935	0.307	6.883166442	0.005
0.5rGO-HAp-0.5Mg	0.2	9.8	1001.45	10.854	0.5118	6.891839913	0.005
0.5rGO-HAp-0.7Mg	0.28	9.72	1000.18328	10.785	0.718	6.90053527	0.005
1rGO-HAp	0	10	1004.6	9.962	0	6.870197093	0.01
1rGO-HAp-0.3Mg	0.12	9.88	1002.70712	10.935	0.307	6.883166442	0.01
1rGO-HAp-0.5Mg	0.2	9.8	1001.45	10.854	0.5118	6.891839913	0.01
1rGO-HAp-0.7Mg	0.28	9.72	1000.18328	10.785	0.718	6.90053527	0.01
1.5rGO-HAp	0	10	1004.6	9.962	0	6.870197093	0.015
1.5rGO-HAp-0.3Mg	0.12	9.88	1002.70712	10.935	0.307	6.883166442	0.015
1.5rGO-HAp-0.5Mg	0.2	9.8	1001.45	10.854	0.5118	6.891839913	0.015
1.5rGO-HAp-0.7Mg	0.28	9.72	1000.18328	10.785	0.718	6.90053527	0.015
3rGO-HAp	0	10	1004.6	9.962	0	6.870197093	0.03
3rGO-HAp-0.3Mg	0.12	9.88	1002.70712	10.935	0.307	6.883166442	0.03
3rGO-HAp-0.5Mg	0.2	9.8	1001.45	10.854	0.5118	6.891839913	0.03
3rGO-HAp-0.7Mg	0.28	9.72	1000.18328	10.785	0.718	6.90053527	0.03

3.4 Experiment set-up.

1. First, eggshell waste is collected and washed with water to clean any protein or liquid residue attached to it. Then, the inner organic shell is removed to only keep the hard white shell which consists of calcium carbonate as shown in the image below.



Figure 20. Raw eggshells.

2. The eggshells are finely crushed to powder by using a ceramic mortar and pestle. The crushing must be very fine because any big particles or chunks will take time for the acid to dissolve it. However, if it was very fine like dust, it will dissolve faster and will not leave any trace in the solution that result in by-product at later stage when producing the hydroxyapatite powder. Thus, this stage is very important, and Eggshells must be crushed to very fine powder as shown below.



Figure 21. (a) Mortar & pestle grinding eggshells, (b) Eggshell powder.

3. Then, CO_2 is removed from egg-shells powder (calcium carbonate) by dissolving the powder in HCl solution. Since we decided that we'll produce 10 grams of each sample, then the solution will be made of 20 mL of hydrochloric acid and 80 mL of distilled water. Then, in a beaker we put the powder and pour the solution on. A foam will be produced at first and by continuous stirring the powder will dissolve in the solution. If any particles are still noticed in the solution, we should add more HCl and continue stirring. Then. We take the beaker and put in ultrasonic to help dissolve the powder and homogenize the solution as shown below.

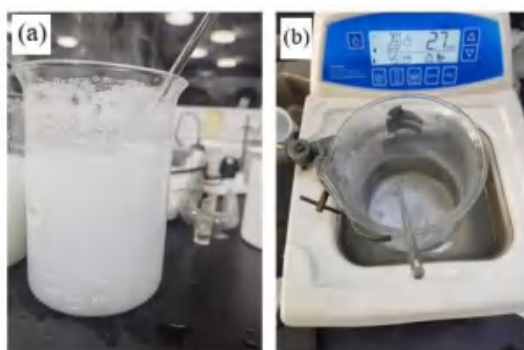


Figure 22. (a) HCl-eggshells solution, (b) Solution in hydro-sonic.

4. If the sample contain rGO, then an rGO solution is prepared by dissolving rGO powder in 20mL of distilled water and then added to the solution dropwise while stirring, and 5 minutes later magnesium is added dropwise using the same method of dissolving the magnesium in 20mL of distilled water, after adding rGO the solution color changes to black as shown below. And lastly, Phosphorous is added in the same way.

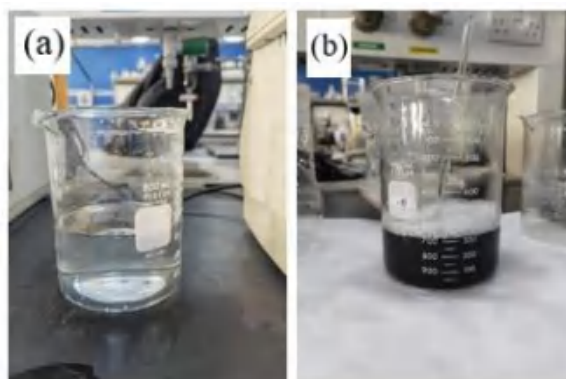


Figure 23. (a) magnesium solution, (b) rGO solution.

5. Then sodium solution is prepared by dissolving sodium hydroxide pellets in 20mL distilled water and then it is added to the solution we prepared earlier. Then, the beaker is moved to pH meter to check the acidity of the solution, the pH must be at 10.

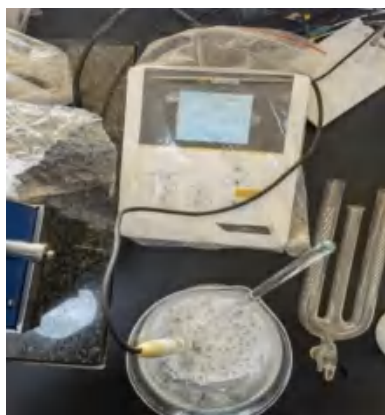


Figure 24. pH meter reading solution pH

6. Finally, the solution is put in the microwave after setting it to 800 W and 100 °C for 30 minutes as shown below.

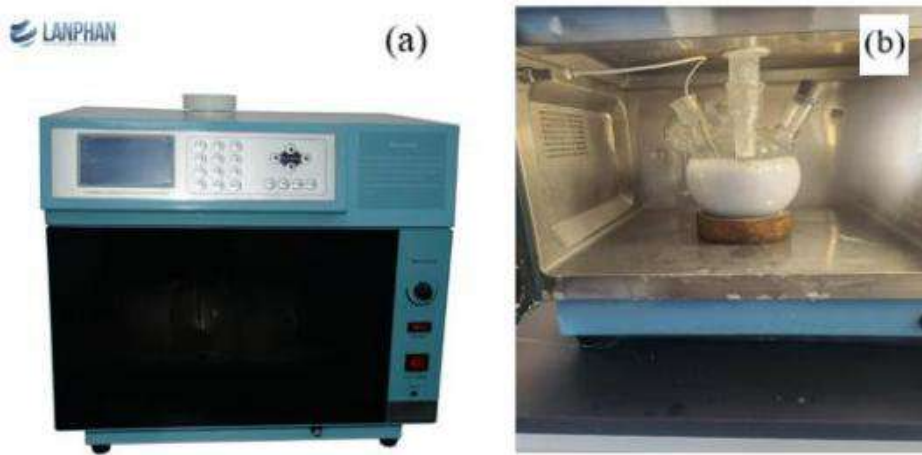


Figure 25. (a) microwave, (b) solution in microwave cabinet.

7. After the microwave heating of the solution, then it is left to cool down. Then filtration set-up is prepared consisting of a vacuum pump, filtration paper, and flask. After filtration, the residue on the filtration paper is moved from the top of the flask.



Figure 26. Filtration set-up.

8. The filter paper filled with residue is heated in an oven, and after that, the product powder is collected and stored as shown below.

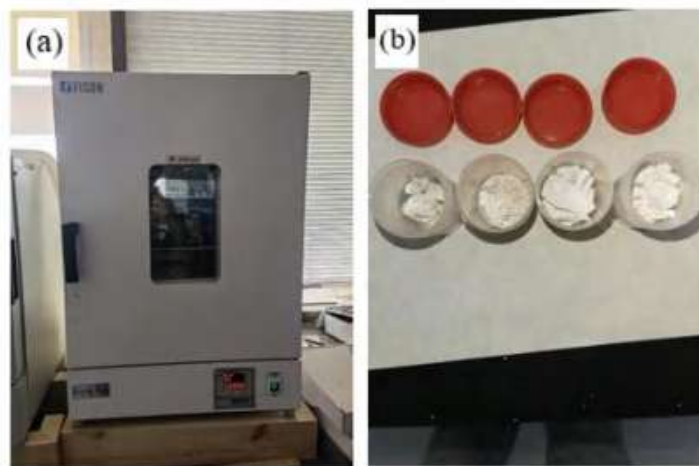


Figure 27. (a) drying oven, (b) final product in containers.

3.5 Characterization

3.6 XRD

XRD analysis for all samples were performed in the CENT, KFUPM on Rigaku Ultima IV and JEOL JDX 3530 -X-ray Diffraction machine with 2° min^{-1} and $0-70^\circ$ range, Cu K_α source, 0.15416 nm wavelength, 10mA current and 30kV voltage. All samples were in small chunks and were crushed to fine powder using mortar and pestle.

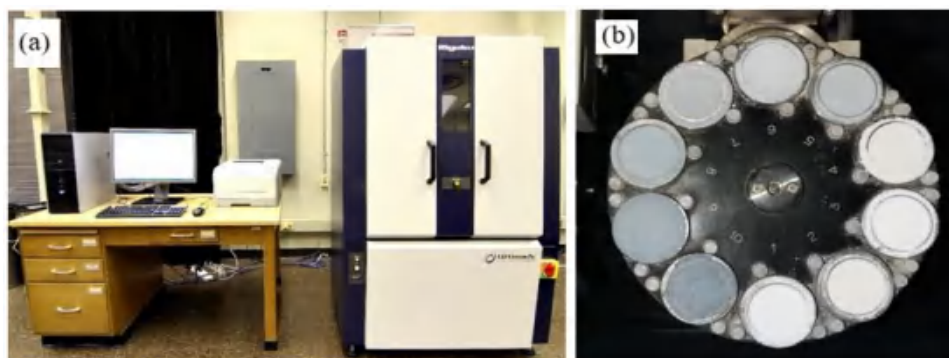


Figure 28. (a) Rigaku Ultima IV and JEOL JDX 3530 machine, (b) samples in holder inside XRD chamber

3.7 FTIR

FTIR analysis was performed only on graphene oxide, group 3 and 4 samples in chemistry department on Smart Nicolet 6700 FT-IR machine. Wavenumber 4000 cm^{-1} to 400 cm^{-1} was scanned. The background spectrum is obtained. % Transmittance spectrum of sample is scanned. Subtraction of background spectrum gives the result.

3.8 SEM

The surface morphology and structure of the samples were studied in JEOL JSM-6610LV scanning electron microscope. Magnification up to 10,000x was used and the results were compared with each other as well as from previous research and literature to compare the outcome of the synthesis. The samples were crushed into fine powder and placed onto the adhesive tape in a pattern of four samples on the stage. The potential of 20kV was utilized to obtain various resolution images.



Figure 29 - Picture of JEOL JSM-6610LV scanning electron microscope and Stage configuration.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 XRD

X-ray diffraction (XRD) is a versatile, nondestructive method that may be used to determine the atom arrangement and identify the crystalline phases of a material based on atomic spacing and symmetry [68]. By using this technique, X-rays with a known wavelength are sent through a material to completely investigate its crystal structure. Since X-rays are waves, a unique type of diffraction occurs in the crystal lattice, resulting in a pattern of "reflection peaks" at different intensities and angles. The diffracted beams from atoms in successive planes cancel unless they are in phase, according to Bragg's law [68]:

$$\lambda = 2 \cdot d \cdot \sin \theta$$

where θ is the diffraction angle, d is the distance between various atomic planes in the crystal lattice, and λ is the wavelength of the X-rays.

Before the XRD analysis, the samples were pulverized with a marble mortar and pestle. 200 mg of the powdered sample powder was then spread across a circular sample holder with a diameter of 10 mm. The powder was carefully metered to ensure that it was equally distributed on the surface of the sample holder. After that, the XRD pattern shown below was created (figure 30).

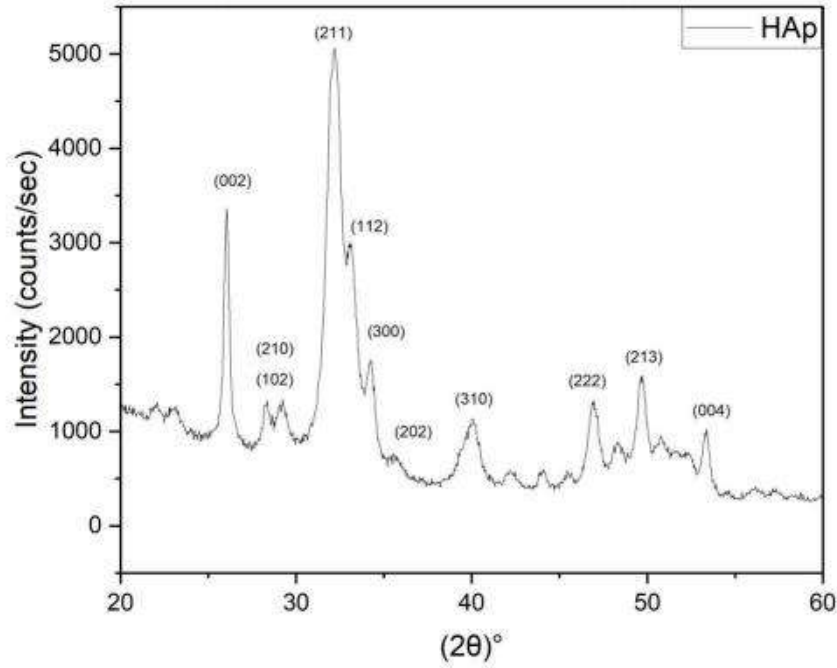


Figure 30. XRD pattern of HAp (experimental)

Crystal size calculations:

Scherrer's formula was used to determine the mean crystalline size of every sample [68],

$$L = \frac{K\lambda}{\beta \cos \theta}$$

Where β is the full width at half-maximum (FWHM) of the diffraction peak, which can be obtained from graphing software or measured manually from the graph, L is crystalline size ($\text{\AA}$) and $\lambda = 1.5406 \text{\AA}$

Scherrer's equation with a constant 0.94 was applied to (002) plane at $26.05^\circ \theta$ observed from figure 30, to calculate the crystal size. The FWHM for HAp samples was measure

manually and then used in the equation $L = K\lambda/\beta \cdot \cos\theta$, the resulting crystal size value calculated for HAp is 25.4 nm.

XRD pattern analysis

XRD pattern of hydroxyapatite from figure 30, were found in agreement with the literature. Planes (002), (102), (210), (211), (112), (300), (202), (310), (222), (213), and (004) are consistent with pure HAp (JCPDS Card #74-0566).

Furthermore, as illustrated in figure 31 below, the pattern conforms to the HAp-Mg card (JCPDS card #09-0432). When Mg ions were added to HA, the XRD pattern considerably shifted to lower 2θ values in compared to the pure HAp pattern. As Mg substitution rises, all peaks ought to widen relative to HAp samples, as the pattern and previously reported studies both demonstrate [69]. A low degree of crystallinity was indicated by the relatively broad, low-intensity XRD peaks seen in the XRD patterns of the Mg-HAp powders. This was explained by the apatite phase's characteristic small crystallite size. The XRD profiles of HAp and Mg-HAp did not reveal any phase changes. The absence of whitlockite and β -tricalcium phosphate (β -TCP) which might have formed as byproducts of synthesis, served as evidence for this. The XRD pattern showed that Mg had been completely absorbed into HAp and that there were generally significant deformations due by the Mg ions present in the HAp structure. The size of the crystal decreased as the concentration of magnesium ions rose. The observed contraction may be explained by the substitution of Ca^{2+} ions (0.099 nm) for the lower ionic radii of Mg^{2+} (0.072 nm) [70].

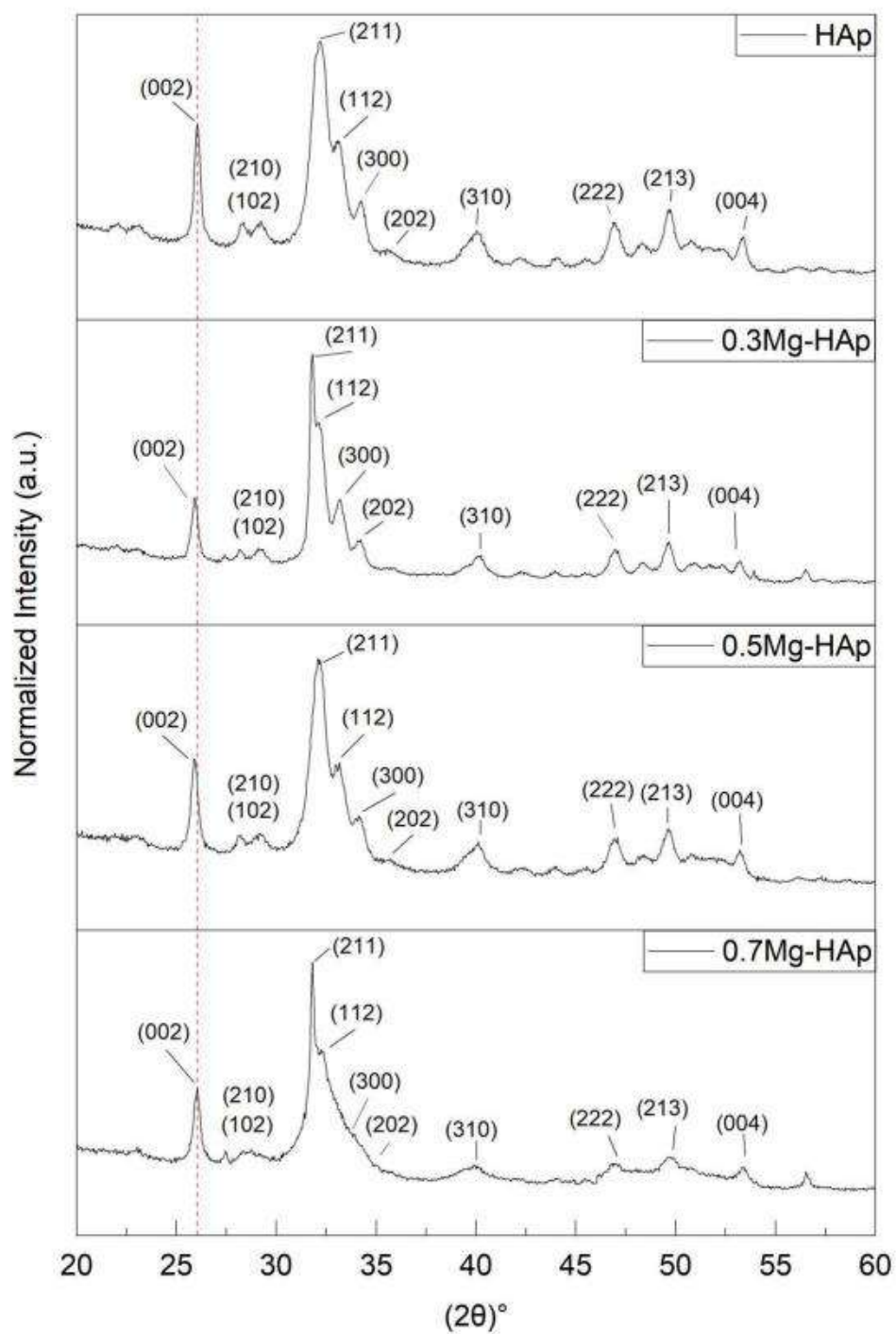


Figure 31. XRD pattern of first group.

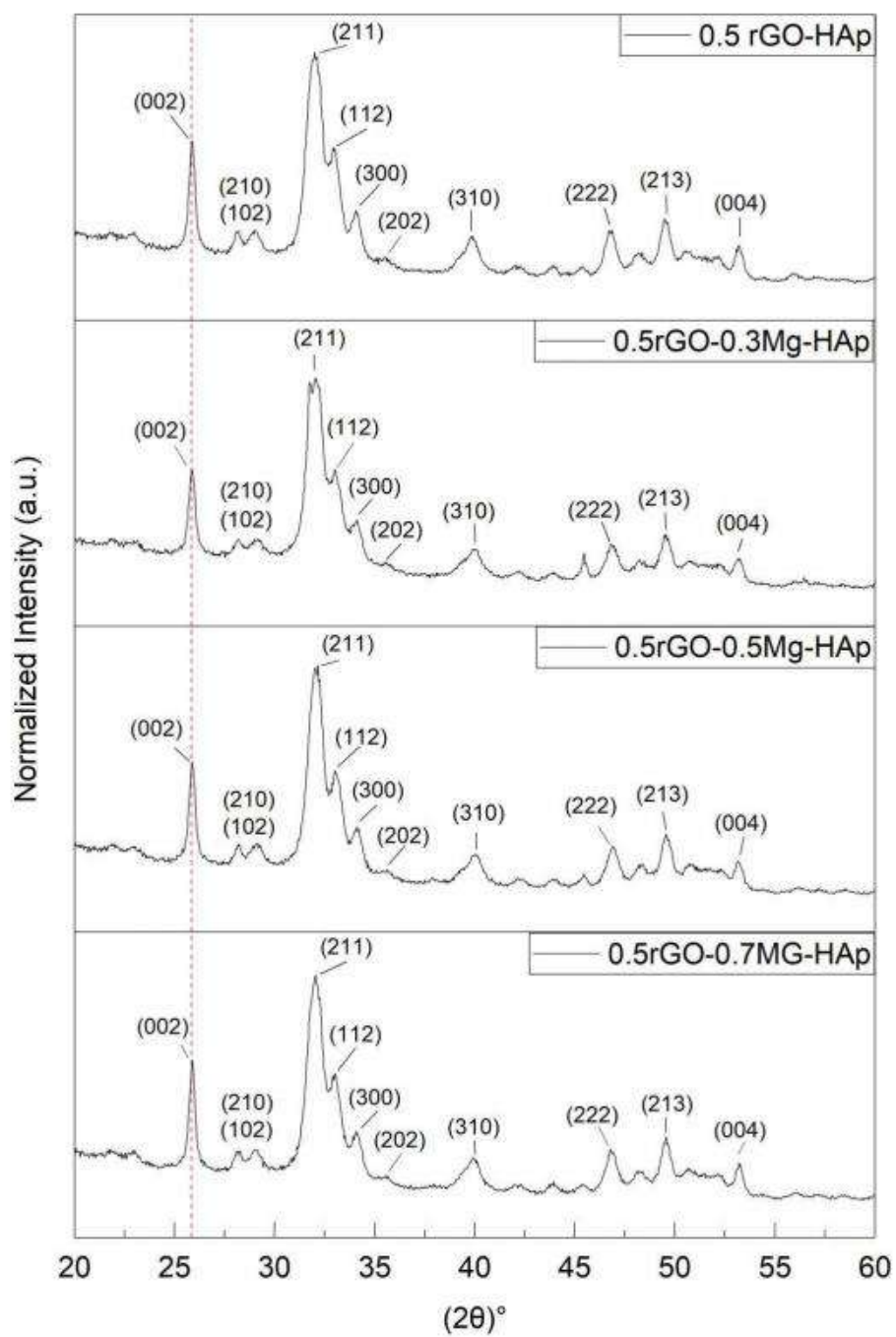


Figure 32. XRD pattern of second group.

Figure 32 illustrates the intensity increase of the main planes (002), (211), and (112). Similarly, the 2θ values in the HAp-Mg-rGO composites differ slightly from those in pure HAp-rGO.

HAp reduces its c axis when Mg is added because Mg atoms are smaller than Ca atoms, and they occupy some of the Ca sites in the HAp lattice [70]. This causes the distance between the adjacent phosphate groups along the c axis to decrease, and thus the c axis length and the unit cell volume to shrink. This reduction of the c axis can affect the mechanical and biological properties of HAp, such as its solubility, bioactivity, and biocompatibility. However, too much Mg substitution can also impair the crystallinity and stability of HAp and alter its phase purity and morphology. Therefore, the optimal amount of Mg substitution depends on the desired properties and applications of HAp. For instance, Mayer et al. reported precipitation of HAp containing up to 1.5 wt.% of magnesium without concurrent carbonate substitution [58]. Since the experiment's highest Mg percentage accounts for 0.7 wt.%, then all Mg ions are expected to substitute Ca in all samples without any phase change or unsubstituted magnesium ions.

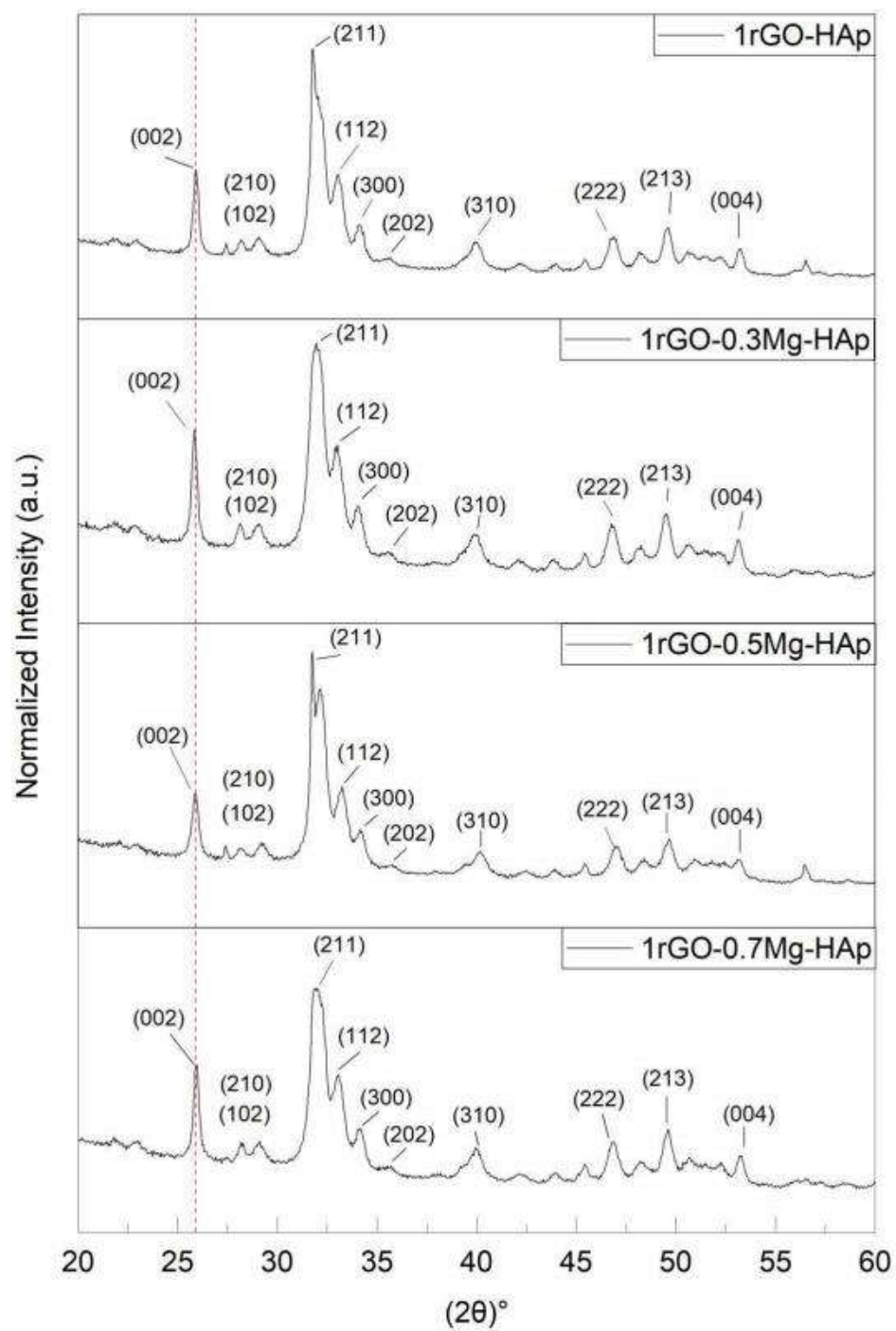


Figure 33. XRD pattern of third group.

The increase of rGO content in figure 33, from 0.5rGO to 1rGO didn't cause much of a change to the composite. No characteristic diffraction peaks of rGO were observed in the patterns of binary rGO/HAP and rGO-Mg-HAP nanocomposites, indicating that exfoliated rGO nanosheets were not restacked during the synthesis process. Since hydroxyapatite and magnesium nanoparticles act as spacers between graphene layers, they cannot be stacked to form a detectable graphitic structure. Thus, the diffraction of single carbon sheet weakens, and it overcomes by HAp and Mg pattern. The noticeable change due to rGO addition is mainly a slight increase in the crystal size that has occurred. This increase may be attributed to one of the following [72], [73]:

- 1- Nucleation and Growth: The presence of rGO may alter the nucleation and growth processes during HAp synthesis. This could lead to larger crystal sizes.
- 2- Surface Interaction: rGO interacts with HAp surfaces, affecting crystal growth kinetics. Enhanced surface interactions may promote larger crystal formation.

In addition, in figure 34, the increase of rGO content from 1rGO to 1.5rGO didn't change the XRD pattern of the composite, only a slight shift to lower 2θ value. And the increase to 3rGO had minimal effects.

Table 7 below gives a summary on all samples calculated crystal size and interplanar spacing.

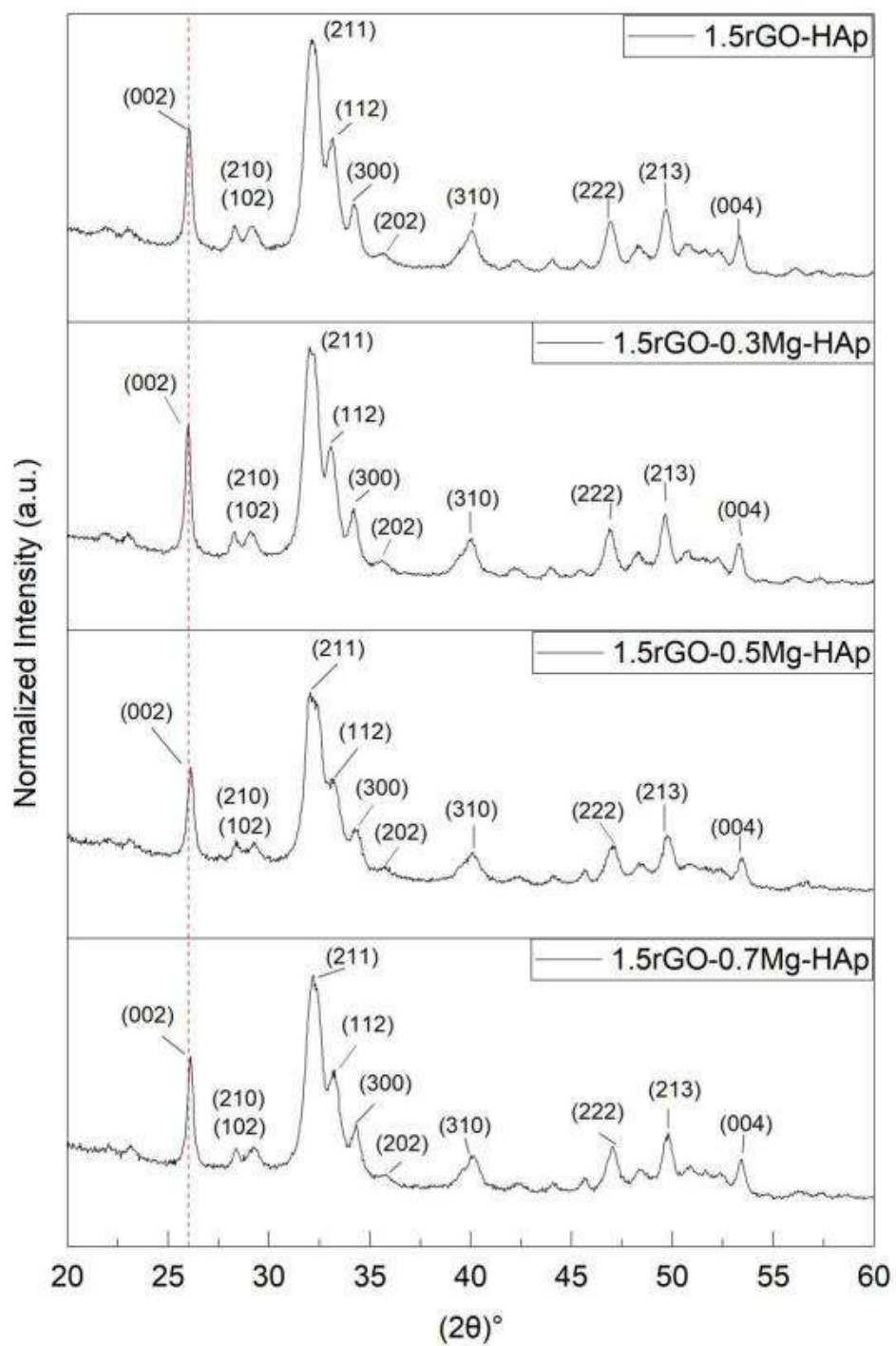


Figure 34. XRD pattern of fourth group.

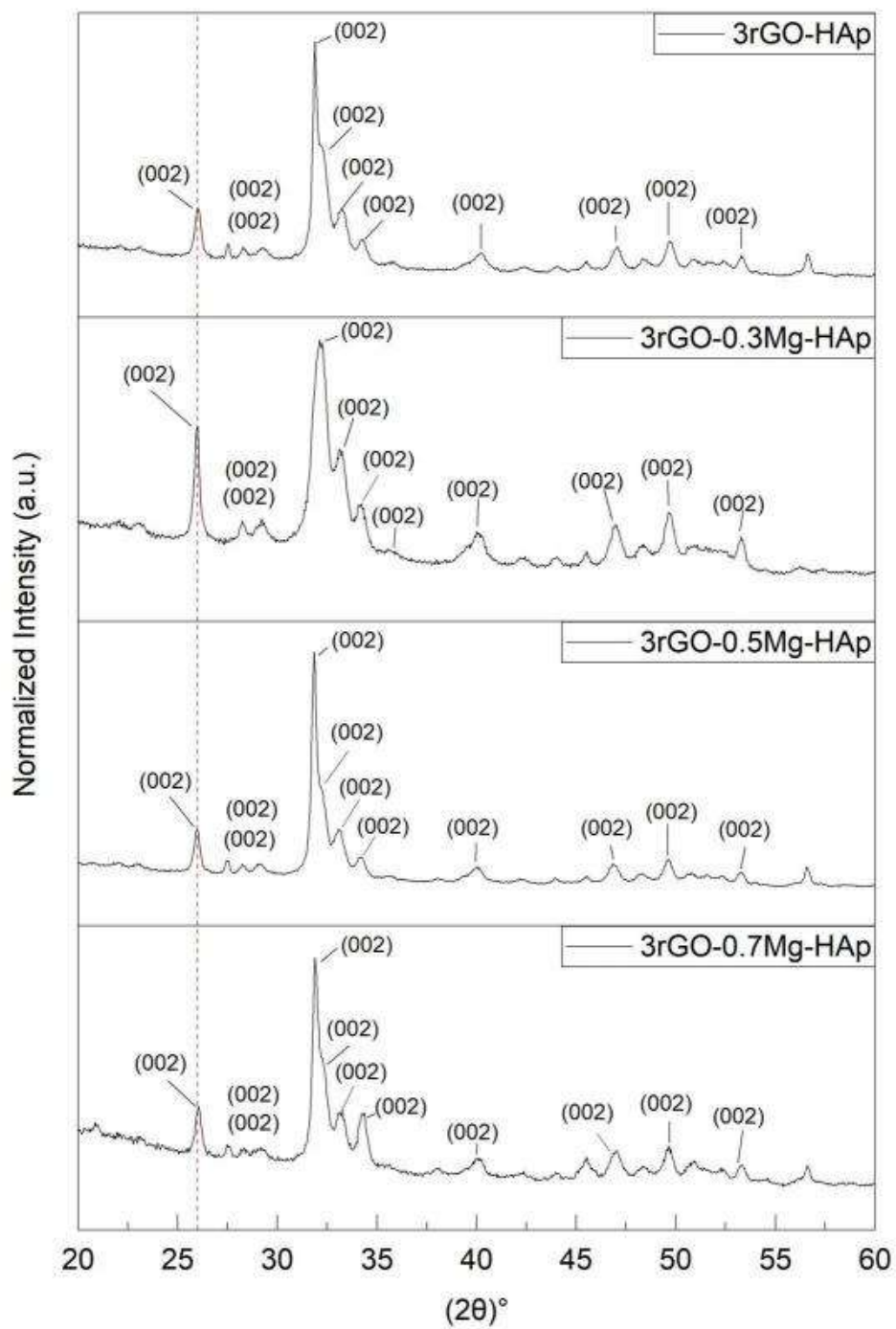


Figure 35. XRD pattern of fifth group.

Table 11. Summary of all sample's crystal size

Sample	2 θ (deg.)	FWHM (deg.)	Crystal Size (nm)	d-spacing (Å) ($d=hkl=\lambda/(2\sin\theta)$)
HAp	26.04	0.32	26.61347	3.4191
0.3Mg-HAp	25.96	0.4	21.28734	3.4295
0.5Mg-HAp	25.88	0.4	21.28392	3.4399
0.7Mg-HAp	26.04	0.44	19.35525	3.4191
0.5rGO-HAp	25.84	0.32	26.60277	3.4451
0.5rGO-HAp-0.3Mg	25.84	0.4	21.28222	3.4451
0.5rGO-HAp-0.5Mg	25.84	0.4	21.28222	3.4451
0.5rGO-HAp-0.7Mg	25.88	0.41	20.7648	3.4399
1rGO-HAp	25.92	0.32	26.60704	3.4347
1rGO-HAp-0.3Mg	25.84	0.36	23.64691	3.4451
1rGO-HAp-0.5Mg	25.92	0.4	21.28563	3.4347
1rGO-HAp-0.7Mg	25.96	0.41	20.76814	3.4295
1.5rGO-HAp	26	0.32	26.61132	3.4243
1.5rGO-HAp-0.3Mg	26	0.36	23.65451	3.4243
1.5rGO-HAp-0.5Mg	26.12	0.38	22.41497	3.4088
1.5rGO-HAp-0.7Mg	26.12	0.4	21.29422	3.4088
3rGO-HAp	26	0.33	25.80492	3.4243
3rGO-HAp-0.3Mg	26	0.36	23.65451	3.4243
3rGO-HAp-0.5Mg	25.96	0.37	23.01335	3.4295
3rGO-HAp-0.7Mg	26.08	0.4	21.2925	3.4140

4.2 Analysis of crystal size changes based on varying magnesium percentage.

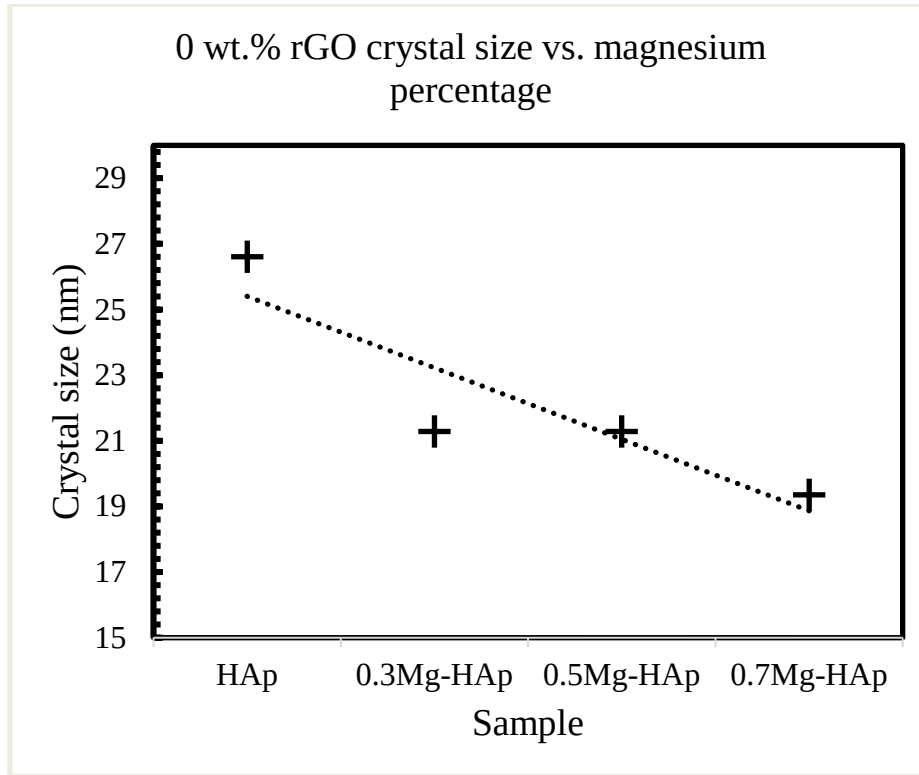


Figure 36. crystal size of 0 wt. % rGO vs. magnesium percentage.

Figure 36 above shows the descending crystal size with increased magnesium percentage in HAp lattice. For the samples HAp, 0.3Mg-HAp, 0.5Mg-HAp, and 0.7Mg-HAp the crystal size calculated was 26.61, 21.28, 21.27, and 19.35 nm, respectively. Generally, Mg ions which were engaged in the HAp structure caused significant deformations in the crystal lattice. As discussed earlier in this chapter, the reason for the observed contraction could be explained by the substitution of the smaller ionic radii Mg^{2+} (0.072 nm) for Ca^{2+} ion (0.099 nm). HAp reduces its c axis when Mg is added because Mg atoms are smaller than Ca atoms, and they occupy some of the Ca sites in the HAp lattice [10]. This causes

the distance between the adjacent phosphate groups along the c axis to decrease, and thus the c axis length and the unit cell volume to shrink.

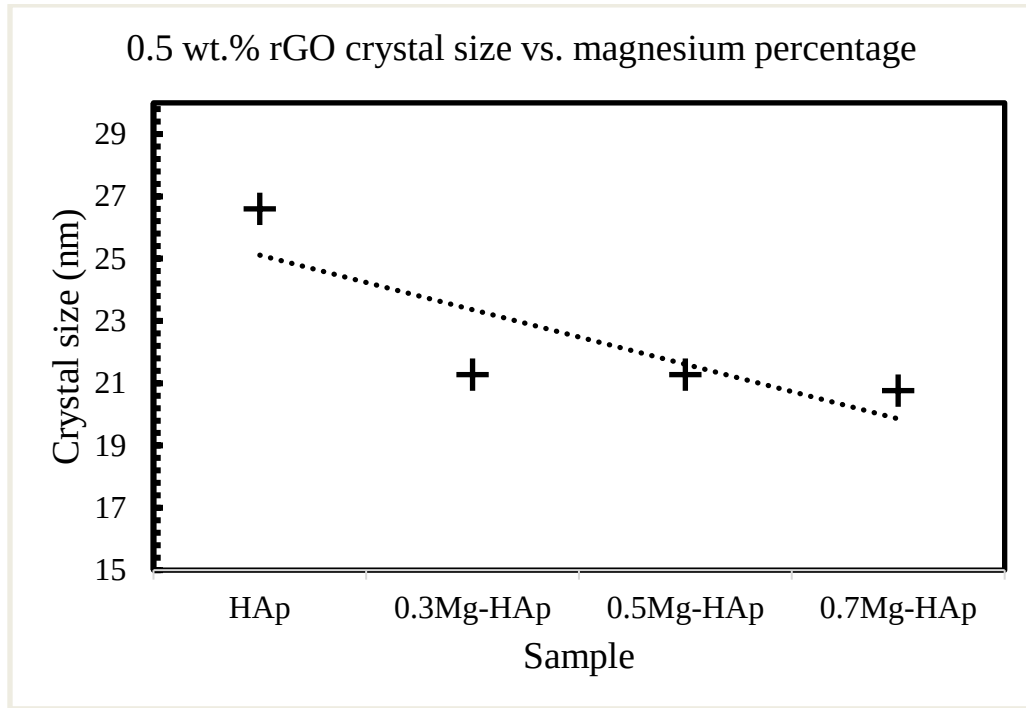


Figure 37. crystal size of 0.5 wt. % rGO vs. magnesium percentage.

As for figure 37. The incorporation of 0.5 rGO to the samples didn't cause much of a change to their crystal size pattern. However, in figure 38, we can notice a slight increase for the sample of 0.5Mg, and 0.7Mg in comparison to the previous samples with lower rGO content. This increase may be due to rGO affecting the nucleation growth of HAp. Similarly, figures 39 and 40 for 1.5 and 3 rGO content had similar effect as for figure 28 for 1 rGO content.

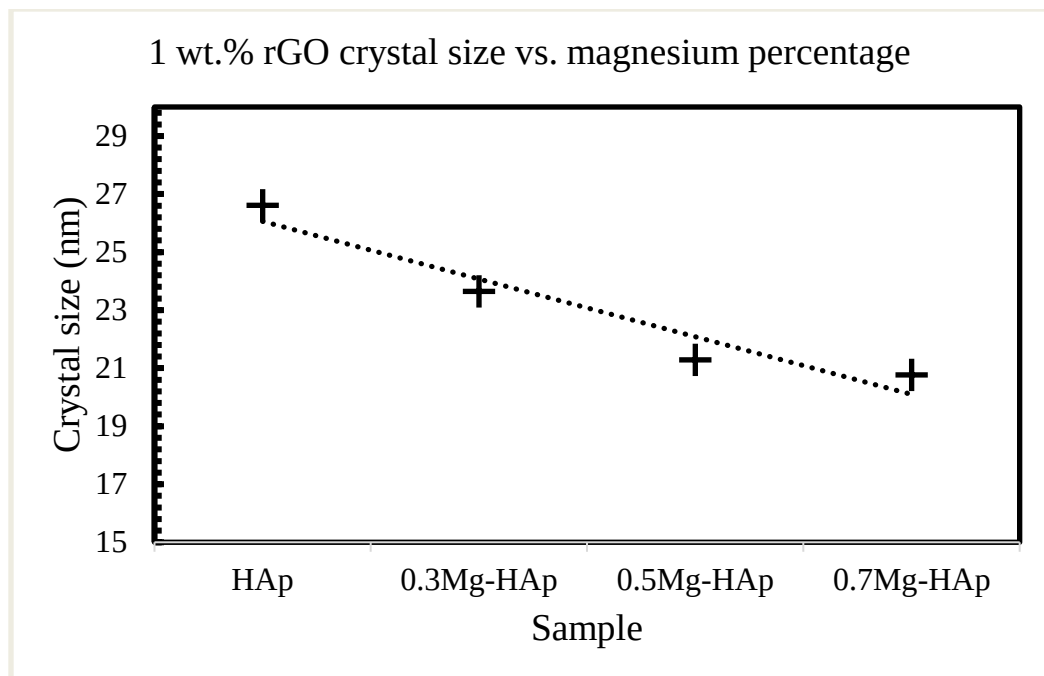


Figure 38. crystal size of 1 wt. % rGO vs. magnesium percentage.

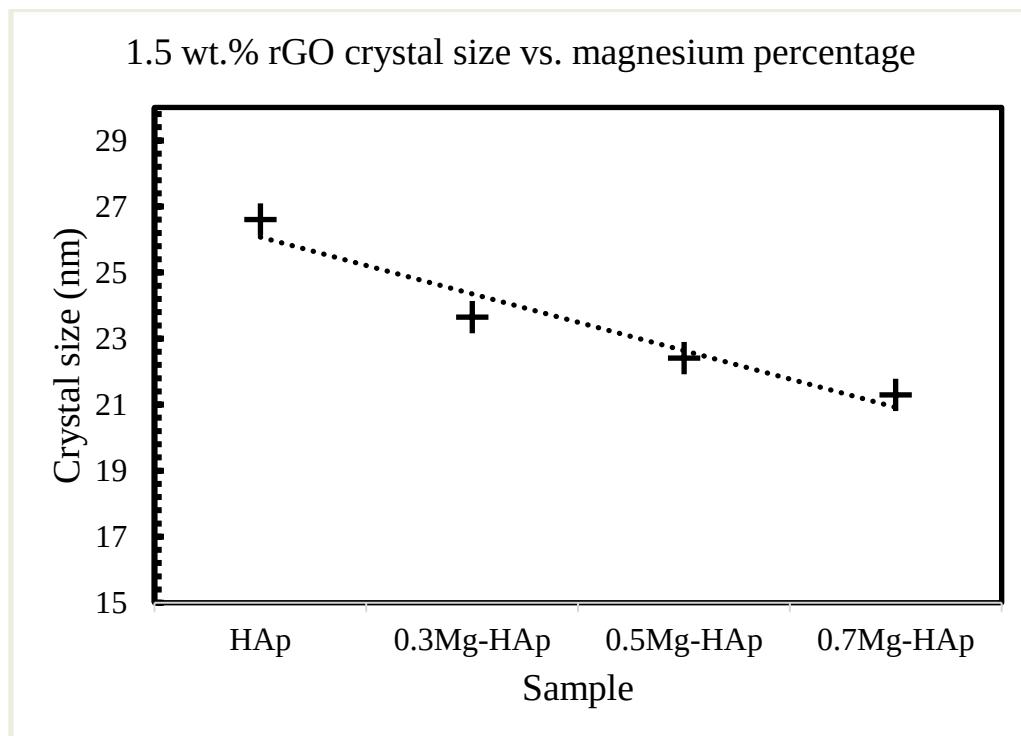


Figure 39. crystal size of 1.5 wt. % rGO vs. magnesium percentage.

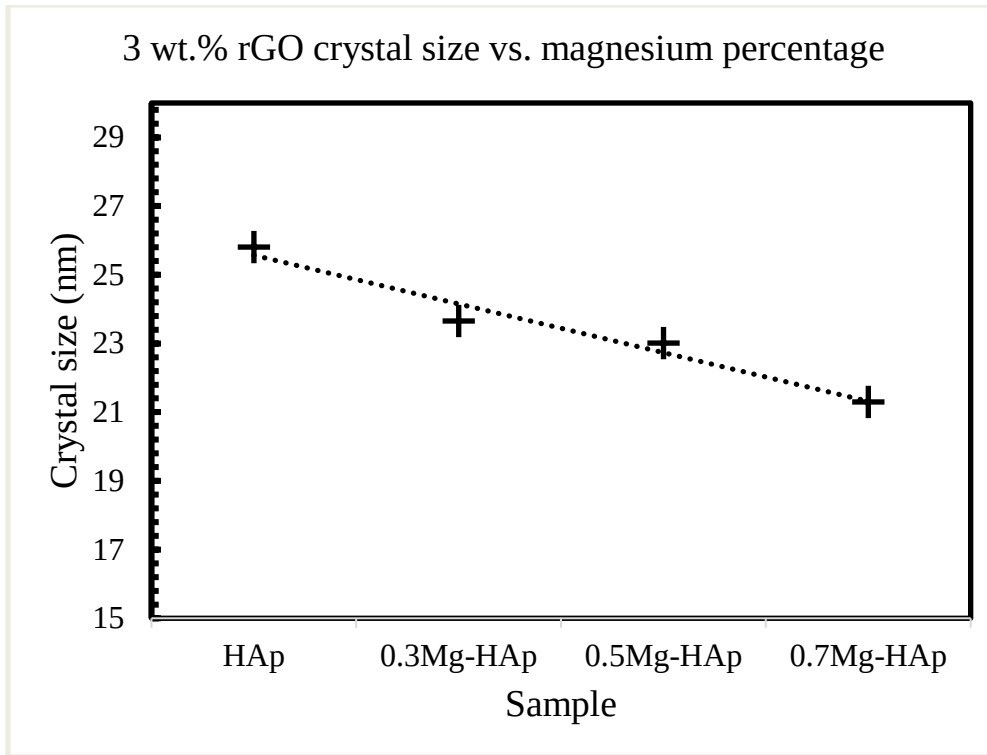


Figure 40. crystal size of 3 wt. % rGO vs. magnesium percentage.

4.3 Analysis of crystal size changes based on varying rGO percentage.

Generally, rGO has minimal effects on the crystal size of HAp since it doesn't impact its internal structure. rGO is chemically bonded with HAp but not incorporated in it as in the case of Mg. Nevertheless, a very slight increase of the crystal size can be noticed for samples with magnesium content. This mechanism needs further research to understand the exact reason for crystal change when Mg is present.

This behavior where the addition of magnesium (Mg) typically decreases the crystal size of hydroxyapatite (HAp) but increases when synthesized with reduced graphene oxide (rGO), can be attributed to several factors [74], [75], [76]:

1. Synergistic Effects: The combination of rGO and Mg ions might lead to synergistic effects that affect the crystal growth of HAp. The presence of rGO could modify the nucleation and growth kinetics of HAp crystals, counteracting the inhibitory effect of Mg ions on crystal growth.
2. Surface Interactions: rGO can interact with both HAp and Mg ions due to its functional groups and large surface area. These interactions may alter the adsorption and distribution of Mg ions on the surface of rGO and HAp, influencing the crystal growth process.
3. Template or Scaffold Effect: rGO might act as a template or scaffold for HAp crystal growth. The presence of rGO sheets could provide nucleation sites for HAp crystals, promoting their growth in certain directions and leading to larger crystal sizes despite the presence of Mg ions.
4. Synthesis Conditions: The specific synthesis conditions, such as pH, temperature, reaction time, and precursor concentrations, can significantly impact the final characteristics of the composite material. The interactions between rGO, Mg ions, and HAp crystals may vary under different synthesis conditions, leading to differences in crystal size.

In summary, the observed increase in HAp crystal size when synthesized with rGO despite the presence of Mg ions likely results from complex interactions between these components, including synergistic effects, surface interactions, template effects, and synthesis conditions. Understanding these interactions requires comprehensive analysis and careful consideration of various factors involved in the synthesis process. The figures below illustrate the increase in crystal size with Mg presence.

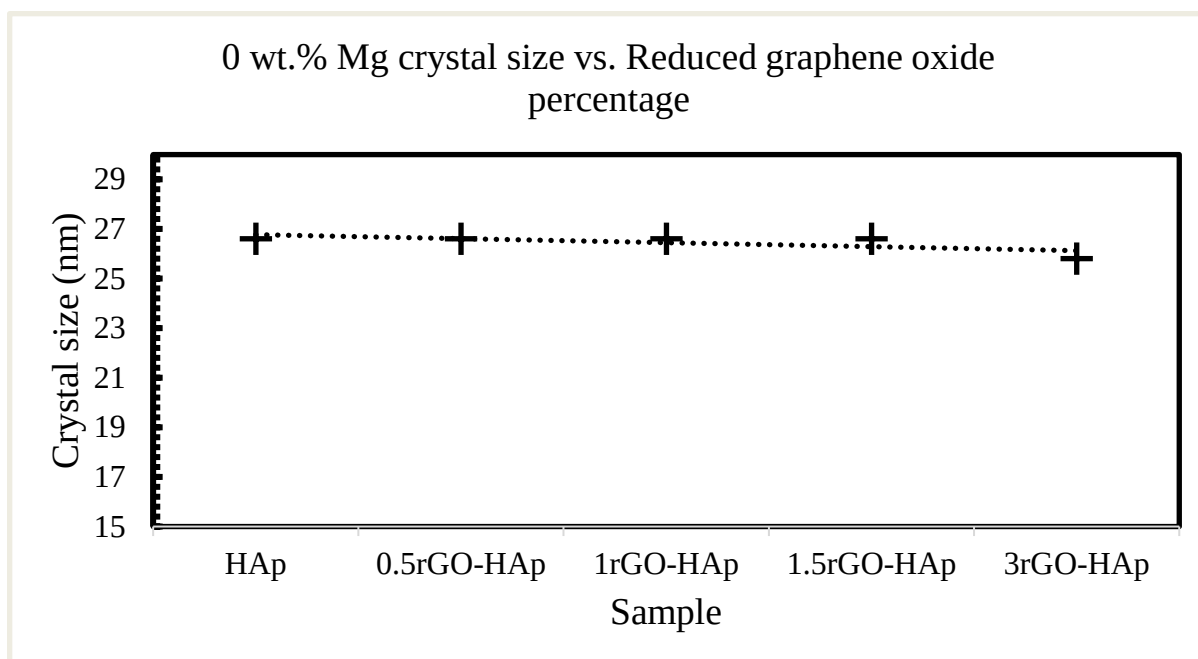


Figure 41. crystal size of 0 wt. % Mg vs. rGO percentage.

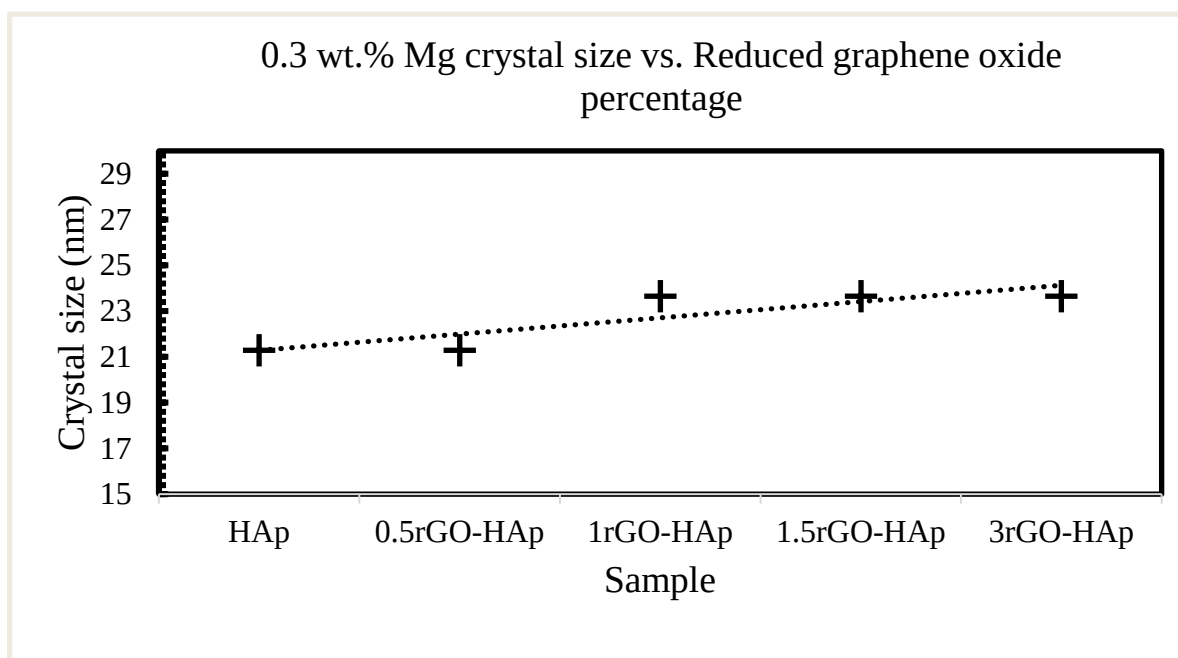


Figure 42. crystal size of 0.3 wt. % Mg vs. rGO percentage.

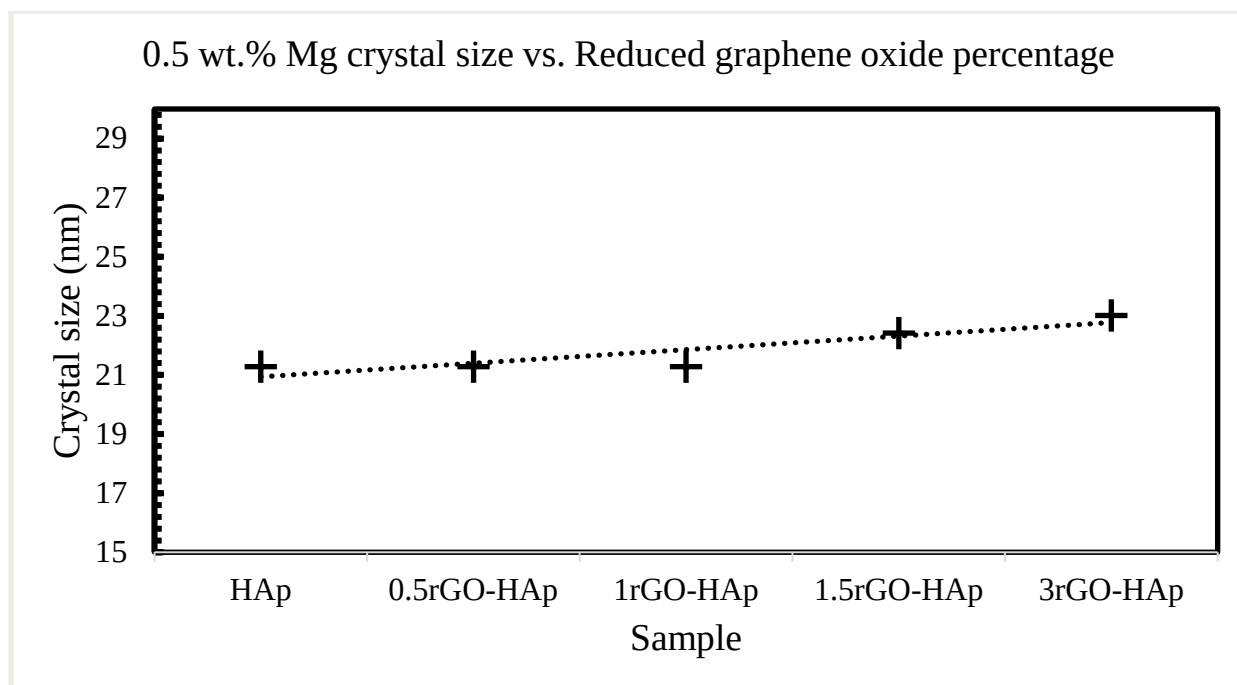


Figure 43. crystal size of 0.5 wt. % Mg vs. rGO percentage.

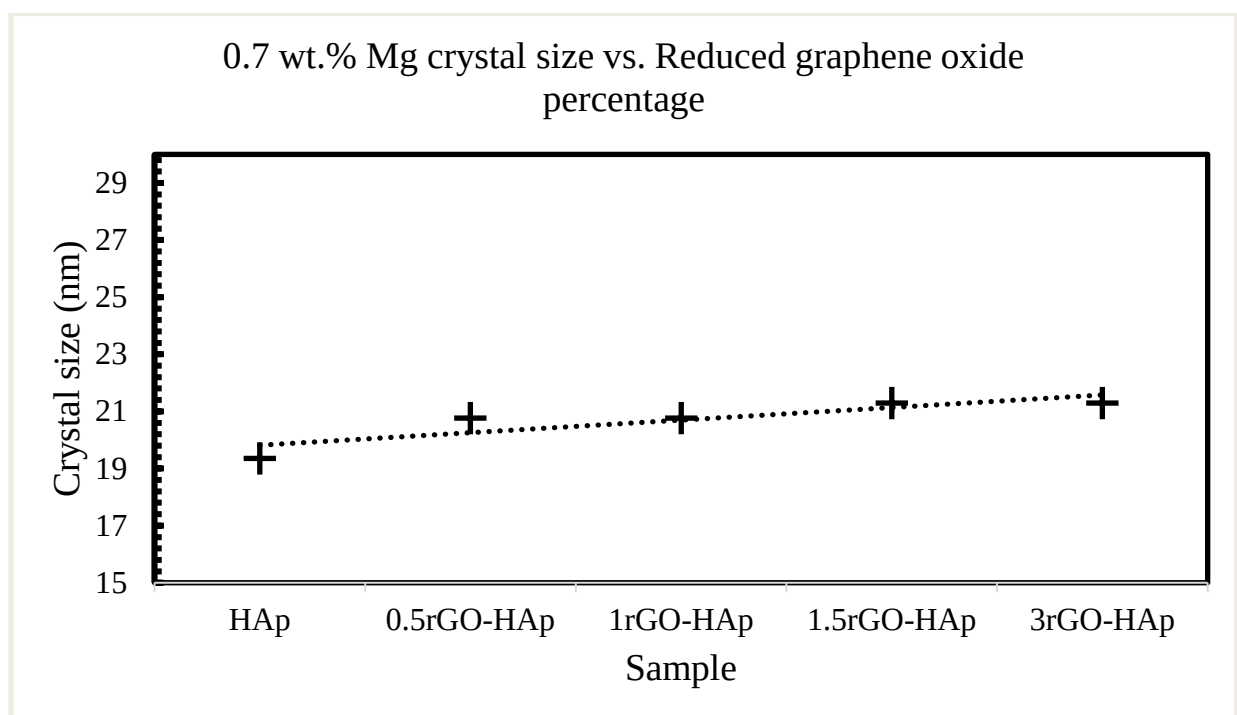


Figure 44. crystal size of 0.7 wt. % Mg vs. rGO percentage.

4.4 FTIR

Fourier transform infrared spectroscopy (FTIR), is a chemical analysis technique that can give the molecular fingerprint of a material. FTIR measures the absorption of infrared light by a sample. When infrared light passes through a sample, specific functional groups in the material absorb energy at characteristic frequencies. These absorption bands provide information about the chemical bonds present in the material. The resulting spectra are characteristic of infrared absorption/emission at various wavelengths. Peaks in the spectrum correspond to specific vibrational modes of functional groups (e.g., O-P-O, O-H, C=O). By comparing the sample spectrum to reference spectra, we can identify the functional groups present. In this experiment, FTIR was used to determine the chemical makeup of the materials. The presence of chemical groups OH and PO₄ was confirmed by FTIR spectroscopy using a clever orbit diamond attenuated total reflectance (ATR) on a Nicolet™ iS™ 10 FTIR Spectrometer. To prepare the samples, two milligrams of each sample were compressed into a tablet, which was then put on the diamond ATR specimen holder together with three hundred milligrams of potassium bromide (KBr). After that, the samples were examined at a resolution of four centimeters, averaging one hundred scans, ranging from 400 to 4000 cm⁻¹. Four spectra readouts were obtained for each synthetic material, and the absorbance spectrum was taken into account. A background reading was acquired in advance and subtracted from the sample spectra in order to produce the sample spectra [78].

Table 12. Summary of all FTIR peaks and assignments for HAp-Mg-rGO composite [77], [78]

Peak	assignment
3572	Stretching mode ν_s , of hydroxyl groups OH^-
3400-3500	O-H stretching related to rGO sheets & HAp
2000	Harmonic overtone 2. ν_3 or combination band $\nu_1 + \nu_{3b}$
1730	C=O stretching vibration
1630	Adsorbed water
1620	C=C stretching vibration
1430	Stretching mode, ν_3 , of B-type CO_3^{2-}
1046, 1032, 1087	Triply degenerated asymmetric stretching mode, ν_{3a} , ν_{3b} , ν_{3c} of the P-O bond of phosphate groups PO_4^{3-}
962	non degenerated symmetric stretching mode, ν_1 , of the P-O bond of phosphate groups PO_4^{3-}
880	characteristic bending mode of A-type CO_3^{2-}
631	Liberational mode, ν_L , of hydroxyl groups OH^-
602	Triply degenerated bending mode, ν_{4a} , of the O-P-O bonds in phosphate groups PO_4^{3-}
574 (shoulder)	Triply degenerated bending mode, ν_{4b} , of the O-P-O bonds in phosphate groups PO_4^{3-}

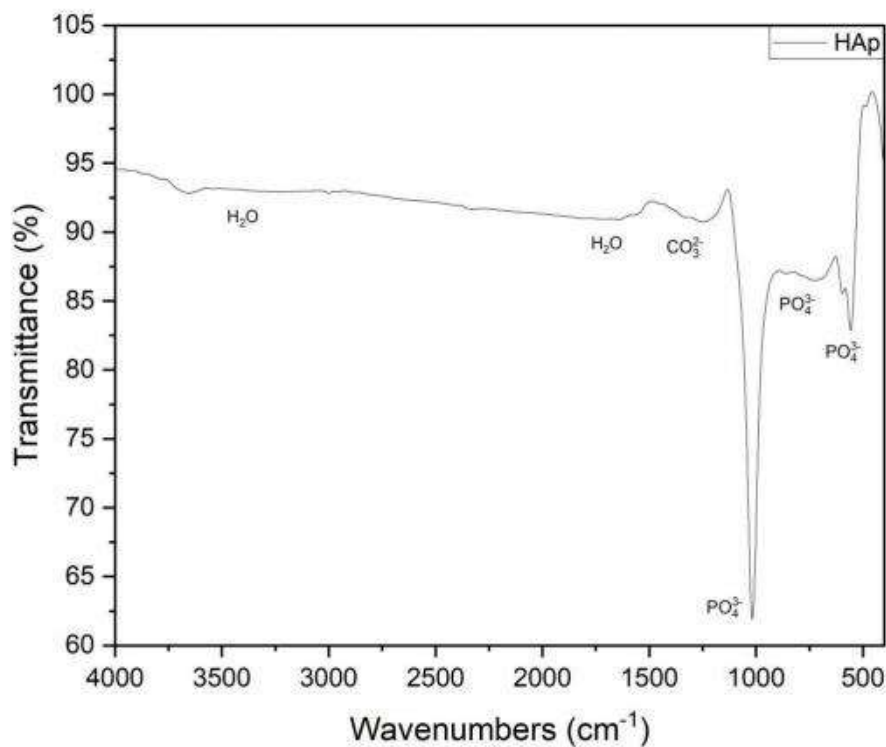


Figure 45. FTIR spectra of HAp (experimental)

Figure 45 above shows the FT-IR spectra of HAp, all FTIR assignments for the HAp sample agree with literature [78]. The OH bond is noticed at 628 and 3576 cm^{-1} and PO_4^{3-} bond is observed at 1113, 1032, 968, 602, 570, and 463 cm^{-1} . As for CO_3^{2-} , it could be seen at 1461 and 1391 cm^{-1} . Finally, the H-O-H bond is seen in 1638 cm^{-1} . This sample was used as a reference to compare other samples' effects of magnesium and reduced graphene oxide to hydroxyapatite FTIR spectra.

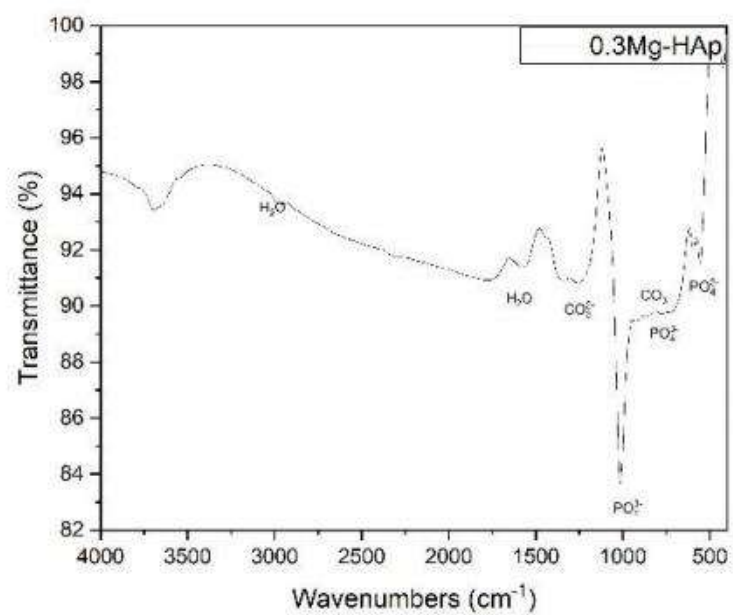


Figure 46. FTIR spectra of 0.3Mg-HAp.

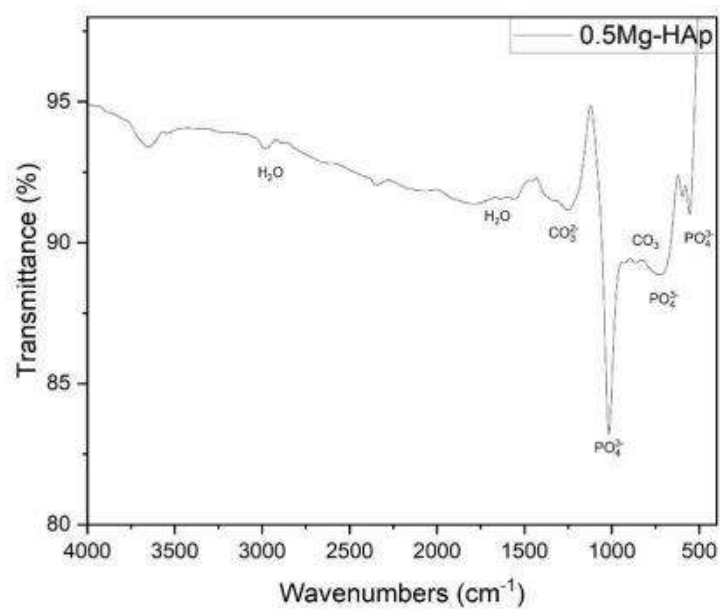


Figure 47. FTIR spectra of 0.5Mg-HAp.

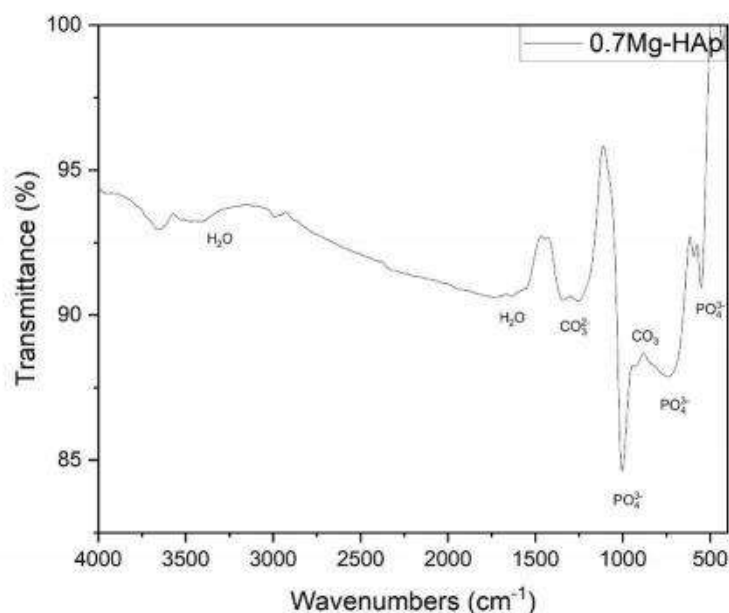


Figure 48. FTIR spectra of 0.5Mg-HAp.

Figures 46, 47, and 48 show the FT-IR spectra of 0.3Mg-HAp, 0.5Mg-HAp, and 0.7Mg-HAp samples. All the FT-IR absorption bands of HAp confirm with data in literature and were present at similar positions. However, the reduction in the intensity of the phosphate group stretching mode for Mg-HAp compared to HAp can be attributed to the substitution of calcium ions by magnesium ions in the hydroxyapatite lattice. When magnesium ions, which are smaller than calcium ions, substitute for some of the calcium ions in the hydroxyapatite structure, it can cause a distortion in the lattice. This distortion can affect the vibrational modes of the phosphate groups, leading to a reduction in the intensity of the phosphate stretching modes [79]. As the magnesium concentration increases, the intensity decreases. This suggests that the degree of lattice distortion and, consequently, the reduction in the intensity of the phosphate stretching modes, is related to the amount of magnesium incorporated into the hydroxyapatite structure [80].

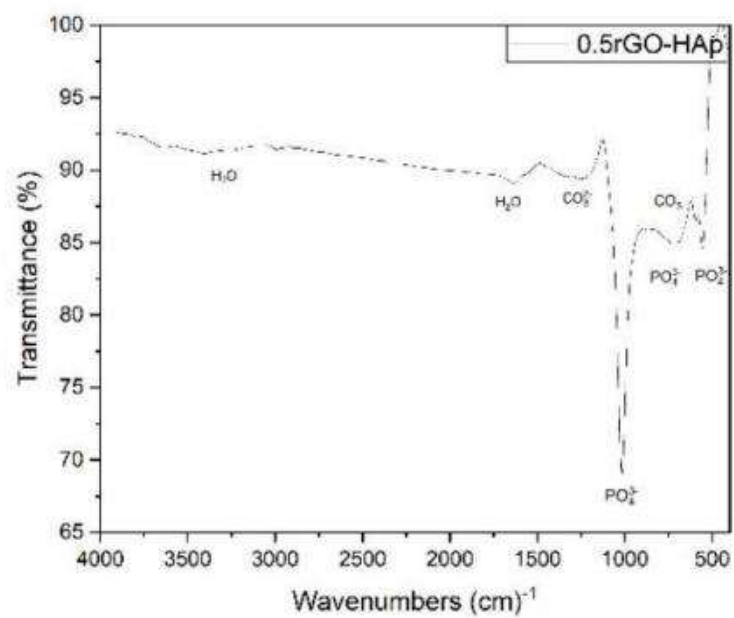


Figure 49. FTIR spectra of 0.5rGO-HAp.

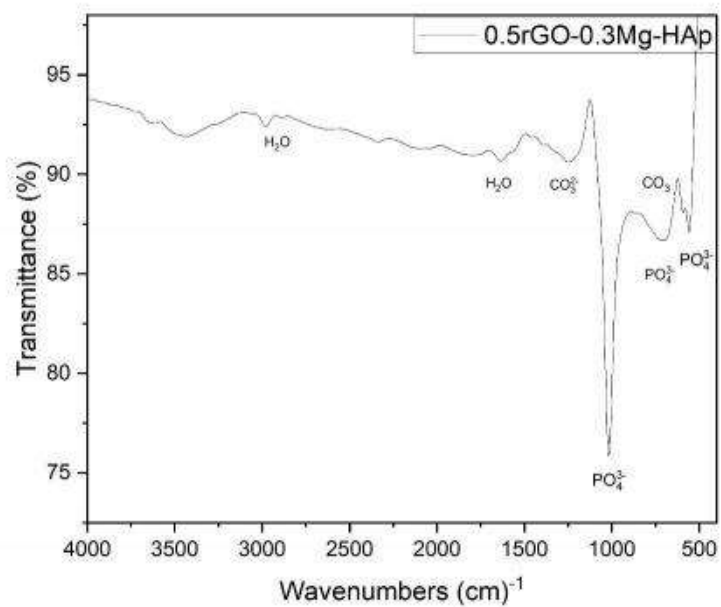


Figure 50. FTIR spectra of 0.5rGO-0.3Mg-HAp.

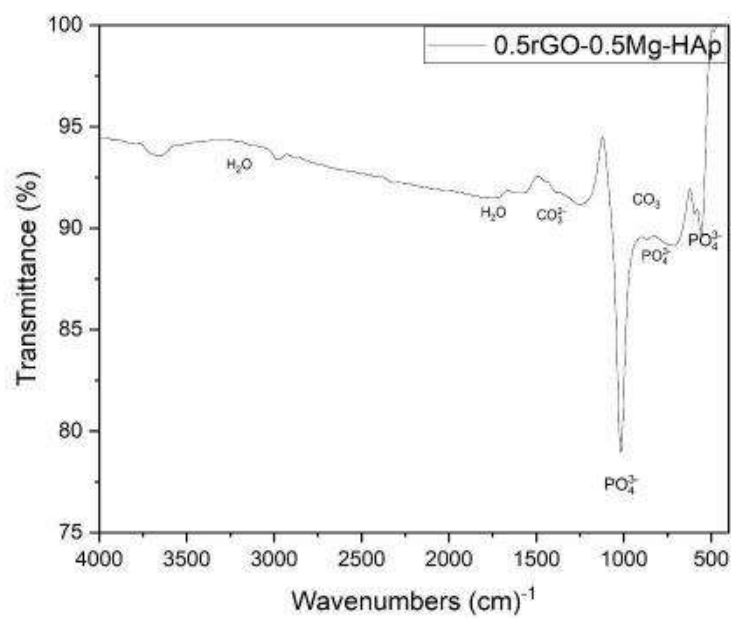


Figure 51. FTIR spectra of 0.5rGO-0.5Mg-HAp.

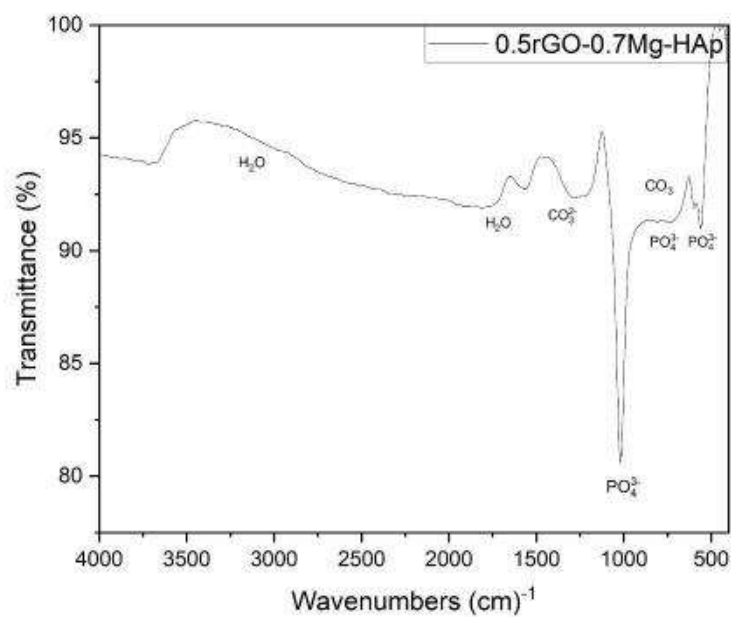


Figure 52. FTIR spectra of 0.5rGO-0.7Mg-HAp.

Figures 49, 50, 51, and 52 above show the FT-IR spectra of 0.5rGO-HAp, 0.5rGO-0.3Mg-HAp, 0.5rGO-0.5Mg-HAp, and 0.5rGO-0.7Mg-HAp samples. When rGO is introduced, it could interact with the phosphate groups in HAp, potentially altering the vibrational frequencies of these groups. This could result in a shift in the absorption peaks observed in the FTIR spectrum, leading to a decrease in the bending mode of the O-P-O mode. Moreover, the presence of rGO could also influence the local chemical environment of the phosphate groups, leading to changes in their vibrational modes. For instance, the introduction of rGO could induce changes in the hydrogen bonding network within the HAp structure, which could in turn affect the vibrational frequencies of the phosphate groups. The composite displays a typical absorption band near 1650 cm^{-1} . This band is associated with the skeletal vibration of aromatic C=C group present in rGO. A very broad peak appeared in the $3100\text{--}3600\text{ cm}^{-1}$ region which corresponds to the hydrogen bonding interaction between the -OH groups of HAp and O- containing groups of rGO (e.g., -OH, -CO-, -COOH and -O-) [78].

Figures 53 54, 55, and 56 below show the FT-IR spectra of 1rGO-HAp, 1rGO-0.3Mg-HAp, 1rGO-0.5Mg-HAp, and 1rGO-0.7Mg-HAp samples. The noticeable difference is for the high peak of the fourth sample in the stretching mode of the P-O band and maybe due to changes in structural features with magnesium addition with rGO.

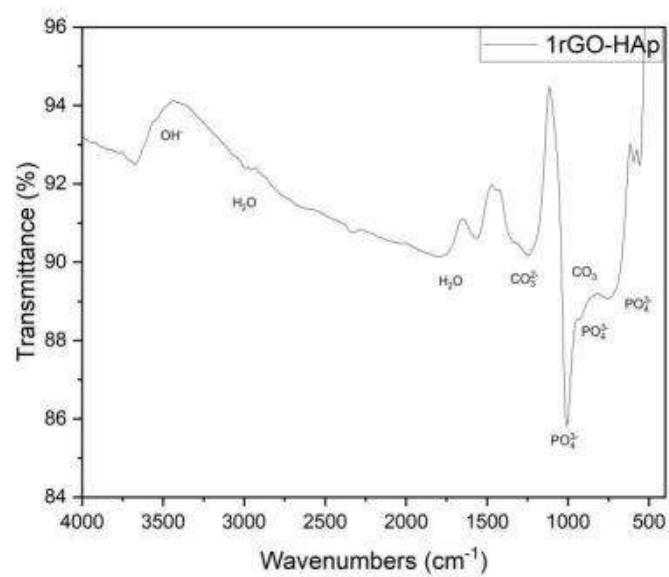


Figure 53. FTIR spectra of 1rGO-HAp.

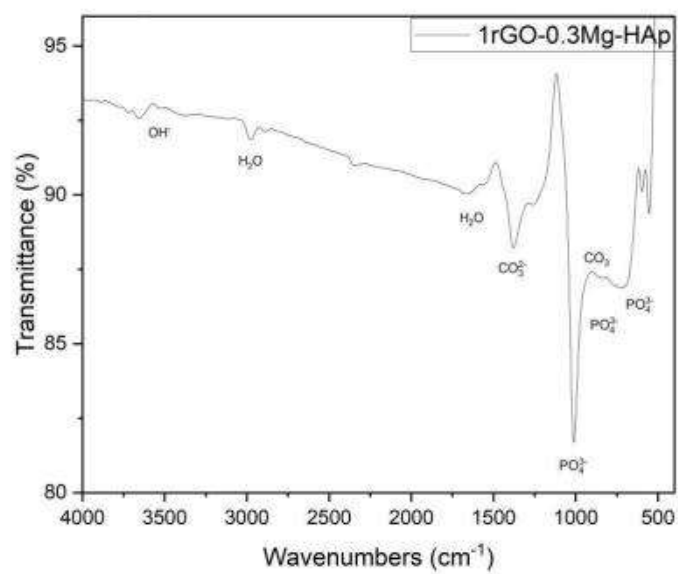


Figure 54. FTIR spectra of 1rGO-0.3Mg-HAp.

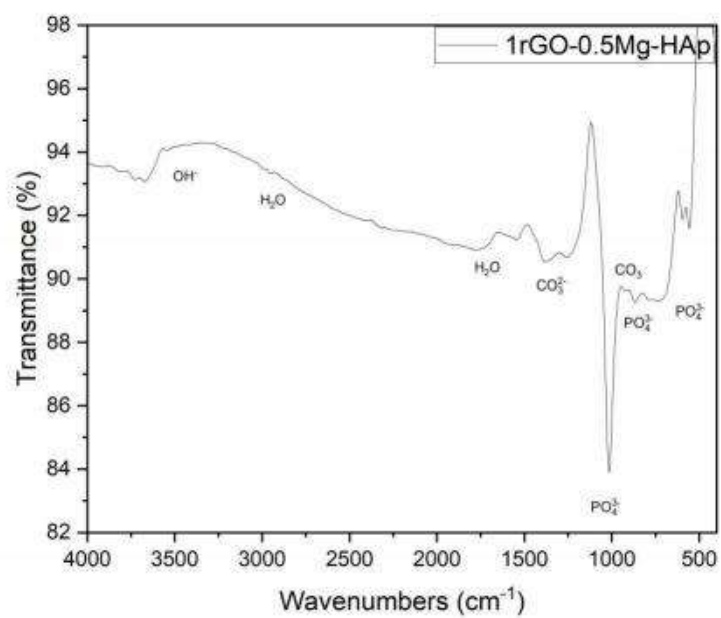


Figure 55. FTIR spectra of 1rGO-0.5Mg-HAp.

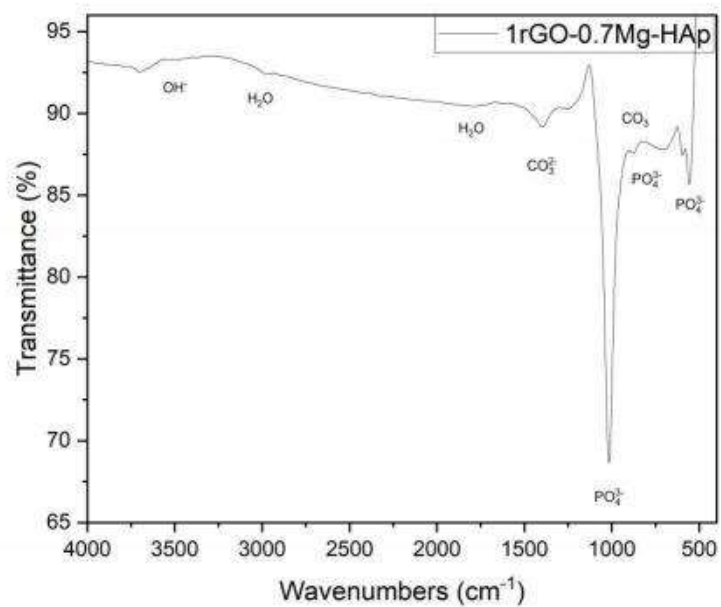


Figure 56. FTIR spectra of 1rGO-0.7Mg-HAp.

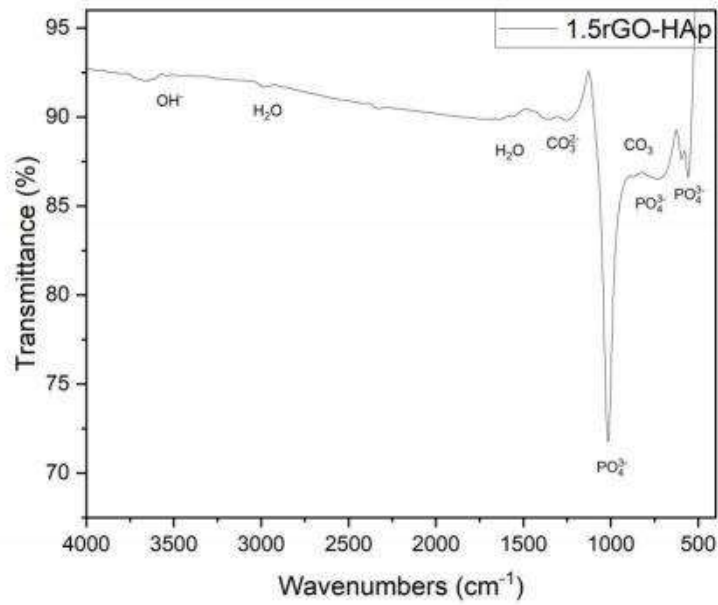


Figure 57. FTIR spectra of 1.5rGO-HAp.

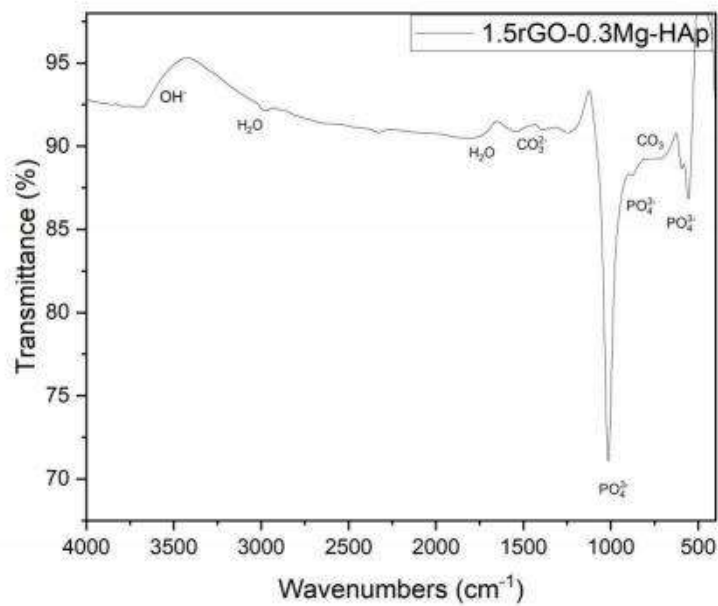


Figure 58. FTIR spectra of 1.5rGO-0.3Mg-HAp

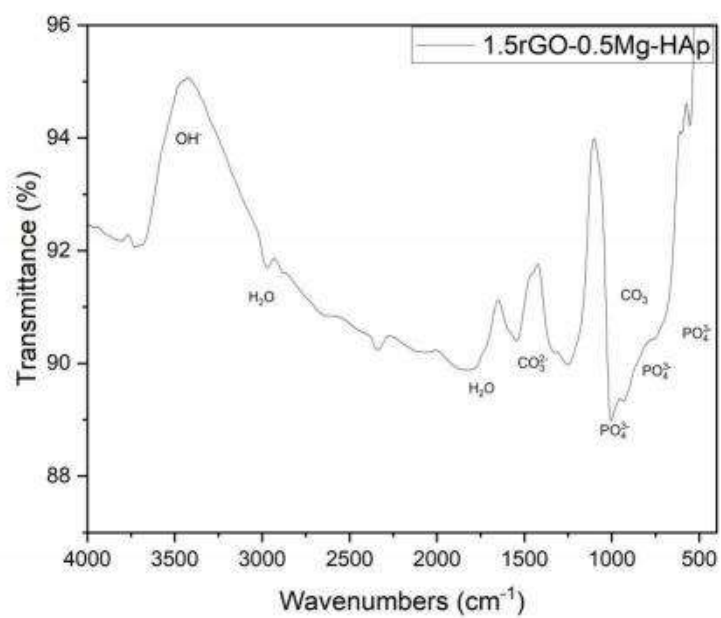


Figure 59. FTIR spectra of 1.5rGO-0.5Mg-HAp.

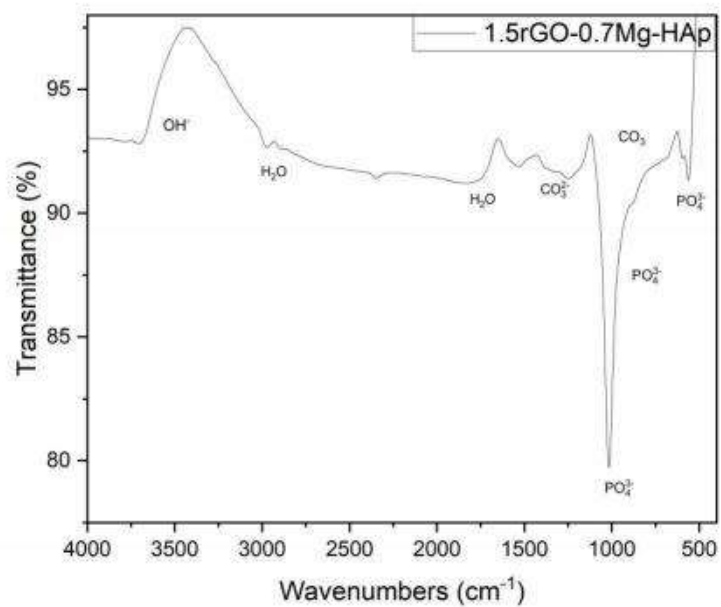


Figure 60. FTIR spectra of 1.5rGO-0.7Mg-HAp.

Figures 57, 58, 59, and 60 show the FT-IR spectra of 1.5rGO-HAp, 1.5rGO-0.3Mg-HAp, 1.5rGO-0.5Mg-HAp, and 1.5rGO-0.7Mg-HAp samples. When the rGO addition increased to a certain percentage, the bonding properties became weak due to their weak wettability. This could potentially lead to an increase in the hydroxyl group at 3500 cm^{-1} .

Additionally, the addition of different reductants to reduce graphene oxide (GO) into reduced graphene oxide (rGO) resulted in changes to the carbon to oxygen ratio, carbon lattice (vacancy), and C sp³ defects with different in-depth distributions of C sp³ because oxygen group reduction proceeded as competing processes at different rates between interstitial layers and in planes. This could also be a factor in the decrease of the phosphate group's stretching mode [80].

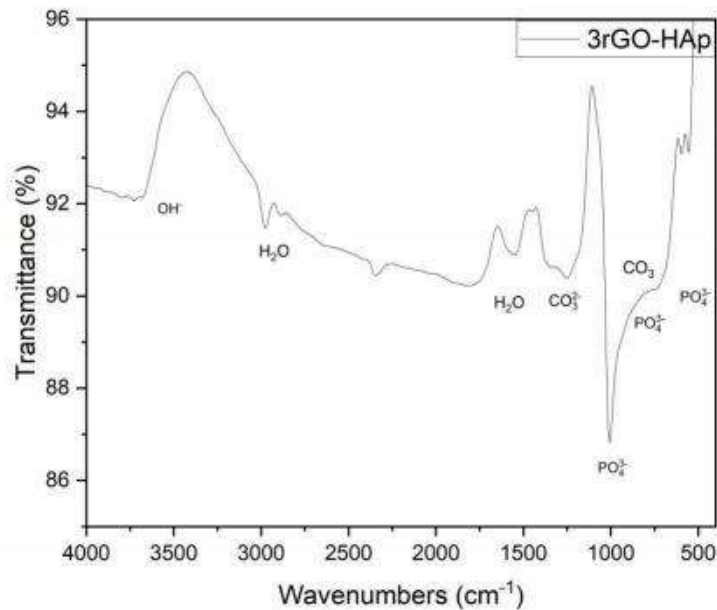


Figure 61. FTIR spectra of 3rGO-HAp.

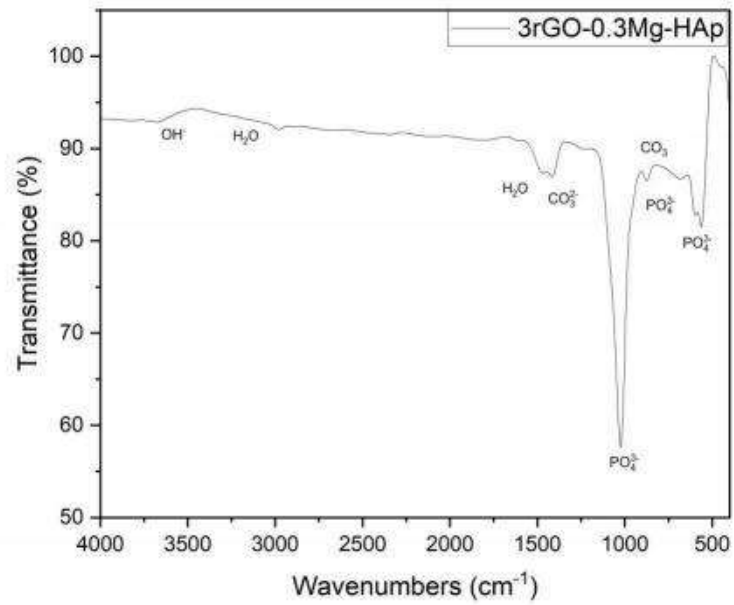


Figure 62. FTIR spectra of 3rGO-0.3Mg-HAp.

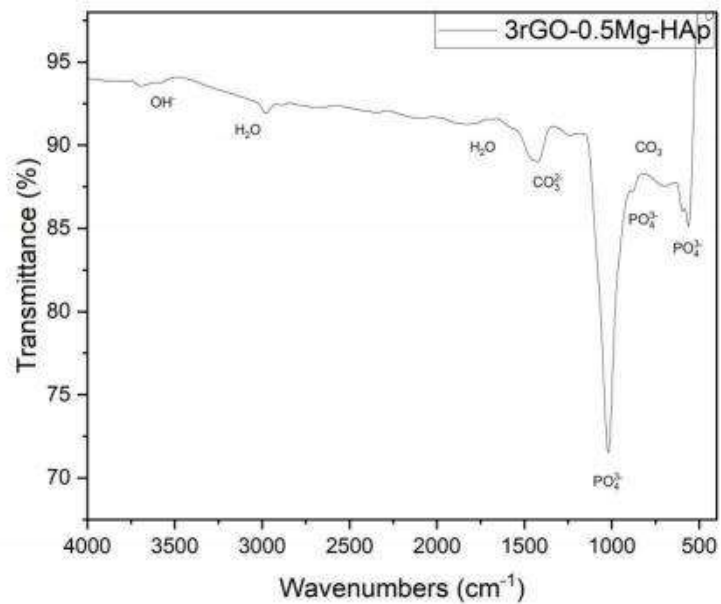


Figure 63. FTIR spectra of 3rGO-0.5Mg-HAp.

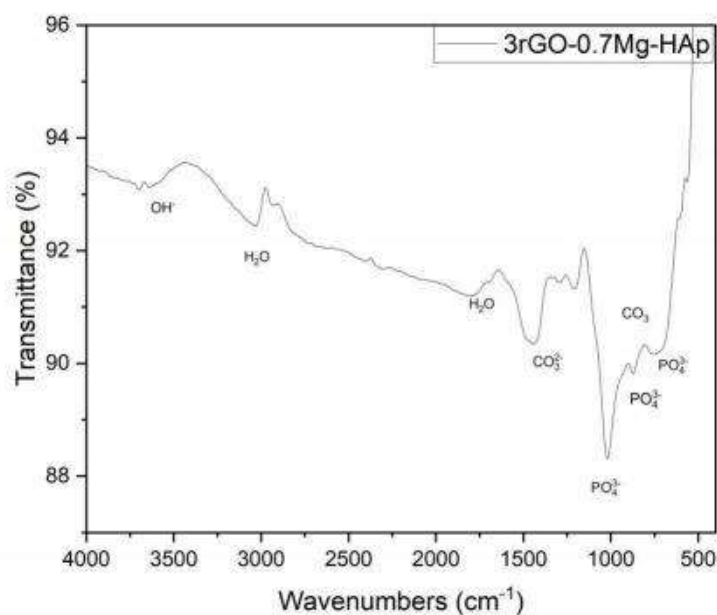


Figure 64. FTIR spectra of 3rGO-0.7Mg-HAp.

Figures 61, 62, 63, and 64 show the FT-IR spectra of 3rGO-HAp, 3rGO-0.3Mg-HAp, 3rGO-0.5Mg-HAp, and 3rGO-0.7Mg-HAp samples. All spectrums have similar pattern as the 1rGO samples discussed earlier except for the first and last sample. The lower intensities notices in the bending mode of the O-P-O and the stretching mode of the P-O bond may be attributed to previously discussed rGO effects on reducing phosphate bonding strength.

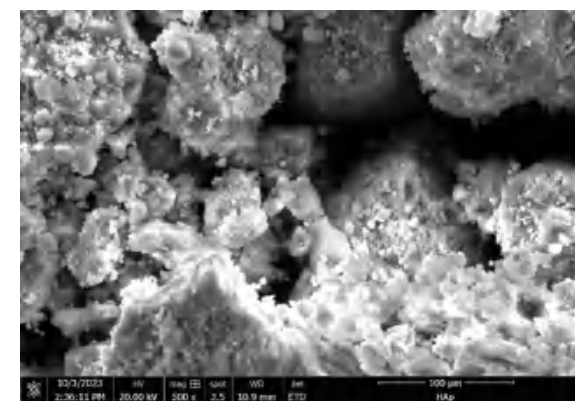
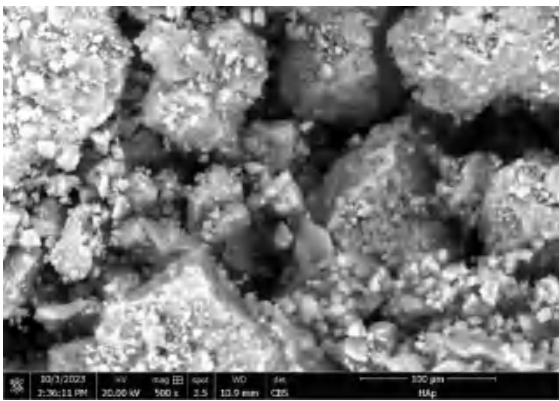
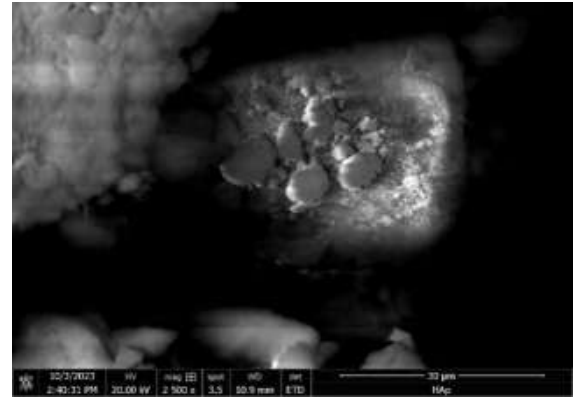
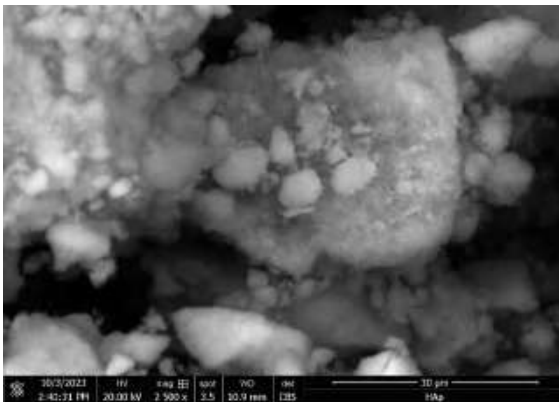
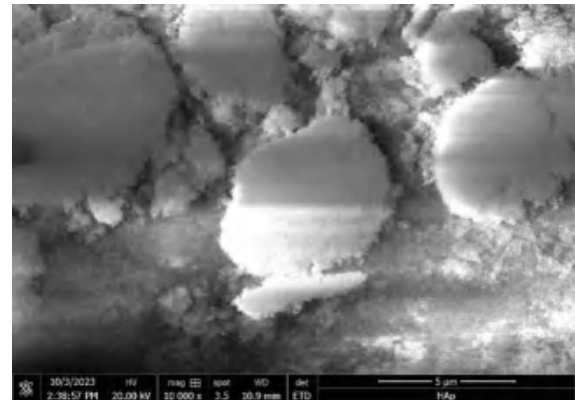
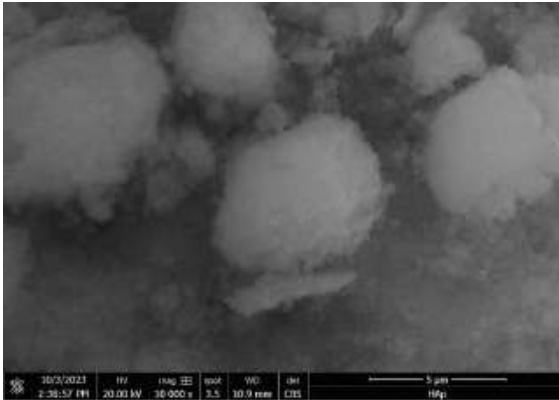
4.5 Scanning electron microscopy (SEM)

SEM reveals the surface morphology and microstructure of the composite, offering high-resolution images that shows the spatial distribution of components. The SEM images obtained at different magnifications unveil details about particle size, agglomeration, and uniformity [81]. The impact of varying rGO and Mg content on the surface morphology becomes evident, providing visual insights into how changes at the microscopic level influence the overall structure of the composite. All samples reading were carried out by Model JEOL 5800LV- Tungsten filament, at 30KeV and magnification from 50 to 5 μm .

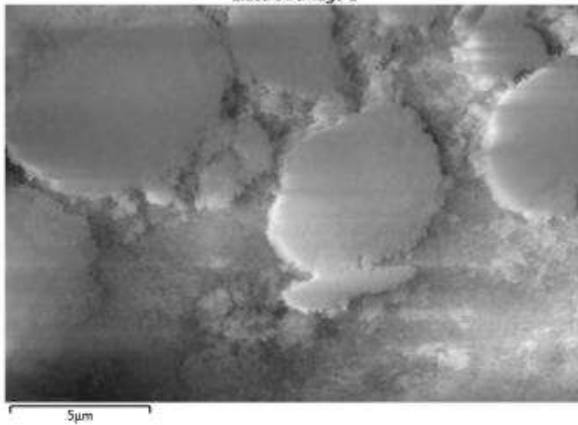
All images showed that the rGO-Mg-HAp shows an irregular surface morphology with a tendency to agglomerate. The composite has a flat & irregular agglomerated structure with different sizes. Size of the HA grains vary between 10-40 μm . At high magnifications agglomeration of small grains that form a grain of higher dimension were observed. When Mg^{2+} is doped in HAp, it influences the formation of an irregular shape as opposed to the anticipated nanorods [81]. This deviation in morphology is credited with the impact of Mg^{2+} joining into HAp, modifying its crystal structure and morphology [82]. With increasing rGO content, it tends to aggregate randomly, forming crumbled/wrinkled structure. rGO may wrap around HAp-Mg nanoparticles creating a layered structure that may be noticed especially for samples 1.5rGO-HAp-0.7Mg and 3rGO-HAp-0.7Mg.

SEM mapping shows the presence of calcium, phosphorus, magnesium, oxygen, and carbon, with changing weight percentages. The uniform circulation of components across the surface supports the homogeneity of the composite which implies that the composite was successfully synthesized. Below all 20 samples SEM images are shown along with mapping and EDS analysis.

HAp



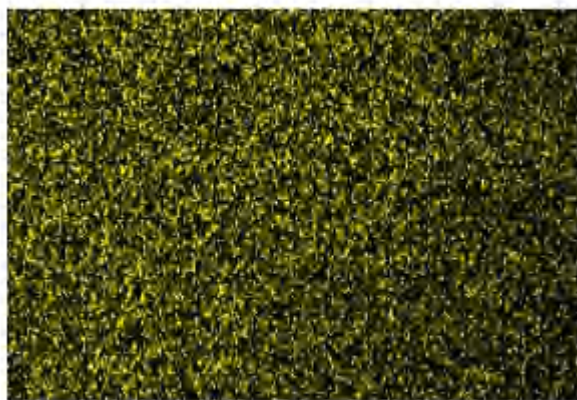
Electron Image 1



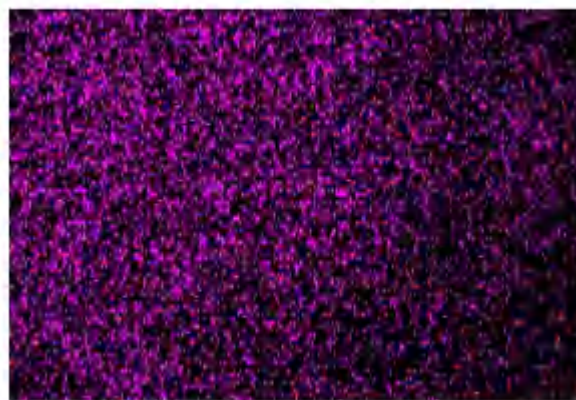
EDS Layered Image 1



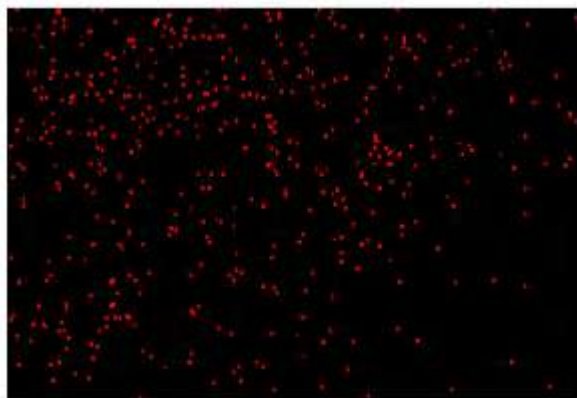
Ca K α 1



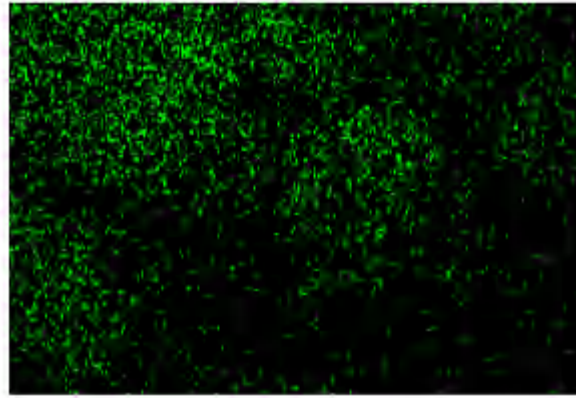
P K α 1

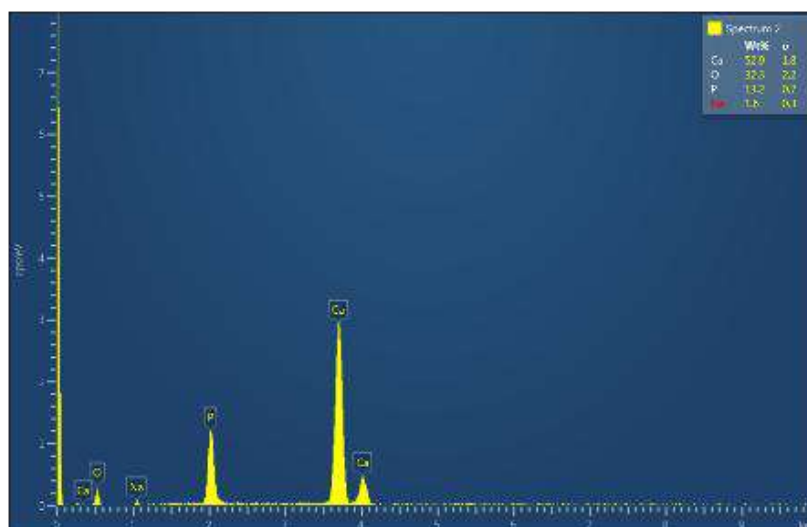
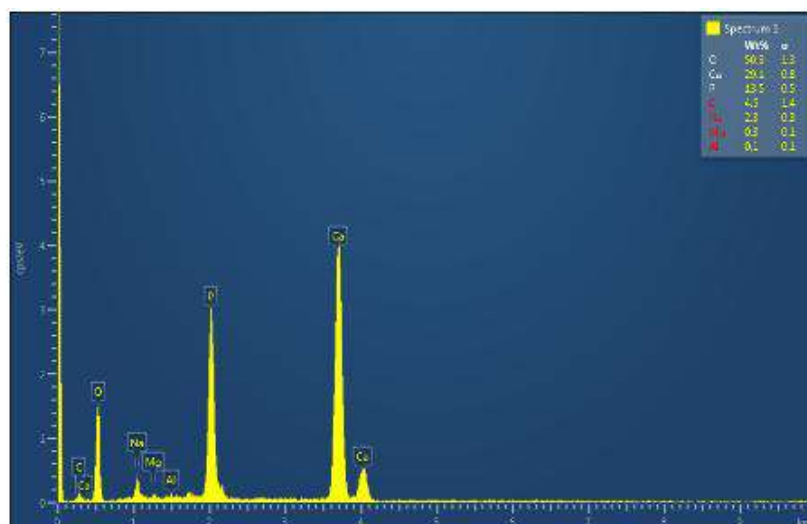
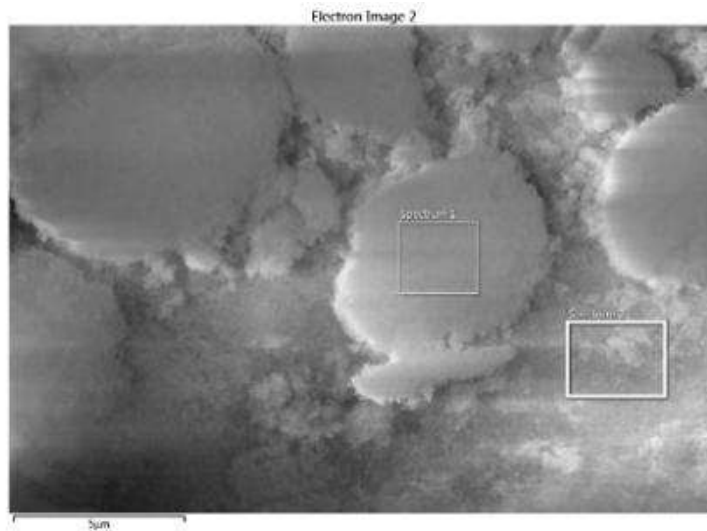


Mg K α 1_2

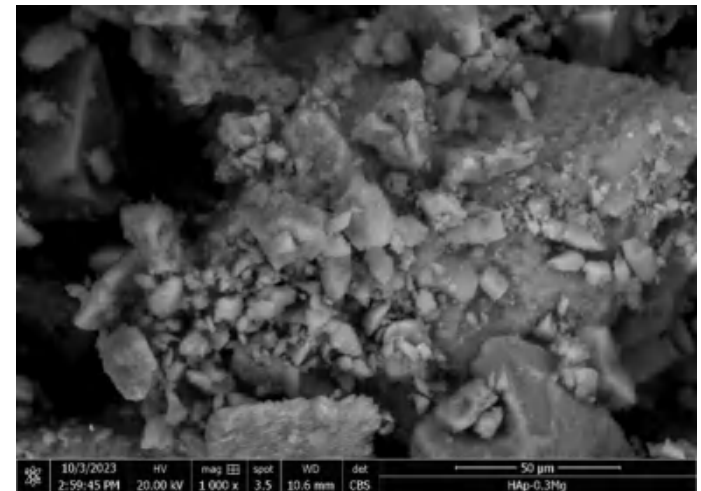
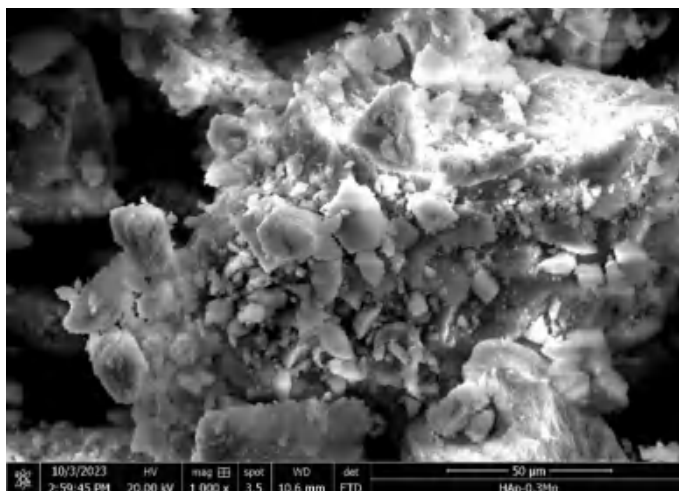
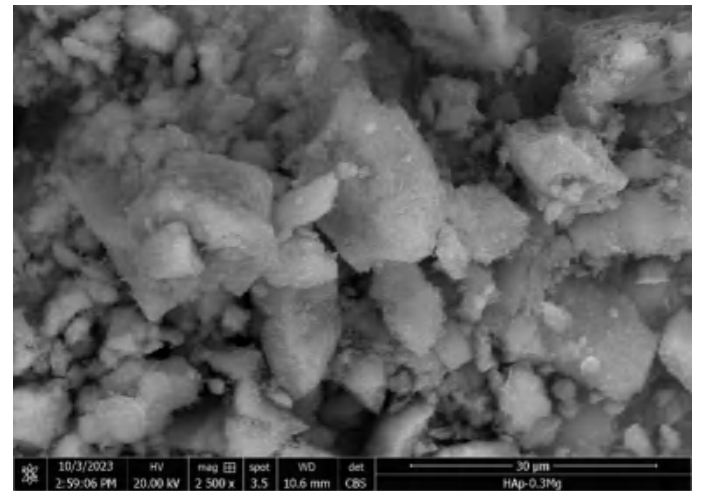
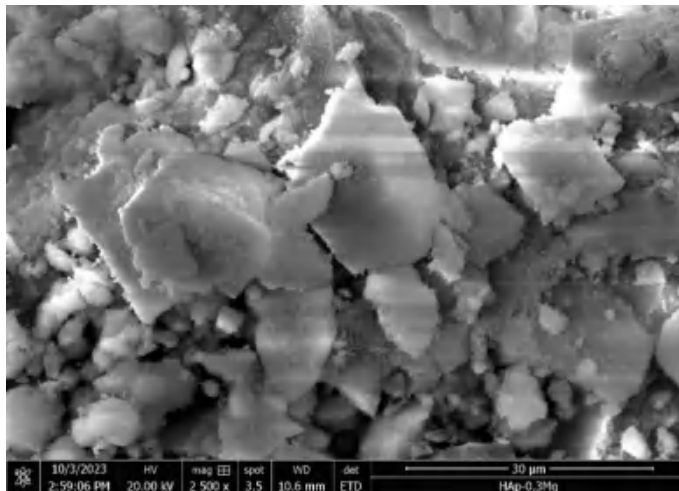
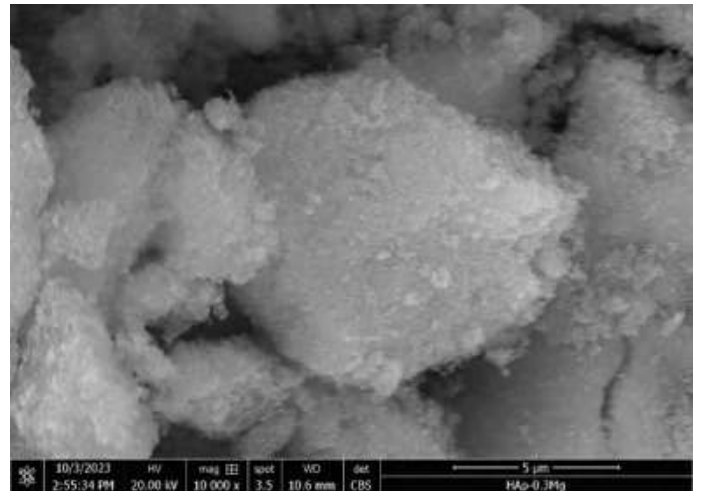
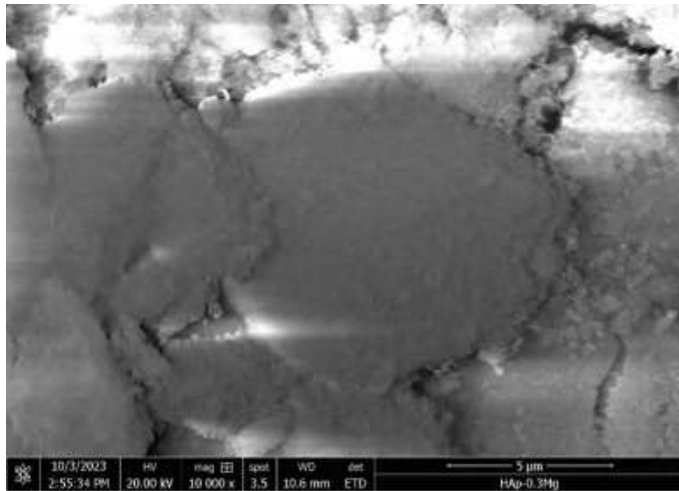


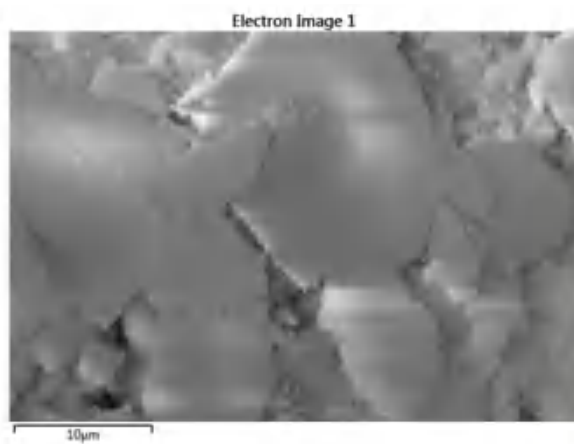
O K α 1



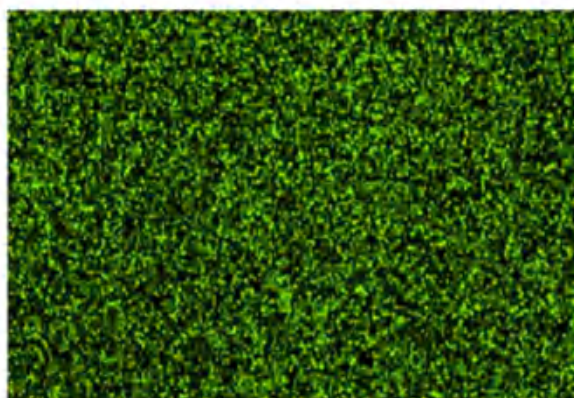


0.3Mg-HAp

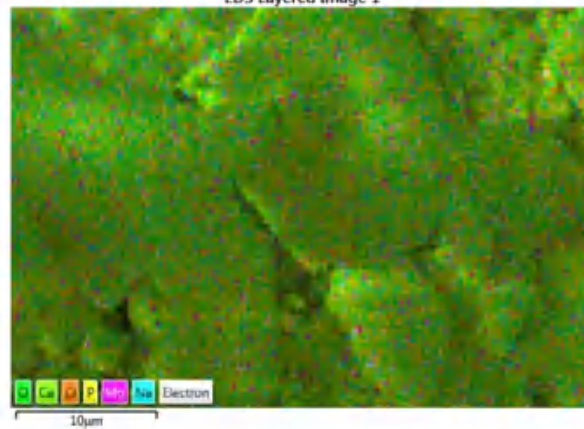




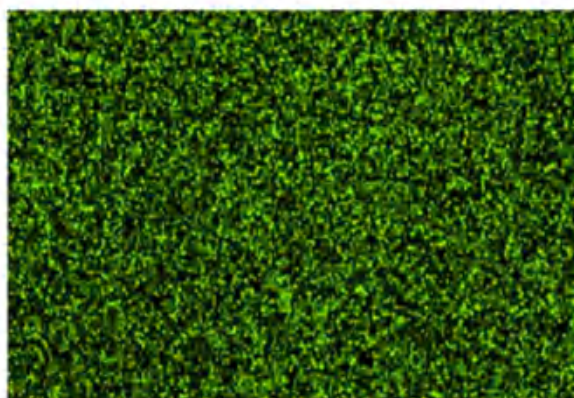
Ca Kα1



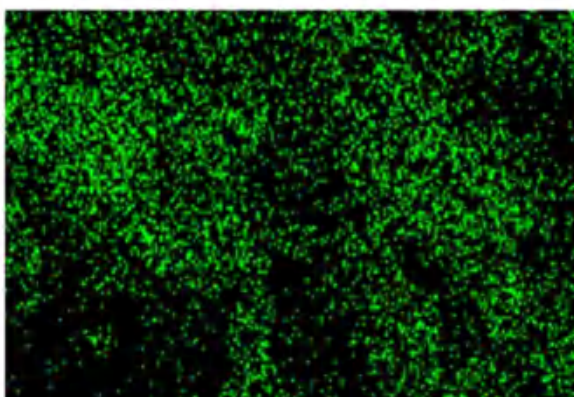
EDS Layered Image 1



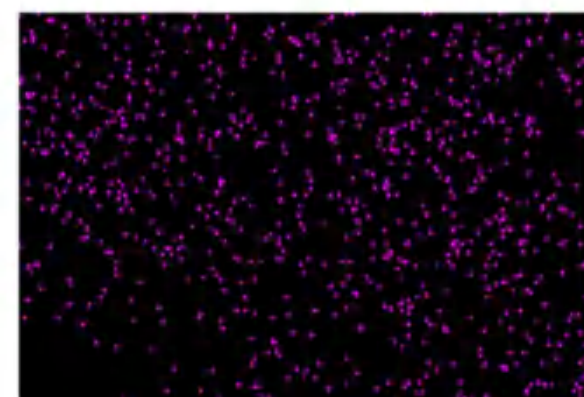
P Kα1

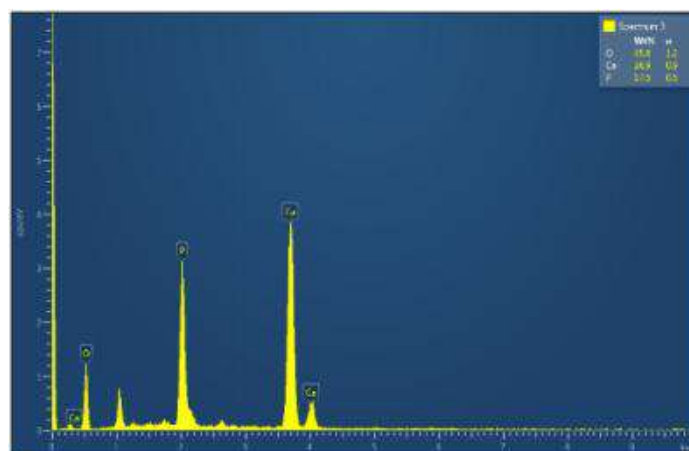
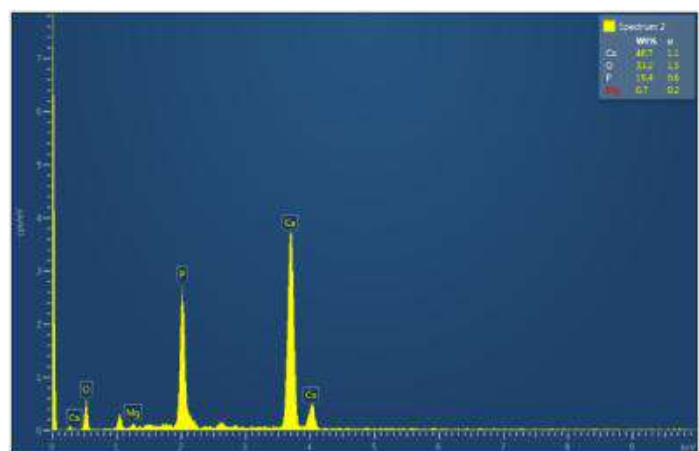
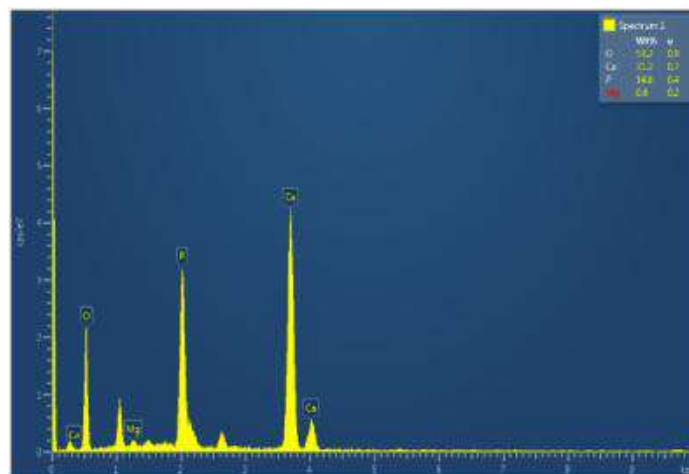
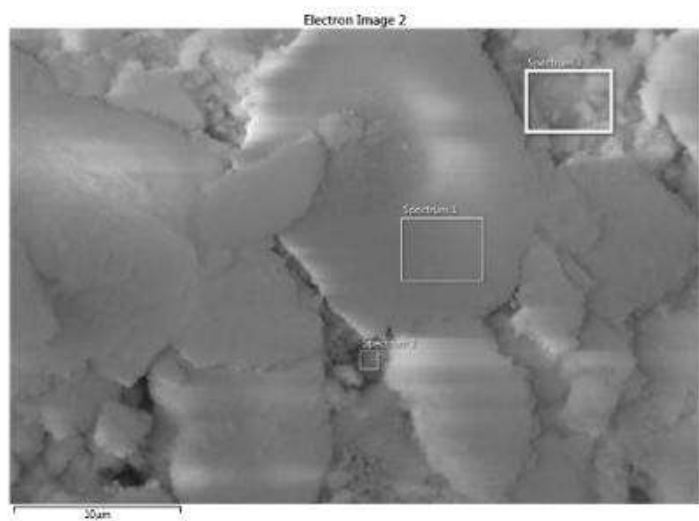


O Kα1

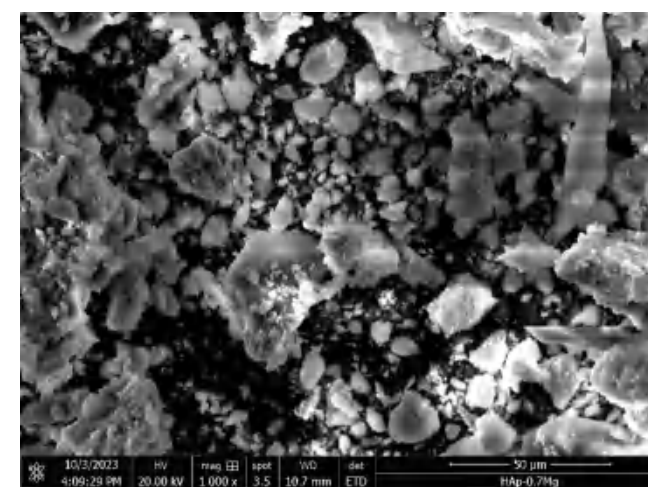
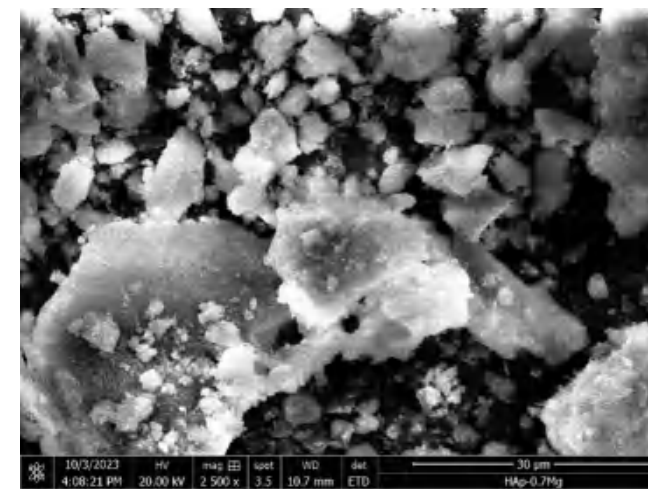
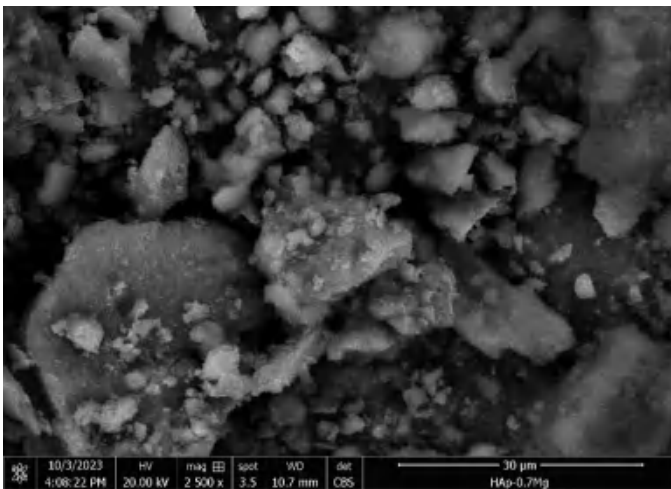
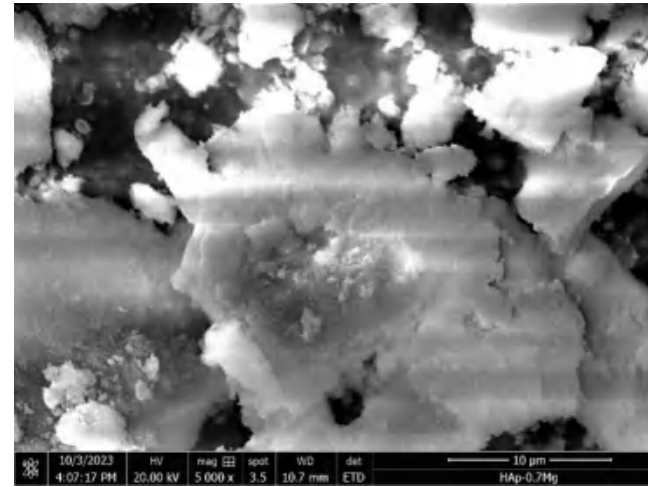
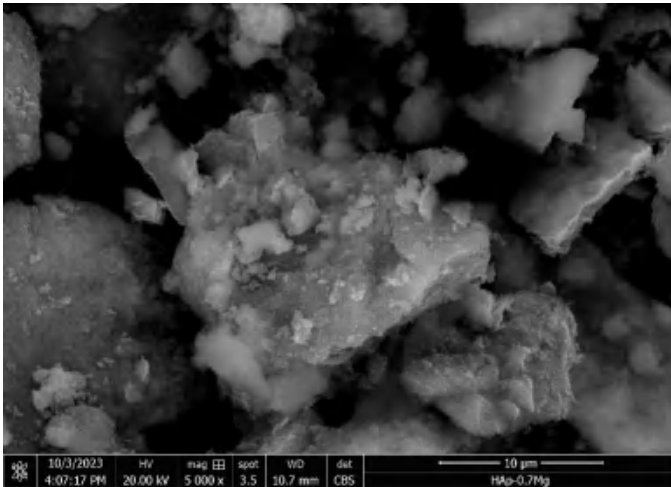


Mg Kα1_2

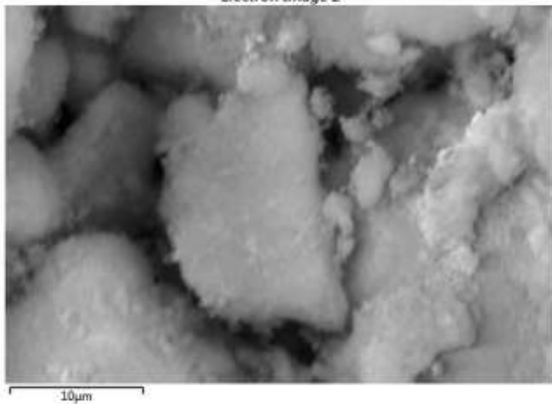




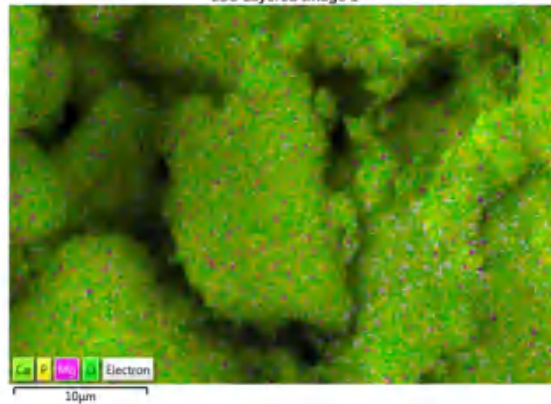
0.5Mg-HAp



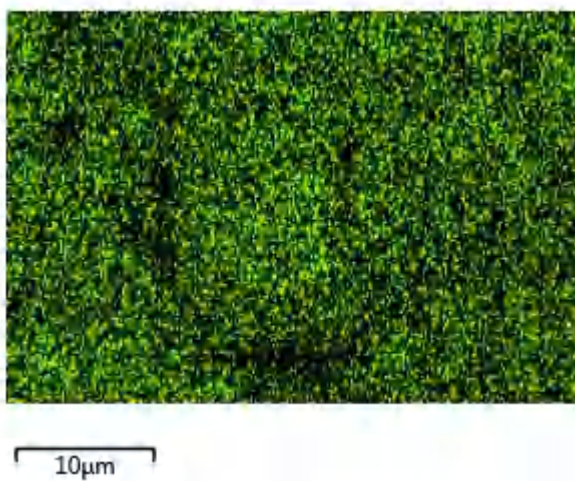
Electron Image 2



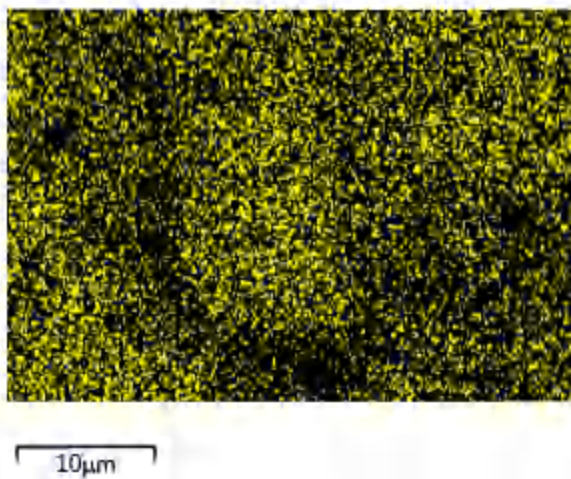
EDS Layered Image 1



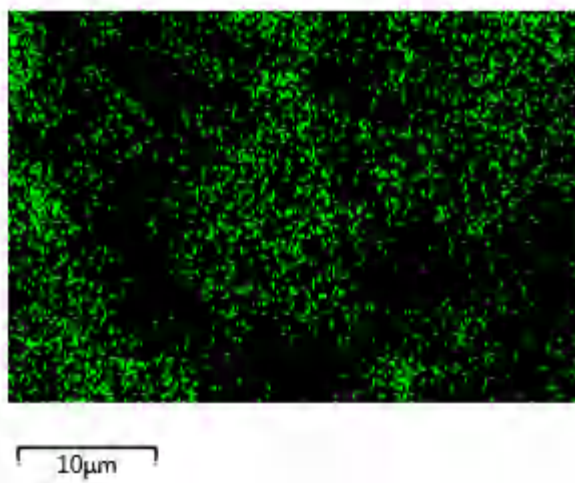
Ca K α 1



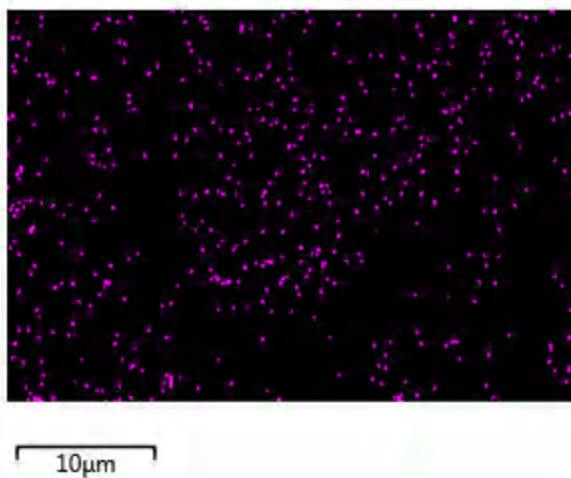
P K α 1

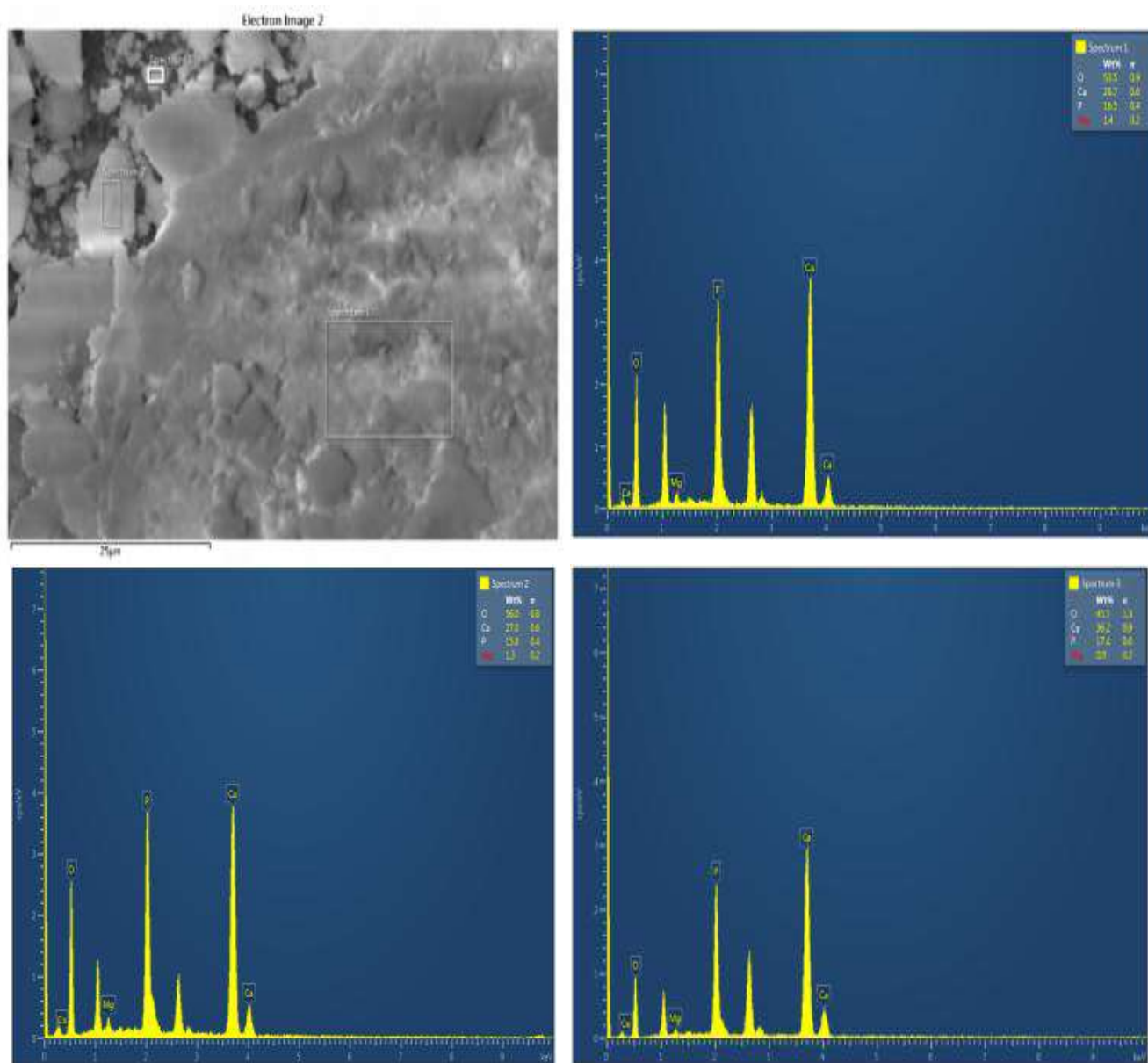


O K α 1

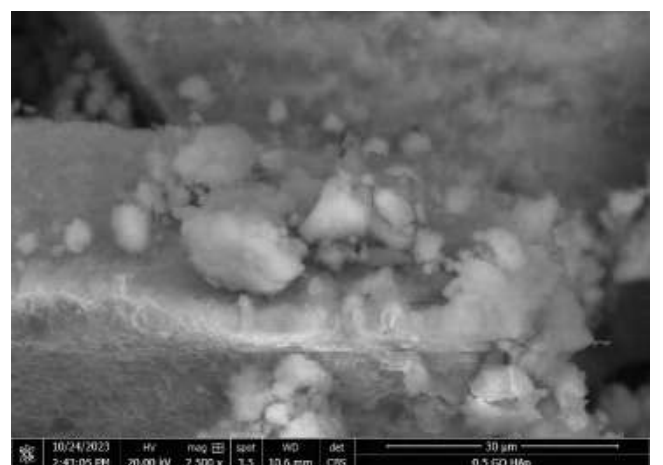
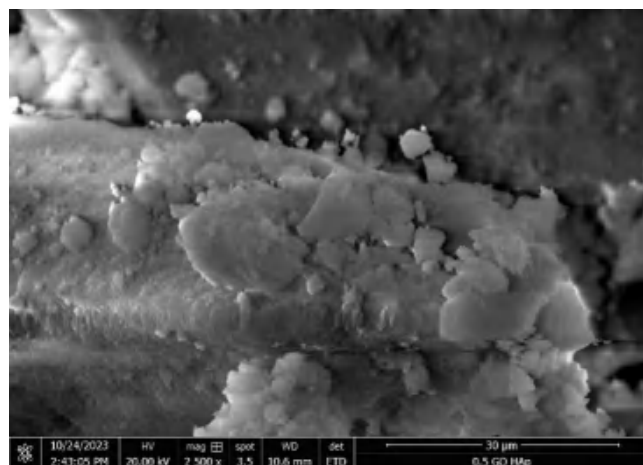
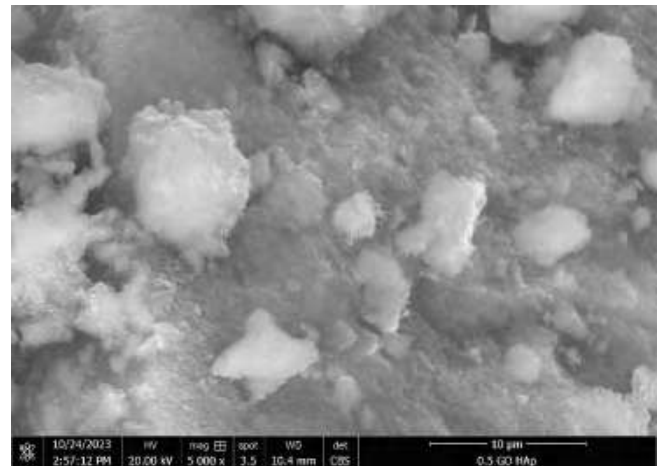
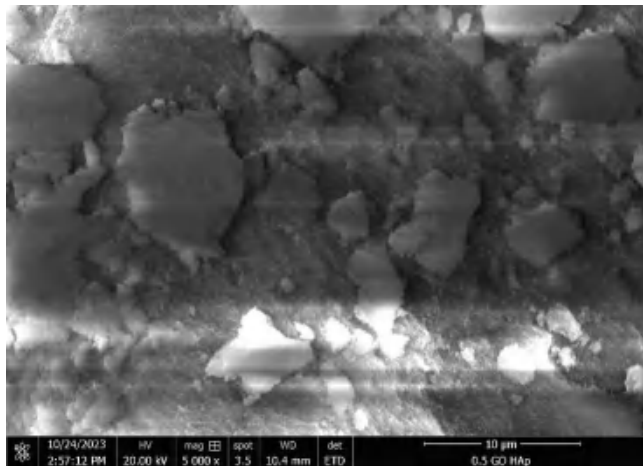
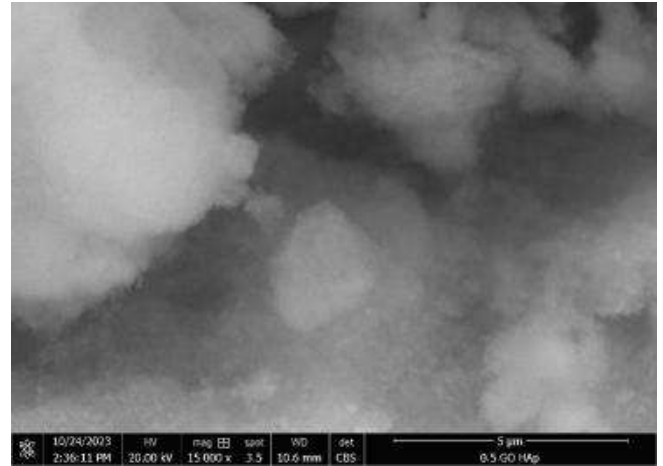
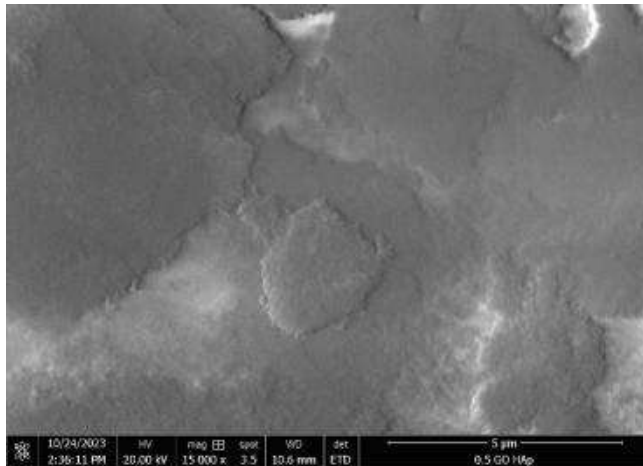


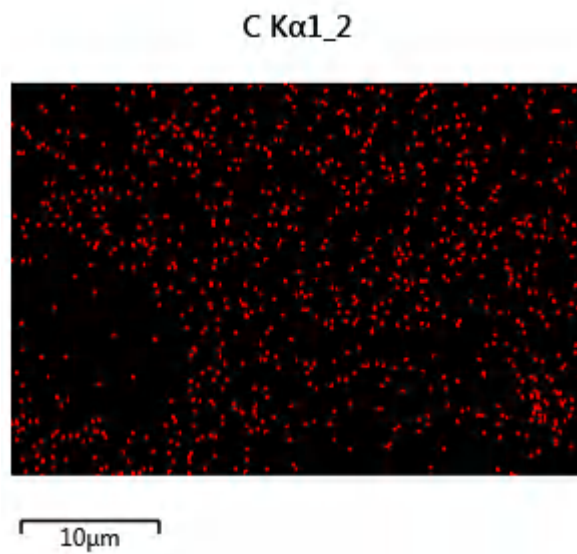
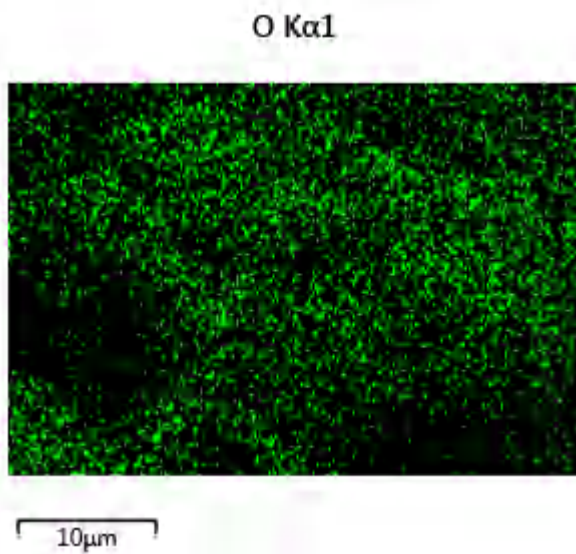
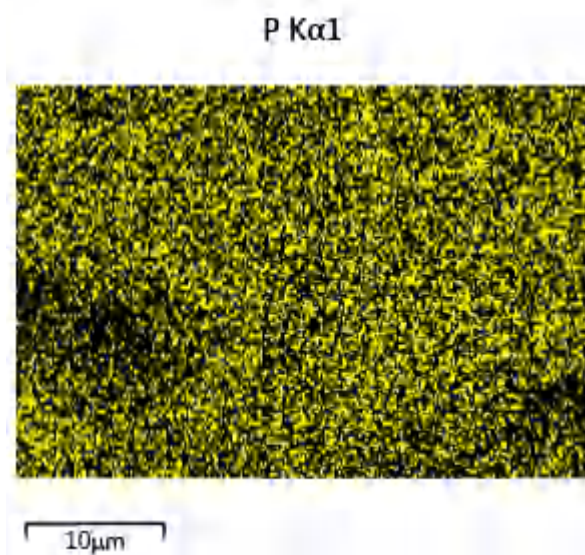
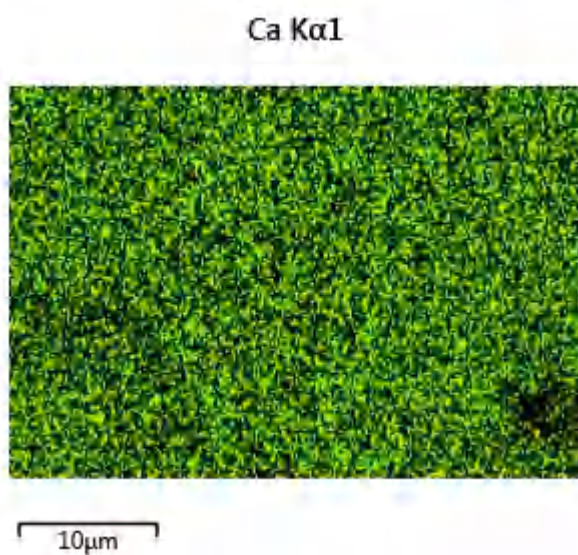
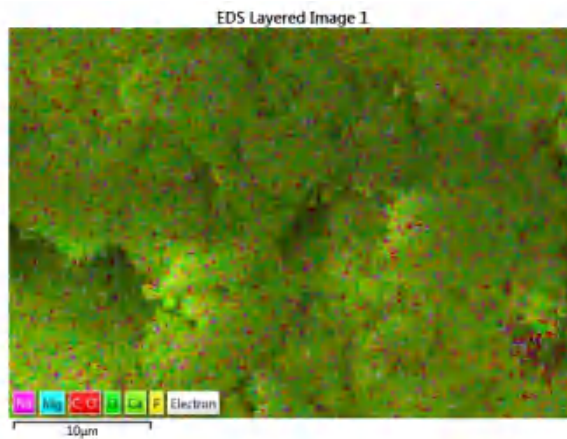
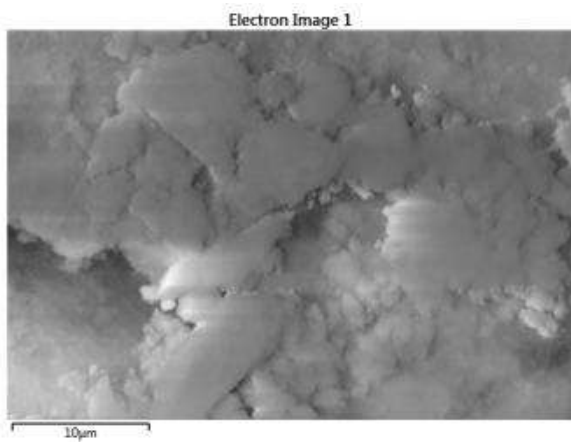
Mg K α 1_2

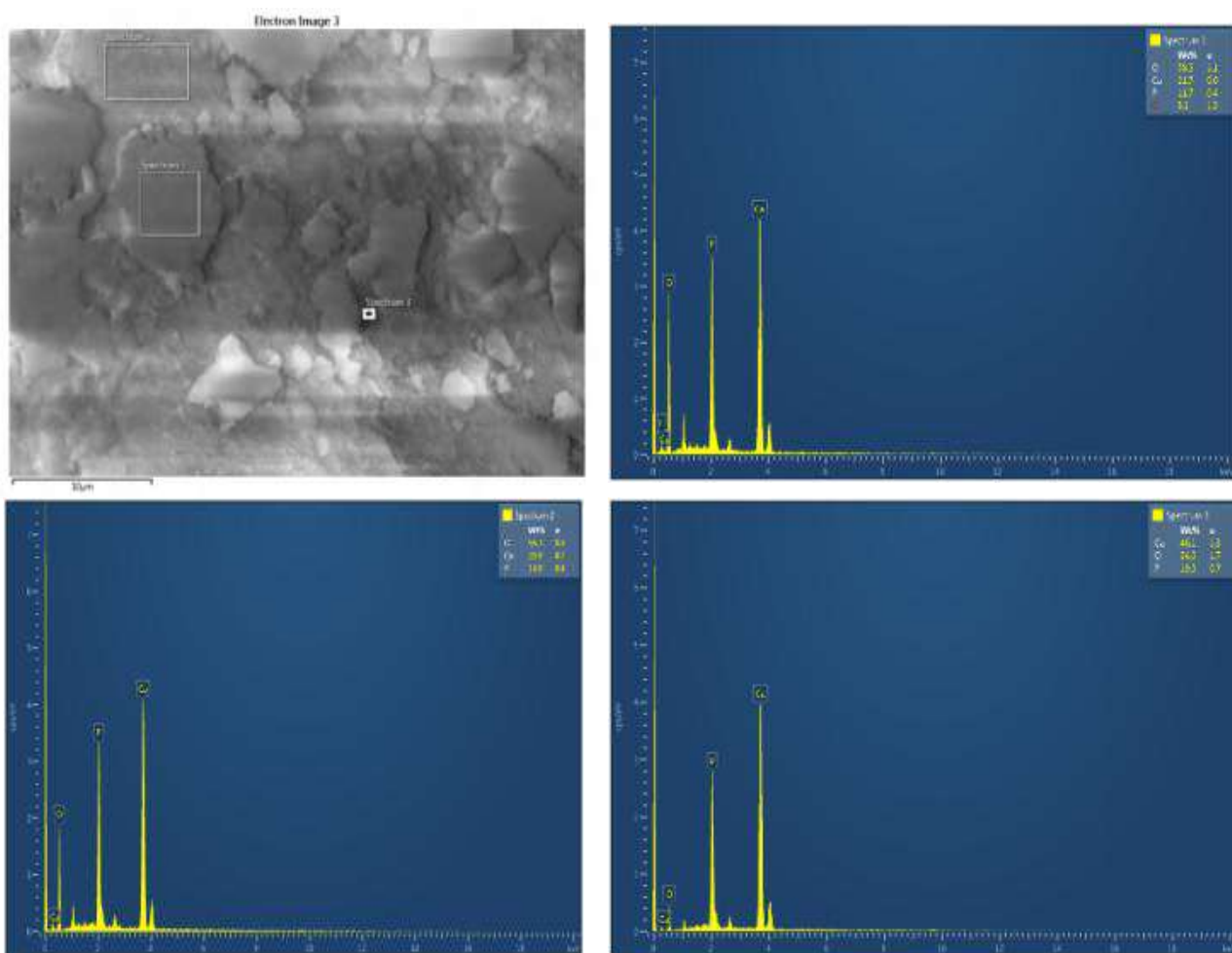




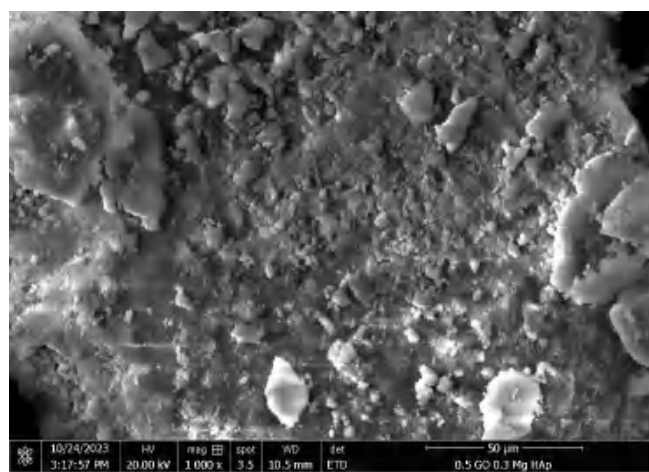
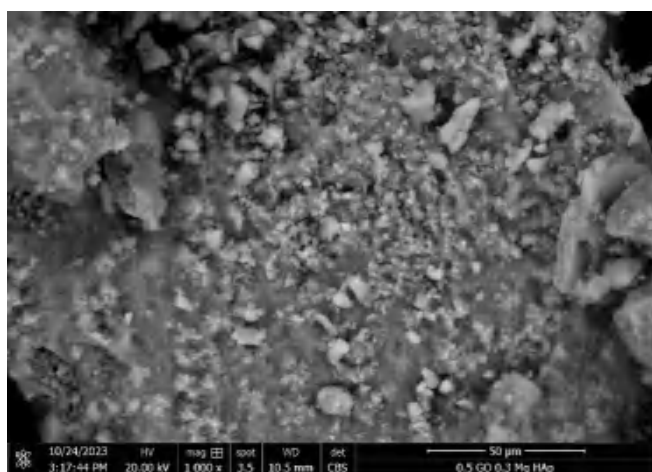
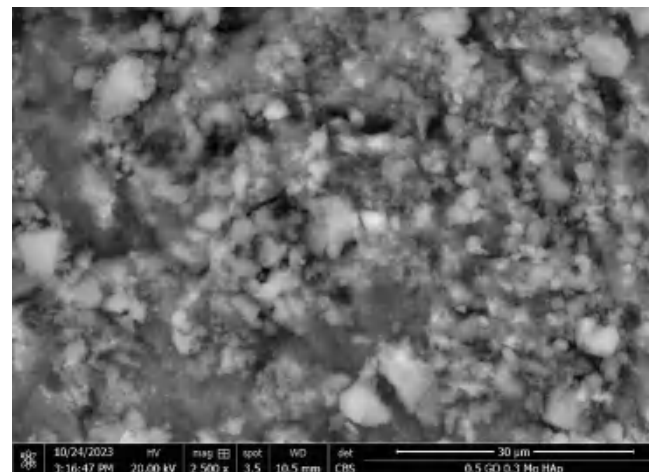
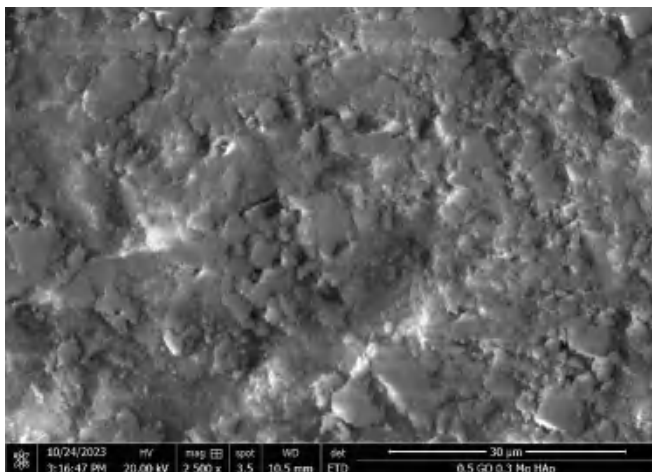
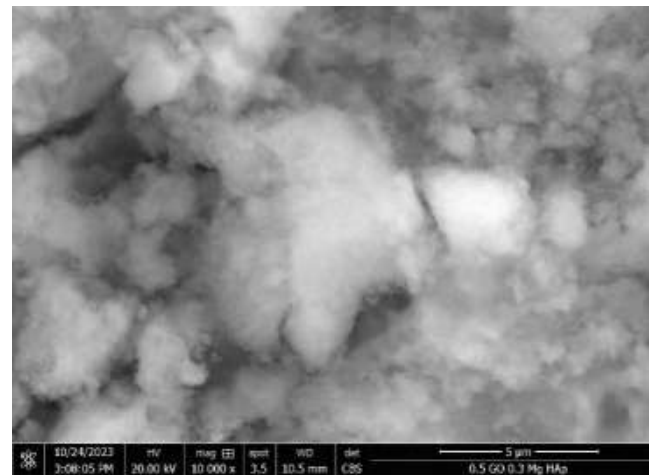
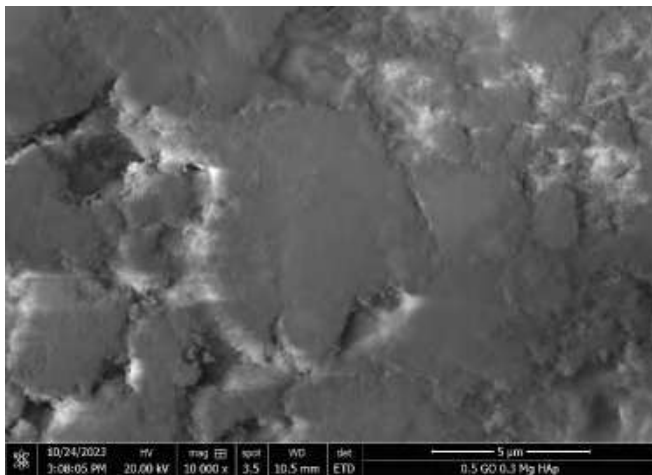
0.5rGO-HAP



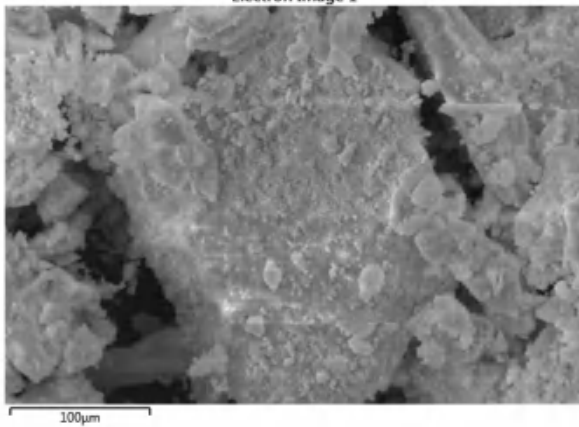




0.5rGO-0.3Mg-Hap



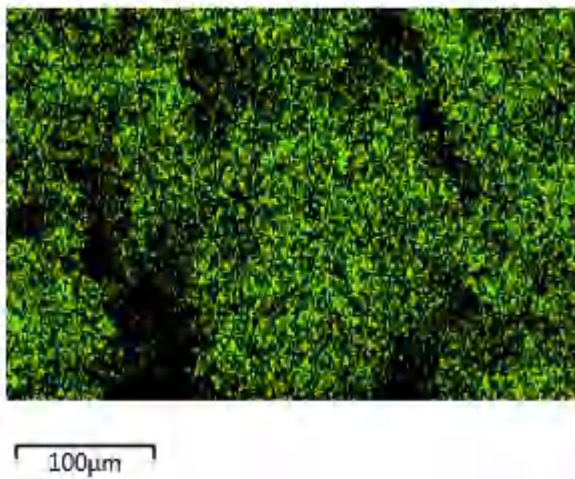
Electron Image 1



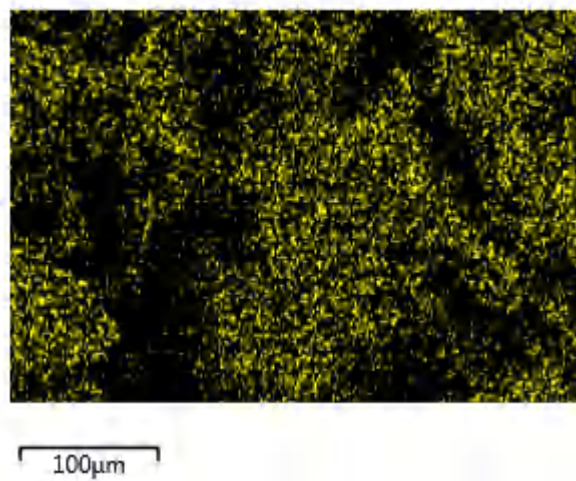
EDS Layered Image 1



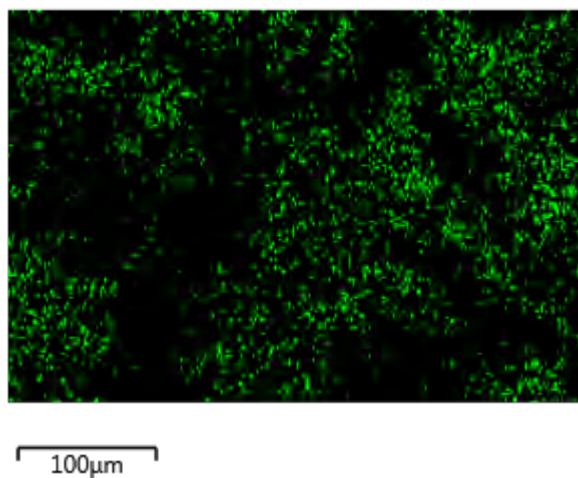
Ca Kα1



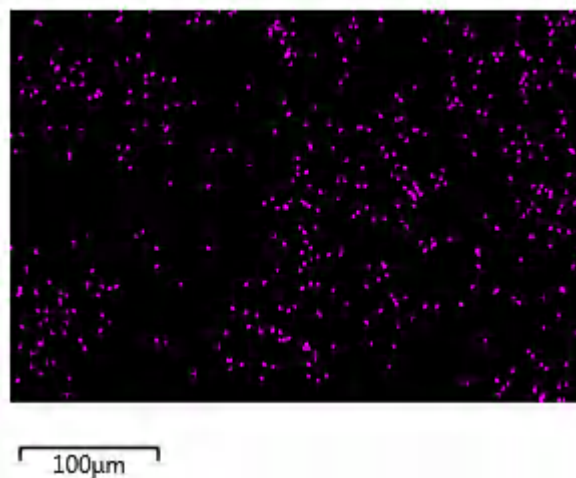
P Kα1



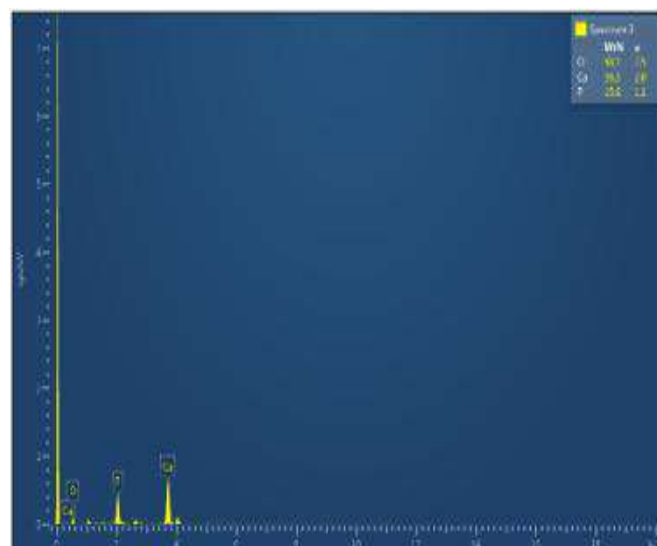
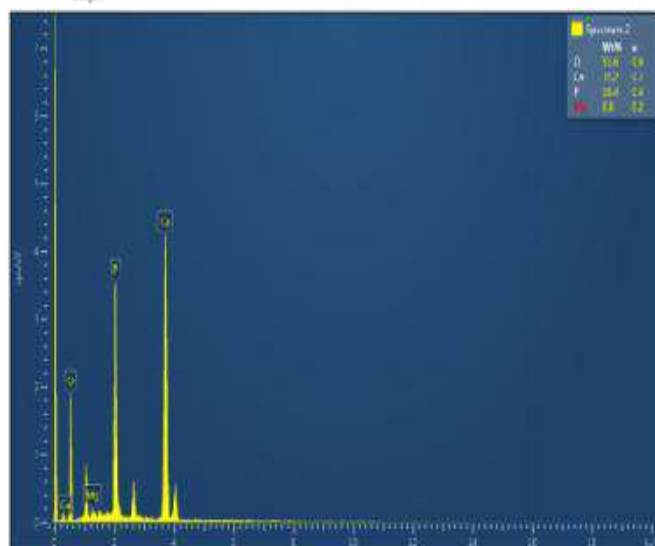
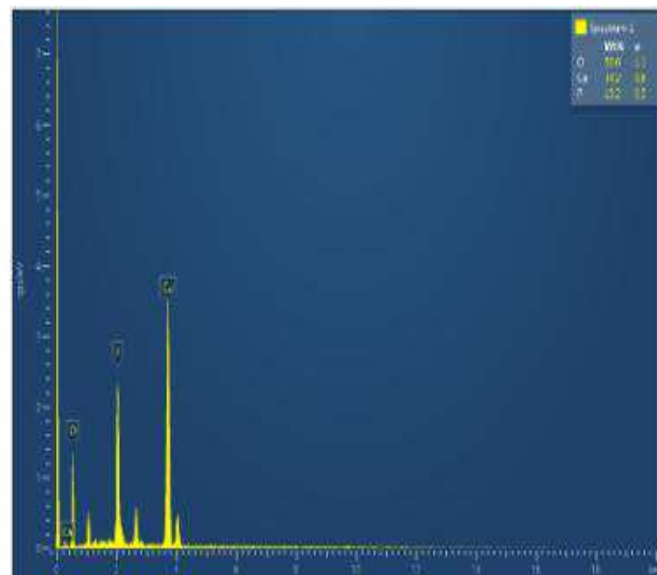
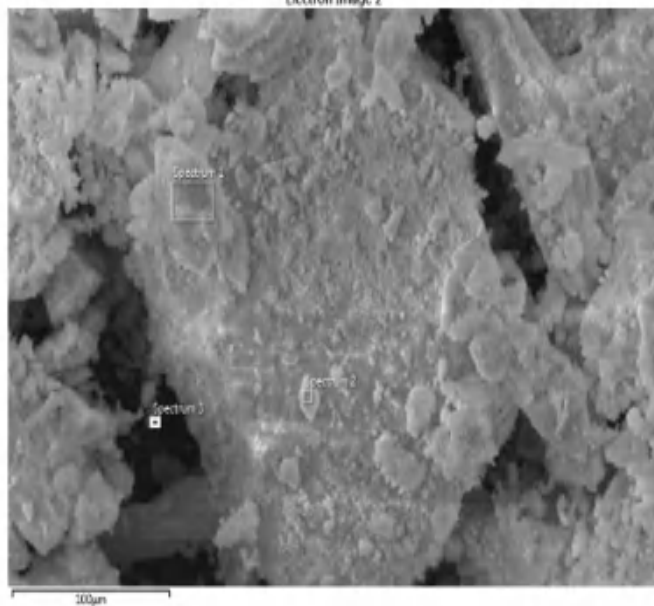
O Kα1



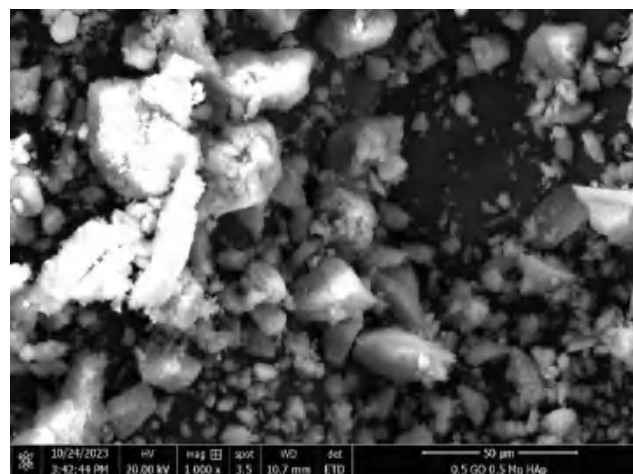
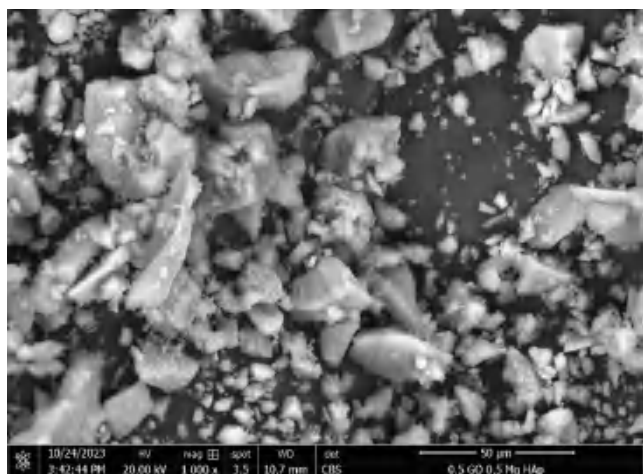
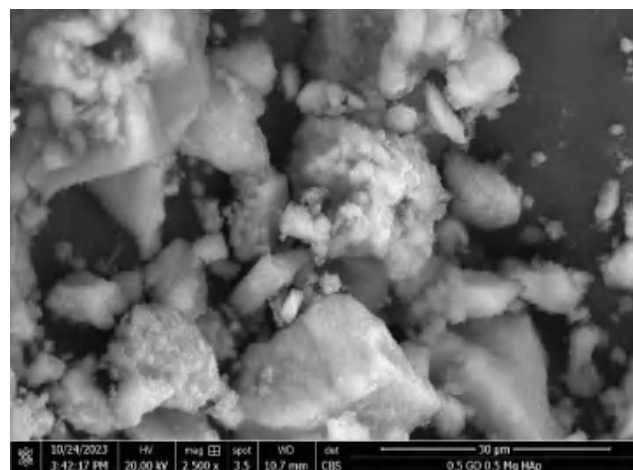
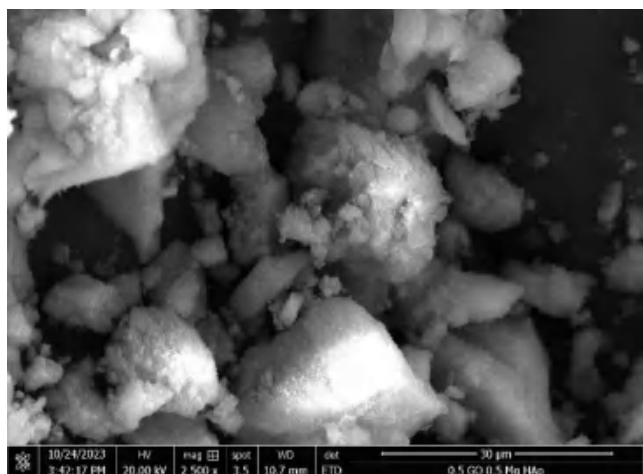
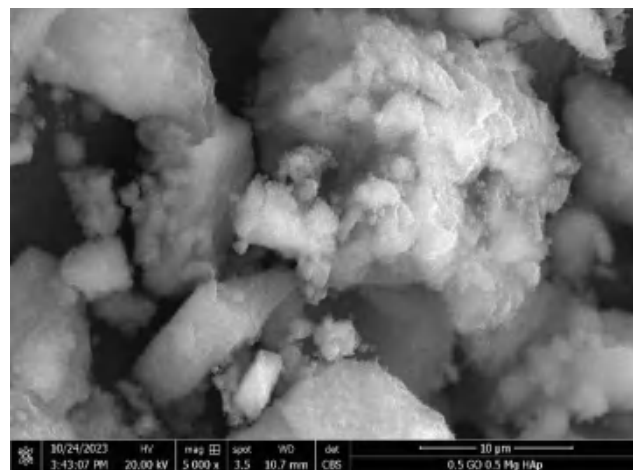
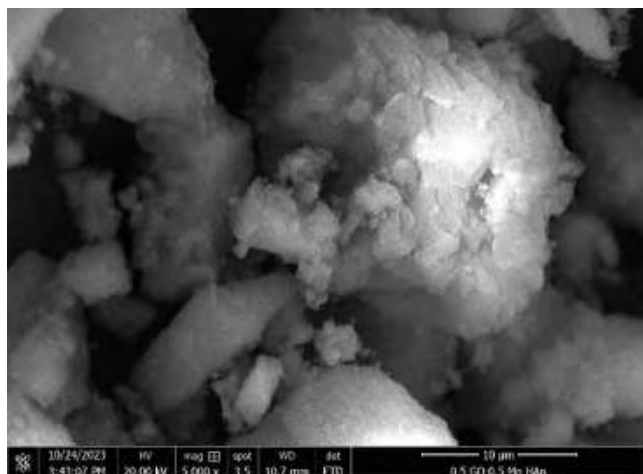
Mg Kα1_2



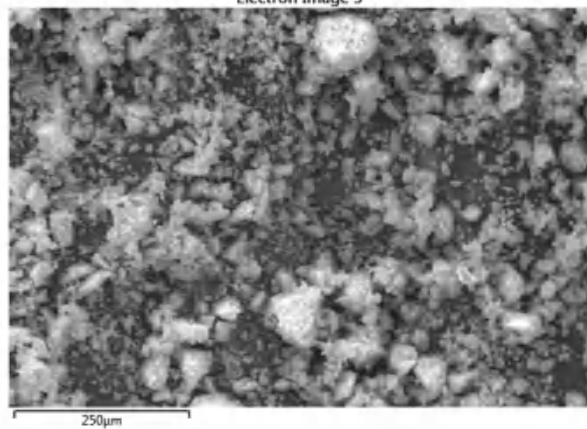
Electron Image 2



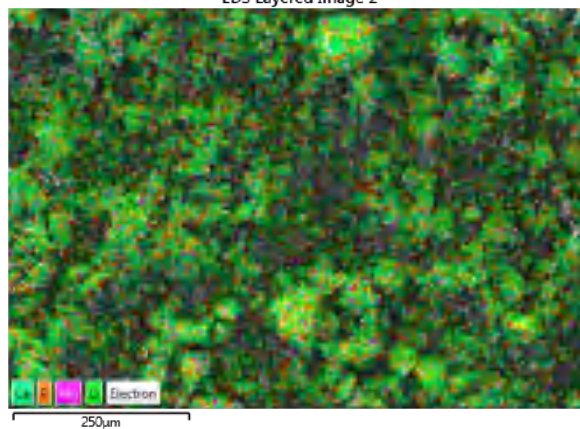
0.5rGO-0.5Mg-HAp



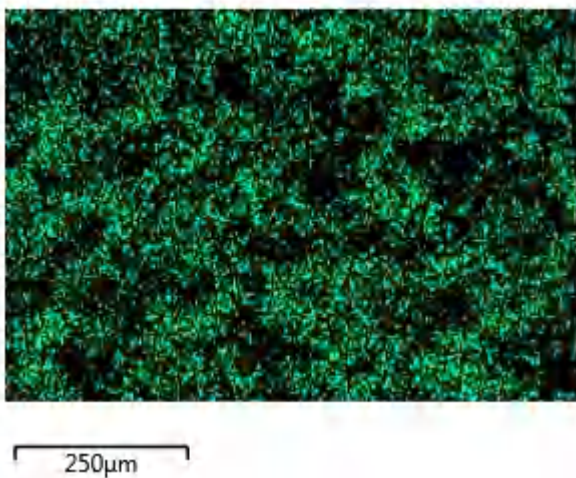
Electron Image 3



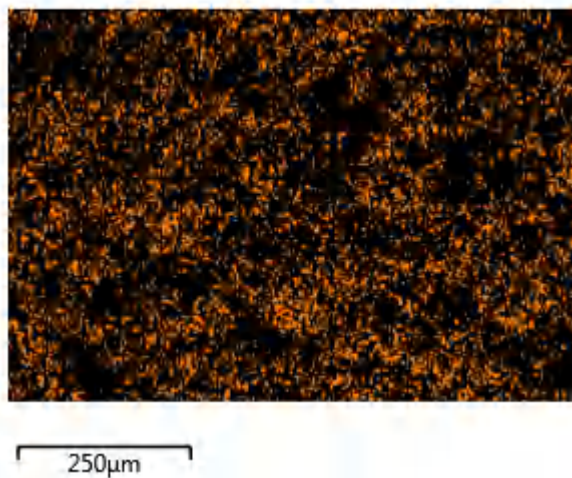
EDS Layered Image 2



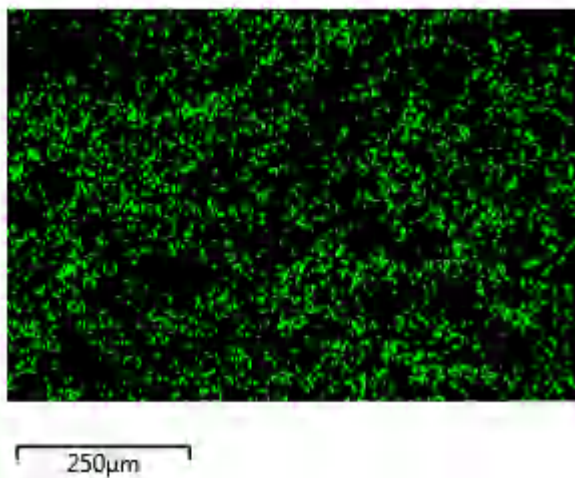
Ca K α 1



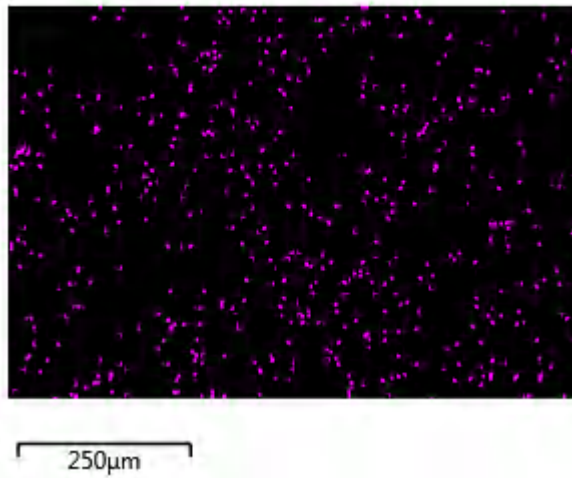
P K α 1

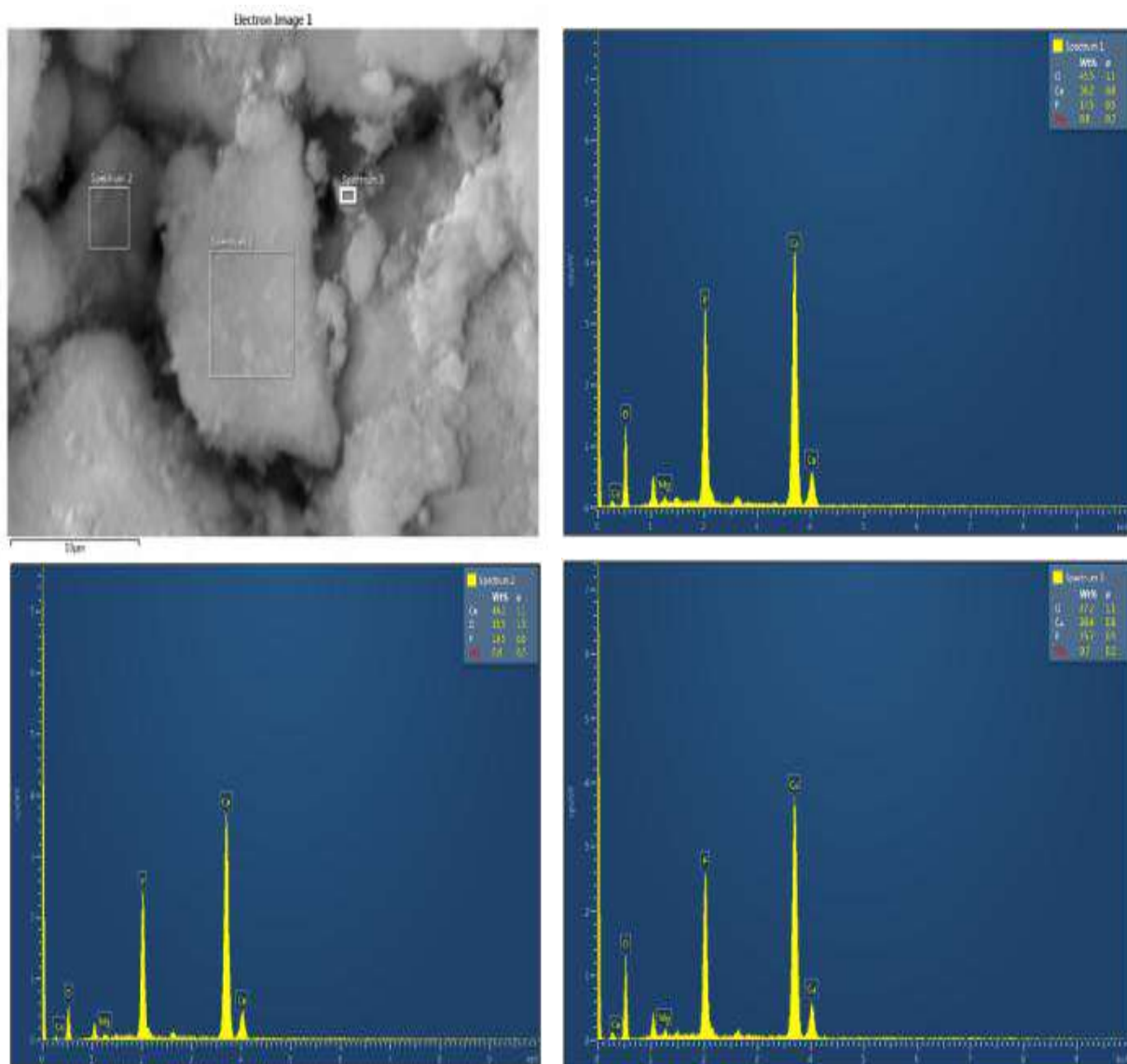


O K α 1

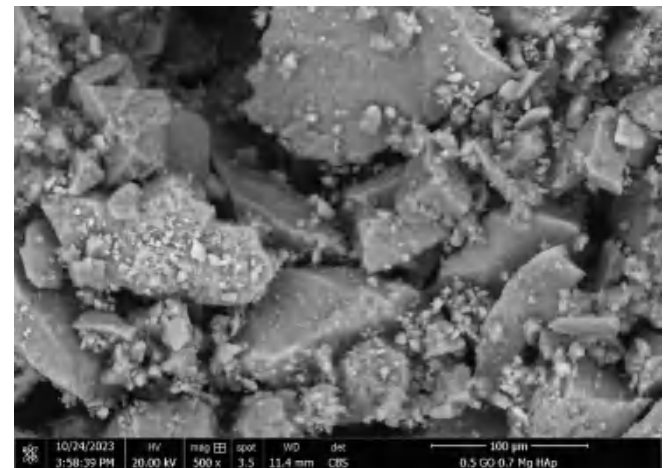
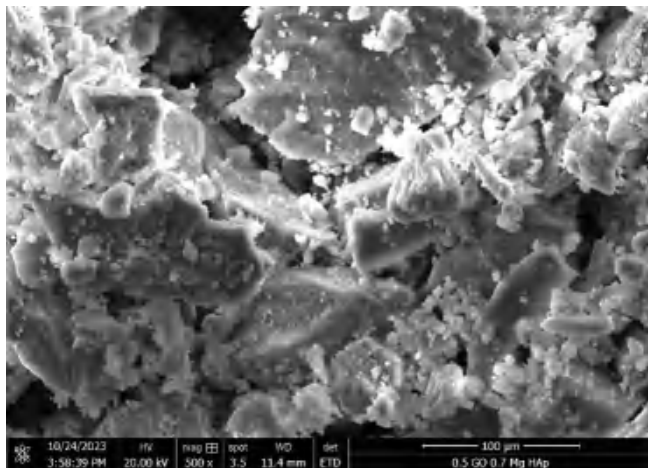
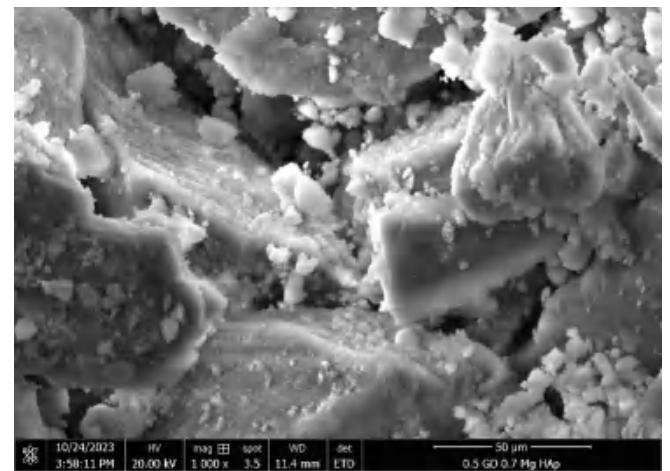
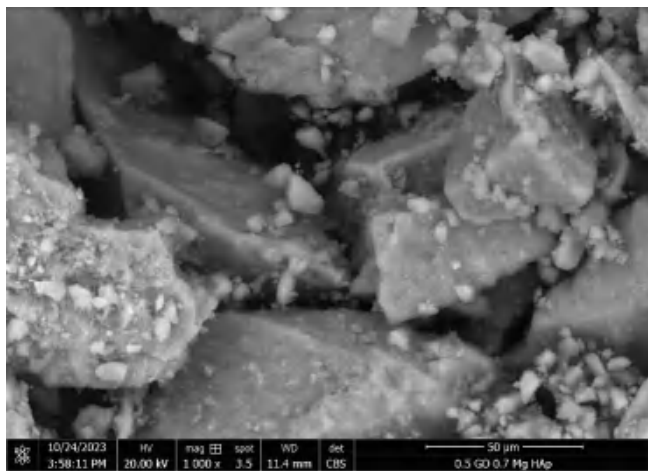
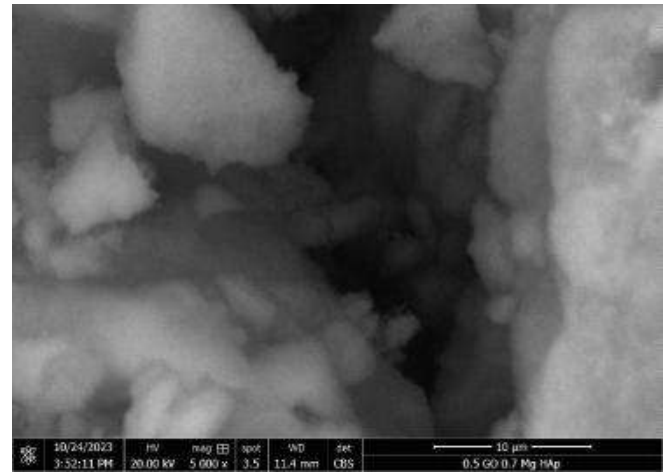
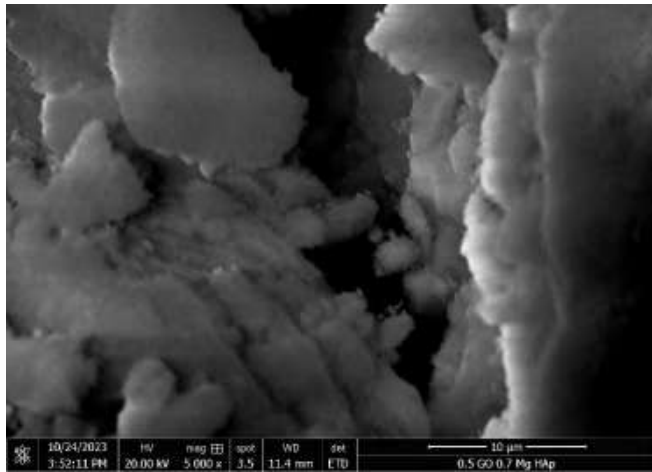


Mg K α 1_2

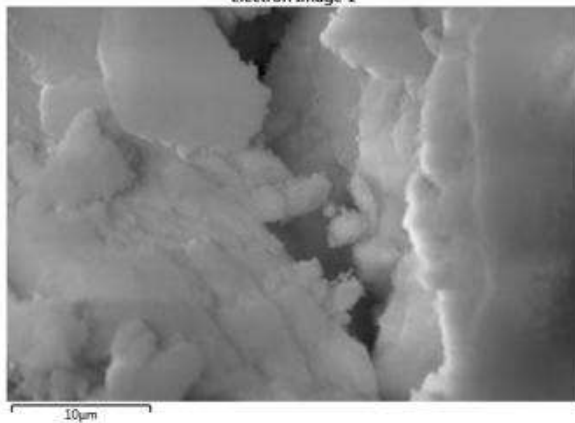




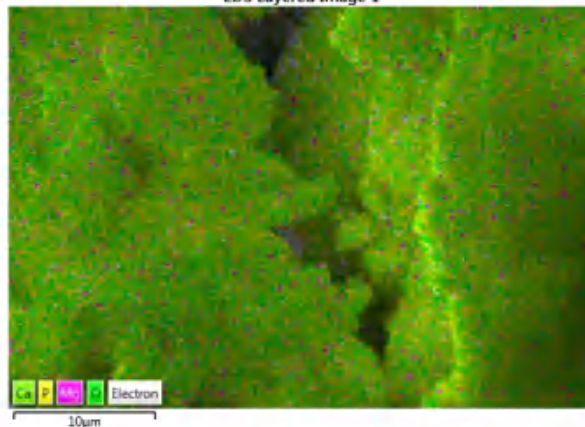
0.5rGO-0.7Mg-HAp



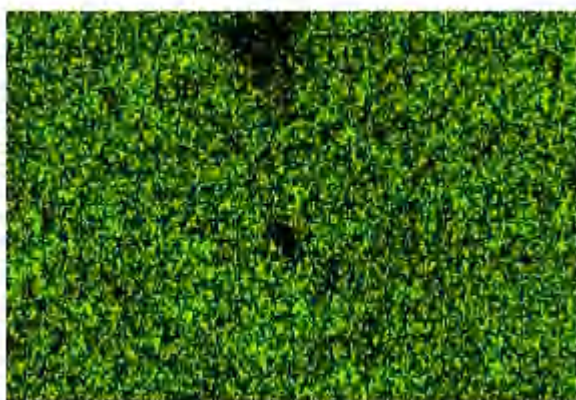
Electron Image 1



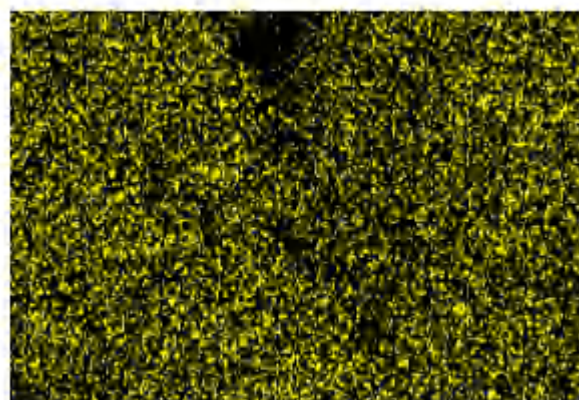
EDS Layered Image 1



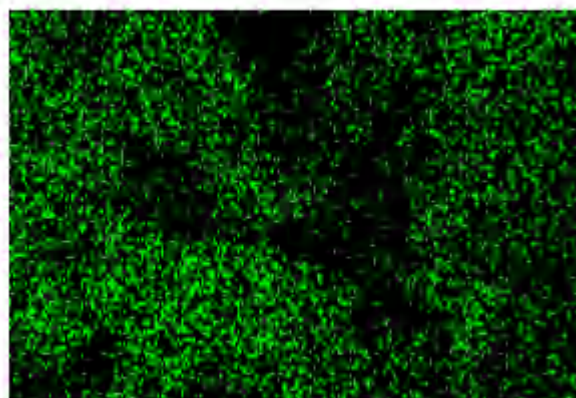
Ca Kα1



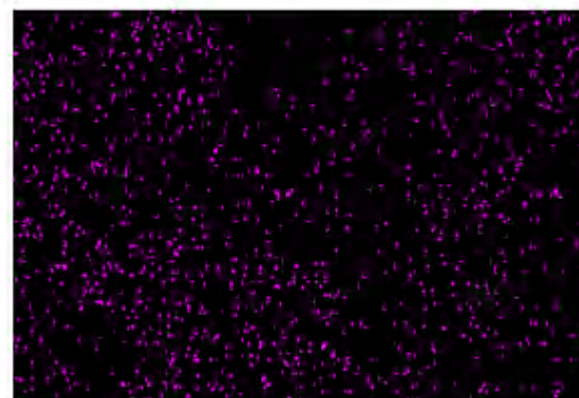
P Kα1

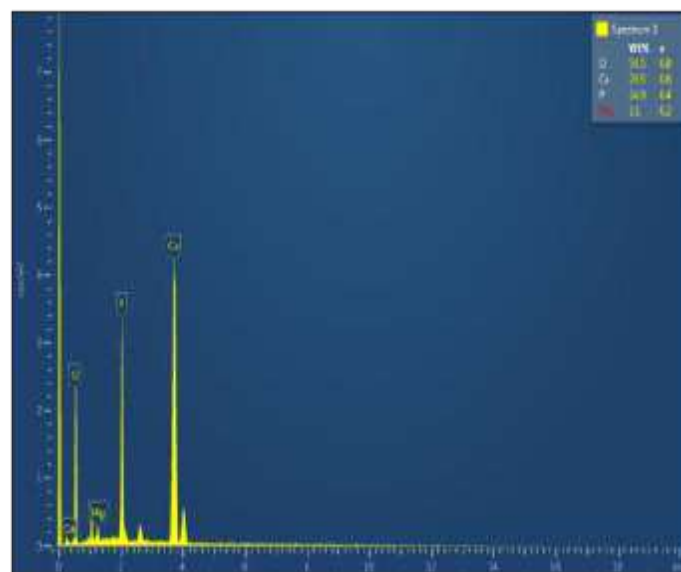
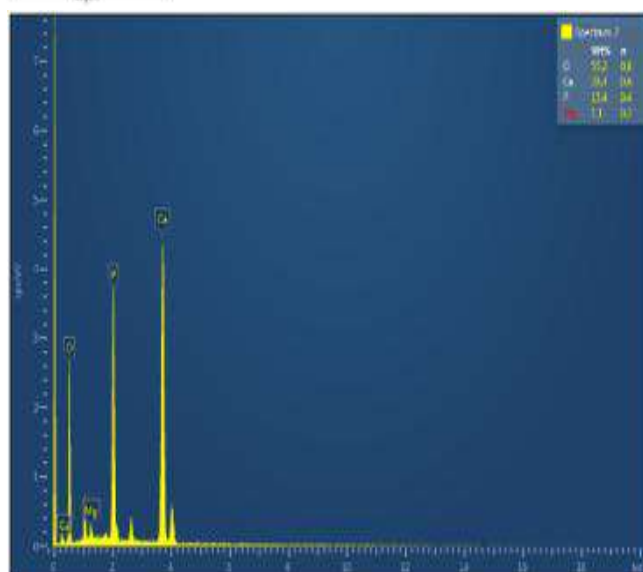
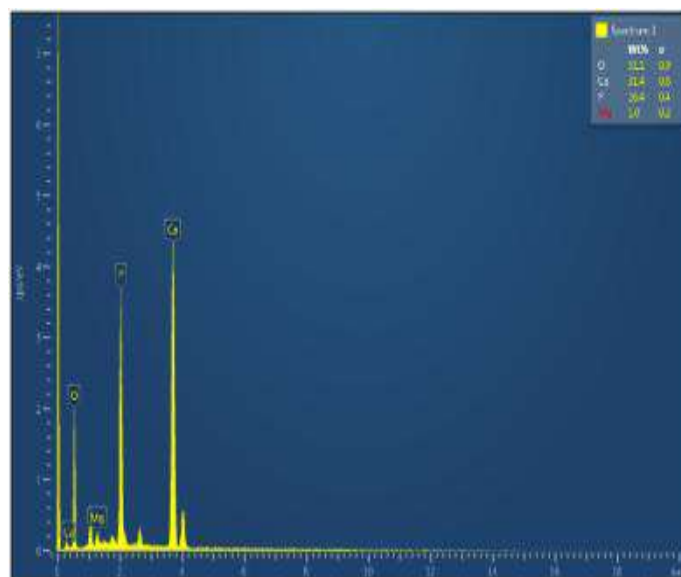
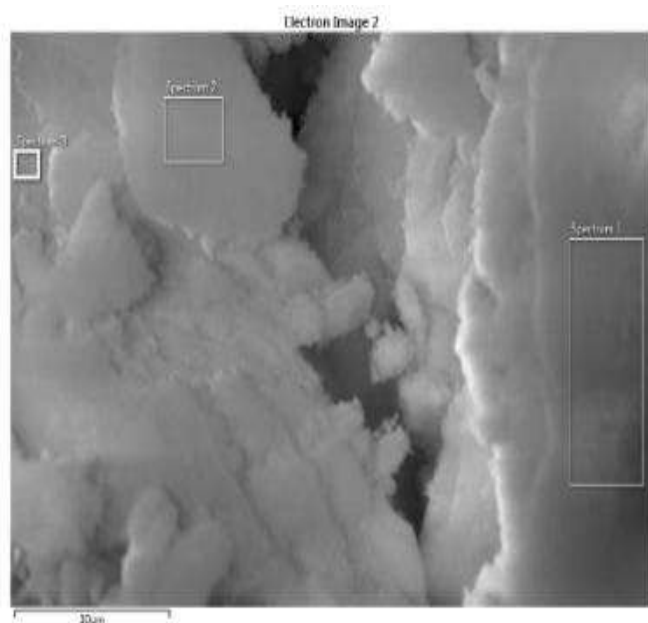


O Kα1

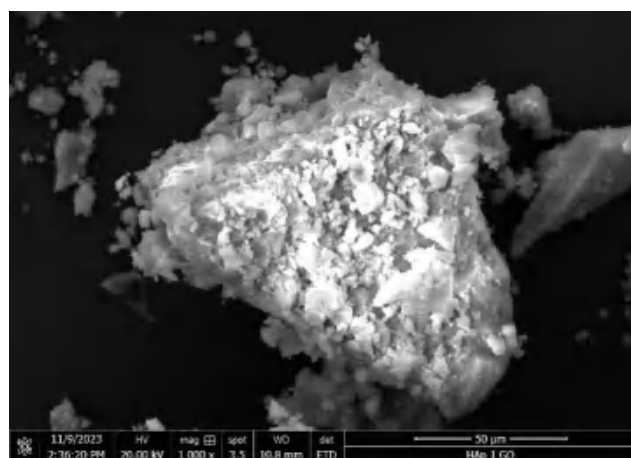
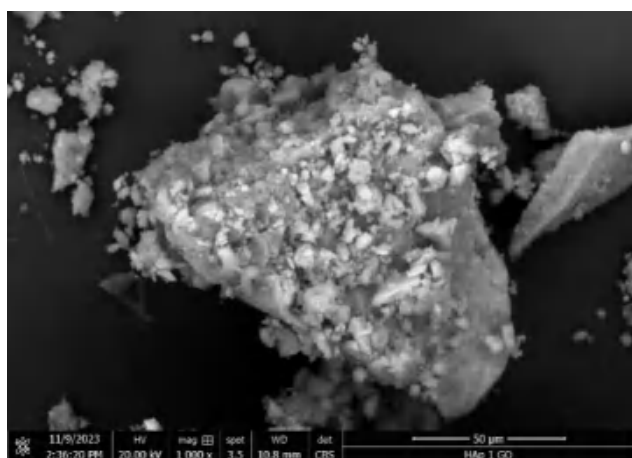
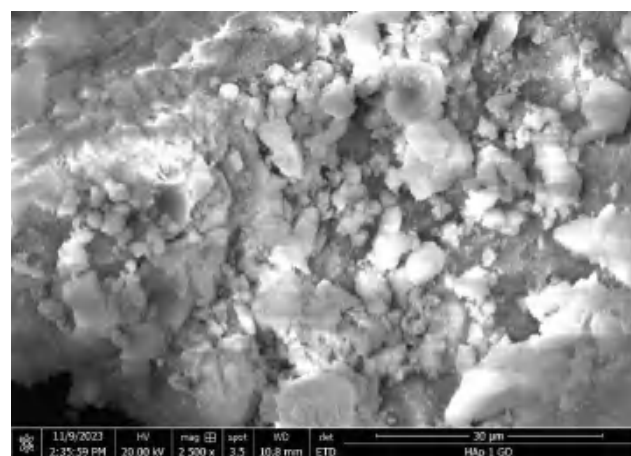
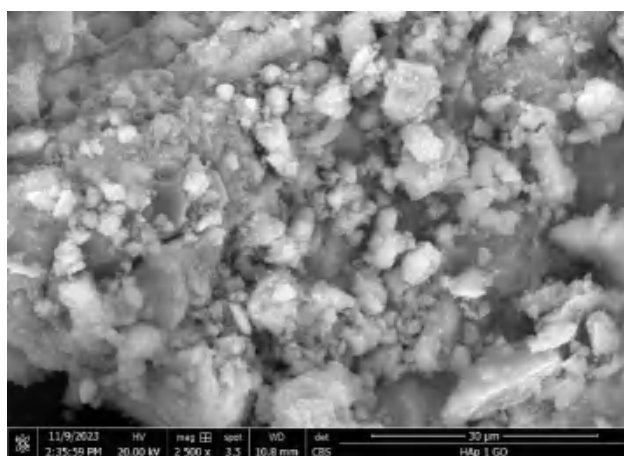
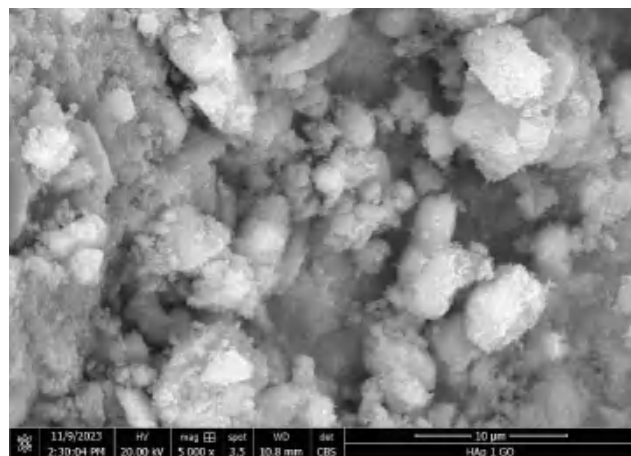
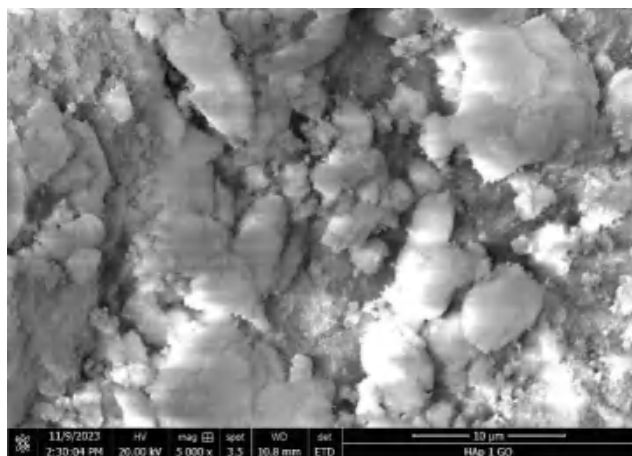


Mg Kα1_2

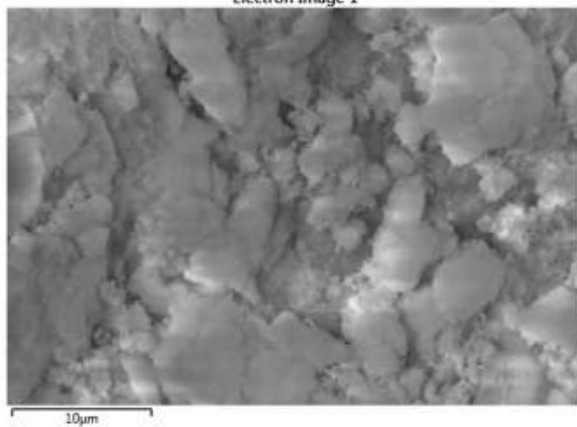




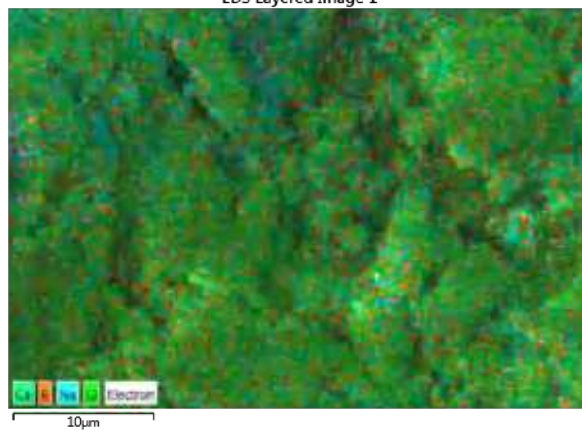
1rGO-HAp



Electron Image 1



EDS Layered Image 1



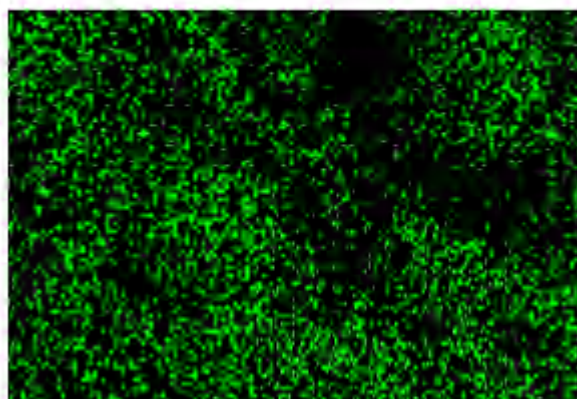
Ca K α 1



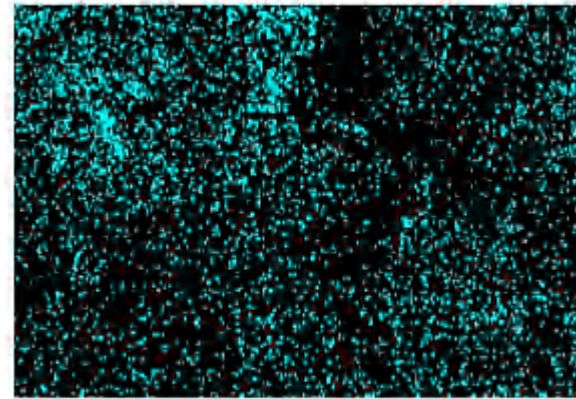
P K α 1



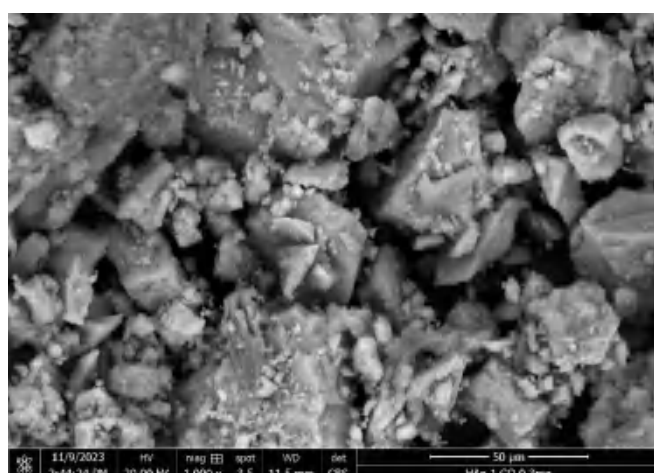
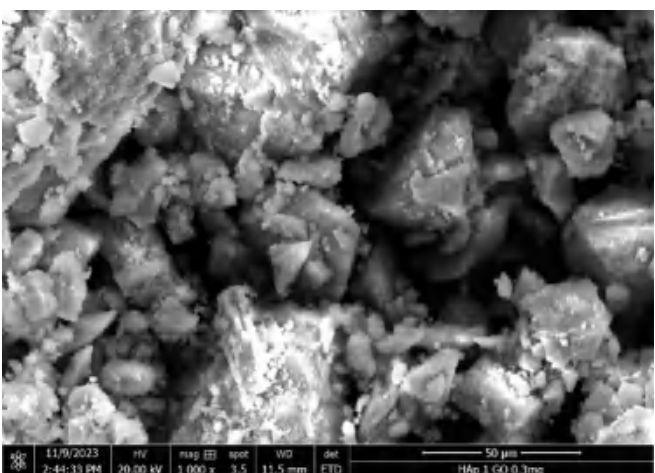
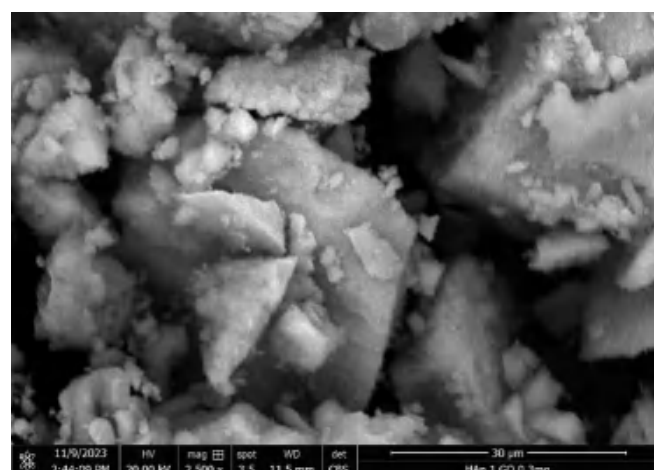
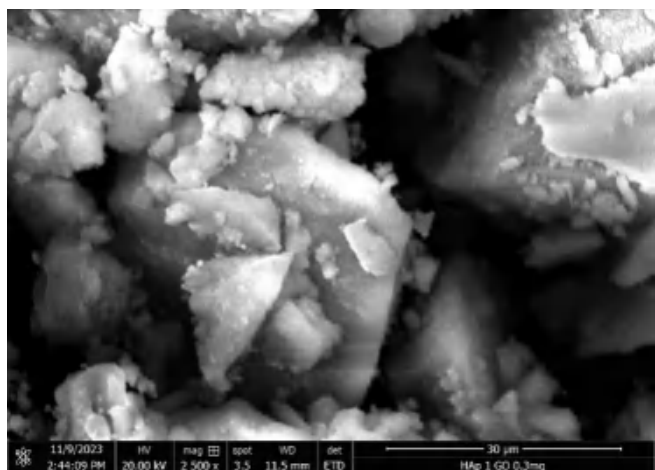
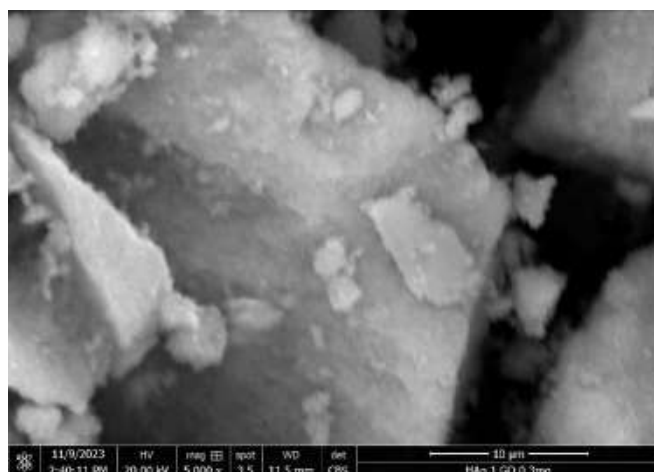
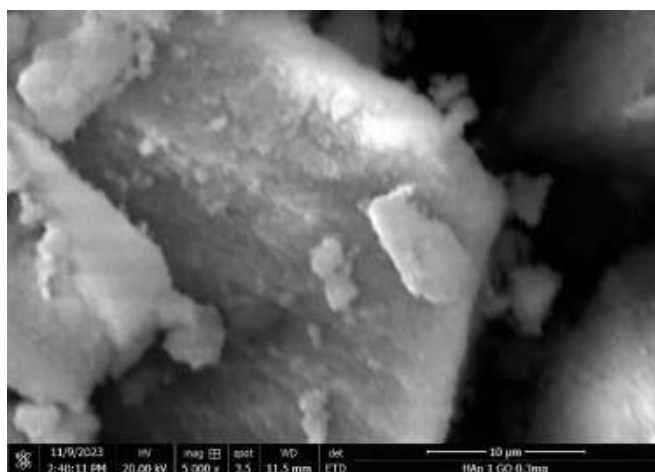
O K α 1



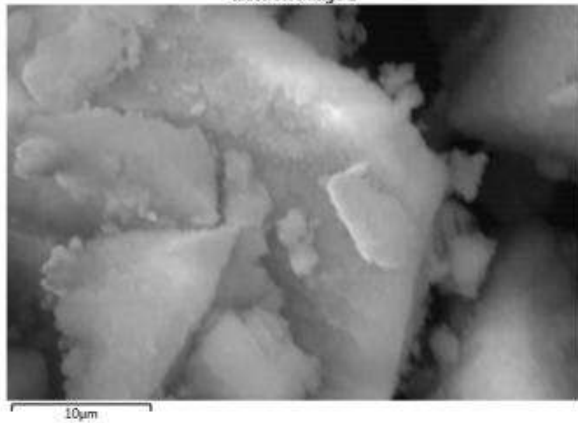
Na K α 1_2



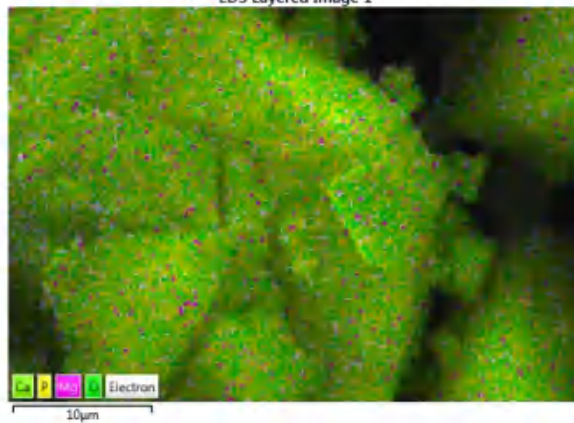
1rGO-0.3Mg-HAp



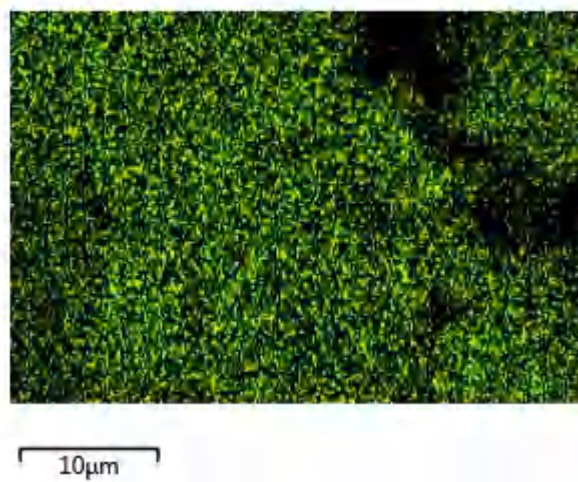
Electron Image 1



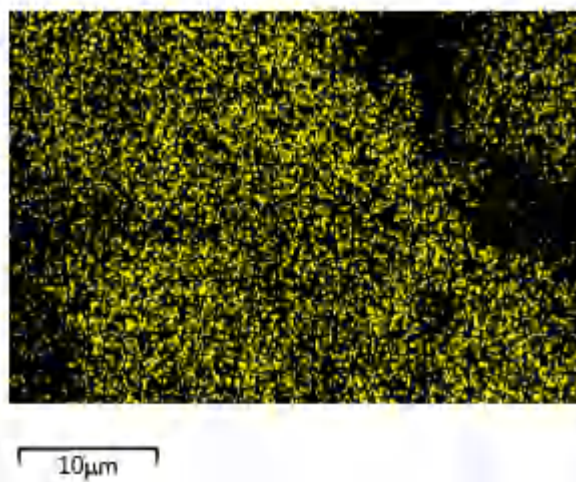
EDS Layered Image 1



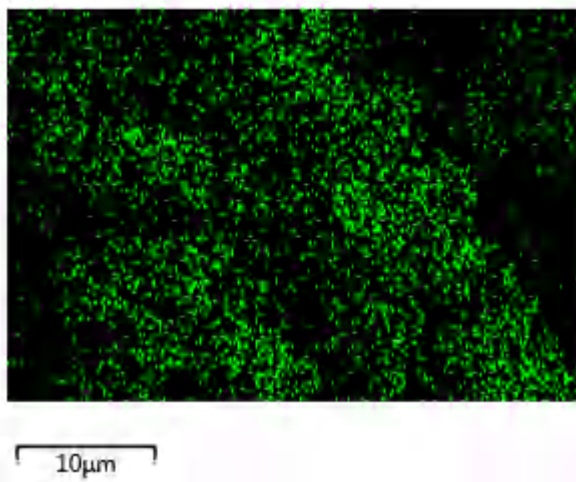
Ca Kα1



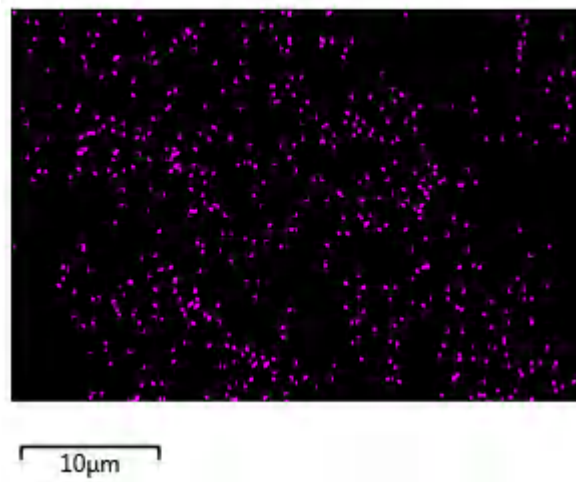
P Kα1



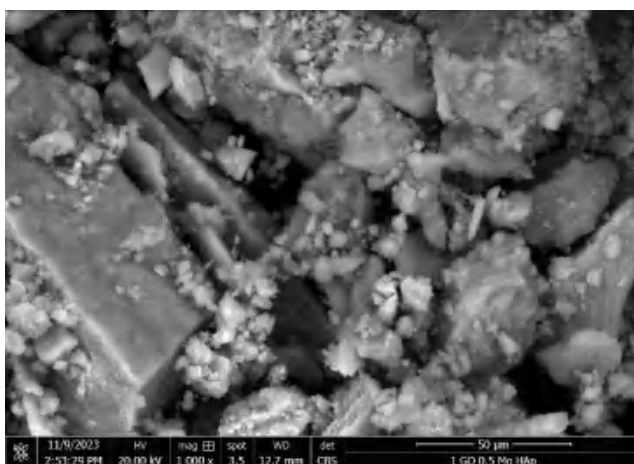
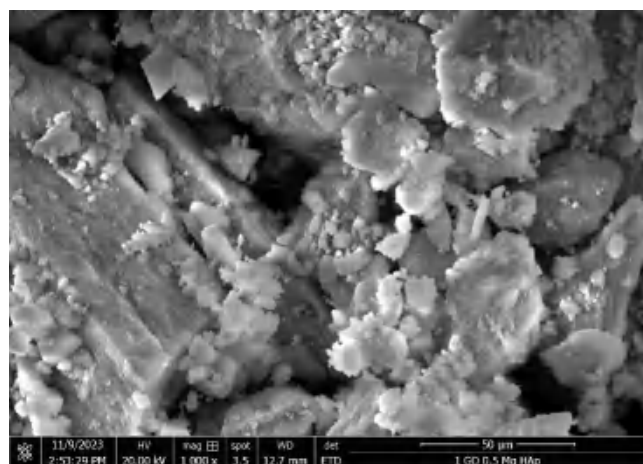
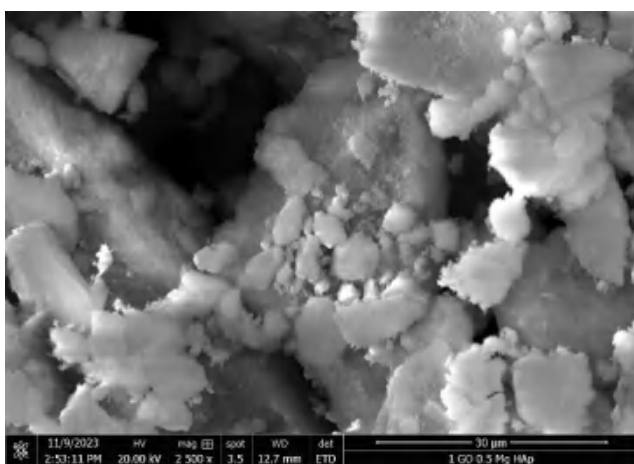
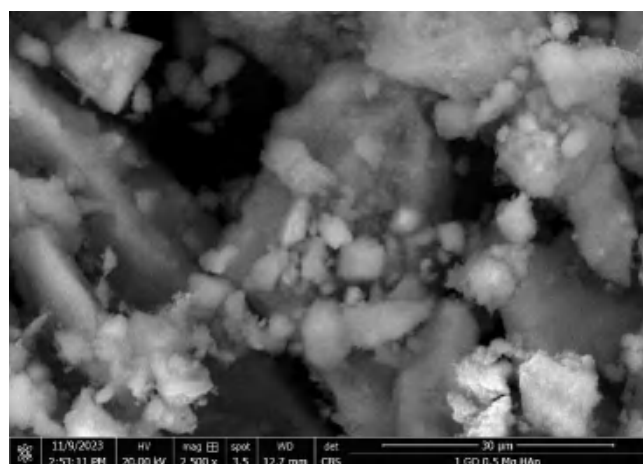
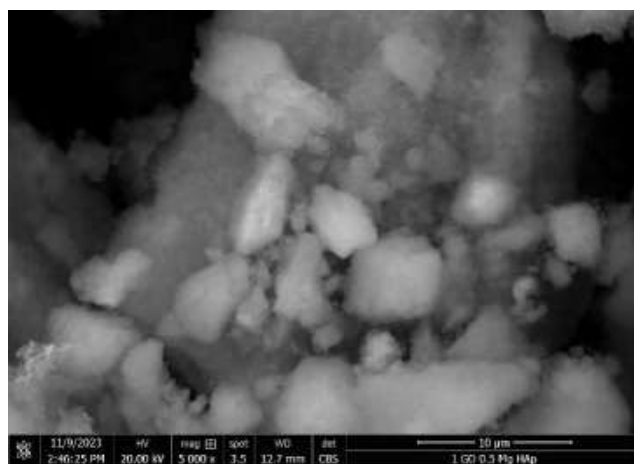
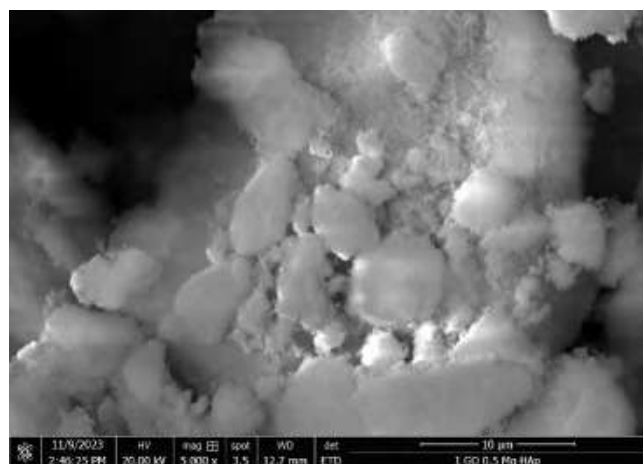
O Kα1



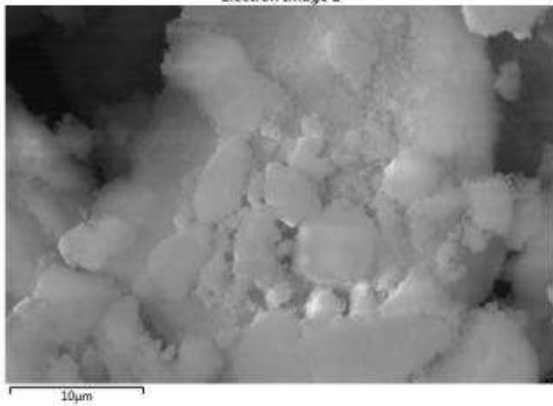
Mg Kα1_2



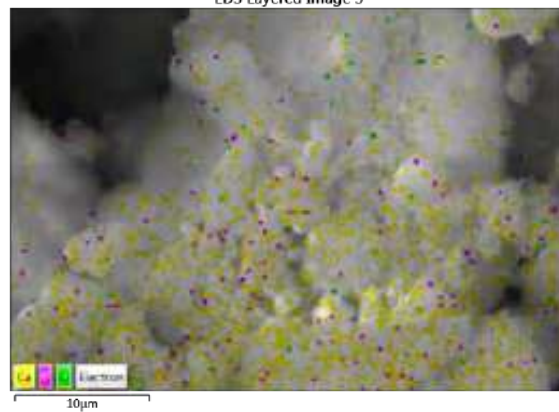
1rGO-0.5Mg-HAp



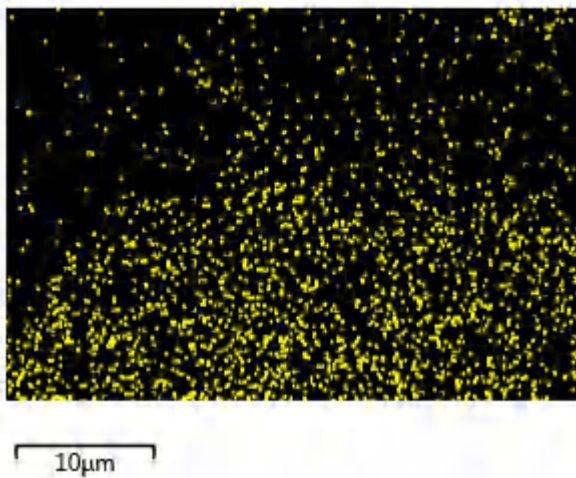
Electron Image 2



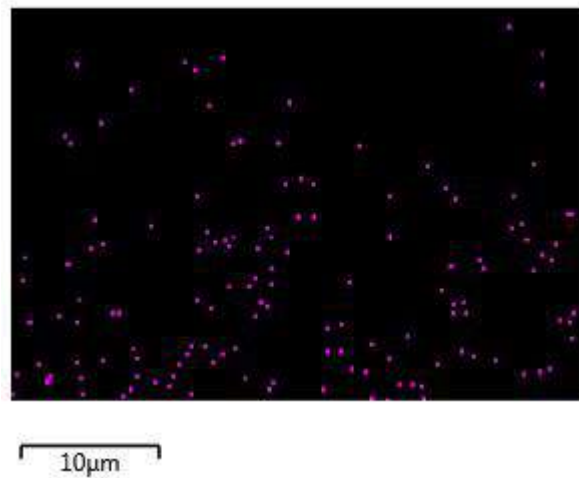
EDS Layered Image 5



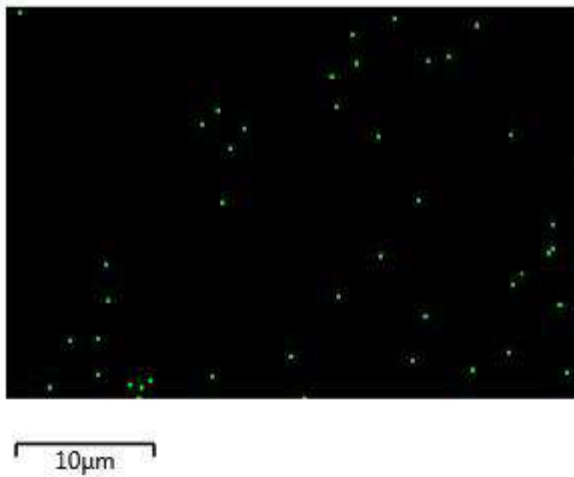
Ca K α 1



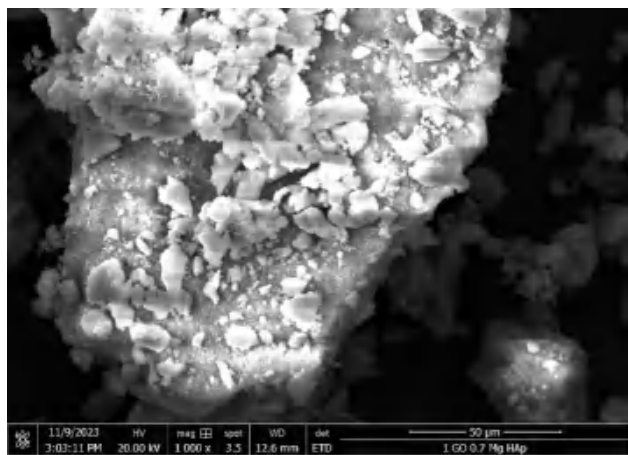
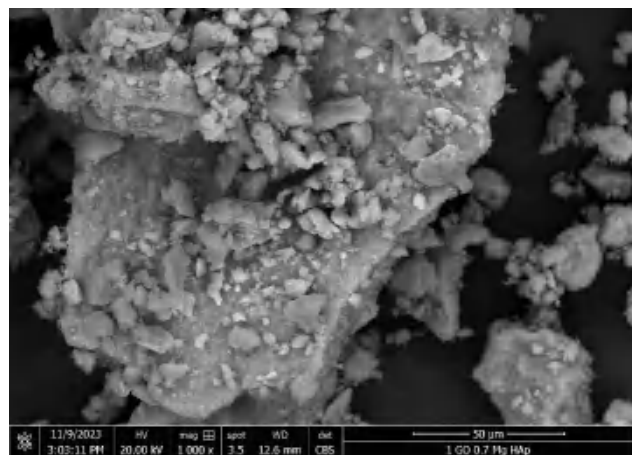
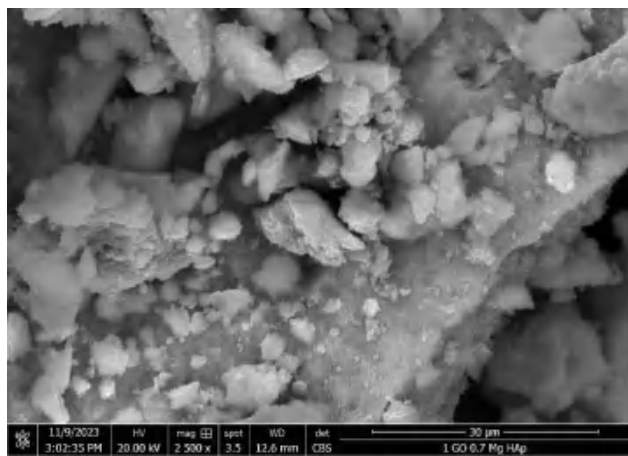
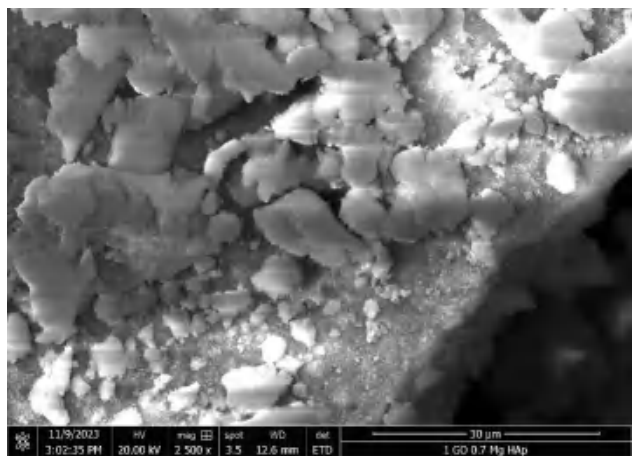
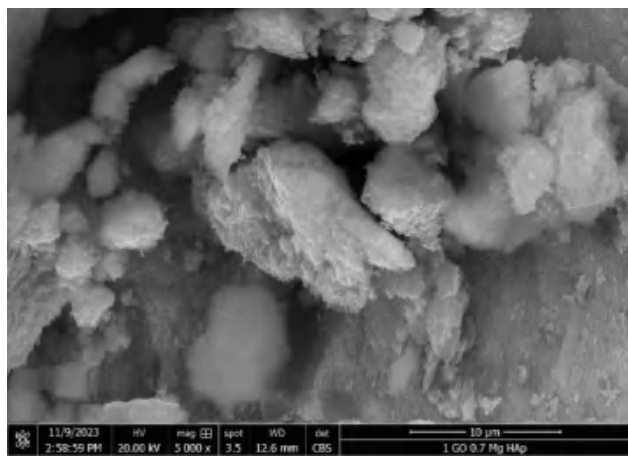
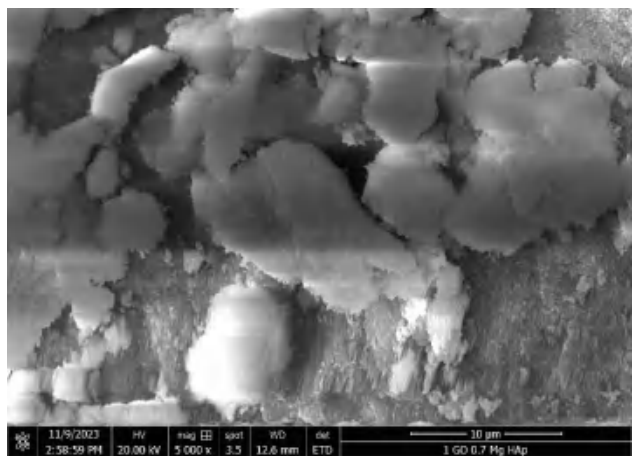
P K α 1



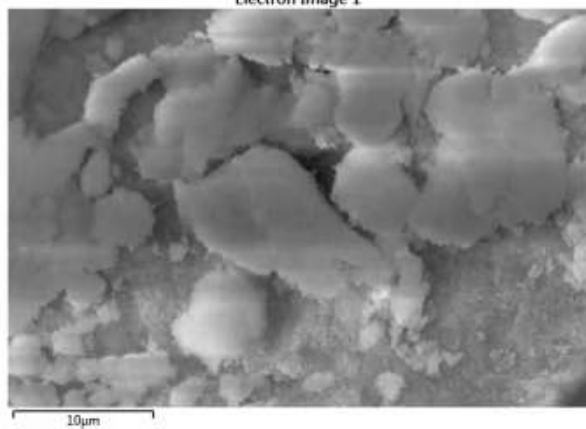
O K α 1



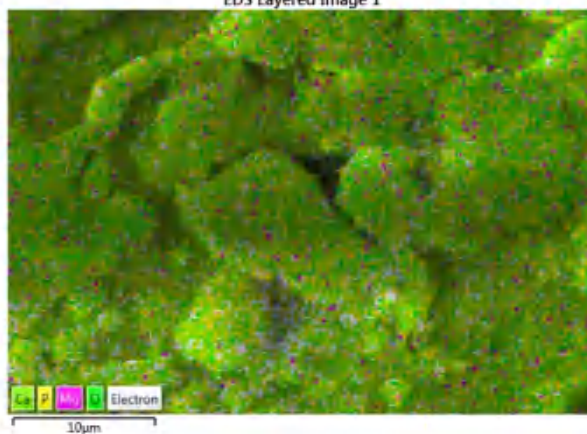
1rGO-0.7Mg-HAp



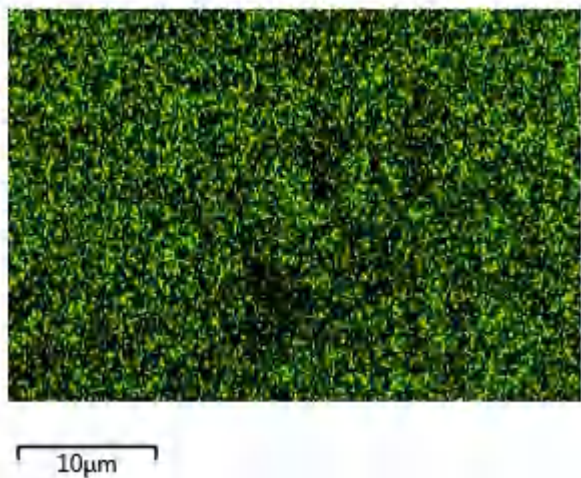
Electron Image 1



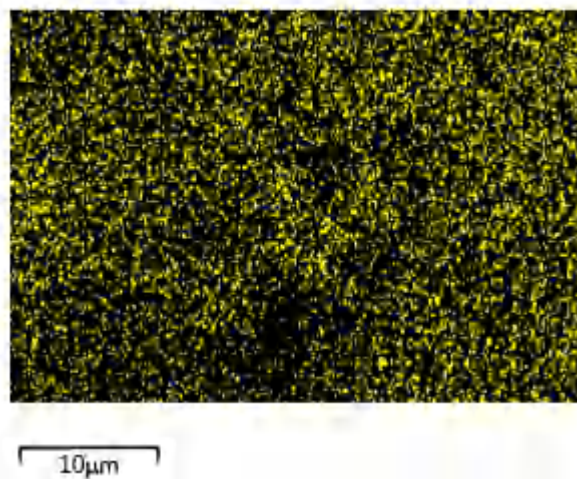
EDS Layered Image 1



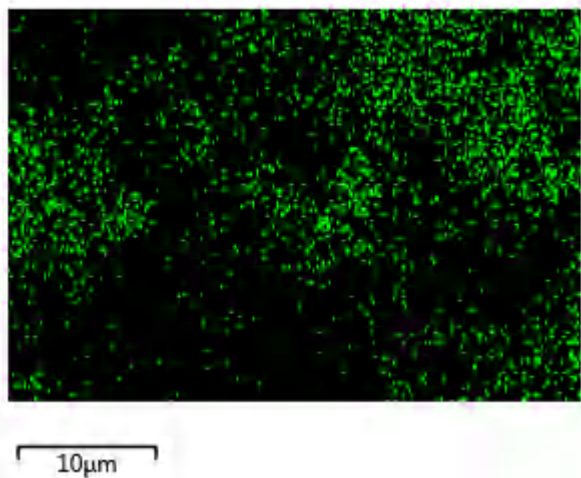
Ca K α 1



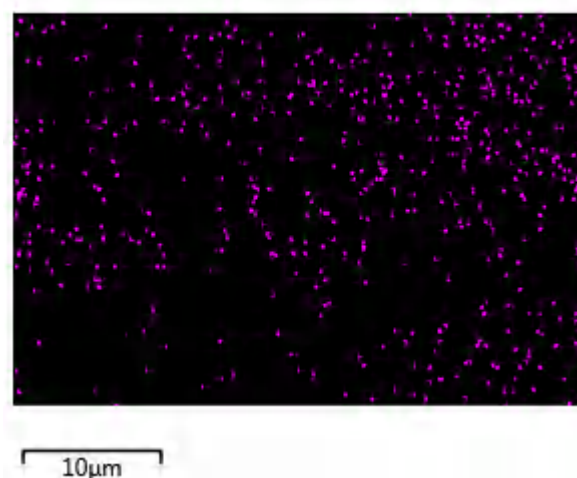
P K α 1



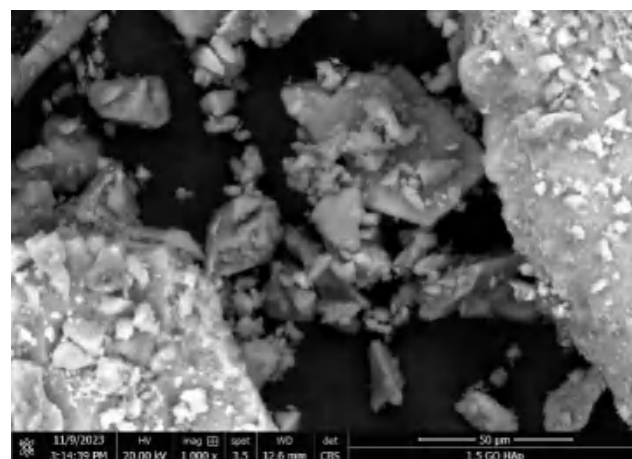
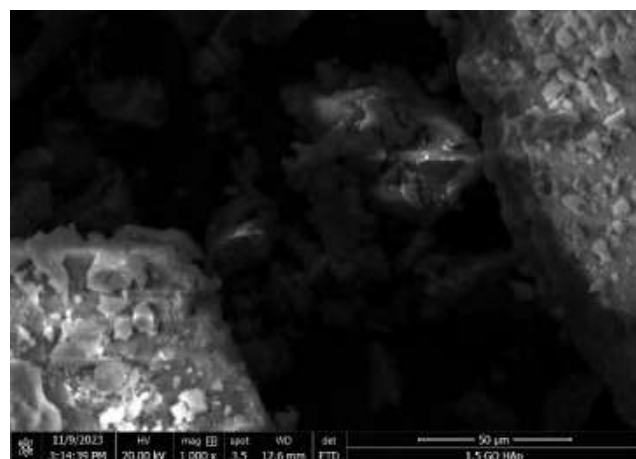
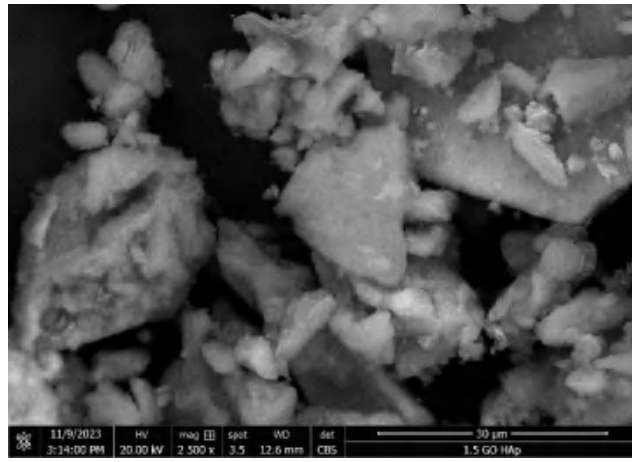
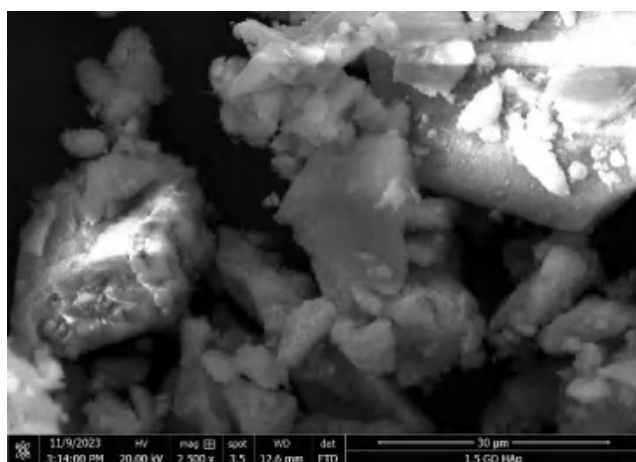
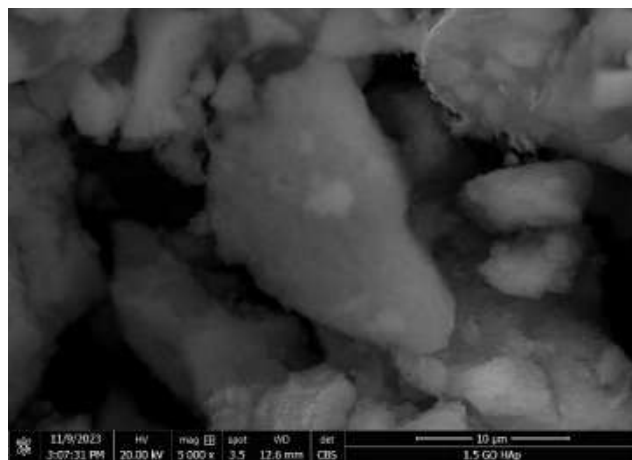
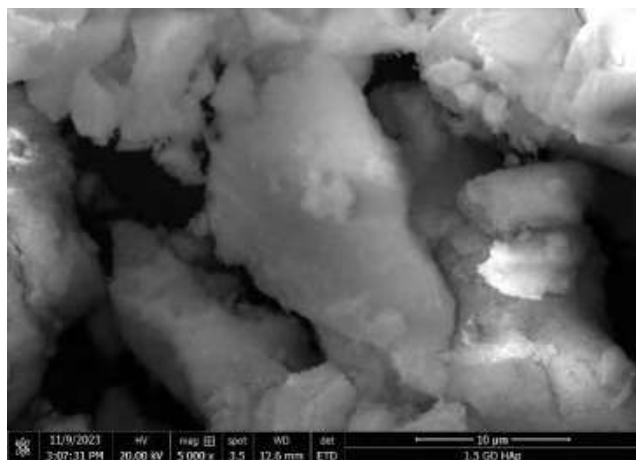
O K α 1



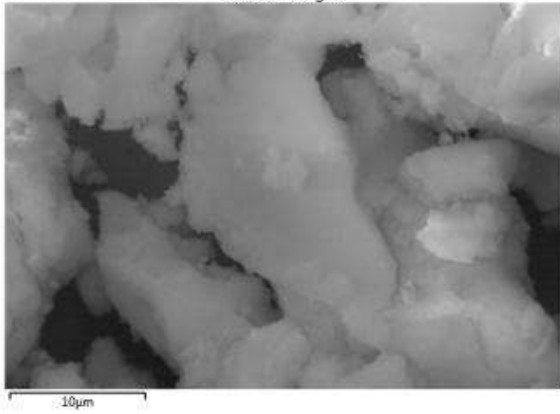
Mg K α 1_2



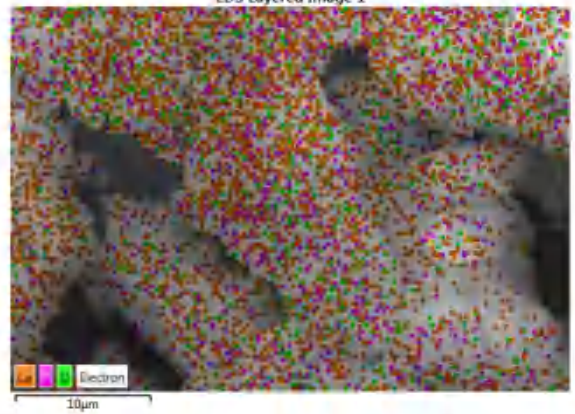
1.5rGO-HAp



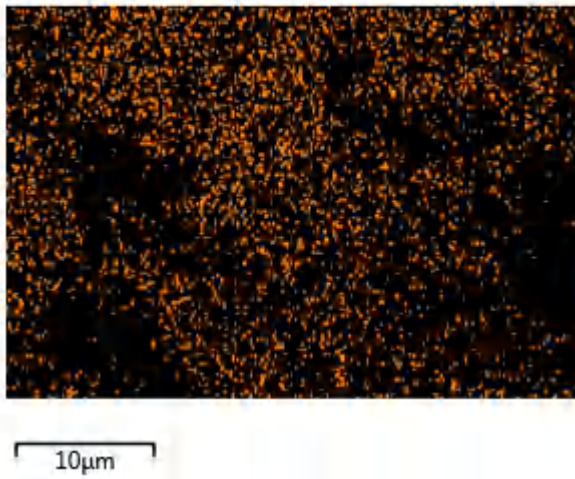
Electron Image 1



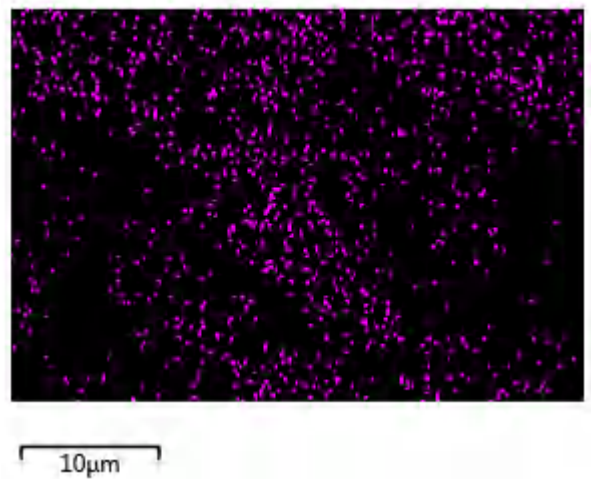
EDS Layered Image 1



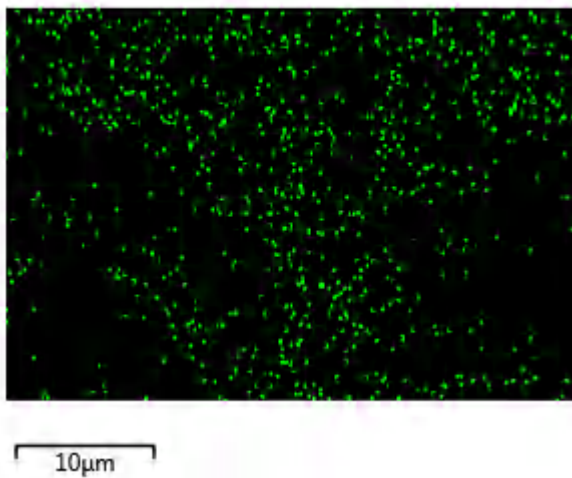
Ca K α 1



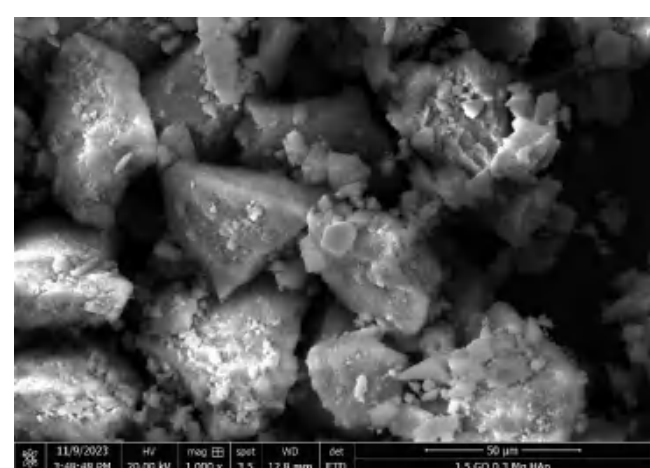
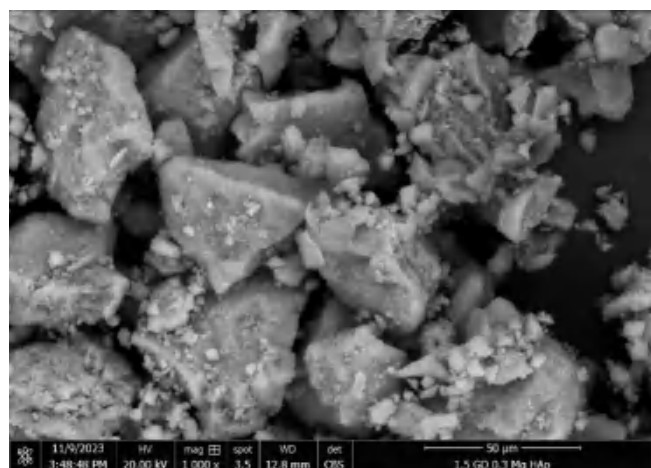
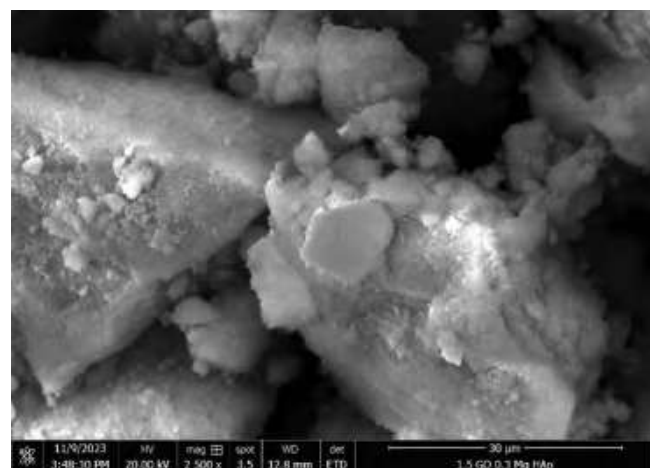
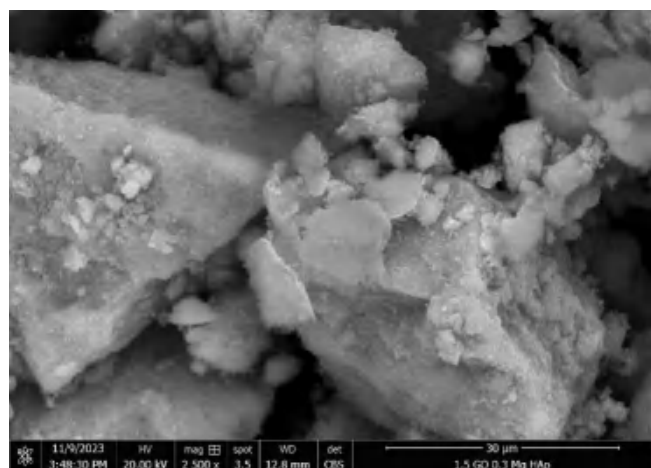
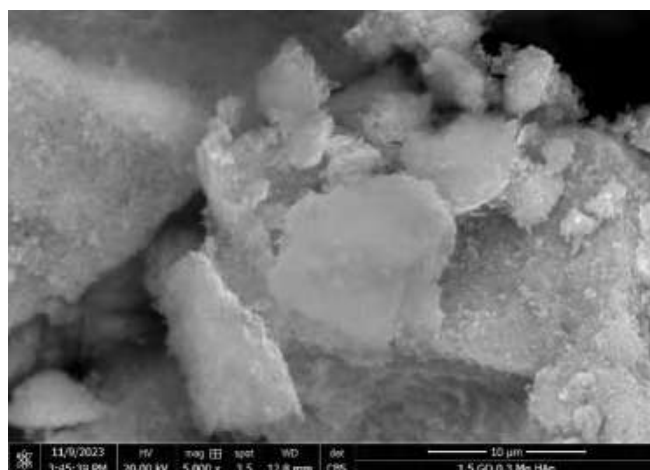
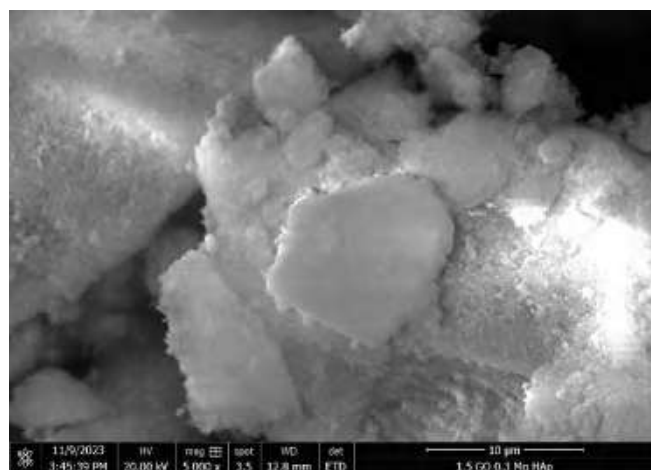
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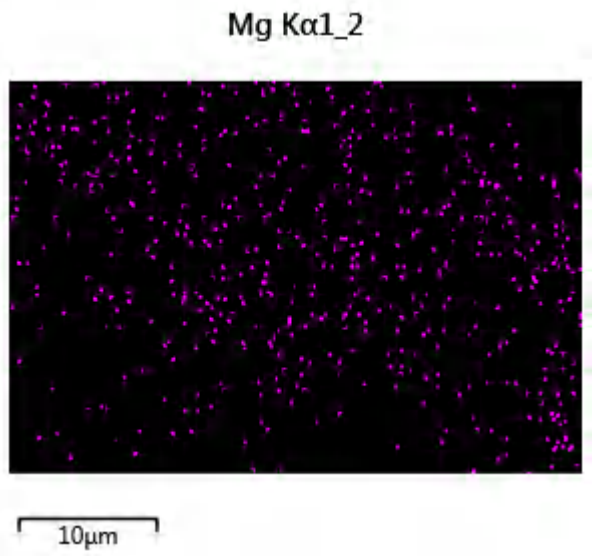
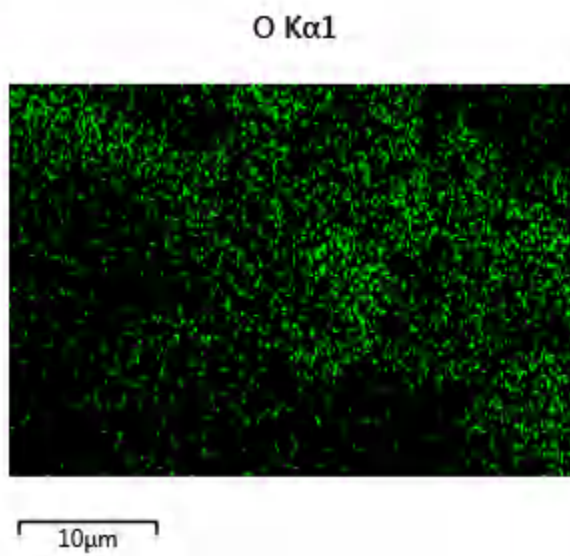
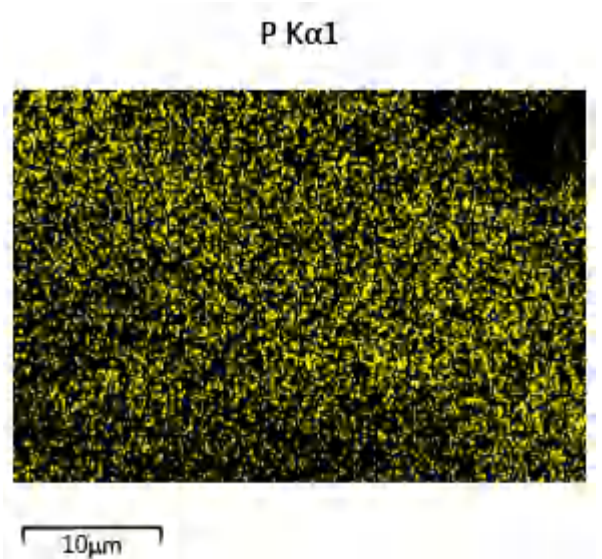
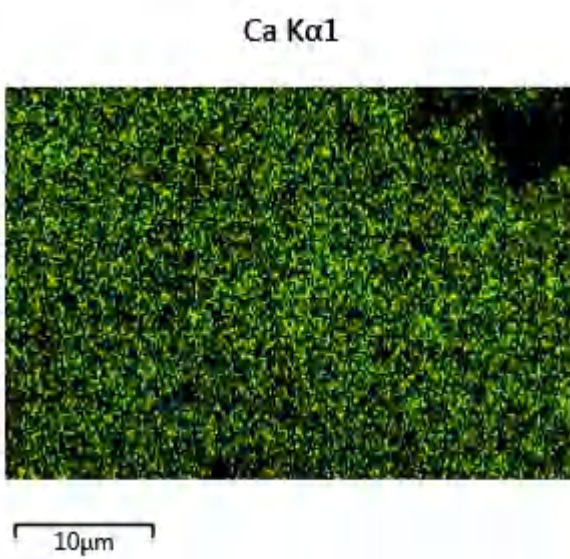
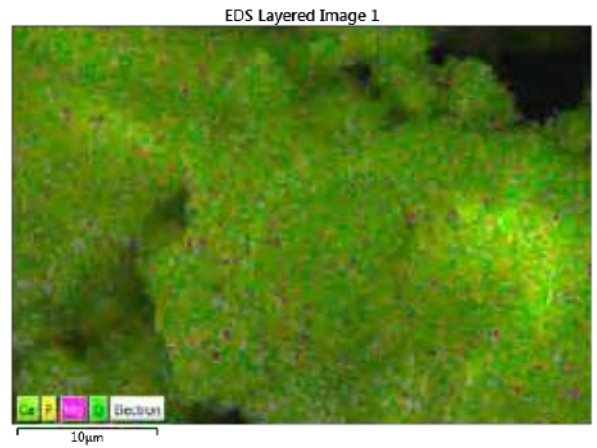
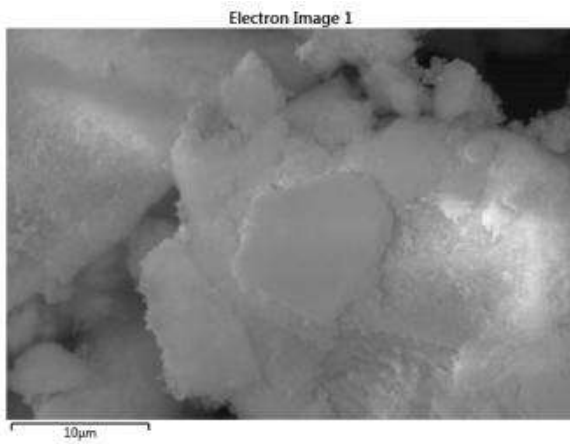


O K α 1

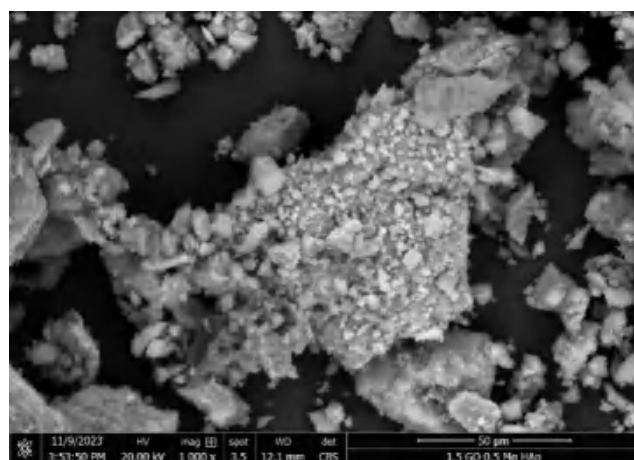
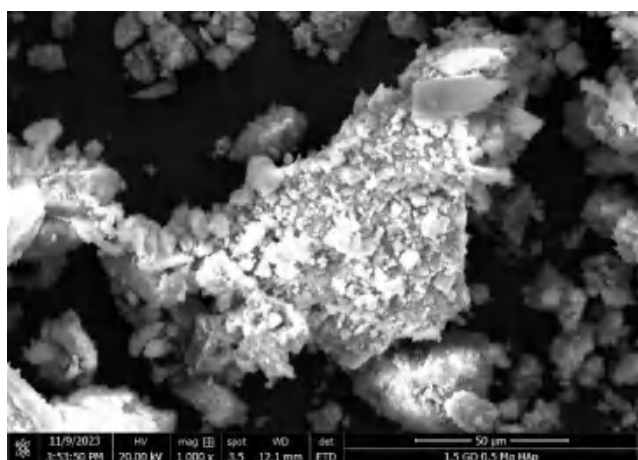
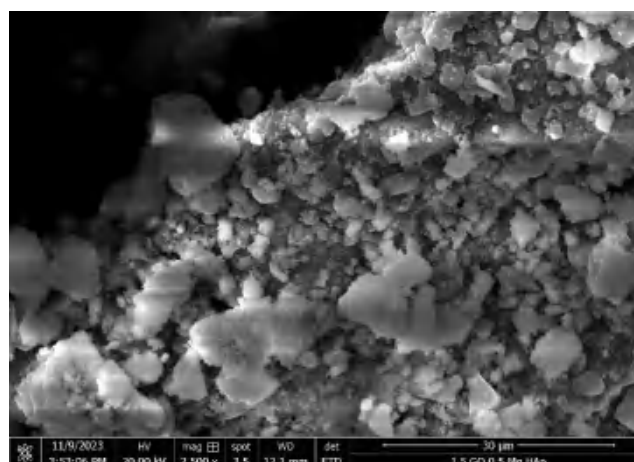
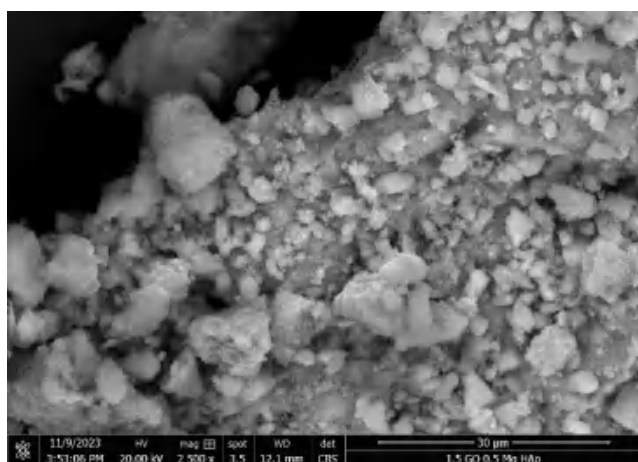
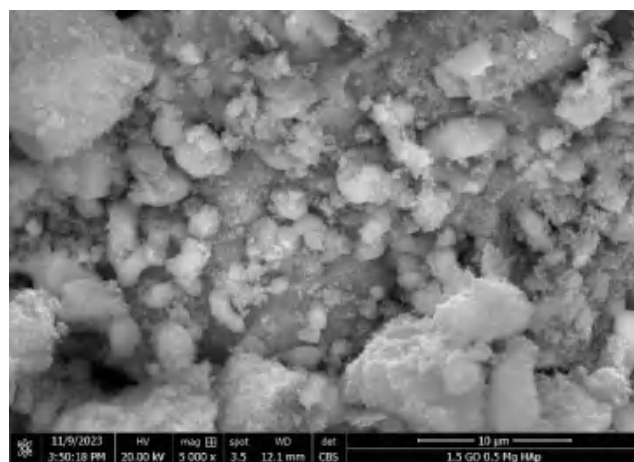
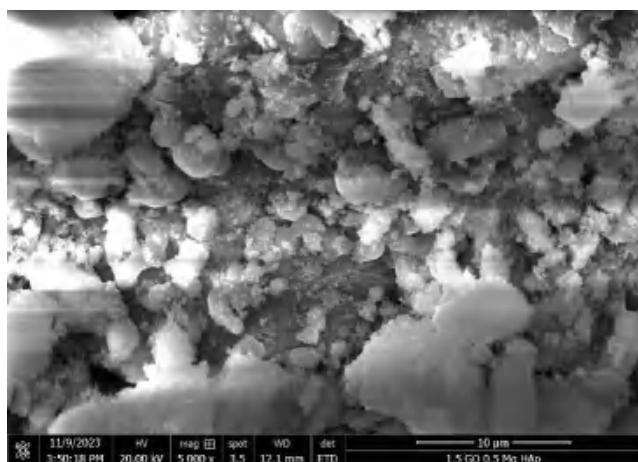


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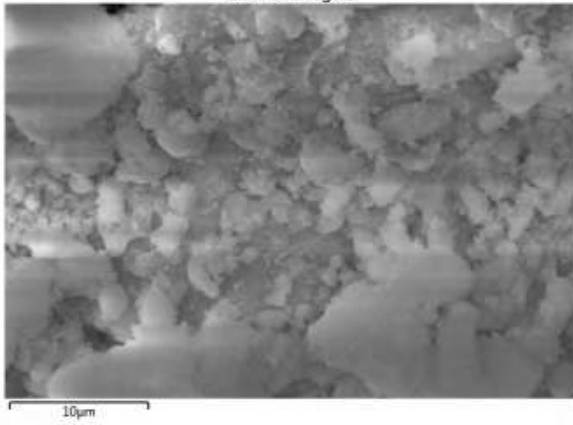




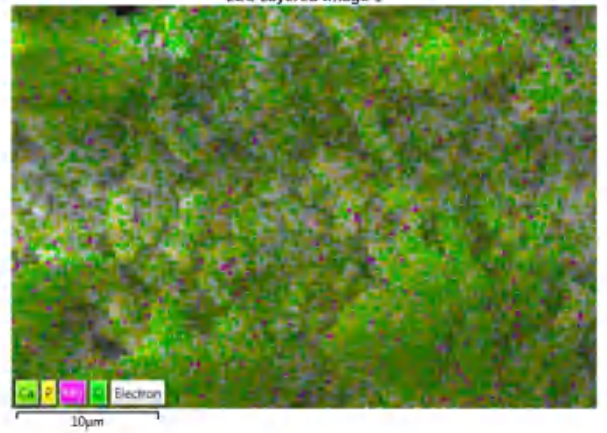
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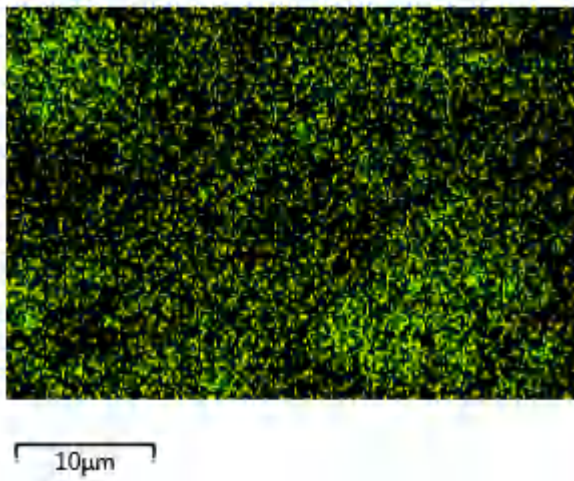
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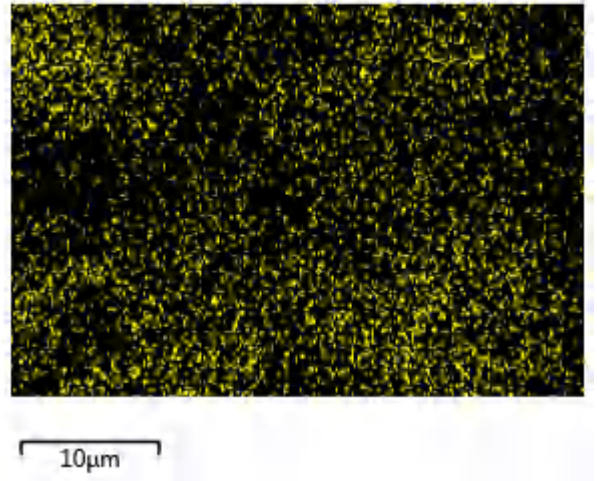
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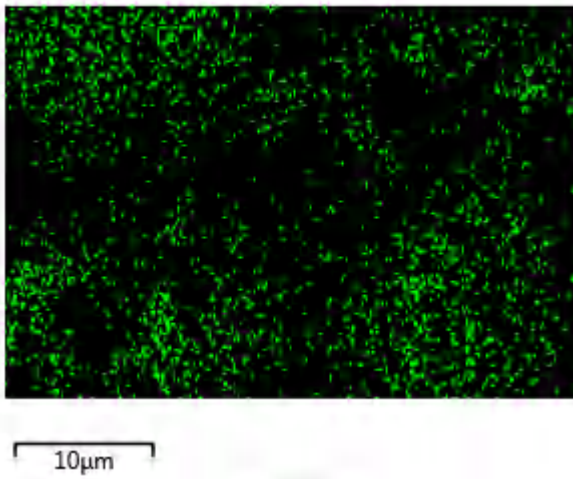
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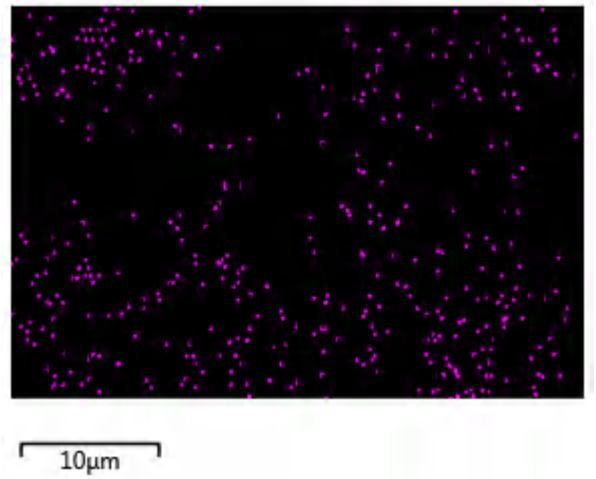
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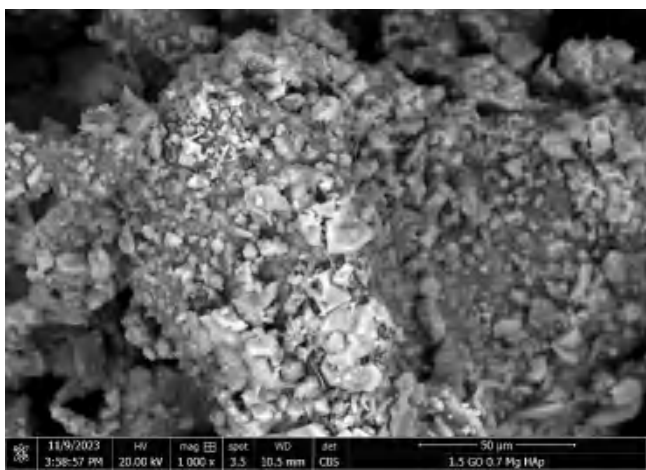
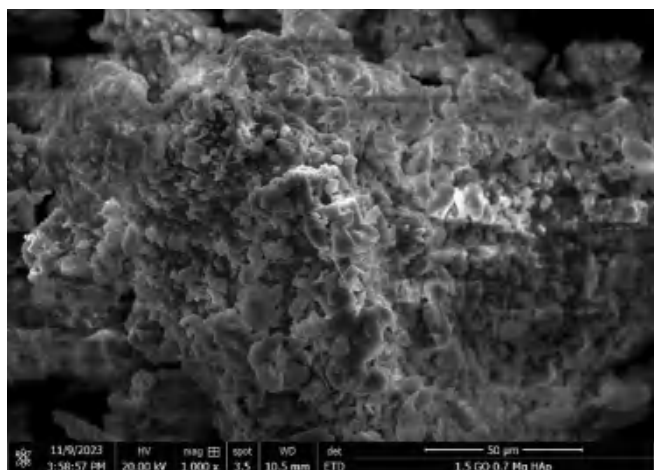
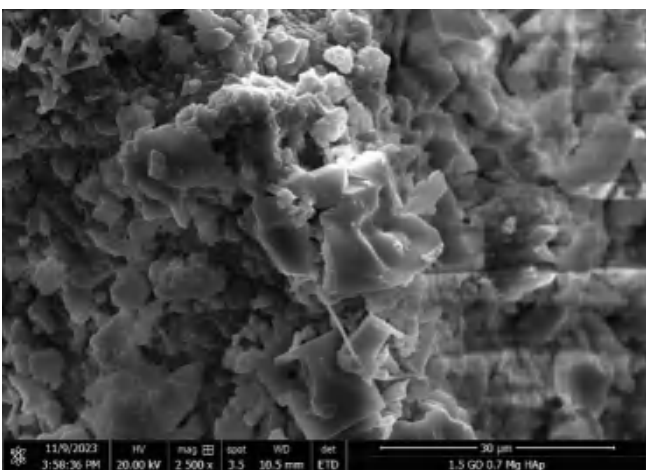
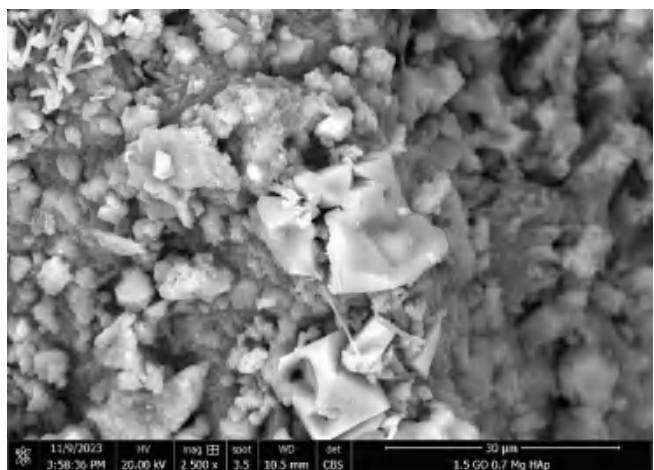
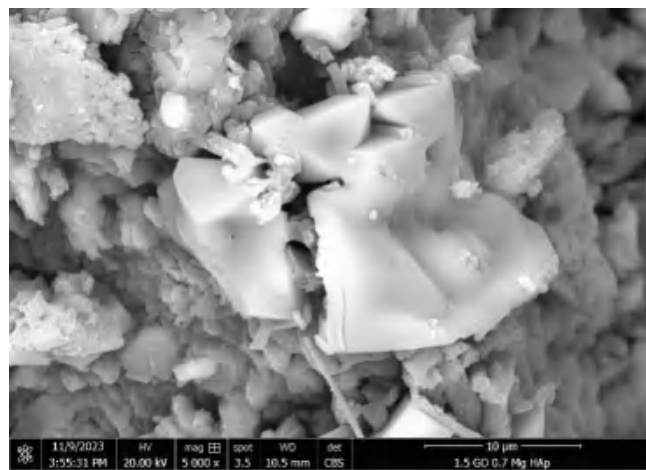
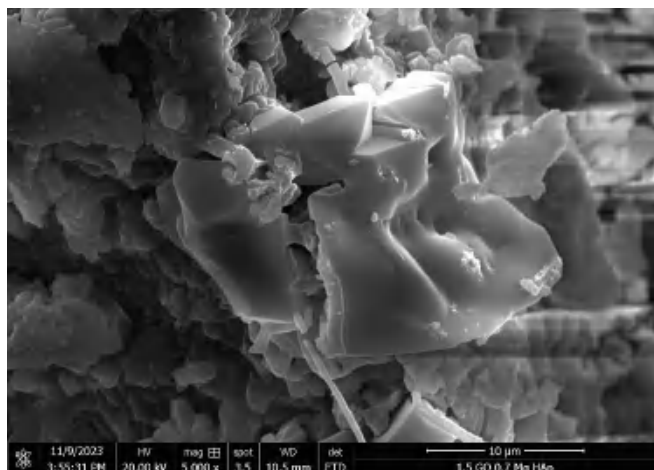
O K α 1



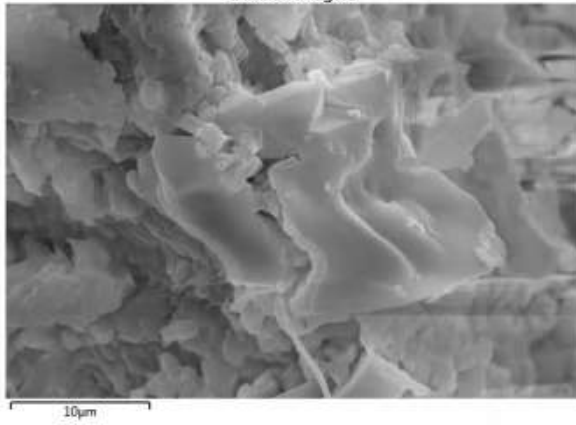
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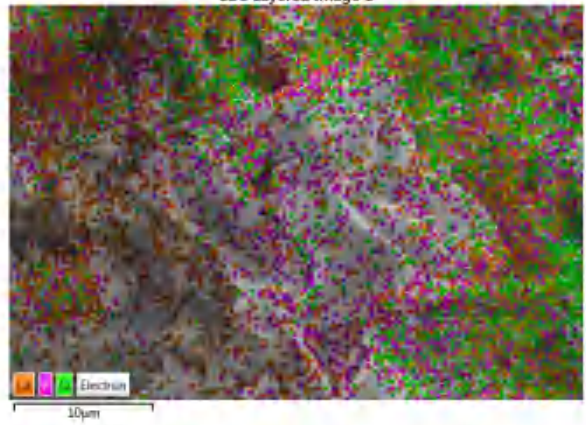
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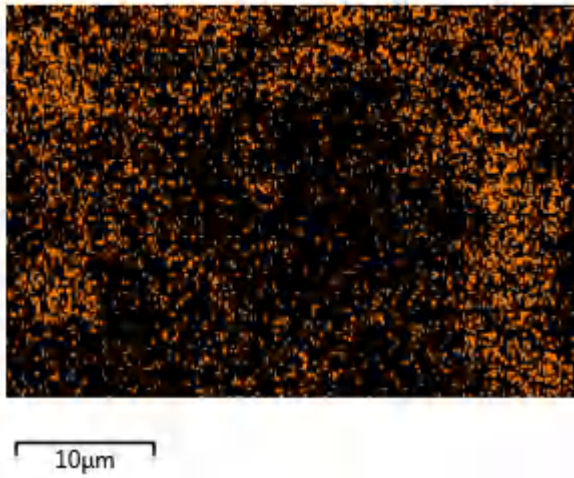
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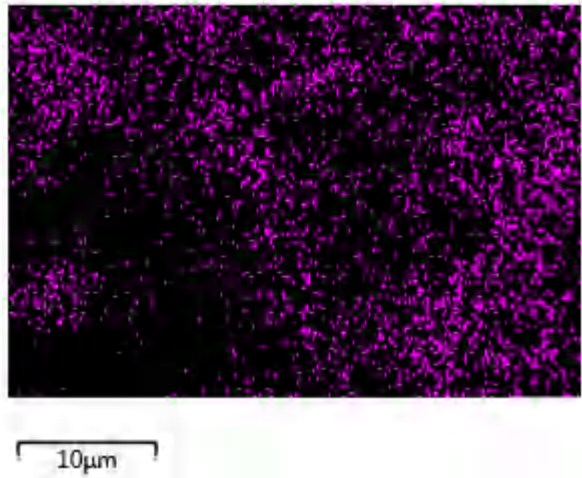
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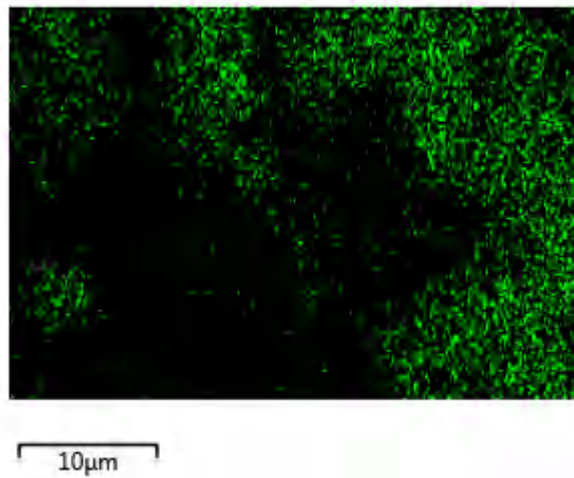
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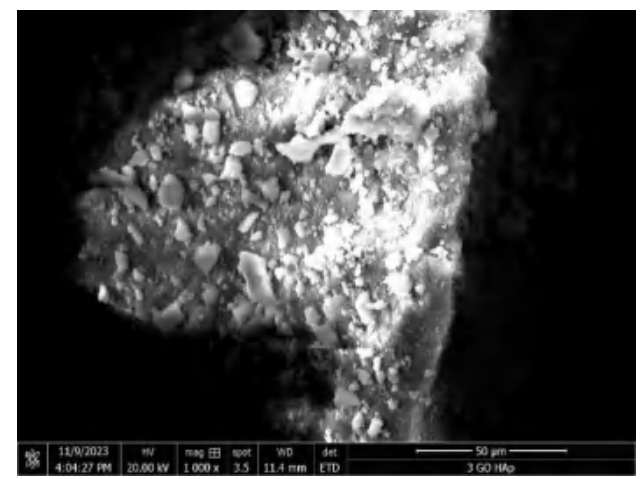
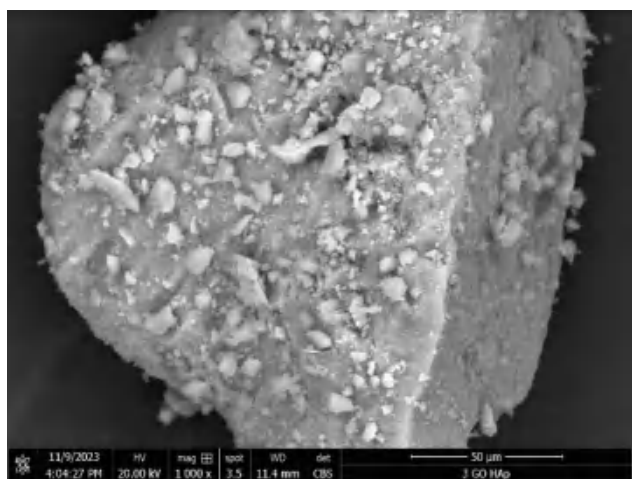
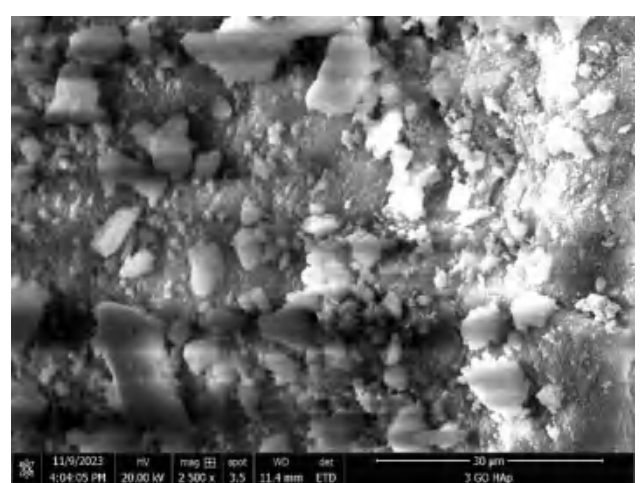
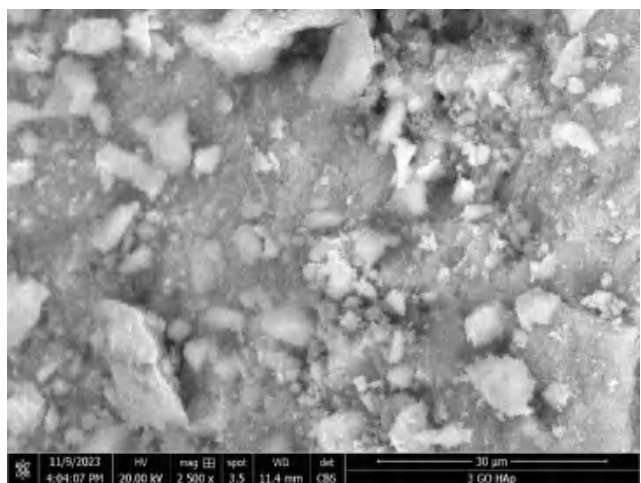
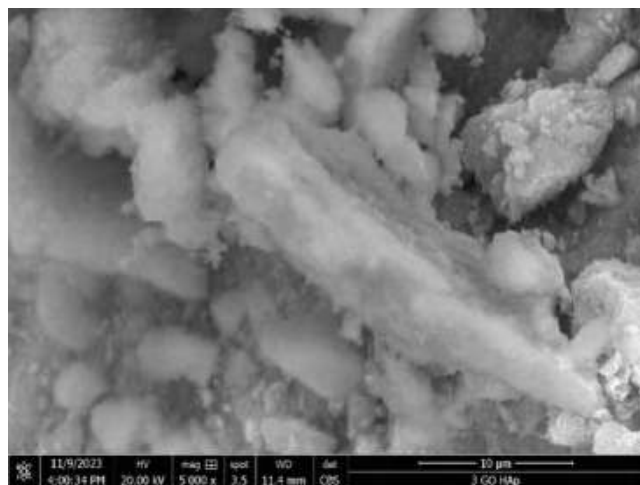
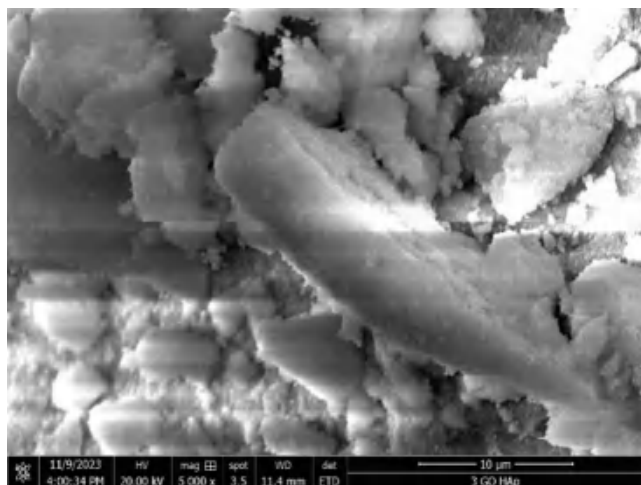
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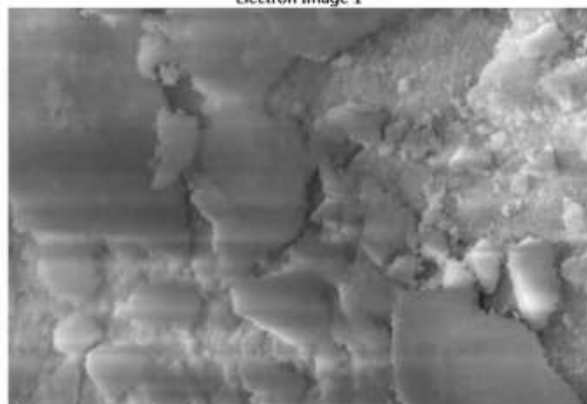
O K α 1



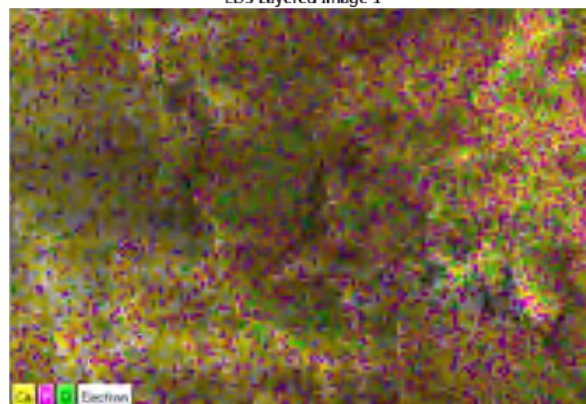
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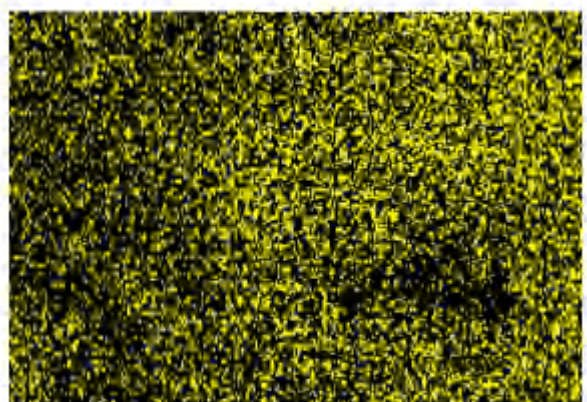
Electron Image 1



EDS Layered Image 1



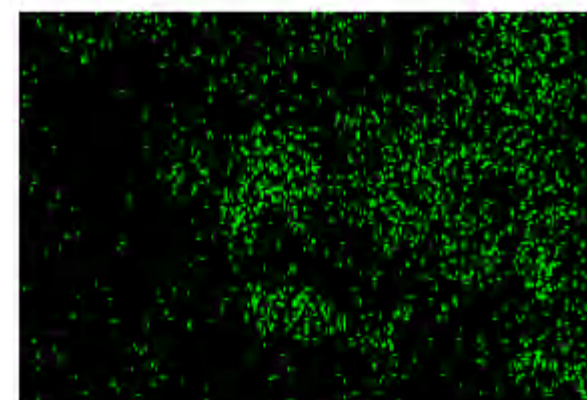
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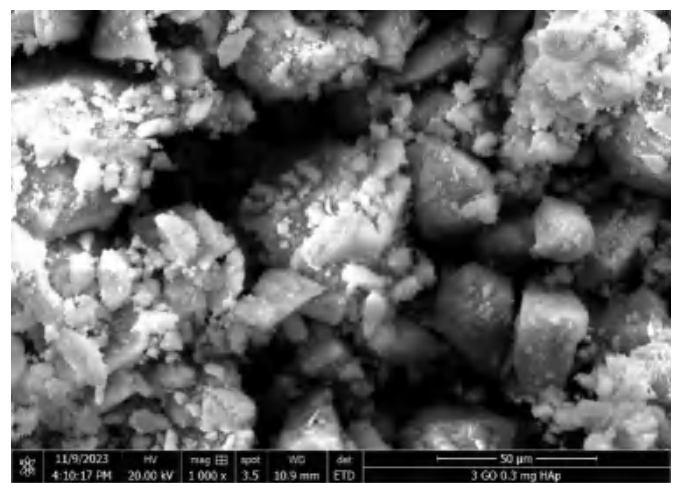
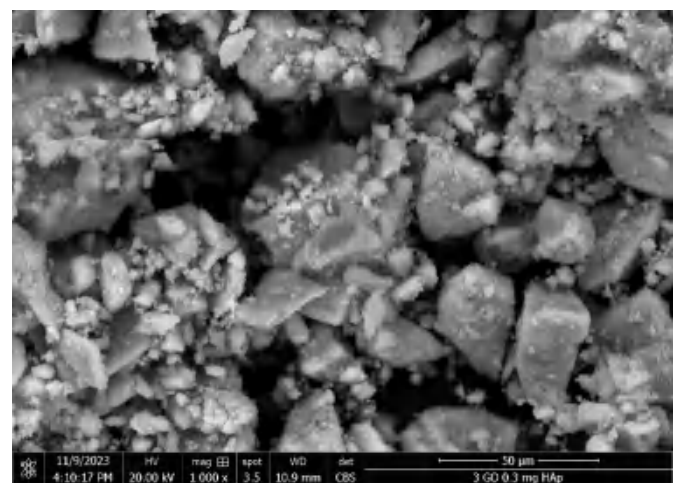
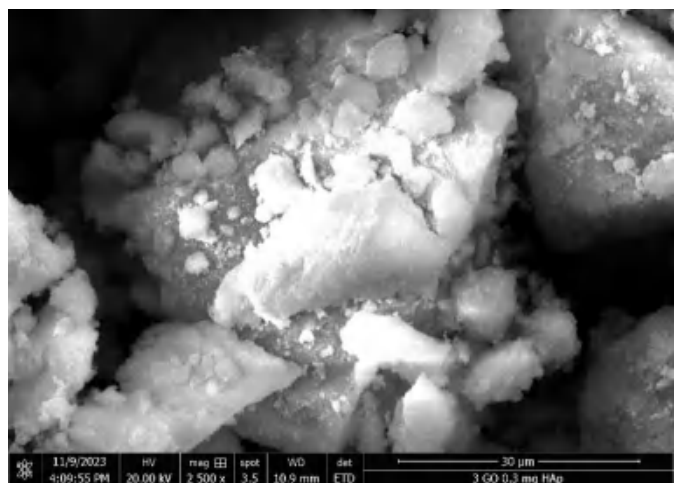
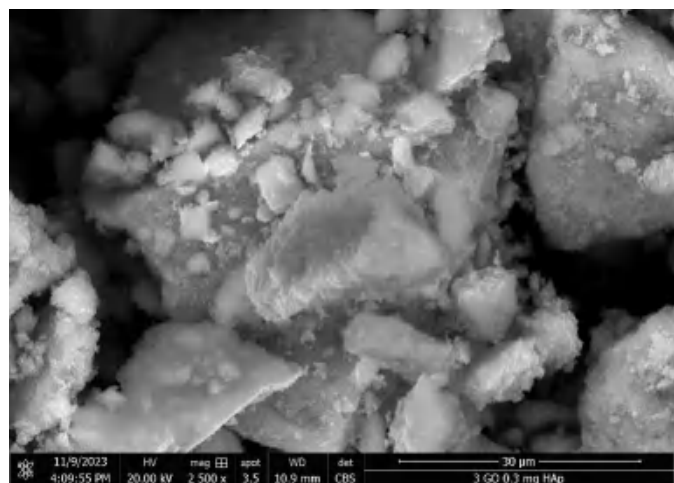
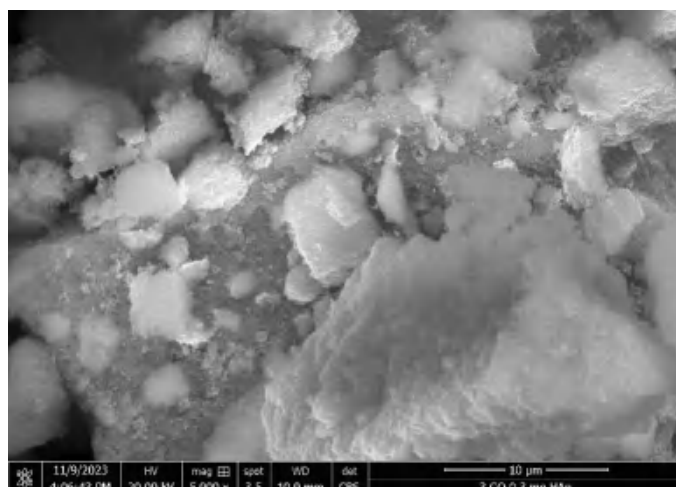
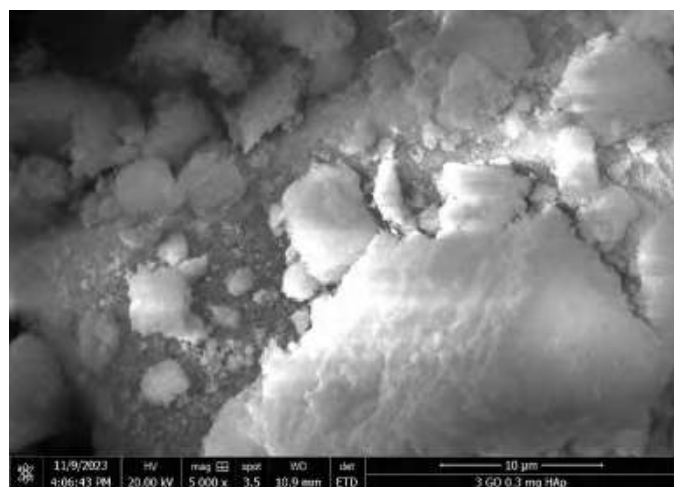
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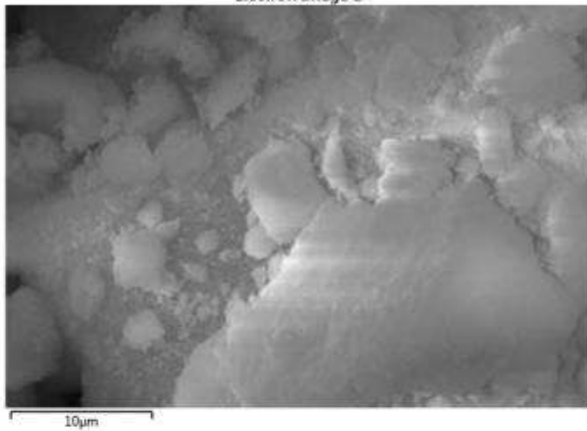
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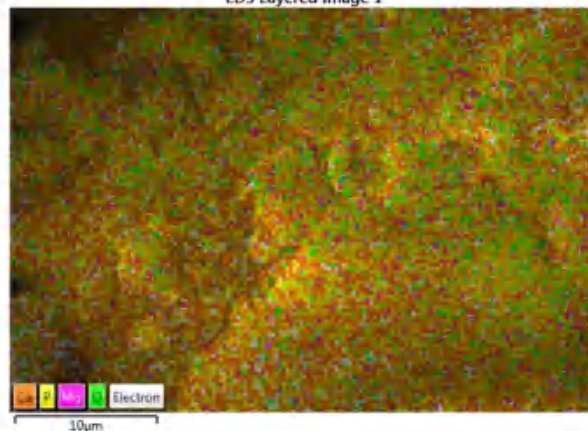
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Electron Image 1



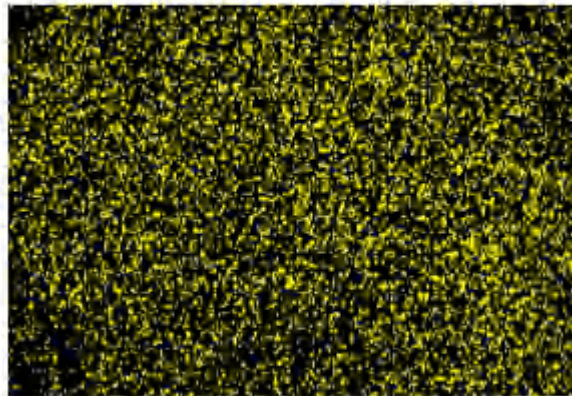
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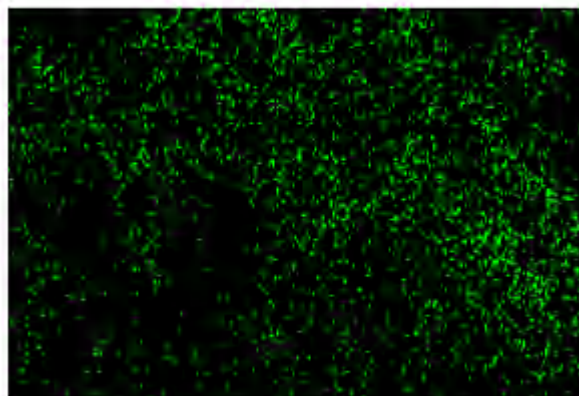
Ca Kα1



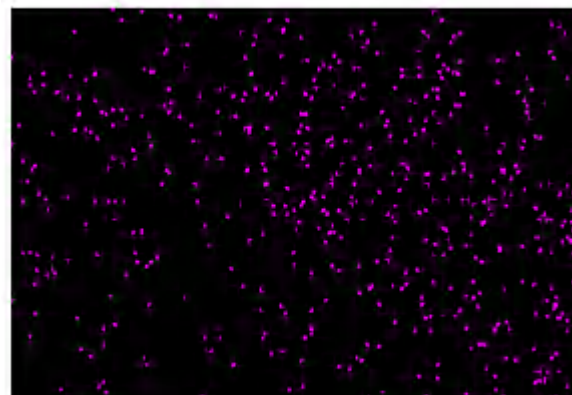
P Kα1



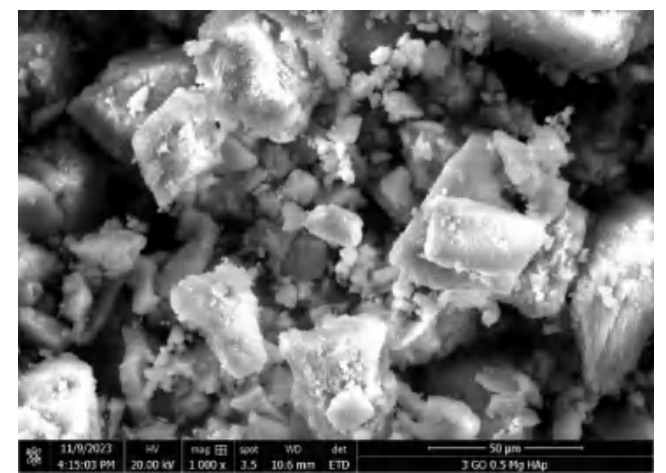
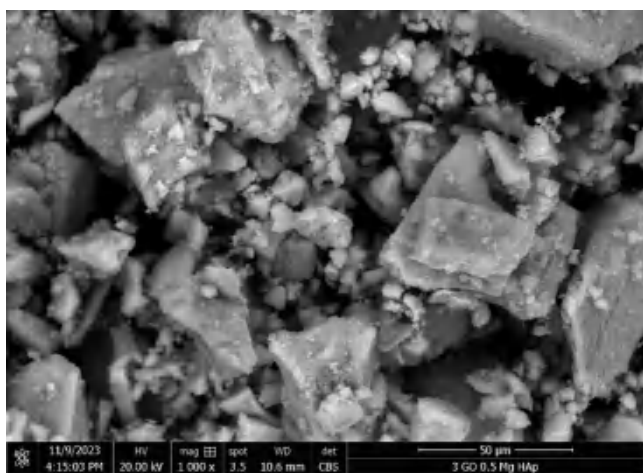
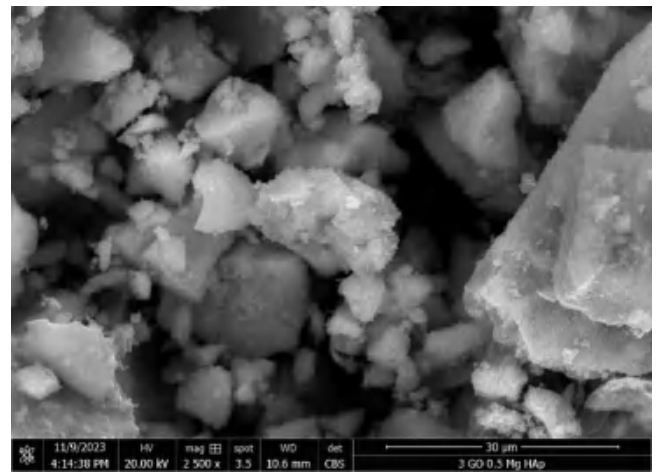
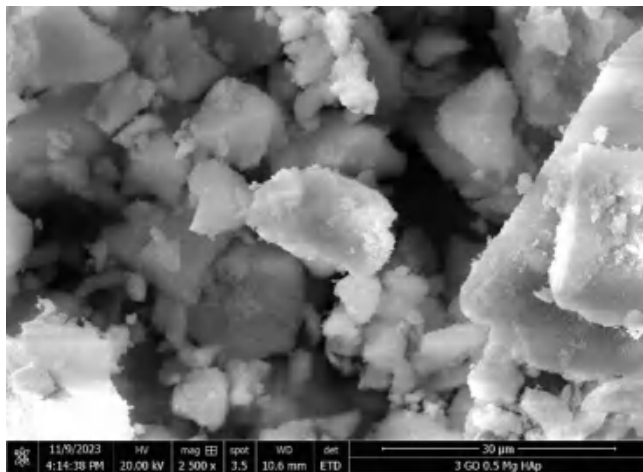
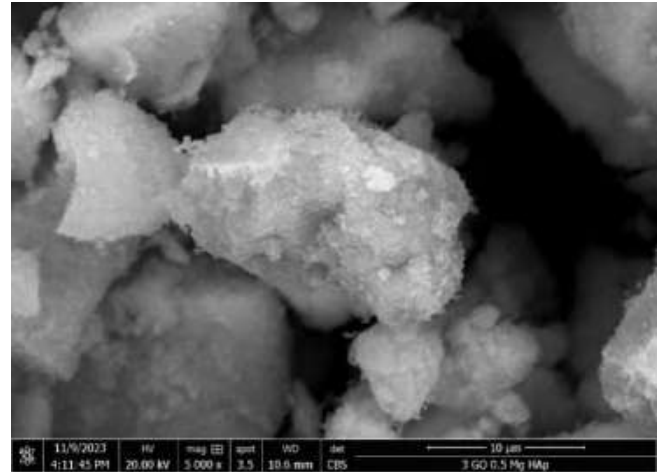
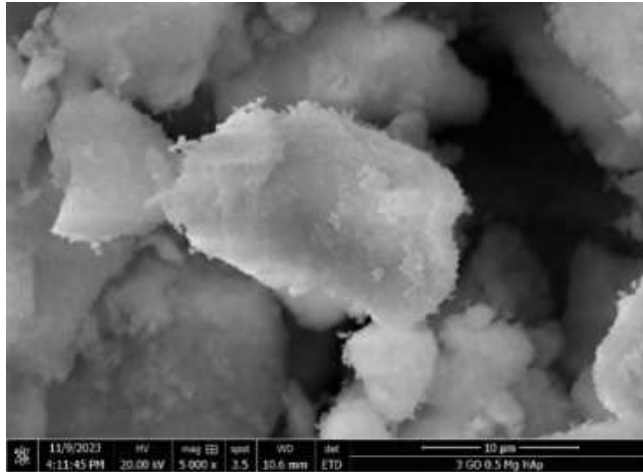
O Kα1



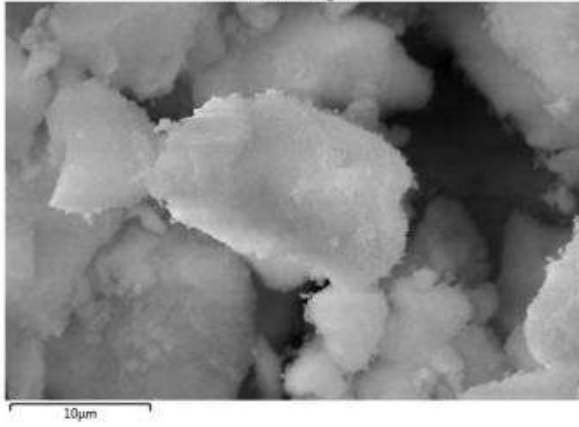
Mg Kα1_2



3rGO-0.5Mg-HAp-



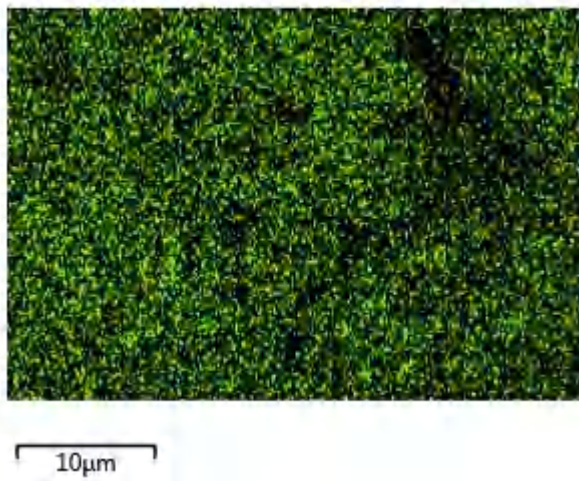
Electron Image 1



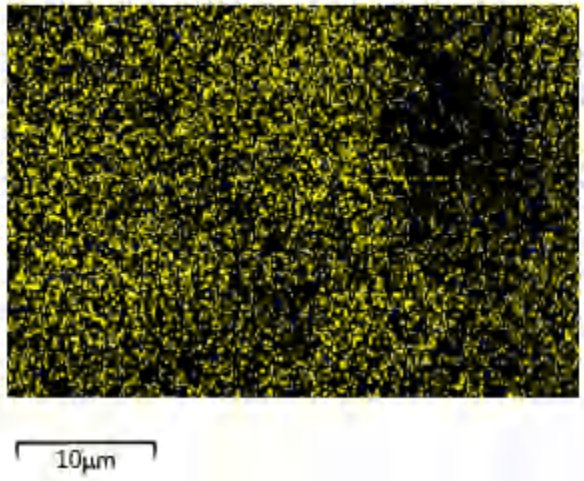
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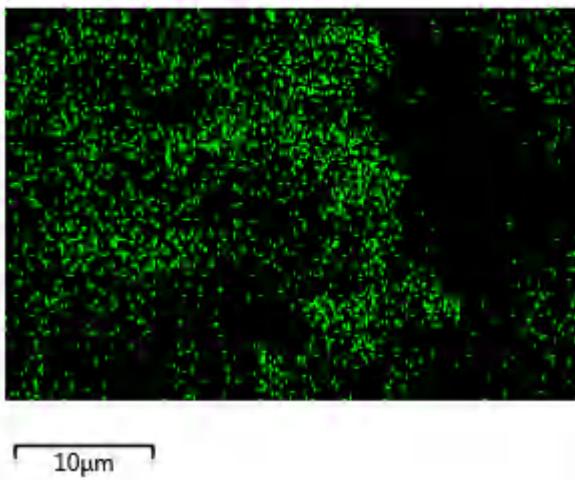
Ca K α 1



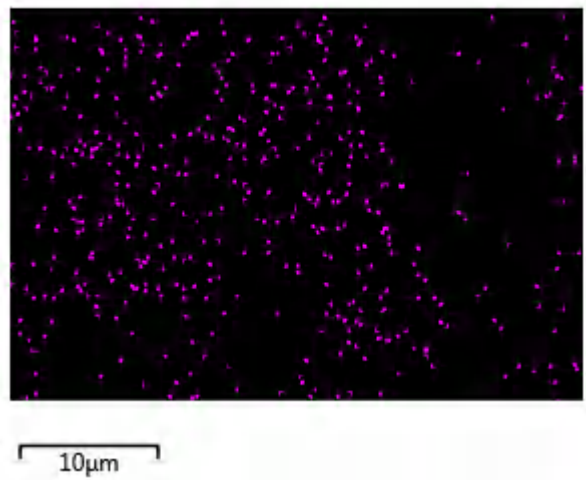
P K α 1



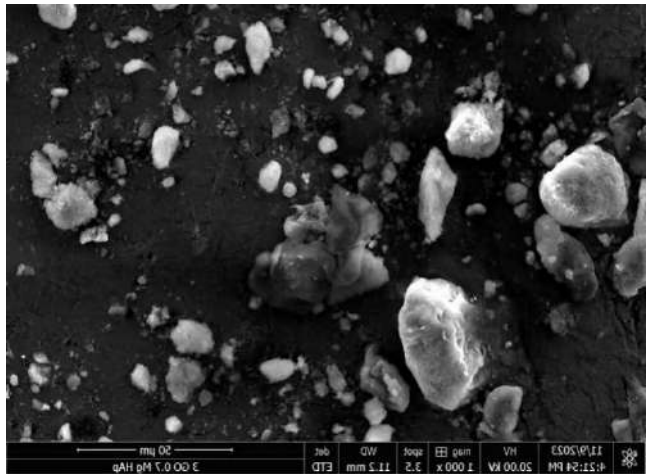
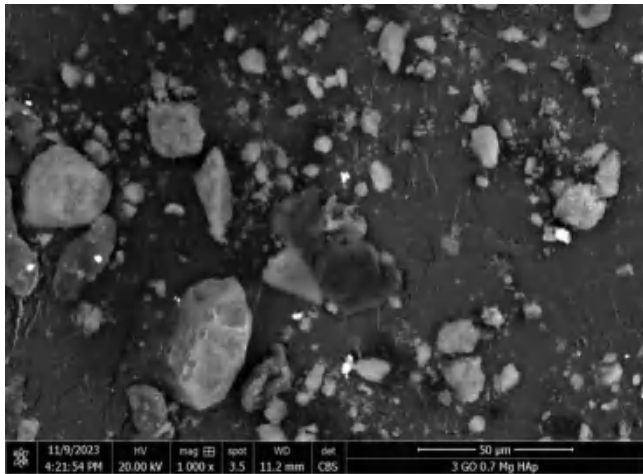
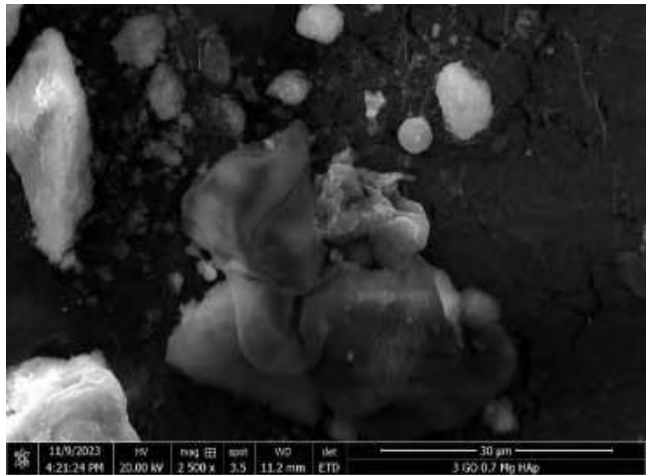
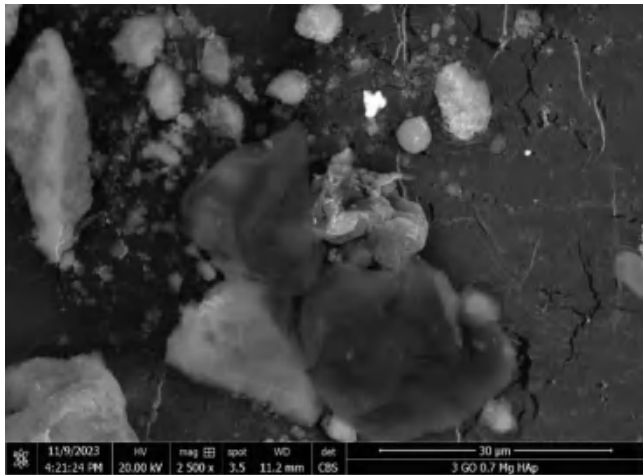
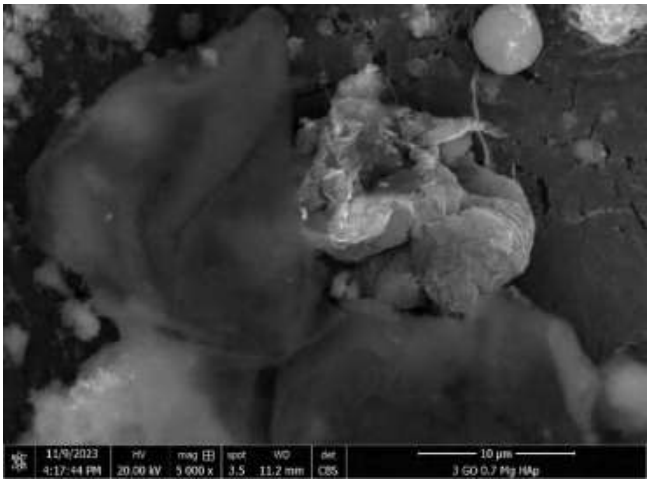
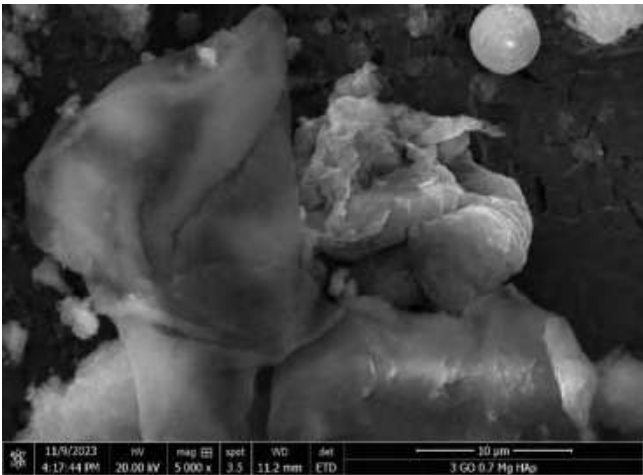
O K α 1



Mg K α 1_2



3rGO-0.7Mg-HAp



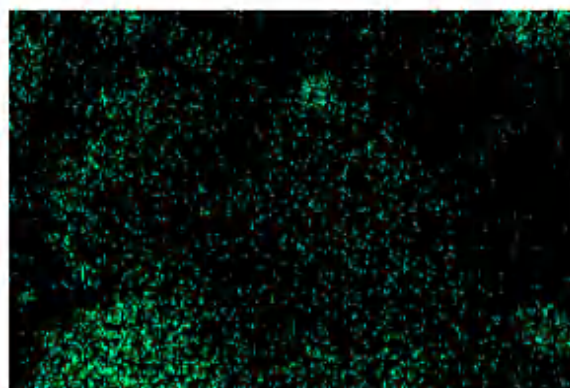
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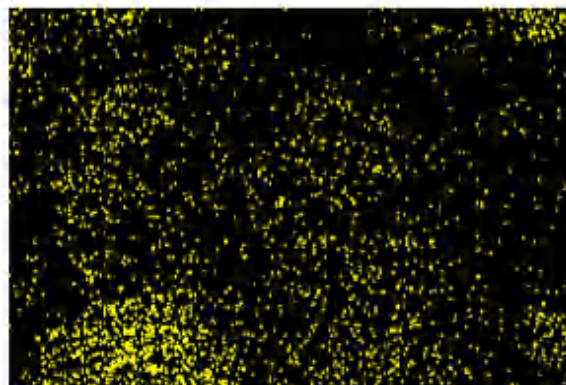
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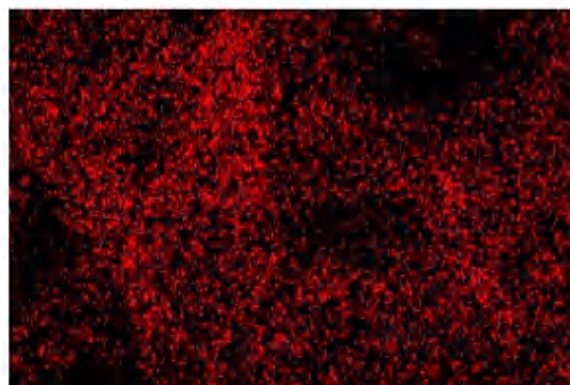
Ca Kα1



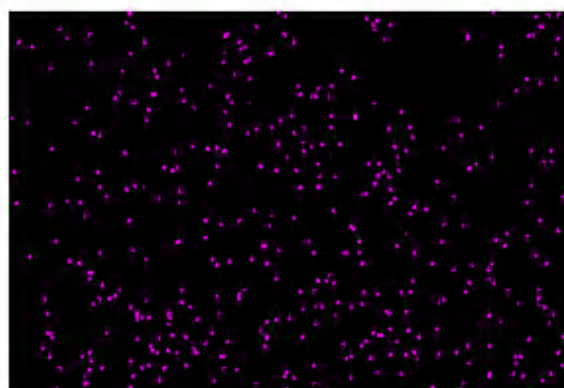
P Kα1



C Kα1_2



Mg Kα1_2



CHAPTER 5

CONCLUSION AND FURTHER RESEARCH

We successfully synthesized magnesium doped hydroxyapatite with reduced graphene composite using the chemical precipitation and microwave irradiation method with eggshells, Ammonium-dihydrogen phosphate ($\text{NH}_4\text{H}_2\text{PO}_4$), Magnesium-nitrate hexahydrate ($\text{Mg}(\text{NO}_3)_2(6\text{H}_2\text{O})$), and Reduced Graphene Oxide (rGO) as precursors for the first time. The XRD analysis matches very closely the material cards with all the peaks matching precisely with the literature as well. The crystal size calculations were performed on the samples and the crystal size decreased with magnesium addition as reported by literature, but the effect of reduced graphene on the crystal size after the magnesium addition wasn't reported before. The rGO addition caused an increase of the crystal size and overcame the Mg tendency to lower the crystal size. The interplanar spacing had similar effects as the crystal size. The XRD profiles of HAp and Mg-HAp did not reveal any phase changes; the absence of unreacted magnesium oxide (MgO) and other byproducts of synthesis, served as evidence for this. The XRD pattern showed that Mg had been completely absorbed into HAp and that there were generally significant deformations due to the Mg ions present in the HAp structure. The size of the crystal decreased as the concentration of magnesium ions rose. The observed contraction may be explained by the substitution of Ca^{2+} ions (0.099 nm) for the lower ionic radii of Mg^{2+} (0.072 nm). The FTIR spectra also agrees with literature and showed all phosphate, carbonate, and water intensities in their literature reported wavenumber. As magnesium ions were added to HAp,

they caused a reduction in intensity of the phosphate group. This suggests that the degree of lattice distortion and, consequently, the reduction in the intensity of the phosphate stretching modes, is related to the amount of magnesium incorporated into the hydroxyapatite structure. Also, rGO had similar effects to the phosphate groups, leading to changes in their vibrational modes. SEM images show irregular shape microstructure with agglomeration for the samples with magnesium addition. When Mg^{2+} is doped in HAp, it influences HAp crystals shape to change as opposed to the anticipated nanorods. Also, the uniform circulation of components across the surface supports the homogeneity of the composite which implies that the composite was successfully synthesized.

In further research, Raman spectroscopy, TEM, and other characterization techniques could be implemented. Also, since our composite is considered a biomaterial, in-vivo, in-vitro, antibacterial and larvicidal activity test could determine certain properties of rGO-HAp-Mg composite. The material can be synthesized using different precursors and the synthesis parameters can be adjusted to observe the effect on crystalline structure, performance, and microstructure of the material. Microwave heating parameters can also be changed as microwave power or irradiation time. Doping with other elements such as Zinc or Strontium could be investigated since Zinc had the same ionic size as magnesium but tends to change the structure of hydroxyapatite from hexagonal to orthorhombic structure [82]. Investigations of the produced material application in the biomedical field and bone tissue engineering specifically could be explored whether as a coating on cobalt, titanium, and magnesium alloys for surgical implants or in dentistry at general. Scaffolding for generating/bone tissue engineering is also an area to investigate our material in.

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