

SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL ACTIVITY OF BIOACTIVE DIOPSIDE FOR BONE REGENERATIVE APPLICATIONS

**Submitted in partial fulfilment of the requirements for the award of
Bachelor of Technology degree in Biotechnology.**

By

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**INSTITUTE OF SCIENCE AND TECHNOLOGY
(DEEMED TO BE UNIVERSITY)**

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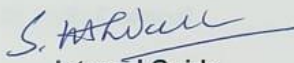
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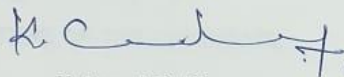
BONAFIDE CERTIFICATE

This is to certify that this Project Report is the bonafide work of VEDHAPRIYA.S (40230048), who carried out the project entitled "SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL ACTIVITY OF BIOACTIVE DIOPSIDE FOR BONE REGENERATIVE APPLICATIONS" under our supervision from September-2023 to April-2024.



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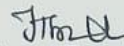
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I, **VEDHAPRIYA.S (40230048)**, hereby declare that the Project Report entitled **“SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL ACTIVITY OF BIOACTIVE DIOPSIDE FOR BONE REGENERATIVE APPLICATIONS”** done by me under the guidance of **Dr. S. USHA NANDHINI** (Internal) and **Dr. K. GOBI SARAVANAN** (External) at **Sathyabama Institute of Science and Technology, Chennai**, is submitted in partial fulfilment of the requirements for the award of **Bachelor of Technology degree in Biotechnology**.

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ABSTRACT

A coprecipitation route was used to synthesize undoped diopside ($\text{CaMgSi}_2\text{O}_6$) powders and doped diopside with cerium nitrate substituted for calcium and lanthanum nitrate as a substitute for magnesium. The pelletizer machine with 105pa pressure was given to make 10mm size diopside pellets and calcinated. The structure, bioactivity, biodegradation, and cell adhesion of the samples were studied by X-ray diffraction, Fourier transform infrared spectroscopy and scanning electron microscopy. According to the results, the Ce-doping process improves the sintering ability and apatite-formation ability of the diopside-based pellets but shows gradual biodegradation. The surface of the pellet showed better adhesion and spreading with typical cell extensions on the Ce-doped pellets in comparison to the undoped diopside and La-doped diopside. It is eventually concluded that cerium nitrate can be further considered as a doping agent to improve the bioactivity and biocompatibility of Mg-Ca silicate pellets.

Keywords: Diopside, Dopants, Calcination, Bioactivity, Cerium nitrate, Lanthanum nitrate, Bio-compatibility and Apatite formation.

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LIST OF ABBREVIATIONS:

FTIR	Fourier Transform Infrared Spectroscopy
XRD	X-Ray Diffraction
SEM	Scanning Electron Microscope
Ca: Ce	Calcium nitrate: Cerium nitrate
Mg: La	Magnesium nitrate: Lanthanum nitrate
SBF	Stimulated Body Fluid
BSA	Bovine Serum Albumin
DPPH	2,2-Diphenyl-1-picrylhydrazyl
Mg	Milli Gram
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide
DMSO	Dimethyl Sulfoxide
HAP	Hydroxy Apatite
HR	Hemolytic Rate
RSA	Radical Scavenging Activity
Nm	Nano Metre
Rpm	Revolutions Per Minute
µg	Micro Gram
ROS	Reactive Oxygen Species

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1. INTRODUCTION

Since the discovery of biologically active glass, a great deal of much research has been carried out to apply glasses and glass–ceramics as human tissue substitutes. Actually, various kinds of bioactive glasses and glass–ceramics have been developed for use as biomedical materials. When we implant glass and glass–ceramics in the human body, we can observe their direct bonding with living bone tissues. Some of them have already been clinically used as bone-repairing materials (Srinath et al., 2021). The bonding of glass and glass–ceramics with living bone is achieved through a bone-like apatite layer formed on the bone surface in the body environment. However, usable parts of these glasses and glass–ceramics in the human body are limited because their mechanical strength, especially the fracture toughness value, is inferior to that of natural bones and teeth (Li et al., 2021).

Metal bone implants are usually made of medical-grade stainless steel, titanium-based or cobalt–chromium-based alloys (Sheikh et al., 2015). The bioinert characteristic of metallic implants may cause formation of a fibrous tissue layer between the implant and surrounding tissue (Kaur et al., 2020). More importantly, the implants made of these metals are non-biodegradable and must stay in the body after the bone heals. However, despite their numerous advantages, metal bone implants also present several notable disadvantages.

Diopside, a naturally occurring mineral belonging to the pyroxene group, has garnered increasing attention in the realm of bone regeneration and tissue engineering (Kumar et al., 2014). With its unique combination of chemical and structural properties, diopside holds significant promise as a biomaterial for promoting bone growth and repair. One of the notable advantages of diopside is its biocompatibility, meaning it can integrate seamlessly with living tissues without eliciting adverse immune responses. Furthermore, diopside possesses excellent osteo-conductivity, facilitating the adhesion and proliferation of osteoblasts—the cells responsible for bone formation—onto its surface.

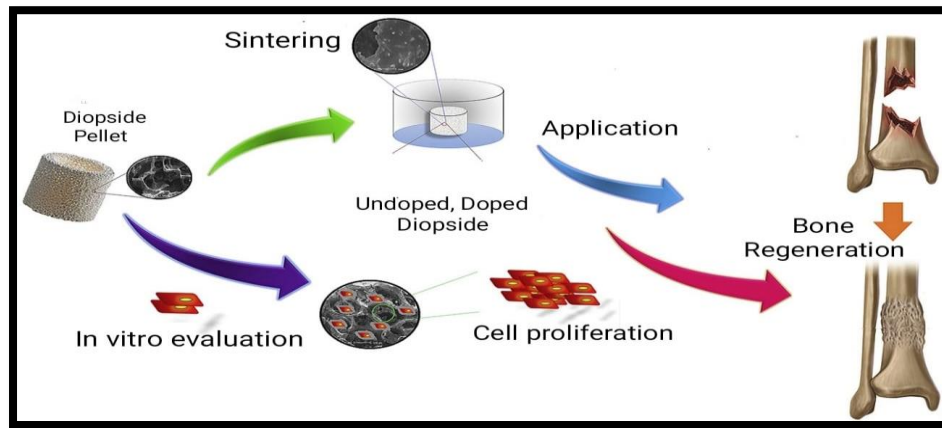


Fig.1.1: Graphical representation of diopside for bone regeneration

This property is crucial for promoting the formation of new bone tissue and accelerating the healing process in bone defects or fractures (Wu et al., 2010). Additionally, diopside exhibits biodegradability, gradually resorbing over time as new bone tissue replaces it, thus eliminating the need for surgical removal. Moreover (Kumar et al., 2014b), diopside has been shown to stimulate osteogenic differentiation of mesenchymal stem cells, further enhancing its regenerative potential (Choudhary et al., 2019). According to a series of reports on diopside, the sintered body of diopside forms a hydroxy-apatite layer on the surface in contact with a simulated body fluid and combines with living bone tissues more rapidly than hydroxyapatite in animal experiments (Shahrouzifar & Salahinejad, 2019).

Doping of proper species can be an efficient approach to improving bioactivity, mechanical properties and anti-bacterial properties (Razavi et al., 2014). The elements that are used for doping in apatite ceramics and glasses in this study are cerium nitrate and lanthanum nitrate (Jafari Baghjehaz & Salahinejad, 2017).

Cerium nitrate, typically used in the form of cerium ions, has been studied for its potential to improve the biological performance of diopside. Cerium ions possess antioxidant properties, which can help mitigate oxidative stress and inflammation at the site of bone injury or defect (Shemshad et al., 2018). Oxidative stress is known to impede the healing process and can lead to tissue damage (Nonami & Tsutsumi, 1999). Additionally, cerium-doped diopside has shown potential in enhancing

osteogenic differentiation of mesenchymal stem cells, further facilitating bone formation.

Nitrate, another dopant used in diopside, it has been found to enhance the bioactivity of ceramic materials and promote cell adhesion and proliferation. Incorporating lanthanum ions into diopside can improve its osteo-conductivity, leading to better integration with surrounding bone tissue and more effective stimulation of bone formation (Fredholm et al., 2011). Furthermore, lanthanum-doped diopside has shown promise in modulating the release kinetics of therapeutic agents, such as growth factors or antibiotics, which can be loaded onto or encapsulated within the biomaterial (Zreiqat et al., 2010).

In conclusion, the undoped diopside, a naturally occurring mineral, and doped diopside, where dopants like cerium nitrate or lanthanum nitrate are incorporated, both hold significant promise as alternatives to metal bone implants in bone regeneration (Sriranganathan et al., 2015). Undoped diopside offers excellent biocompatibility, osteo-conductivity, and biodegradability, making it an ideal scaffold material for promoting bone growth and repair. Its natural composition mimics that of bone, facilitating seamless integration with surrounding tissue. Doped diopside enhances these properties further by introducing specific functionalities (Jafari Baghjehaz & Salahinejad, 2017b). For instance, cerium-doped diopside exhibits antioxidant properties, reducing oxidative stress and inflammation, while lanthanum-doped diopside enhances bioactivity and controlled release of therapeutic agents (Hosseini et al., 2016). These materials offer a versatile platform for developing implants and scaffolds tailored to the unique needs of each patient, ultimately providing a more effective and sustainable solution for bone regeneration compared to traditional metal implants (Palazzo et al., 2014).

2. LITERATURE SURVEY

2.1 DIOPSIDE

(Shemshad et al., 2018) A study developed chitosan-hydroxyapatite-diopside (CS-HA-DP) scaffolds, combining biodegradable polymers and bioactive ceramics for tissue engineering. Diopside nanoparticles were synthesized via solgel method. Scaffolds, cross-linked with tripolyphosphate (TPP), showed enhanced mechanical strength. Varying ratios of diopside-hydroxyapatite-chitosan affected scaffold properties, with 5% diopside demonstrating highest mechanical strength. Porosity decreased from 92% to 76% with increasing diopside content. Cytocompatibility tests with human osteoblast cells revealed no toxicity and cell attachment within scaffolds, indicating potential for tissue engineering.

(Choudhary et al., 2019) Mesoporous nano diopside ($\text{CaMgSi}_2\text{O}_6$) was synthesized via a cost-effective sol-gel combustion route using eggshell, glycine, magnesium nitrate, and nitric acid. Optimization at 1100°C yielded pure diopside. Rietveld refinement confirmed phase purity, with 36 nm particles and $51 \text{ m}^2/\text{g}$ specific surface area. EDX analysis verified elemental composition, while dissolution in simulated body fluid indicated apatite deposition. FT-IR spectra showed phosphate bands, suggesting hydroxyapatite formation. Diopside exhibited superior mechanical stability to cancellous bone. Release of Ca^{2+} and Mg^{2+} ions increased pH, inhibiting bacterial growth, suggesting potential antibacterial activity.

(Wu et al., 2010) This study focuses on preparing and evaluating bioactive diopside ($\text{CaMgSi}_2\text{O}_6$) scaffolds for bone repair. Analysis via X-ray diffraction, SEM, micro-CT, and energy-dispersive spectrometry confirmed composition, microstructure, and pore interconnectivity. Mechanical properties and degradation were assessed through compressive strength testing and silicon ion release. Diopside scaffolds exhibited high porosity (97%) with varied compressive strength, surpassing bio-glass and CaSiO_3 scaffolds. Apatite-forming ability in simulated body fluids and support for human osteoblastic-like cell attachment, proliferation, and differentiation were demonstrated. Results highlight diopside scaffolds' potential for bone tissue

engineering, offering excellent pore structure, improved mechanical strength, and bioactivity.

(Kumar et al., 2014) In this study, rice straw-derived Diopside ($\text{CaMgSi}_2\text{O}_6$) particles were synthesized economically and incorporated into chitosan (CS) matrix to create CS/Dp scaffolds for bone tissue engineering. Physico chemical properties were evaluated via SEM, EDS, XRD, and FT-IR, showing decreased water retention but unchanged degradation properties. CS/Dp scaffolds exhibited protein adsorption affinity and demonstrated bioactivity through apatite formation. Compatibility with human osteoblastic cells (MG-63) was observed, with cells attaching, proliferating, and expressing osteoblast differentiation markers. In vivo rat model confirmed biocompatibility and potential for cell recruitment via collagen deposition. Overall, CS/Dp scaffolds show promise for bone tissue engineering applications.

2.2 DOPANTS USED FOR DIOPSIDE

(Razavi et al., 2014) Magnesium alloys are promising for orthopedic implants but can degrade too quickly. To enhance corrosion resistance and bioactivity, a nanostructured diopside ($\text{CaMgSi}_2\text{O}_6$) film was coated on AZ91 magnesium alloy via combined micro-arc oxidation (MAO) and electrophoretic deposition (EPD). XRD, SEM, and EDS characterized coated and uncoated substrates, revealing enhanced corrosion resistance and bioactivity with the diopside coating. Polarization, impedance spectroscopy, and SBF immersion tests validated these improvements. The study demonstrates that the diopside coating on MAO layer enhances corrosion resistance and in vitro bioactivity of biodegradable magnesium alloys, making them more suitable for bone healing procedures.

(Jafari Baghjeghaz & Salahinejad, 2017) Diopside ($\text{CaMgSi}_2\text{O}_6$) was doped with fluoride at a 1 mol% level using a wet chemical precipitation method, resulting in the absence of any secondary phase formation. The sintered structure of the synthesized nano powders was analysed using X-ray diffraction, Fourier transform infrared spectroscopy, and field-emission scanning electron microscopy. In vitro assessment of apatite-forming ability in simulated body fluid was conducted through electron microscopy, inductively coupled plasma spectroscopy, and Fourier

transform infrared spectroscopy. Results indicated improved sinter ability with fluoride doping, evidenced by enhanced development of sintering necks. Furthermore, the doped sample exhibited a higher deposition of apatite compared to the undoped bio-ceramic. The study concludes that fluoride doping in magnesium-containing silicates has the potential to enhance biological properties, particularly bioactivity.

(Shahrouzifar & Salahinejad, 2019) Nanostructured diopside powders were synthesized using inorganic-salt coprecipitation, with 2 mol% strontium substitution for calcium. The sponge replication technique produced highly porous scaffolds. Characterization via XRD, FTIR, SEM/EDS, densitometry, and ICP analyses revealed improved sinter ability and apatite formation with Sr-doped scaffolds, albeit slower biodegradation. Mesenchymal stem cells exhibited enhanced adhesion and spreading on Sr-doped scaffolds compared to pure ones, indicating improved bioactivity and biocompatibility. Strontium doping emerges as a viable strategy to enhance Mg-Ca silicate scaffold properties for biomedical applications.

(Kaur et al., 2020) The study investigates the effect of SrO doping in $\text{SiO}_2\text{--CaO--MgO--P}_2\text{O}_5$ sol-gel derived bio-ceramics, varying SrO content from 0 to 5 mol%. Structural and biological properties were assessed using XRD, FTIR, degradability in SBF, BET, BJH, and antioxidant activity against H_2O_2 -induced oxidative stress via cell culture studies. FESEM-EDX was used for morphological studies. Results show that strontium doping does not hinder hydroxyapatite precipitation, and samples possess negative surface charge conducive to osteoblast binding. MTT assay on MG-63 cells indicates significant cell survival, while samples with higher strontium concentration exhibit maximum protection against oxidative stress. Overall, SrO-doped bio-ceramics demonstrate promising physical and biological properties, highlighting their potential as successful implant materials.

(Gao et al., 2019) DFT analysis explored RE (La, Ce, Pr, Nd) doping effects on $\text{CaMgSi}_2\text{O}_6$ diopside. RE atoms preferentially replace Ca, contributing more valence electrons, enhancing charge accumulation. Mechanical properties, assessed via elastic constants, show La decreases while others increase bulk, shear, and Young's moduli. Anisotropy reduction from RE doping suppresses micro-crack generation.

2.3 BONE REGENERATIVE APPLICATION OF DIOPSIDE

(Srinath et al., 2021) The study aims to produce low-temperature diopside ($\text{CaMgSi}_2\text{O}_6$) ceramics from rice husk ash and eggshell waste, investigating their physico-mechanical and in vitro biological properties. XRD, TG-DTA, SEM, FTIR, and EDS analyzed crystalline phase, thermal behavior, microstructure, functional groups, and composition. Degradation and mechanical stability were assessed in dynamic SBF mode, while bioactivity was tested by apatite mineralization in static SBF mode. Cytocompatibility with human osteoblast-like cells and proliferation were evaluated using an MTT assay. Results showed successful attainment of pure phase diopside at 800°C with mechanical properties akin to human cortical bone and enhanced stability. Apatite growth on the diopside surface in SBF and excellent biocompatibility with MG-63 cells were observed, indicating potential for cost-effective orthopedic applications.

(Sheikh et al., 2015) This highlights recent advancements in biodegradable materials for bone repair, emphasizing the careful consideration needed when choosing between degradable and non-degradable devices for orthopedic and maxillofacial applications. While traditional biodegradable devices have shown success in low to mild load-bearing scenarios, ongoing research in material science has led to the development of biomaterials with enhanced strength and mechanical properties. Polymers, ceramics, and magnesium alloys have emerged as promising options for osteologic repair. Future biodegradable materials must focus on improving cell-material interactions and better control of interfacing with surrounding bone tissue. Additionally, enhancements in mechanical properties and degradation profiles are crucial to expand their clinical utility and achieve better outcomes in bone repair and regeneration applications.

(Li et al., 2021) Osteoporosis, driven by osteoclast activation, leads to bone defects, highlighting the need for biomaterials with antioxidant properties to modulate osteoclast activity. Manganese (Mn) supplementation has shown potential in treating osteoporosis, yet its effect on osteoclasts and osteoporotic bone defects remains unclear. In this study, Mn-contained β -tricalcium phosphate (Mn-TCP) bio-

ceramics were developed, demonstrating improved scavenging of oxygen and nitrogen radicals. In vitro and in vivo results revealed that Mn^{2+} ions released from Mn-TCP inhibited osteoclast formation and function while promoting osteoblast differentiation and bone regeneration under osteoporotic conditions. Mechanistically, Mn-TCP inhibited osteoclastogenesis and enhanced bone regeneration through ROS scavenging via Nrf2 activation. These findings suggest that Mn-containing bio-ceramics with antioxidant and osteoconductive properties could serve as an effective biomaterial for treating osteoporotic bone defects.

(Wu et al., 2010) This study aimed to produce low-temperature diopside ceramics using rice husk ash and eggshells, evaluating physio-mechanical and biological properties. Techniques such as XRD, TG-DTA, SEM, FTIR, and EDS were employed for characterization. Diopside ceramics exhibited pure phase at 800°C, demonstrating good mechanical properties akin to human cortical bone and enhanced stability. Surface apatite mineralization in simulated body fluid (SBF) indicated bioactivity, while cytocompatibility with MG-63 cells was confirmed through MTT assay. The findings suggest the potential of these cost-effective diopside ceramics for orthopedic applications.

3. AIM AND SCOPE

3.1 AIM

To enhance the regenerative potential of diopside pellet through the incorporation of dopants such as cerium nitrate and lanthanum nitrate for bone regeneration applications.

3.2 SCOPE

- Evaluate the osteo-conductivity of diopside pellets in promoting cell adhesion, proliferation, and differentiation.
- Investigate the degradation kinetics of diopside and its influence on new bone formation.
- Explore the mechanical properties of undoped diopside scaffolds and their suitability for load-bearing applications in bone regeneration.
- Examine the in vivo performance of undoped diopside implants in preclinical models of bone defects or fractures.
- Investigate the effects of dopants on the biological properties of diopside pellets.
- Evaluate the biocompatibility and degradation behaviour of undoped and doped diopside pellets in vitro and in vivo.

4. MATERIALS AND METHODS

4.1 MATERIALS

- Calcium nitrate (CaNO_3)
- Magnesium nitrate (MgNO_3)
- Cerium nitrate (CeNO_3)
- Lanthanum nitrate (LaNO_3)
- Tetraethyl orthosilicate (TEOS)
- Ethanol
- Distilled water
- Ammonia solution
- Stimulating body fluid (SBF)
- Bovine serum albumin (BSA)
- 2% SDS
- DPPH
- Methanol
- Blood
- Saline solution
- Nutrient agar
- DMSO
- UV-Vis Spectrophotometer
- Rotary shaker
- Magnetic stirrer
- Petri plate
- Well plates

4.2 METHODOLOGY

4.2.1 SYNTHESIS OF UNDOPED DIOPSIDE

Synthesis of the undoped diopside powder was done by mixing an equimolar amount of CaNO_3 (0.125 mol) and MgNO_3 (0.125 mol). CaNO_3 and MgNO_3 were dissolved in 300ml ethanol for 30 minutes and kept in an ice-water bath. After 30 minutes, 0.25 mol of TEOS was added to the mixture. The required amount of ammonia was added to the above solution which was kept under magnetic stirring to reach the $\text{pH}=10$ (Rahmani & Salahinejad, 2018). After adding the ammonia solution, a white precipitate was obtained. The solution was kept in a hot air oven for 24 hours to get dry powder. Then the obtained powder was calcinated at 700°C for 2 hours. The dried powder was compressed with a hydraulic pressure of 300 MPa to make a 10 mm pellet using a pelletizer machine (Rahmani & Salahinejad, 2018).

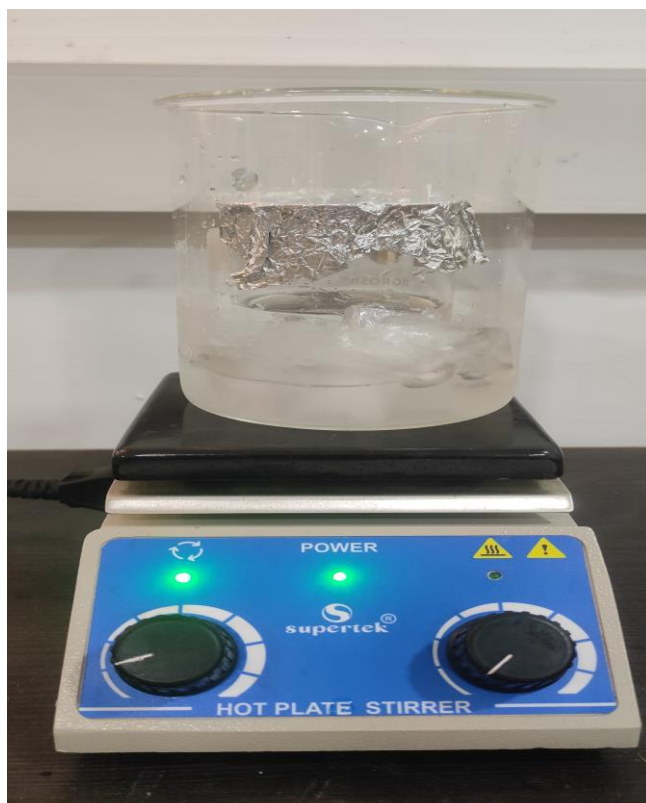


Fig.4.1: Synthesis of Undoped diopside in ice water bath



Fig.4.2: Undoped diopside solution

4.2.2 SYNTHESIS OF DOPED DIOPSIDE

Synthesis of the doped diopside was made using two dopants such as cerium nitrate (CeNO_3) and lanthanum nitrate (LaNO_3) in three different ratios.

4.2.2.1 Synthesis of Cerium nitrate doped diopside

Synthesis of the doped diopside powder with cerium nitrate was done in three different ratios such as (Ca: Ce - 20:80), (Ca: Ce - 50:50), and (Ca: Ce - 80:20). For making doped diopside in (20:80) ratio, 0.125 mol of CaNO_3 and 0.1 mol of CeNO_3 were taken. For making doped diopside in (50:50) ratio, 0.0625 mol of CaNO_3 and 0.0625 mol of CeNO_3 were taken. For making doped diopside in (80:20) ratio, 0.1 mol of CaNO_3 and 0.125 mol of CeNO_3 were taken. CaNO_3 and CeNO_3 were dissolved in 300ml ethanol for 30 minutes and kept in an ice-water bath (Gao et al., 2019). Then 0.125 mol of MgNO_3 was added to the mixture. After 30 minutes, 0.25 mol of TEOS was added to the mixture. The required amount of ammonia was added to the above solution which was kept under magnetic stirring to reach the $\text{pH}=10$. After adding the ammonia solution, a white precipitate was obtained. The solutions were kept in a hot air oven for 24 hours to get dry powder. Then the obtained powder was calcinated at 700°C for 2 hours. The dried powder was

compressed with a hydraulic pressure of 300 MPa to make a 10 mm pellet using a pelletizer machine (Titorenkova et al., 2022).



Fig.4.3: (Ca: Ce - 20:80) diopside solution



Fig.4.4: (Ca: Ce - 50:50) diopside solution

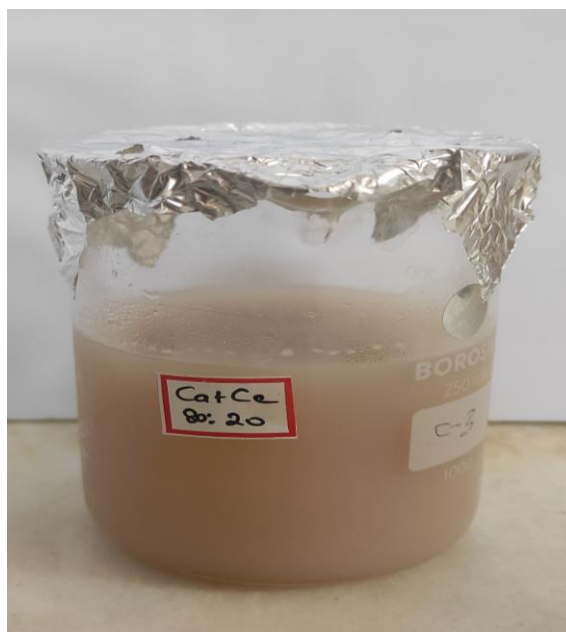


Fig.4.5: (Ca: Ce - 80:20) diopside solution

4.2.2.2 Synthesis of Lanthanum nitrate doped diopside

Synthesis of the doped diopside powder with lanthanum nitrate was done in three different ratios such as (Mg: La - 20:80), (Mg: La - 50:50), and (Mg: La - 80:20). For making doped diopside in (20:80) ratio, 0.125 mol of MgNO_3 and 0.1 mol of LaNO_3 were taken (Ghosh et al., 2023). For making doped diopside in (50:50) ratio, 0.0625 mol of MgNO_3 and 0.0625 mol of LaNO_3 were taken. For making doped diopside in (80:20) ratio, 0.1 mol of MgNO_3 and 0.125 mol of LaNO_3 were taken. MgNO_3 and LaNO_3 were dissolved in 300ml ethanol for 30 minutes and kept in an ice-water bath. Then 0.125 mol of CaNO_3 was added to the mixture. After 30 minutes, 0.25 mol of TEOS was added to the mixture. The required amount of ammonia was added to the above solution which was kept under magnetic stirring to reach the $\text{pH}=10$. After adding the ammonia solution, a white precipitate was obtained. The solutions were kept in a hot air oven for 24 hours to get dry powder. Then the obtained powder was calcinated at 700°C for 2 hours. The dried powder was compressed with a hydraulic pressure of 300 MPa to make a 10 mm pellet using a pelletizer machine (Deng et al., 2019).

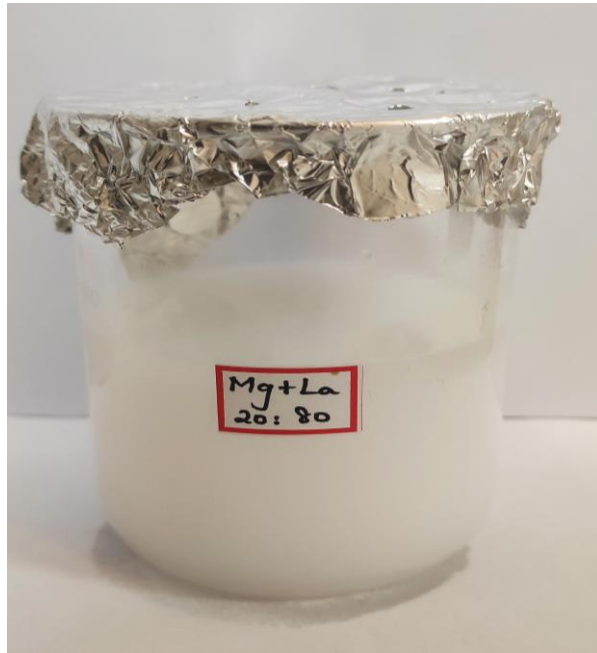


Fig.4.6: (Mg: La - 20:80) diopside solution

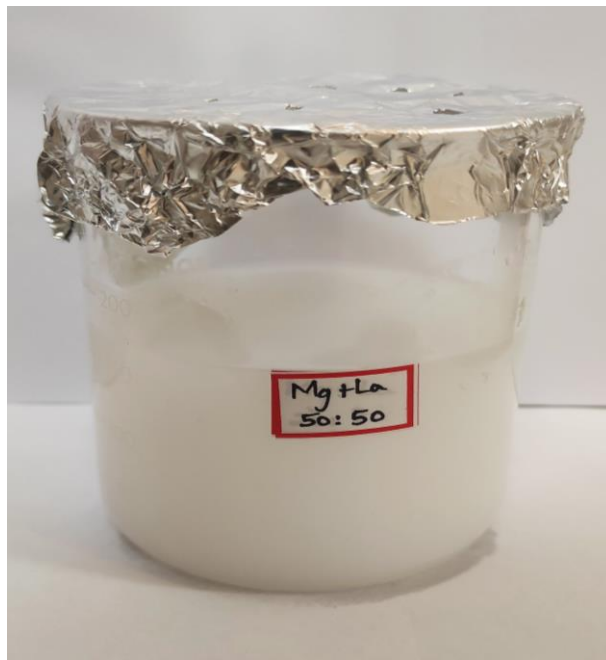


Fig.4.7: (Mg: La - 50:50) diopside solution

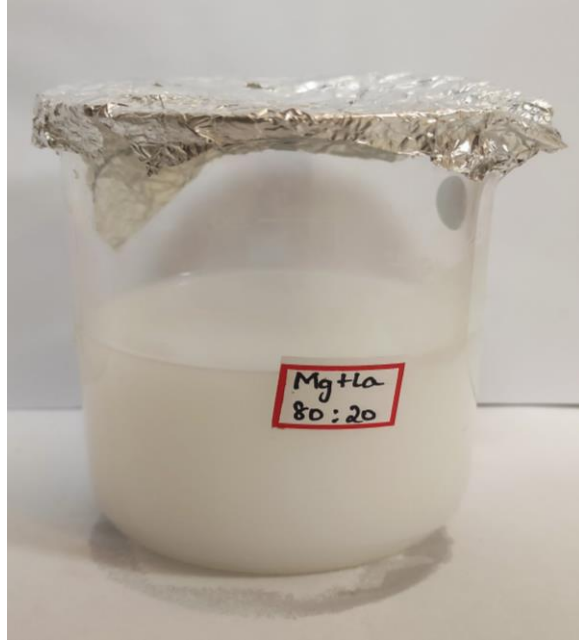


Fig.4.8: (Mg: La - 80:20) diopside solution

4.2.3 BIODEGRADATION TEST

The biodegradation test was done by using the undoped and doped diopside pellets. The pellets were immersed in simulated body fluid (SBF) and then were incubated at 37°C in a closed well plate for 42 days (Juraski et al., 2017). The SBF was changed after every 7 days of incubation. The degradation rate was measured once a week by using the following equation where W_o was denoted as the initial weight of the pellets and W_t was denoted as the weight of pellets after soaking in SBF (Razavi et al., 2014b).

$$\text{Weight loss (\%)} = \frac{W_o - W_t}{W_o} \times 100$$



Fig.4.9: Biodegradation test of pellets in SBF

4.2.4 BIOMINERALIZATION TEST

The biomineralization of undoped and doped diopside pellets was done by immersing the pellets in the simulated body fluid (SBF) solution and then were incubated at 37°C in a closed well plate for 30 days in a CO₂ incubator. The SBF solution was prepared according to a report by Kokubo et al (Collin et al., 2021). After the mentioned time, the pellets were washed three times with deionized water for the removal of the adsorbed minerals and were later dried in an air oven and characterized using SEM to determine the deposition of the minerals and hydroxyapatite formation on the surface of the pellets (Salahinejad & Jafari Baghjehaz, 2017).



Fig.4.10: Pellets immersed in SBF for 30 days

4.2.5 PROTEIN ADSORPTION TEST

The undoped and doped diopside pellet surfaces were equilibrated in isotonic Tris-buffer, pH 7.4, for 20 hours. The adsorption experiments were carried out under static conditions at room temperature. 2 ml of bovine serum albumin (BSA) was taken into a 5 ml sterile screw cap bottle and immersed 10 mm diameter pellets of undoped, (Ca: Ce - 20:80), (Ca: Ce - 50:50), (Ca: Ce - 80:20), (Mg: La - 20:80), (Mg: La - 50:50), and (Mg: La - 80:20) for 3 hours (Karimi et al., 2018). Then the pellets were rinsed with Tris-buffer, following rinsing, pellets were exposed to 2% sodium dodecyl sulfate (SDS) solution for 24 hours to elute the adsorbed proteins. The eluates were stored at -20°C. The eluted protein concentration was estimated by

Lowry's method or by nanodrop spectrophotometer and was done in duplicates (Choudhary et al., 2016).

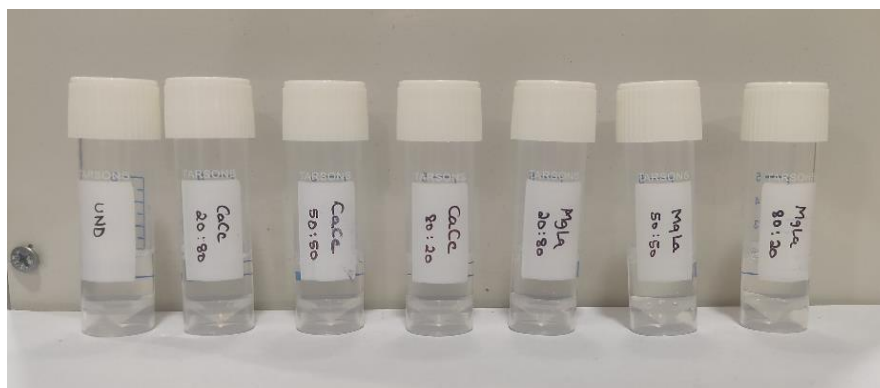


Fig.4.11: Diopside pellets elutes for protein adsorption

4.2.6 ANTIOXIDANT ASSAY

An antioxidant test was performed using a DPPH radical scavenging assay for the diopside pellets. A modified method of (Kumar et al., 2018) was used. In brief, equal-weight pellets were added to each test tube. 1 ml of 0.1 mM DPPH solution was added to all the test tubes and made up to 2 ml with methanol. They were allowed to stand for 30 min in the dark before the absorbance was read at 517 nm. This experiment was carried out in triplicate with different concentration to find out the IC₅₀ value. The percentage of radical scavenging activity was determined with the following equation (Kapoor et al., 2015).

$$\text{RSA\%} = \left[\frac{(\text{Control absorbance} - \text{Test absorbance})}{\text{Control absorbance}} \right] \times 100$$

Where blank is methanol, control absorbance corresponds to the absorbance of the DPPH solution and the test absorbance represents the absorbance of the test samples (Mahheidari et al., 2024).

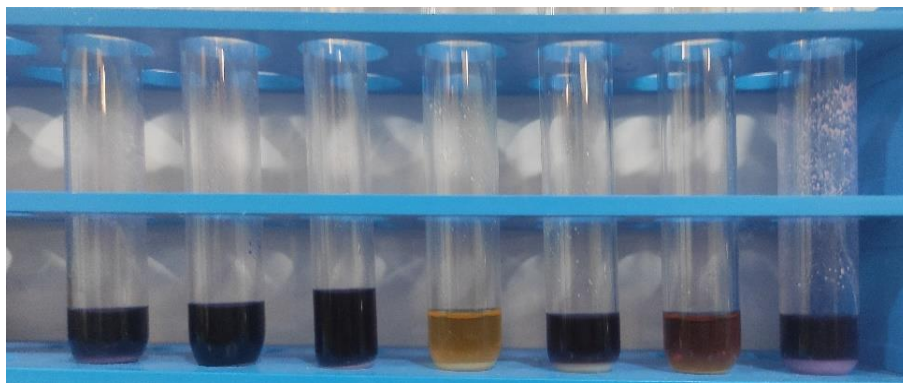


Fig.4.12: Diopside pellets incubated in DPPH solution to determine antioxidant activity

4.2.7 HEMOLYSIS ASSAY

The hemolytic activity of the pellets was investigated by the following protocol. A sterile saline solution (1.25 ml) was added to 1 mg of both undoped and doped diopside pellets and 4 ml blood was collected from a healthy human and diluted with 5 ml of sterile saline solution; 20 μ l of the diluted blood was then added to the tubes containing 1 mg/ml of pellets in sterile saline solution and incubated at 37°C for 30 min. Subsequently, the tubes were incubated for 60 min in a water bath shaker at 37°C and centrifuged at 7000 rpm for 10 min (Alagarsamy et al., 2018). Sterile saline solution and distilled water were used as the negative and positive controls, respectively. The amount of free haemoglobin was determined by measuring the absorbance of the supernatant at 540 nm by using a UV Spectrophotometer (JASCO J657 made in Japan). This experiment was carried out in duplicate with different concentration. The hemolysis rate (HR) was calculated using the formula (Mothilal et al., 2024)

$$\text{HR \%} = \frac{\text{Dt} - \text{Dnc}}{\text{Dpc} - \text{Dnc}} \times 100$$

Where Dt, Dnc, and Dpc are the absorbance of the test sample, negative control, and positive control, respectively.

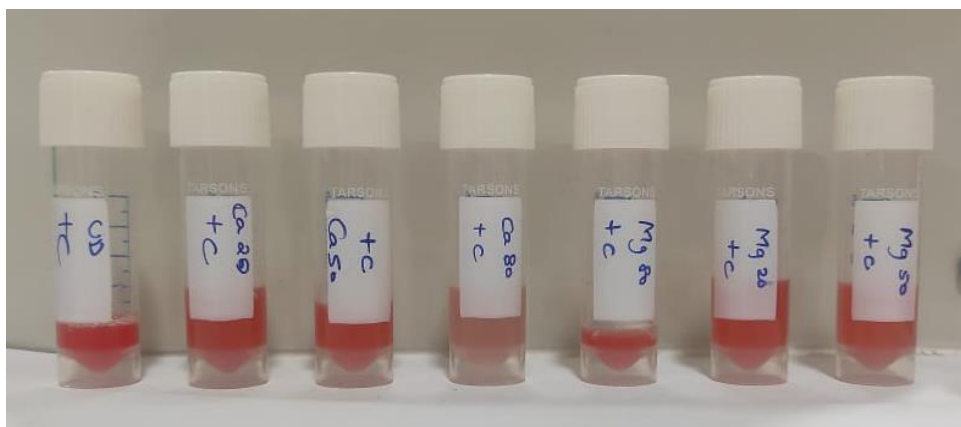


Fig.4.13: Diopside pellets incubated in blood for hemolysis test

4.2.8 ANTIMICROBIAL TEST

50 μ g and 100 μ g concentrations of undoped, (Ca: Ce - 20:80), (Ca: Ce - 50:50), (Ca: Ce - 80:20), (Mg: La - 20:80), (Mg: La - 50:50), and (Mg: La - 80:20) powders were used for testing the antimicrobial activity. The Agar disk diffusion test was performed by Muller–Hinton Agar (Shuai et al., 2014). This technique was carried out with 4 mm thick layers of nutrient agar wherein two different bacterial cultures *E.Coli* and *Staphylococcus aureus* were swabbed on the agar plates. Petri plates were left for 10 min to dry and after drying, the different concentrations of pellets were arranged on the Agar and incubated for 24 hours at 37 °C. After incubation, the zone of inhibition was measured and tabulated (Ghosh et al., 2024).

4.2.9 CYTOTOXICITY TEST

The fibroblast cells procured from NCCS, Pune, India, were used for in vitro cell proliferation and MTT assay. MTT (3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyl tetrazolium bromide) was procured from Sigma Aldrich (Alagarsamy et al., 2022). Fibroblast cultures were maintained in Minimal Essential Medium (MEM) supplemented with 10% Fetal Bovine Serum (FBS), 200 mM Glutamine, 2 mg/ml sodium bicarbonate, and 1X antibiotic and antimycotic solution and incubated at 37 °C in a humidified atmosphere of 5% CO₂. Periodically, the medium was replaced. The cells were seeded at the density of 0.5×10^6 cells per well and incubated at 37 °C. 0.010 grams of pellets were weighed and soaked in 500 μ l of the MEM medium for 24 hours. The supernatant as a conditioned medium was taken and it was added

to the cells-containing wells at 50 μ l and they were incubated for up to 3 days. After 24 hours of incubation, the media of each well were replaced with MTT diluted in a serum-free medium, and the plates were incubated at 37 °C for 24 hours, 48 hours and 72 hours (Grela et al., 2018). After removing the MTT solution, Dimethyl Sulfoxide (DMSO) was used to dissolve the formazan crystals and optical densities (OD) were determined using the spectrophotometer at 570 nm. Each experiment was performed in triplicates (Rittidach et al., 2023).

4.2.10 CHARACTERIZATION OF DIOPSIDE

4.2.10.1 FTIR ANALYSIS

FTIR (Fourier Transform Infrared Spectroscopy) analysis of diopside powder is a common technique used to characterize its chemical composition and structural properties (Kansal et al., 2010). Diopside powder exhibits a characteristic infrared absorption band corresponding to the vibrational modes of the bonds within its crystal lattice. KBR press was used to make thin pellets containing diopside powder and scanned over a range of 4000 to 450 cm^{-1} . The spectra were recorded using the instrument from American Perkin Elmer Co (Kazemi et al., 2017).

4.2.10.2 XRD ANALYSIS

The powder method of XRD was adopted in the present study by Smart Lab, Rigaku (9 kW) with an X-ray source of Cu K α radiation ($\lambda=1.5406$ Å). XRD is one of the vital techniques for the investigation of crystalline compounds (Otto et al., 2013). XRD analysis of the powder samples of different weight percentages of synthesized undoped, (Ca: Ce - 20:80), (Ca: Ce - 50:50), (Ca: Ce - 80:20), (Mg: La - 20:80), (Mg: La - 50:50), and (Mg: La - 80:20) were carried out at the range of 2θ angle 20° to 80°. The standard JCPDS database for the XRD pattern was used for phase identification for a large variety of crystalline phases in undoped and doped diopside (Salama et al., 2005).

4.2.10.3 SEM ANALYSIS

The size and morphological features of undoped and doped diopside particles were observed by SEM analysis. The prepared diopside particles were dispersed in ethanol, sonicated, coated on a cover slip, and subjected to SEM analysis (HR SEM Quanta 200 FEG Instrument, the Netherlands) (Goel et al., 2007). The energy dispersive X-ray spectroscopy (EDS) was performed using JEOL JSM 6490 LA. The samples were placed on a carbon tape-coated stub and coated with platinum using JEOL JFC 1600 for 2 min at 10 mA (Wu et al., 2010b).

5. RESULTS AND DISCUSSION

5.1 PELLETIZING OF DIOPSIDE POWDER AND SINTERING

The synthesised diopside solution had a wet gel like nature which was kept in hot air oven at 100°C for 24 hours. Then the powder was grinded using mortar and pestle to make fine powders of diopside. Then using a pelletizer instrument, the diopside powder was made into pellets of 10mm size and calcinated in heating furnace at 700°C for 2 hours.



Fig.5.1: Diopside solution kept in hot air oven



Fig.5.2: Pelletizing the diopside powder and sintering

5.2 BIODEGRADATION TEST

The degradation kinetic was expected to synchronize the newly formed bone tissue. Hence, the evaluation of degradation of diopside pellets was of importance before further clinical application. The weight loss of the pellets was measured to estimate the biodegradability level by immersing the scaffolds in SFB for a period of 7, 14, 21, 28, 35 and 42 days. The pellets showed a weight gain after the 1st week of immersion. The same weight was maintained for 4 weeks and no sign of degradation. For all the pellets immersed in SBF, it showed degradation after the 5th week of immersion. The diopside pellets showed a decreased level of deterioration throughout the subsequent time, which could be advantageous since bone replacement material must biodegrade gradually at a slow rate during the regeneration period. This slow degradation of pellets confirms the stability of the pellets. From the statistical analysis table, the p-value of an ANOVA test is 0.045 for a biodegradation test of diopside pellets, it suggests that there is a statistically significant difference in biodegradation among the different diopside pellets being compared. There is enough evidence to reject the null hypothesis. Therefore, the conclusion would be that the type of diopside pellets does have a significant effect on biodegradation.

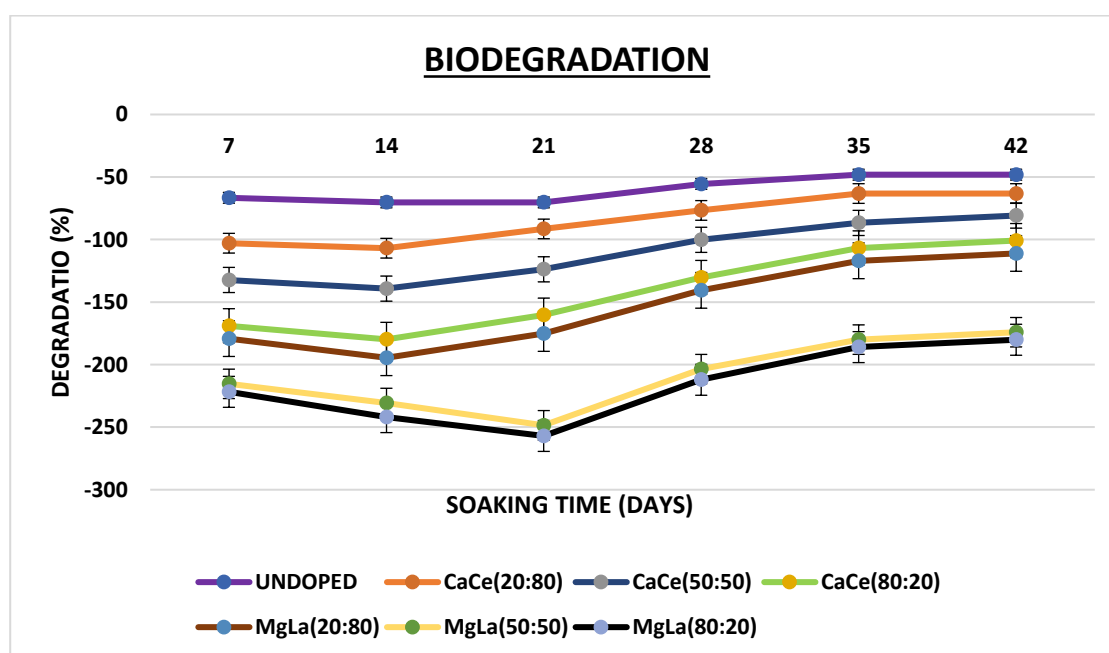


Fig.5.3: Graphical representation of biodegradation rate of undoped and doped diopside pellets in SBF

Table.5.1: Statistical analysis of Biodegradation test of diopside pellets

SUMMARY				
Groups	Count	Sum	Average	Variance
Undoped	6	-358.9	-59.8167	111.8017
CaCe20:80	6	-145.6	-24.2667	93.61867
CaCe50:50	6	-185	-30.8333	78.26667
CaCe80:20	6	-159.4	-26.5667	30.51867
MgLa20:80	6	-71.4	-11.9	4.724
MgLa50:50	6	-335.2	-55.8667	245.0777
MgLa80:20	6	-46.97	-7.82833	4.441217

ANOVA						
Source of Variation	SS	Df	MS	F	P-value	F crit
Between Groups	14493.04	6	2415.507	29.7450	0.0456	2.371781
Within Groups	2842.243	35	81.20694			
Total	17335.28	41				

5.3 BIOMINERALIZATION TEST

The biomineralization potential of diopside bio-ceramic revealed upon exposure to simulated biological fluid. Diopside exhibited mineral formation on the surface of the pellets after immersing in SFB for 30 days. This suggests a significant interaction between the diopside and the surrounding environment, conducive to mineral deposition. This confirms that when the diopside pellets are used as a bone implant it enhances the bone forming by the deposition of hydroxy apatite layer formation on the surface.

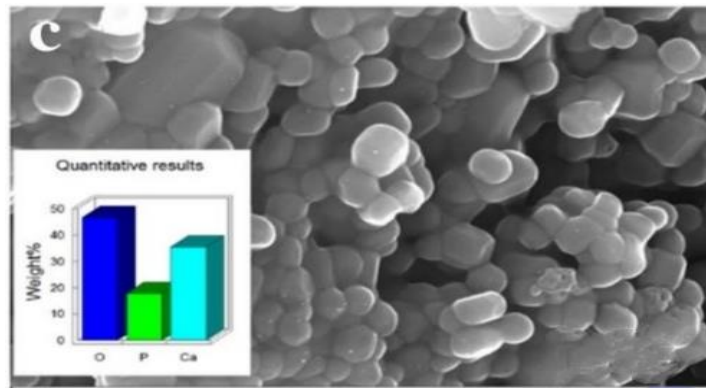


Fig.5.4: SEM analysis of HAP deposition on diopside pellets

5.4 PROTEIN ADSORPTION

The most vital protein albumin is accountable for several processes, including the transfer of molecules, cell adherence, and proliferation. Albumin protein is advantageous in the process of regeneration in bone. Hence, the capacity to absorb the active proteins is essential for the attachment of cells and their development in the extracellular matrix. It determines the amount of protein absorbed in the different diopside pellet samples at a time duration of 24 hours. From the result, the diopside pellets doped with cerium nitrate in the ratio (Ca: Ce – 20:80) showed a greater level of protein adsorption when compared to the other pellets. This is because of the incorporation of higher amount of cerium ions which absorbed more protein on the pellet surface. The (Ca: Ce – 20:80) diopside showed 1.352 mg/ml as maximum protein absorption at 24 hours. From the statistical analysis table, the p-value of an ANOVA test is 0.012379 for a protein adsorption test of diopside pellets, it indicates that there is a statistically significant difference among the means of the groups being compared. It suggests that there are significant differences in protein adsorption among the diopside pellets under the conditions tested.

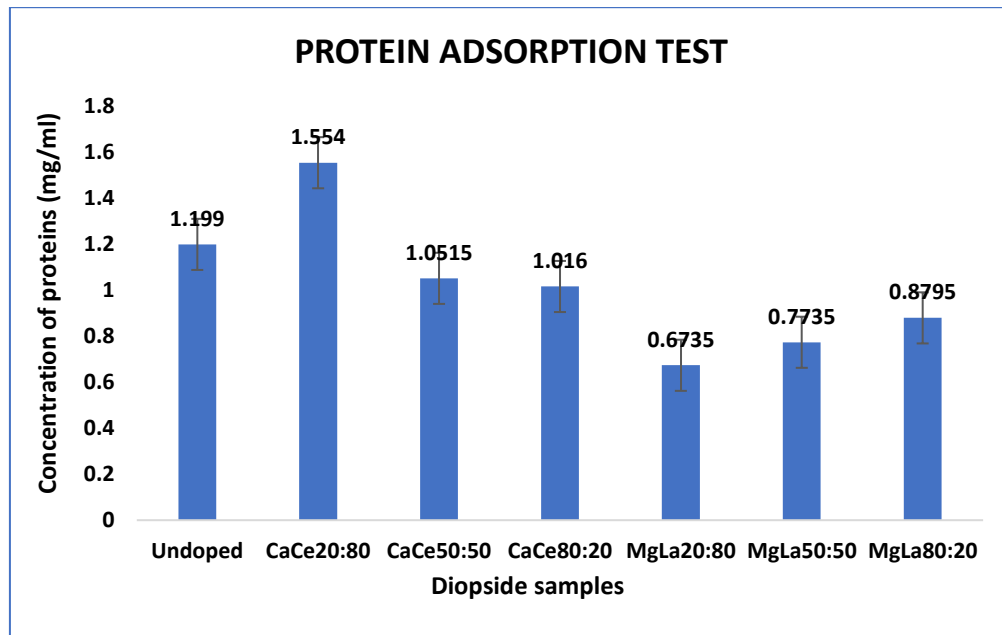


Fig.5.5: Graphical representation of Protein adsorption test of undoped and doped diopside pellets

Table.5.2: Statistical analysis of Protein adsorption test of diopside pellets

		SUMMARY		
Groups	Count	Sum	Average	Variance
Undoped	2	2.398	1.199	0.00245
CaCe20:80	2	3.108	1.554	0.081608
CaCe50:50	2	2.103	1.0515	0.008321
CaCe80:20	2	2.032	1.016	0.0242
MgLa20:80	2	1.347	0.6735	0.025313
MgLa50:50	2	1.547	0.7735	0.016744
MgLa80:20	2	1.759	0.8795	0.023113

ANOVA						
<i>Source of Variation</i>	<i>SS</i>	<i>Df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
Between Groups	1.037526	6	0.172921	6.660029	0.012379	3.865969
Within Groups	0.181748	7	0.025964			
Total	1.219274	13				

5.5 ANTIOXIDANT ASSAY

The antioxidant activity of undoped and doped diopside were assessed using DPPH as a free radical, which measures the reduction in absorbance as an indicator of antioxidant capacity. These results indicate that diopside exhibited a significant antioxidant effect, with a percentage of radical scavenging activity of 28.06%, 32.17%, 27.89%, 30.56%, 24.37%, 20.63% and 29.52% for undoped, (Ca: Ce - 20:80), (Ca: Ce - 50:50), (Ca: Ce - 80:20), (Mg: La - 20:80), (Mg: La - 50:50), and (Mg: La - 80:20) pellet samples. This suggests that diopside possesses antioxidant properties, potentially contributing to neutralize the free radical release which can prevent further damage to the tissues. From the statistical analysis table, the p-value of an ANOVA test is 0.000125 for an antioxidant test of diopside pellets, it indicates that there is a statistically significant difference in antioxidant activity among the different groups of diopside. In other words, there is strong evidence to reject the null hypothesis, which typically states that there are no differences among the groups being compared. With such a low p-value, the observed differences in antioxidant activity are unlikely to have occurred by random chance alone. Therefore, the results suggest that there are meaningful differences in antioxidant effectiveness between the diopside pellets being tested under different conditions.

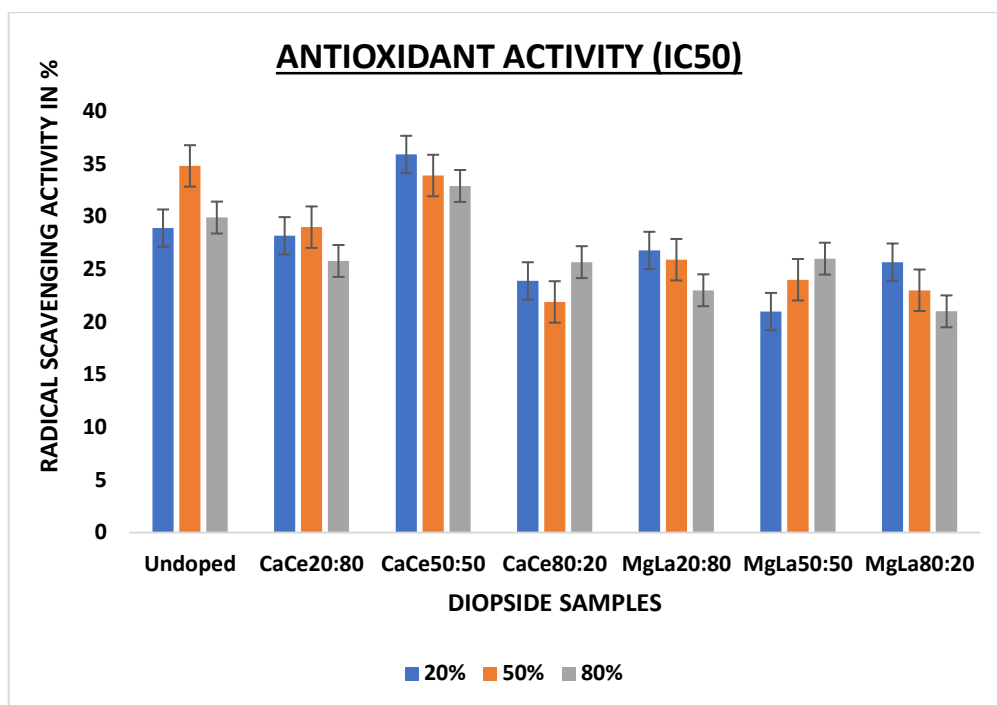


Fig.5.6: Graphical representation of Antioxidant assay of undoped and doped diopside pellets

Table.5.3: Statistical analysis of Antioxidant test of diopside pellets

SUMMARY				
Groups	Count	Sum	Average	Variance
Undoped	3	93.6	31.2	9.97
CaCe20:80	3	82.94	27.64667	2.781433
CaCe50:50	3	102.68	34.22667	2.320033
CaCe80:20	3	71.45	23.81667	3.576133
MgLa20:80	3	75.68	25.22667	3.912133
MgLa50:50	3	70.98	23.66	6.3868
MgLa80:20	3	69.67	23.22333	5.489633

ANOVA						
<i>Source of Variation</i>	<i>SS</i>	<i>Df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
Between Groups	326.9401	6	54.49001	11.0764	0.000125	2.847726
Within Groups	68.87233	14	4.919452			
Total	395.8124	20				

5.6 HEMOLYSIS TEST

The hemolytic percentage of the undoped, (Ca: Ce - 20:80), (Ca: Ce - 50:50), (Ca: Ce - 80:20), (Mg: La - 20:80), (Mg: La - 50:50), and (Mg: La - 80:20) pellets were found to be 1.2%, 1.7%, 0.8%, 1.3%, 1.6%, 1.0% and 0.9% respectively. The sample with a hemolytic rate of less than 2% is considered a non-hemolytic sample which doesn't cause any damage to the red blood cells. From the result, the undoped and doped diopside pellets were determined to be hemocompatible in nature and proved as non-hemolytic samples. From the statistical analysis of the table the p-value of an ANOVA test is 0.488687 for a hemolysis test of diopside pellets, it indicates that there is no statistically significant difference in hemolysis among the different groups being compared. In other words, there is insufficient evidence to reject the null hypothesis, which suggests that the mean hemolysis rates are equal across the groups being studied. Therefore, the conclusion would be that the type of diopside pellets does not have a significant effect on hemolysis.

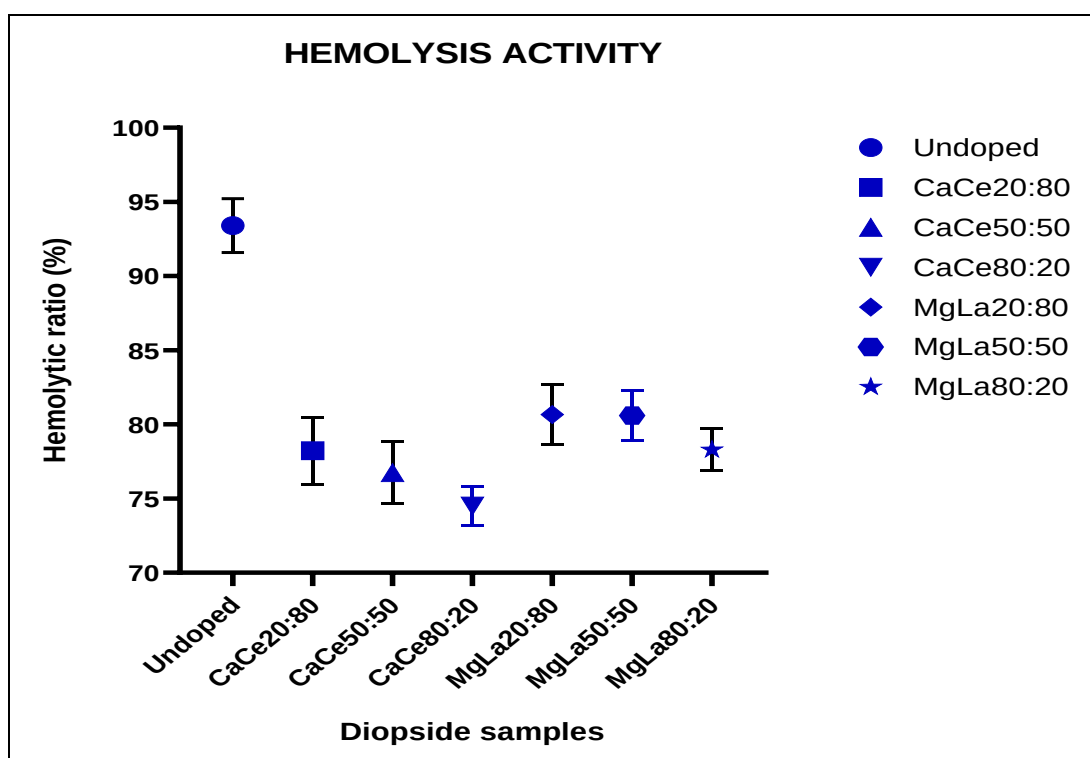


Fig.5.7: Graphical representation of Hemolysis test of undoped and doped diopside pellets

Table.5.4: Statistical analysis of hemolysis test of diopside pellets

		SUMMARY		
Groups	Count	Sum	Average	Variance
Undoped	2	2.9	1.45	0.005
CaCe20:80	2	1.9	0.95	0.605
CaCe50:50	2	2.1	1.05	0.045
CaCe80:20	2	2.8	1.4	0.08
MgLa20:80	2	1.3	0.65	0.045
MgLa50:50	2	2.4	1.2	0.18

MgLa80:20	2	2.2	1.1	0.08
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ANOVA						
<i>Source of Variation</i>	<i>SS</i>	<i>Df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
Between Groups	0.897143	6	0.149524	1.0064	0.488687	3.865969
Within Groups	1.04	7	0.148571			
Total	1.937143	13				

5.7 ANTIMICROBIAL TEST

The antimicrobial activity of the undoped, cerium nitrate doped and lanthanum nitrate doped diopside samples were evaluated against a gram positive *Staphylococcus* bacteria and a gram negative *E.Coli* bacteria by disk diffusion method. The diameter of the zone of inhibition (mm) around the diopside loaded well was recorded. The diopside powders were diluted in 50 μ g and 100 μ g concentrations to determine its efficiency against the pathogens. the undoped, cerium nitrate doped and lanthanum nitrate doped diopside samples displayed significant activity against *E.Coli* and *Staphylococcus* with a zone of inhibition of 11mm, 2mm, 2 mm and 9mm, 5mm, 5mm respectively. This inherent antimicrobial activity stems from cerium and lanthanum ability to generate reactive oxygen species (ROS) upon interaction with oxygen molecules, leading to oxidative stress and subsequent cell damage in microbial organisms.



Fig.5.8: Undoped diopside in E.Coli cultured plate

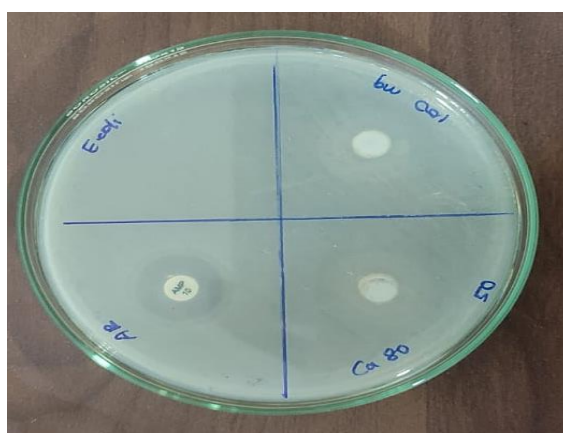


Fig.5.9: Cerium nitrate doped diopside in E.Coli cultured plate

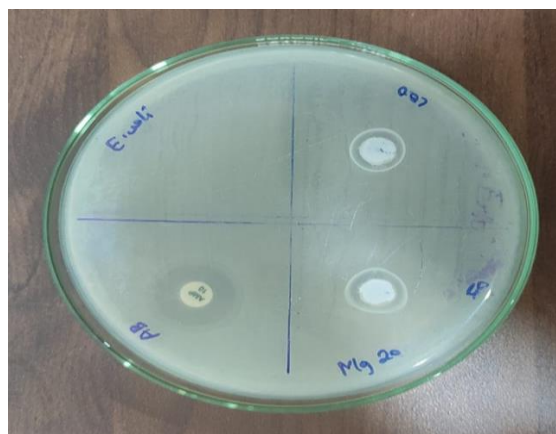


Fig.5.10: Lanthanum nitrate doped diopside in E.Coli cultured plate



Fig.5.11: Undoped diopside in Staphylococcus cultured plate

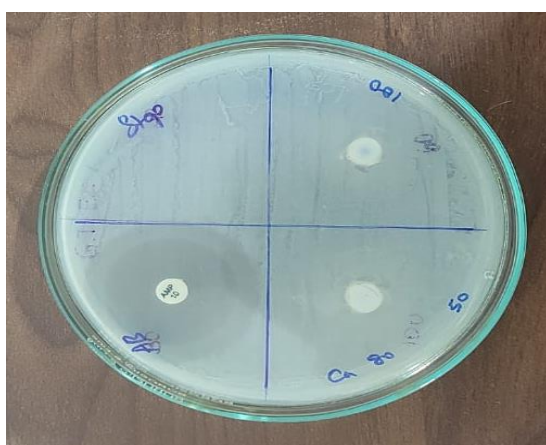


Fig.5.12: Cerium nitrate doped diopside in Staphylococcus cultured plate

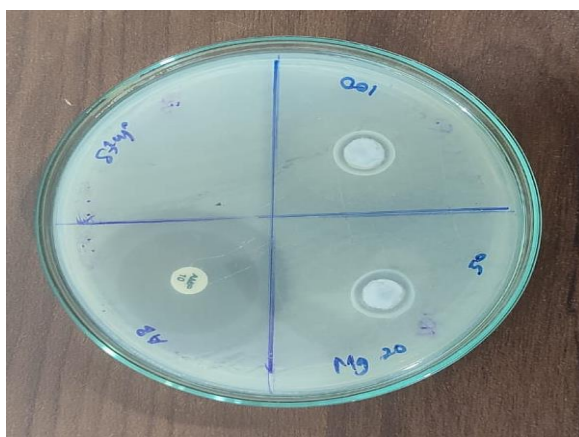


Fig.5.13: Lanthanum nitrate doped diopside in Staphylococcus cultured plate

Table.5.5: Zone of inhibition of diopside against pathogens

SAMPLES	<i>E.COLI</i> ZONE OF INHIBITION IN (mm)	<i>STAPHYLOCOCCUS</i> ZONE OF INHIBITIONIN (mm)
Undoped	11	9
Cerium nitrate doped	2	5
Lanthanum nitrate doped	2	5

5.8 CYTOTOXICITY TEST

Diopside pellets have emerged as promising materials for various biomedical applications due to their favourable mechanical properties and biocompatibility. However, comprehensive evaluation of their cytotoxicity is essential to ensure their safety for clinical use. In this study, we investigated the cytotoxicity of diopside bio-ceramics pellet using the MTT assay, aiming to provide valuable insights into their biocompatibility. Fibroblasts were cultured in the presence of diopside pellets in different concentration and cell viability was assessed using the MTT assay. The results demonstrate that diopside exhibit minimal cytotoxic effects after 24 hours, 48 hours and 72 hours, with cell viability comparable to or even higher than control samples. These findings suggest that diopside are safe and biocompatible materials for biomedical applications. From the statistical analysis from the table, the p-value of an ANOVA test for a cell viability test of diopside pellets is 0.3679, it indicates that there is no statistically significant difference in cell viability among the different diopside being compared. In other words, there is insufficient evidence to reject the null hypothesis, suggesting that the mean cell viability rates are equal across the groups being studied. Therefore, the conclusion would be that the type of undoped diopside and doped diopside pellets does not have a significant effect on cell toxicity.

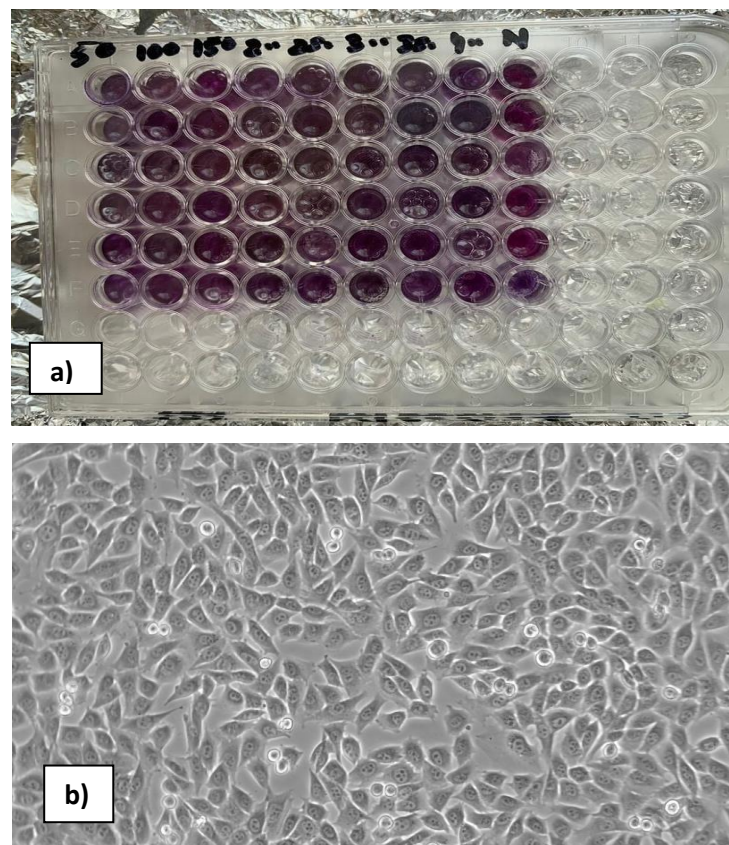


Fig.5.14: a) Cells in MTT after 24hours before taking absorbance
b) Microscopic image of cells after 24hours in MTT

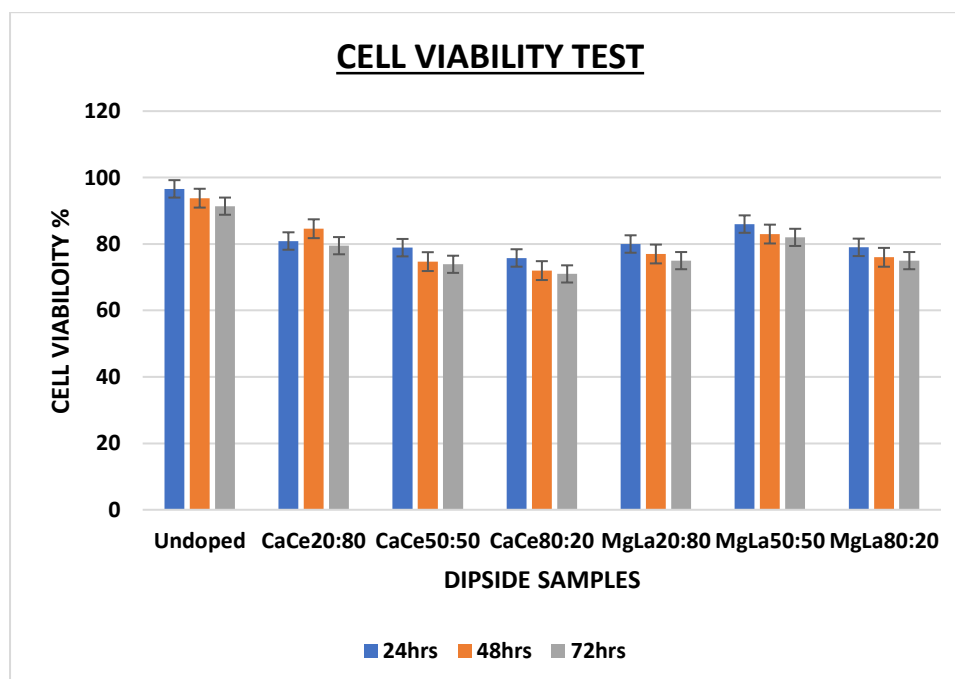


Fig.5.15: Graphical representation of Cell viability rates of diopside pellets

Table.5.6: Percentage of cell viability of the undoped and doped diopside pellets

SAMPLES	UNDOPED	CaCe 20:80	CaCe 50:50	CaCe 80:20	MgLa 20:80	MgLa 50:50	MgLa 80:20
PERCENTAGE OF CELL VIABILITY	96.2	83	81	80.4	80	83.23	80.4

Table.5.7: Statistical analysis of Cell viability test of diopside pellets

		SUMMARY		
<i>Groups</i>	<i>Count</i>	<i>Sum</i>	<i>Average</i>	<i>Variance</i>
Undoped	3	281.8	93.93333	6.773333
CaCe20:80	3	245	81.66667	6.943333
CaCe50:50	3	227.5	75.83333	7.213333
CaCe80:20	3	218.8	72.93333	6.413333
MgLa20:80	3	232	77.33333	6.333333
MgLa50:50	3	251	83.66667	4.333333
MgLa80:20	3	230	76.66667	4.333333

ANOVA						
<i>Source of Variation</i>	<i>SS</i>	<i>Df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
Between Groups	885.8714	6	147.6452	24.40801	0.3679	2.847726
Within Groups	84.68667	14	6.049048			
Total	970.5581	20				

5.9 CHARACTERIZATION

5.9.1 FTIR ANALYSIS

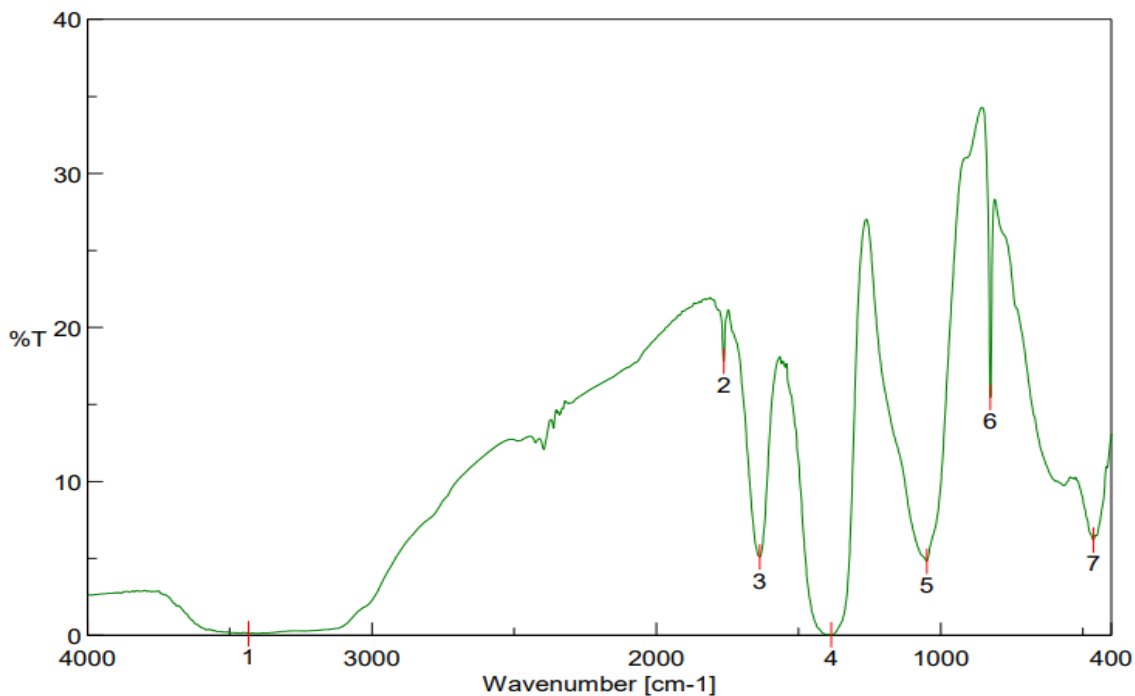


Fig.5.16: FTIR spectrum of undoped diopside

Table.5.8: FTIR analysis of Undoped diopside with functional group

PEAK NUMBER	POSITION	INTENSITY	COMPOUND NAME	BOND VIBRATION
1	3434.6	0.107673	Hydroxyl group	O-H stretching
2	1762.62	17.7977	Carbonate group	CO ₃ Stretching
3	1636.3	5.08796	Carbonate group	CO ₃ Stretching
4	1384.64	0.0276846	Si-O	Si-O stretching
5	1049.09	4.81411	Phosphate	stretching

6	825.384	15.4429	Si-O	Si-O stretching
7	462.832	6.18852	Si-O	Si-O bending

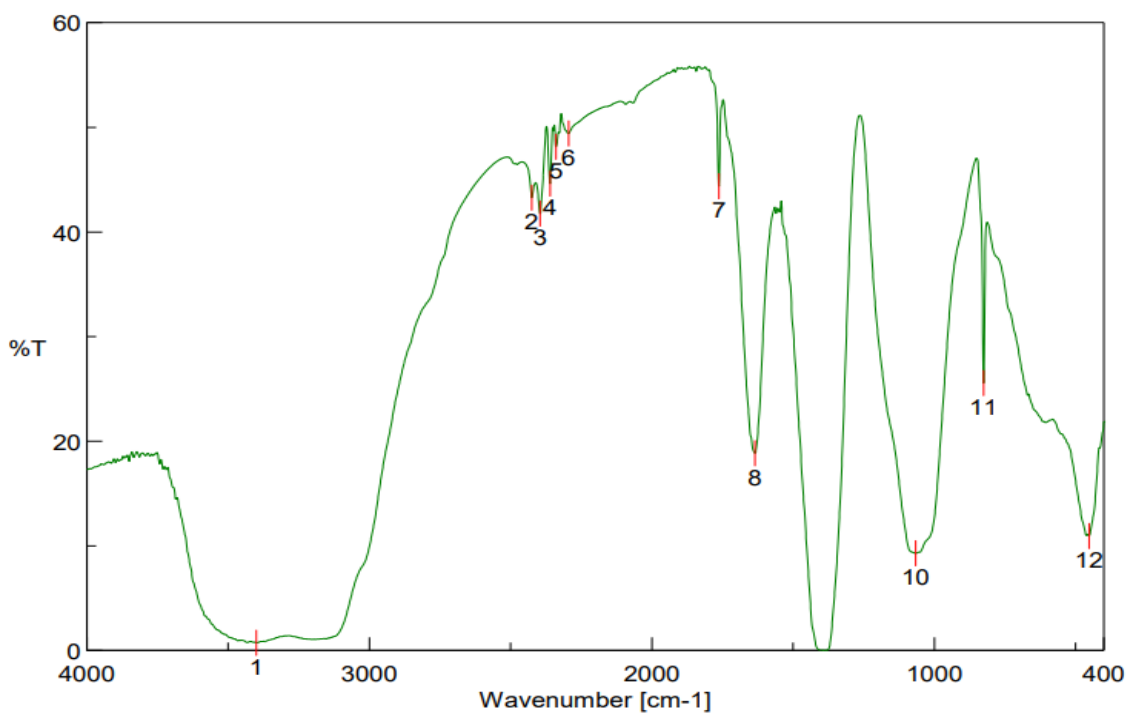


Fig.5.17: FTIR spectrum of (Ca: Ce – 20:80) doped diopside

Table.5.9: FTIR analysis of (Ca: Ce – 20:80) doped diopside with functional group

PEAK NUMBER	POSITION	INTENSITY	COMPOUND NAME	BOND VIBRATION
1	3400.85	0.732487	Hydroxyl group	O-H stretching
2	2425.05	43.2522	-	-
3	2396.12	41.7273	-	-
4	2360.44	44.6161	-	-
5	2339.23	48.1132	-	-
6	2294.88	49.3885	-	-

7	1762.62	44.3604	Si-O, CeO	Si-O, CeO stretching
8	1635.34	18.8386	Si-O, CeO	Si-O, CeO stretching
9	1390.42	0.001644	Si-O	Si-O stretching
10	1066.44	9.27437	Phosphate	stretching
11	825.384	25.5348	Si-O	Si-O stretching
12	452.225	10.9382	Si-O, CeO	Si-O bending CeO stretching

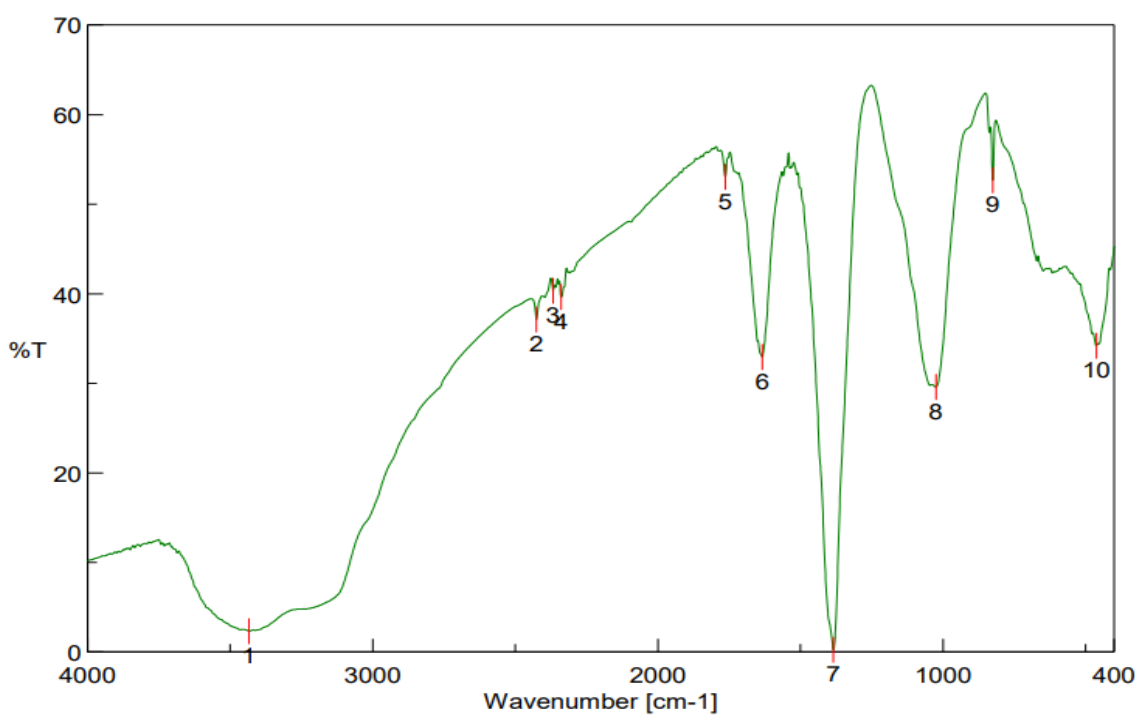


Fig.5.18: FTIR spectrum of (Ca: Ce – 50:50) doped diopside

Table.5.10: FTIR analysis of (Ca: Ce – 50:50) doped diopside with functional group

PEAK NUMBER	POSITION	INTENSITY	COMPOUND NAME	BOND VIBRATION
1	3434.6	2.30718	Hydroxyl group	O-H stretching
2	2426.01	37.1158	-	-
3	2367.19	40.3094	-	-
4	2339.23	39.6054	-	-
5	1763.58	53.0256	Si-O, CeO	Si-O, CeO stretching
6	1634.38	32.9256	Si-O, CeO	Si-O, CeO stretching
7	1384.64	0.235318	Si-O	Si-O stretching
8	1024.98	29.5601	Phosphate	stretching
9	825.384	52.6668	Si-O	Si-O stretching
10	461.868	34.1708	Si-O, CeO	Si-O bending CeO stretching

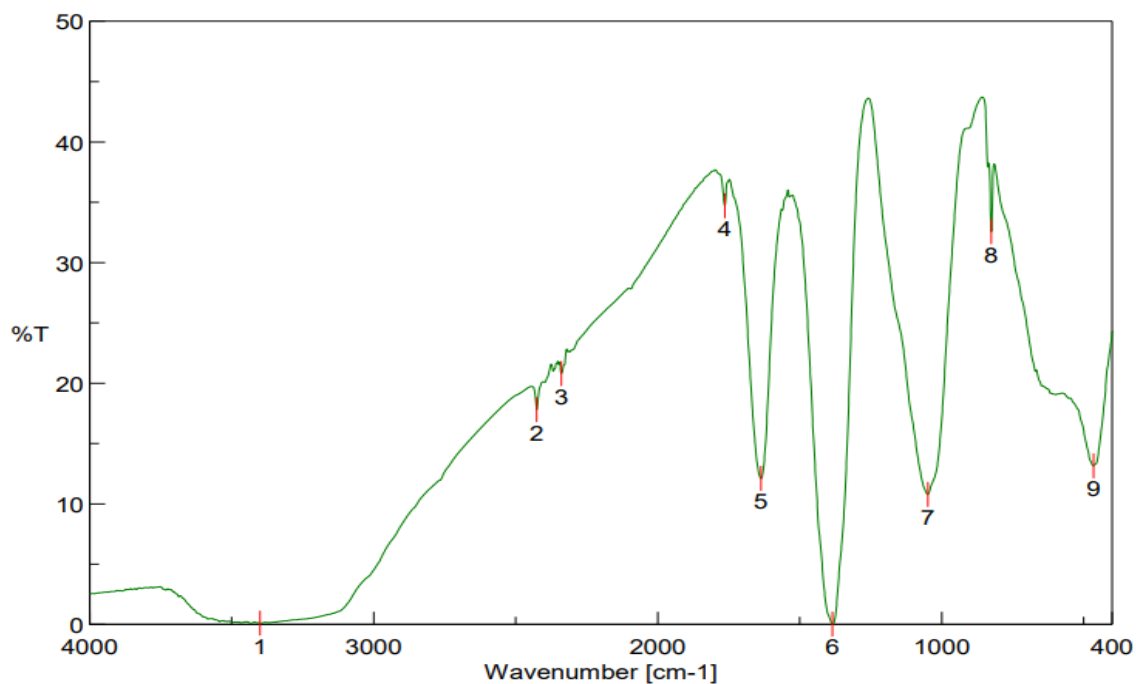


Fig.5.19: FTIR spectrum of (Ca: Ce – 80:20) doped diopside

Table.5.11: FTIR analysis of (Ca: Ce – 80:20) doped diopside with functional group

PEAK NUMBER	POSITION	INTENSITY	COMPOUND NAME	FUNCTIONAL GROUP
1	3400.85	0.101636	Hydroxyl group	O-H stretching
2	2426.01	17.7953	-	-
3	2339.23	20.7985	-	-
4	1763.58	34.7057	Si-O, Ce-O	Ce-O, Si-O stretching
5	1636.3	12.0938	Si-O, Ce-O	Ce-O, Si-O stretching
6	1384.64	0.0109406	Si-O	Si-O stretching
7	1049.09	10.7592	Phosphate	stretching

8	8325.384	32.5626	Si-O	Si-O stretching
9	465.725	13.1296	Si-O, Ce-O	Si-O bending Ce-O stretching

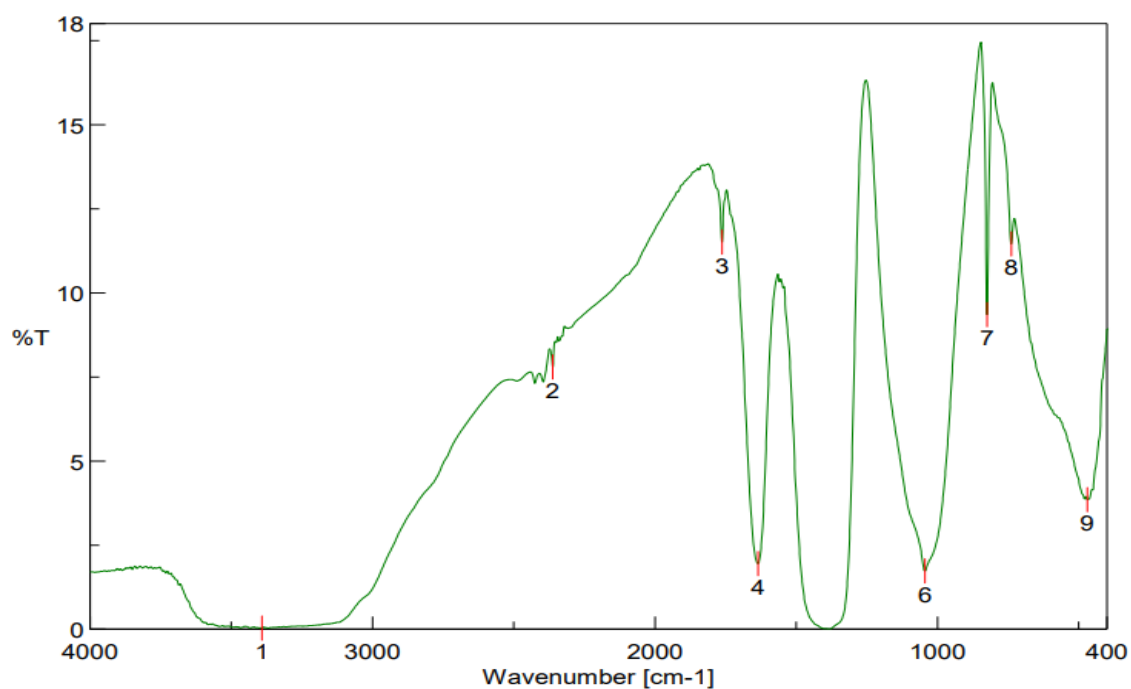


Fig.5.20: FTIR spectrum of (Mg: La – 20:80) doped diopside

Table.5.12: FTIR analysis of (Mg: La – 20:80) doped diopside with functional group.

PEAK NUMBER	POSITION	INTENSITY	COMPOUND NAME	FUNCTIONAL GROUP
1	3392.17	0.0293029	Hydroxyl group	O-H Stretching
2	2362.37	7.78956	-	-
3	1762.62	11.5001	Si-O, La-O	Si-O, La-O stretching
4	1635.34	1.94523	Si-O, La-O	Si-O, La-O stretching
5	1384.64	0.00228653	Si-O	Si-O stretching

6	1044.26	1.72546	Phosphate	stretching
7	824.42	9.34451	Si-O	Si-O stretching
8	738.603	11.4529	-	-
9	469.582	3.84887	Si-O, La-O	Si-O bending La-O stretching

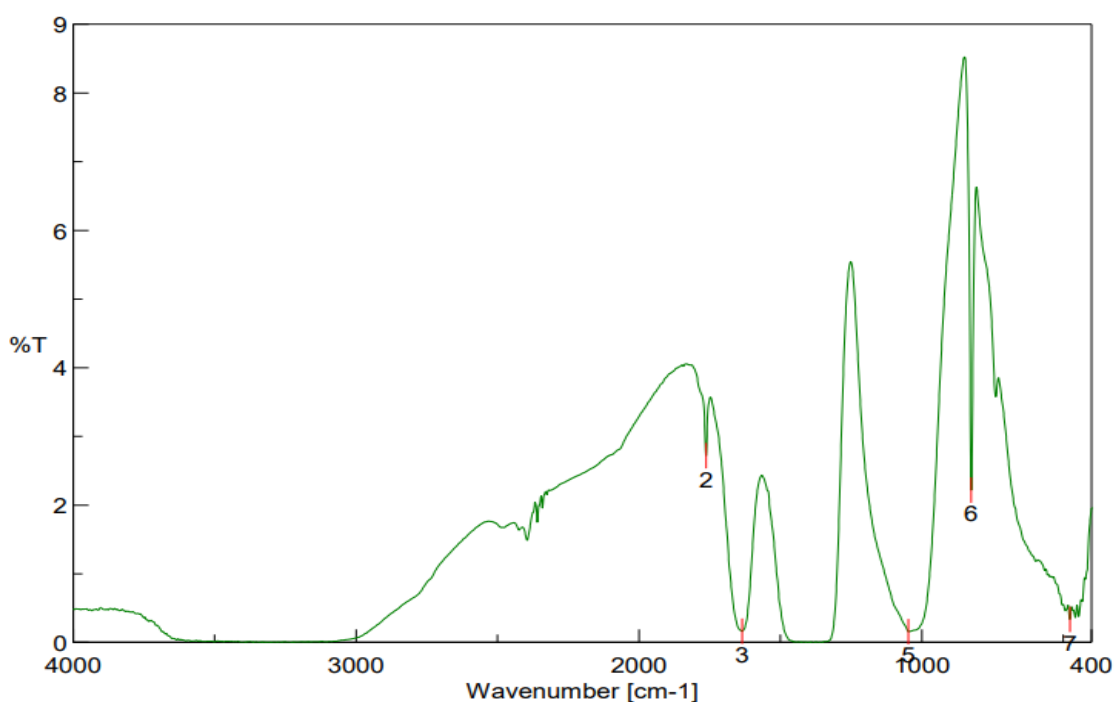


Fig.5.21: FTIR spectrum of (Mg: La – 50:50) doped diopside

Table.5.13: FTIR analysis of (Mg: La – 50:50) doped diopside with functional group

PEAK NUMBER	POSITION	INTENSITY	COMPOUND NAME	FUNCTIONAL GROUP
1	3226.33	0	Hydroxyl group	O-H Stretching
2	1762.62	2.71567	Si-O, La-O	Si-O, La-O stretching
3	1635.34	0.161443	Si-O, La-O	Si-O, La-O stretching

4	1353.78	0	Si-O	Si-O stretching
5	1047.16	0.151835	Phosphate	Stretching
6	825.384	2.21723	Si-O	Si-O stretching
7	476.331	0.33461	Si-O, La-O	Si-O bending La-O stretching

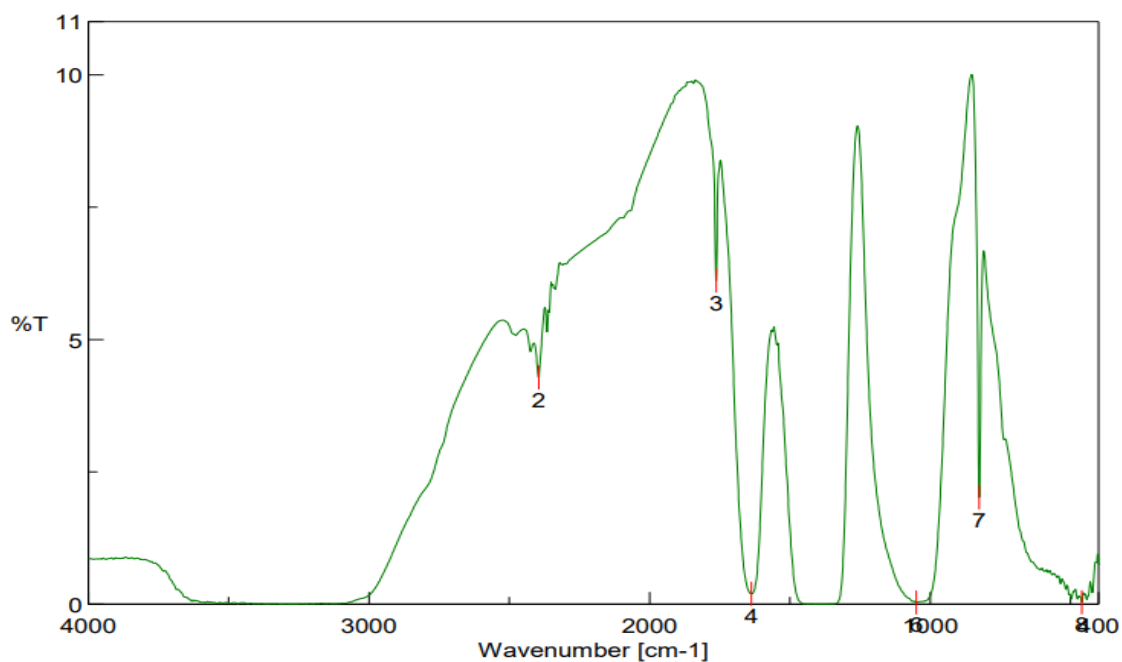


Fig.5.22: FTIR spectrum of (Mg: La – 80:20) doped diopside

Table.5.14: FTIR analysis of (Mg: La – 80:20) doped diopside with functional group

PEAK NUMBER	POSITION	INTENSITY	COMPOUND NAME	FUNCTIONAL GROUP
1	3256.22	0	Hydroxyl group	O-H Stretching
2	2395.16	4.28105	CH group	CH stretching
3	1762.62	6.11205	Si-O, La-O	Si-O, La-O stretching
4	1637.27	0.196455	Si-O, La-O	Si-O, La-O stretching
5	1402.96	0	Si-O, La-O	Si-O, La-O Stretching

6	1050.05	0.0336334	Phosphate	Stretching
7	825.384	2.01549	Si-O	Si-O stretching
8	459.939	0.0367006	Si-O, La-O	Si-O bending La-O stretching

The FTIR spectra of the undoped and doped diopside pellets showed the adsorption band of Si group, lanthanum group, cerium group, carbonate group and hydroxyl group. The bending vibration observed at 400 to 600 cm^{-1} were Si-O group. The peak observed at 1600 to 1800 cm^{-1} were because of the stretching vibration of carbonate group. The stretching vibration observed at 400 to 600 cm^{-1} were because of lanthanum and cerium bond. The stretching vibration observed at 3000 to 3600 cm^{-1} were hydroxyl group. The nitrates were observed at the peak of 1300 to 1600 cm^{-1} with stretching vibration. The peak at 1049 cm^{-1} showed phosphate group.

5.9.2 XRD ANALYSIS

In this study, analysed XRD to investigate the crystallographic properties of diopside pellets, which are potential candidates for biomedical and engineering applications. Diopside pellets were prepared using standard procedures, and their crystal structure was analysed using a high-resolution X-ray diffractometer. It showed a highest peak at a 2θ value of undoped diopside were 21.723, 26.698, 30.584 and 37.974. The peaks of cerium doped diopside were 21.635, 38.033, 29.78, 47.69, 56.45, 61.387, 27.996 and 47.679. The peaks of lanthanum doped diopside were 10.47, 21.579, 38.97, 27.69, 26.598, 35.344 and 37.980. The obtained diffraction patterns were analysed to identify the phases present in the samples and to determine the crystallographic parameters. This result reveal characteristic diffraction peaks corresponding to the crystal structure of diopside, confirming the purity and crystallinity of the pellets. Additionally, minor peaks corresponding to potential impurities or secondary phases were also identified and analysed.

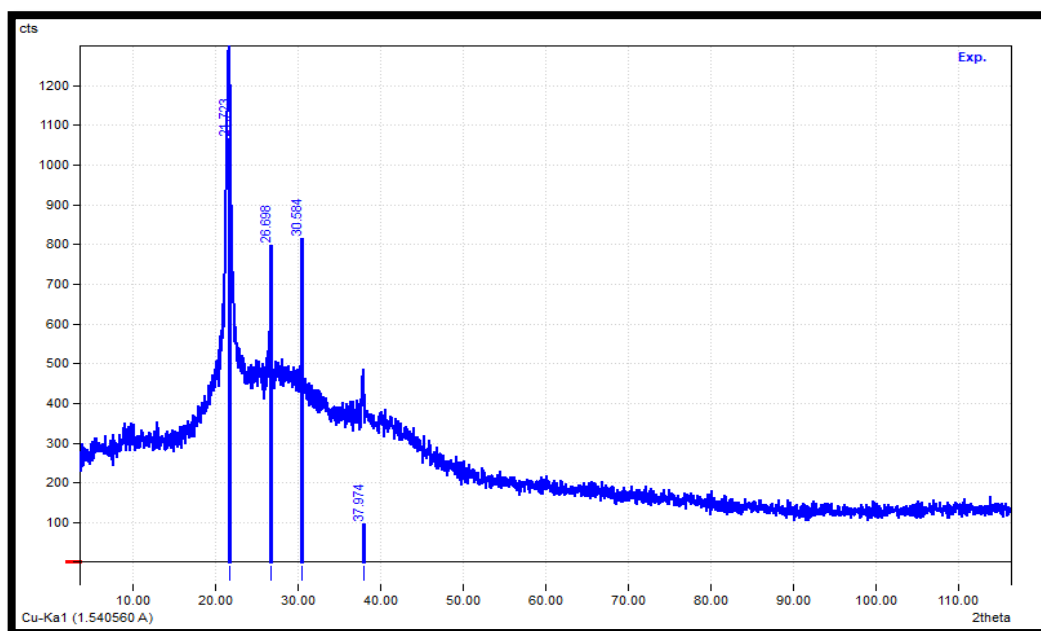


Fig.5.23: XRD analysis of undoped diopside

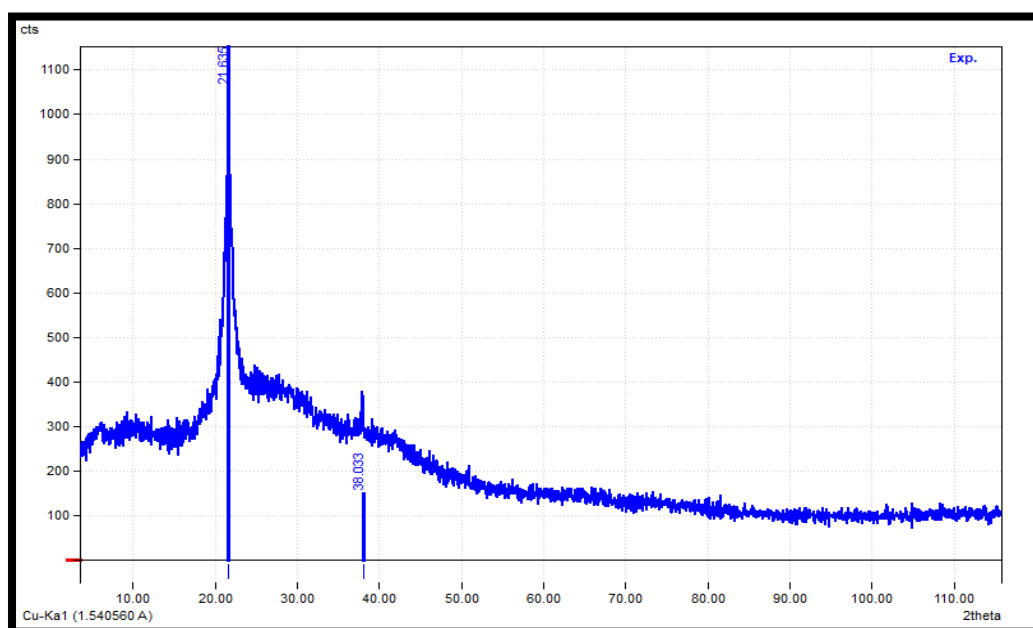


Fig.5.24: XRD analysis of (Ca:Ce-20:80) diopside

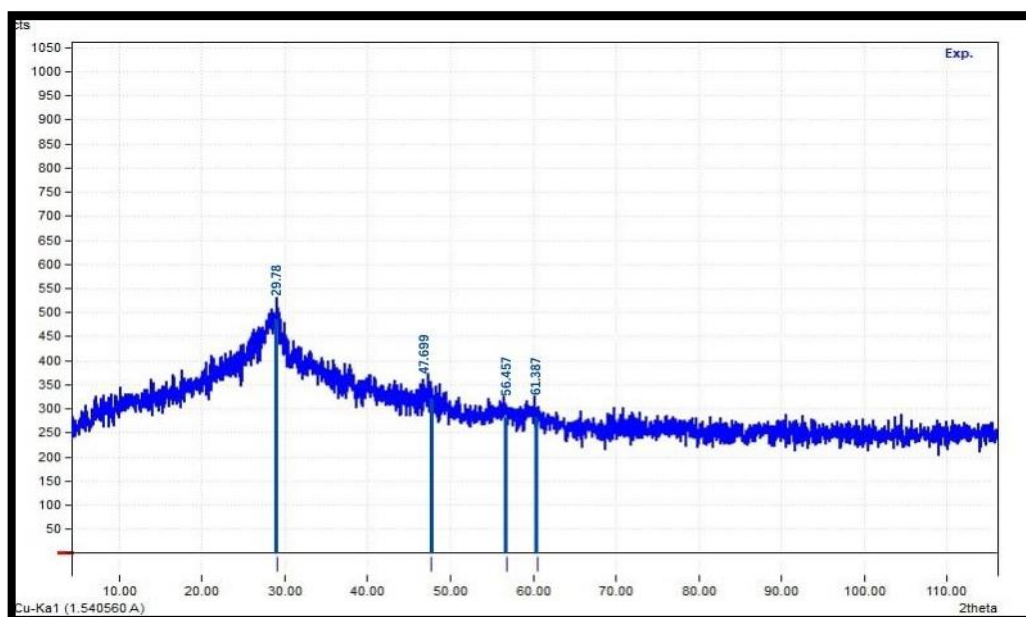


Fig.5.25: XRD analysis of (Ca:Ce-50:50) diopside

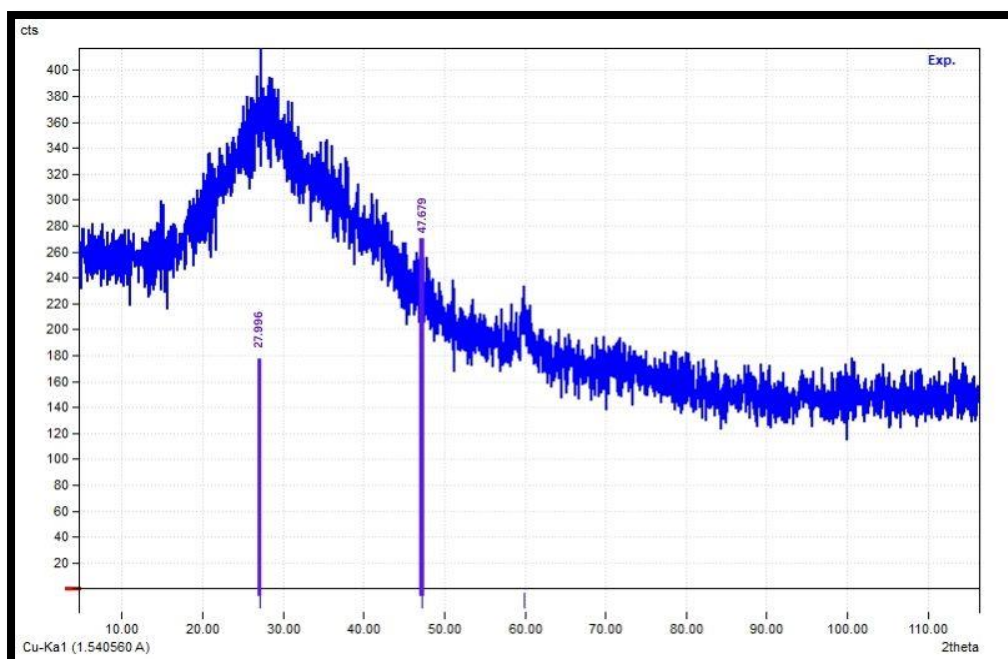


Fig.5.26: XRD analysis of (Ca:Ce-80:20) diopside

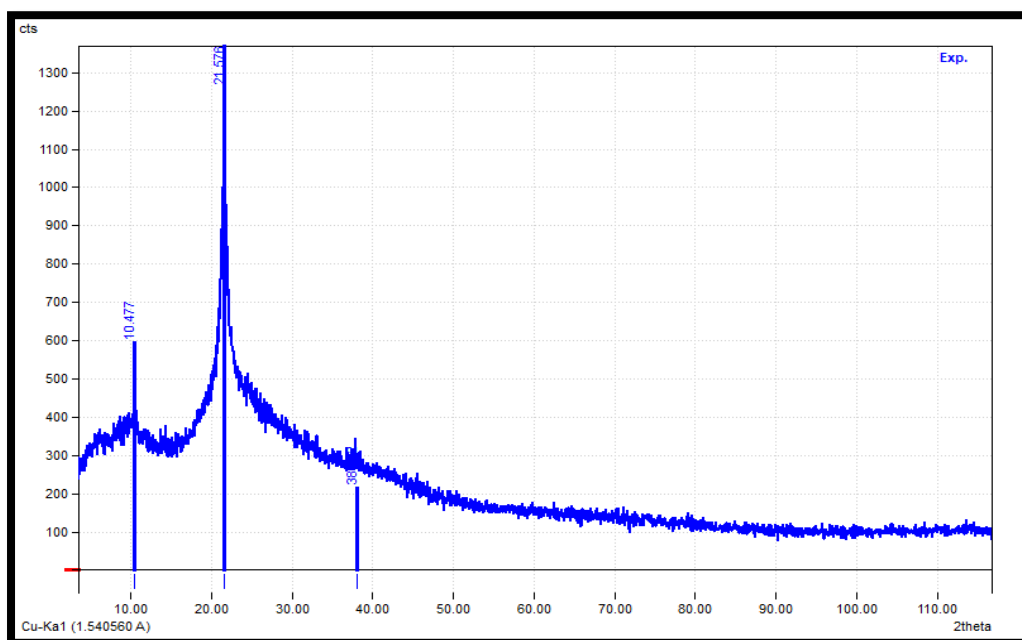


Fig.5.27: XRD analysis of (Mg:La-20:80) diopside

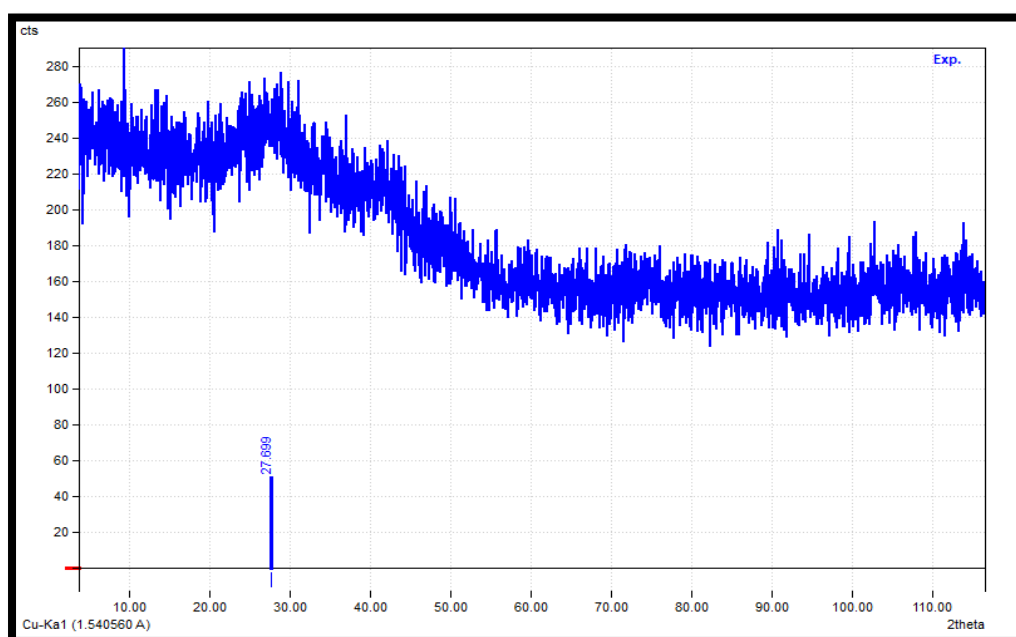


Fig.5.28: XRD analysis of (Mg:La-50:50) diopside

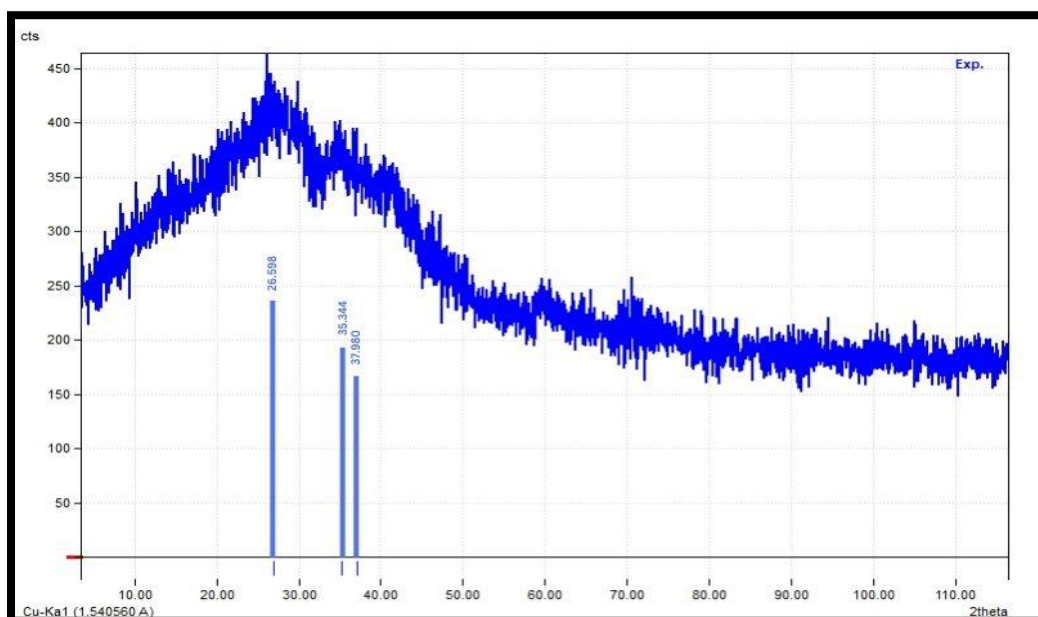


Fig.5.29: XRD analysis of (Mg:La-80:20) diopside

5.9.3 SEM ANALYSIS

The morphology of the synthesised undoped, cerium doped diopside and lanthanum doped diopside were shown in the below images. It was observed that there were agglomerated hexagonal grains of hydroxy-apatite layer on the pellet surface. Furthermore, a small spherical agglomeration was observed on the hexagonal grains on the pellets. The presence of small spherical agglomerations is because of deposition of minerals like cerium, lanthanum, phosphorous, calcium, magnesium and other ions in the SBF solution. The addition of Ce and La diopside pellets also gained hydroxy apatite formation on the surface. After soaking for 30 days in SBF, diopside pellets maintained their rigid structure. Higher-magnification SEM showed that bone-like apatite particles were deposited on surface of the pellets. EDAX spectrum and elemental map of pellets confirmed the presence of calcium (Ca), phosphate (P), oxygen (O), cerium (Ce), lanthanum (La) and magnesium (Mg) respectively. The percentages of the elements are given in the below table.

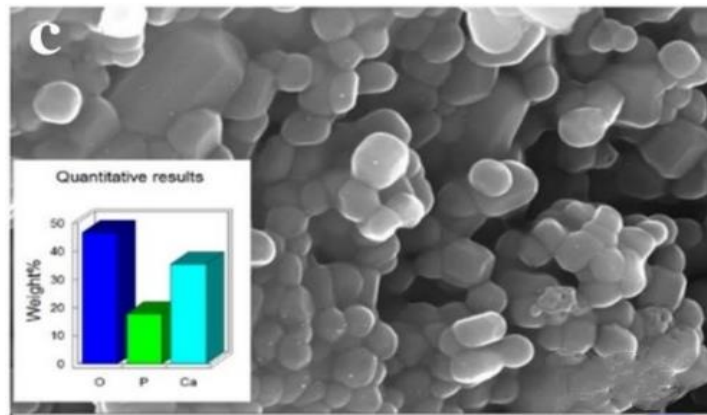


Fig.5.30: SEM analysis of undoped diopside pellet

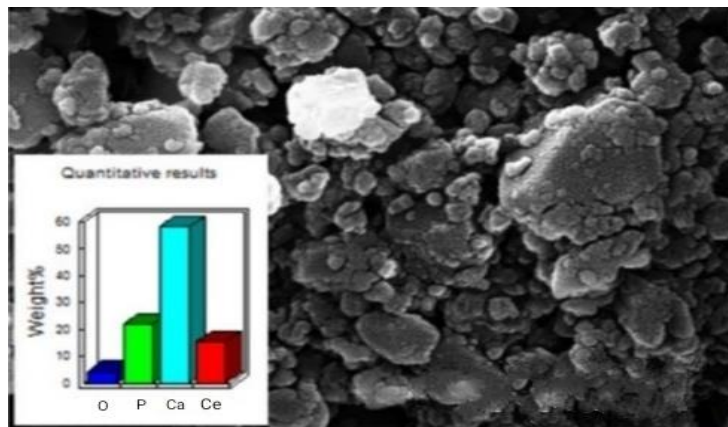


Fig.5.31: SEM analysis of (Ca: Ce - 20:80) diopside pellet

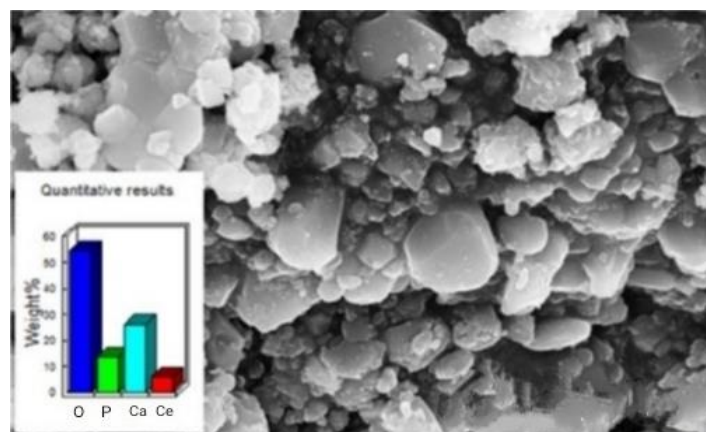


Fig.5.32: SEM analysis of (Ca: Ce - 50:50) diopside pellet

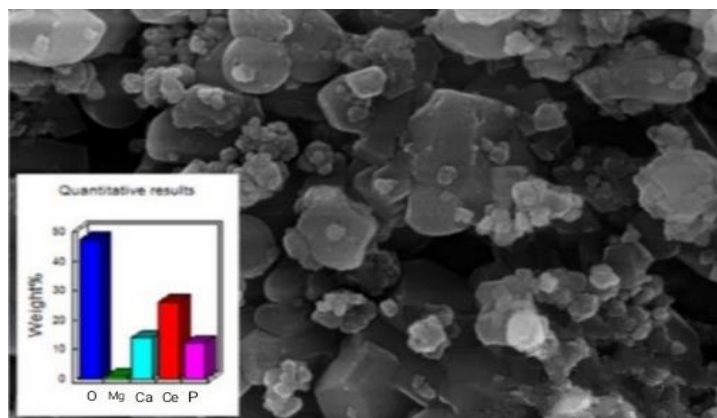


Fig.5.33: SEM analysis of (Ca: Ce - 80:20) diopside pellet

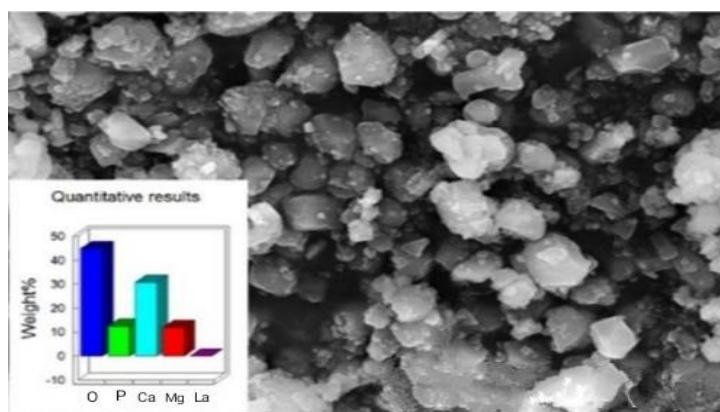


Fig.5.34: SEM analysis of (Mg: La - 20:80) diopside pellet

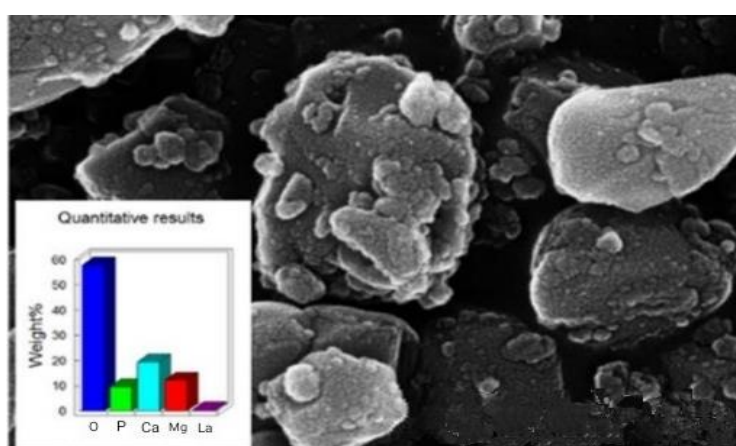


Fig.5.35: SEM analysis of (Mg: La - 50:50) diopside pellet

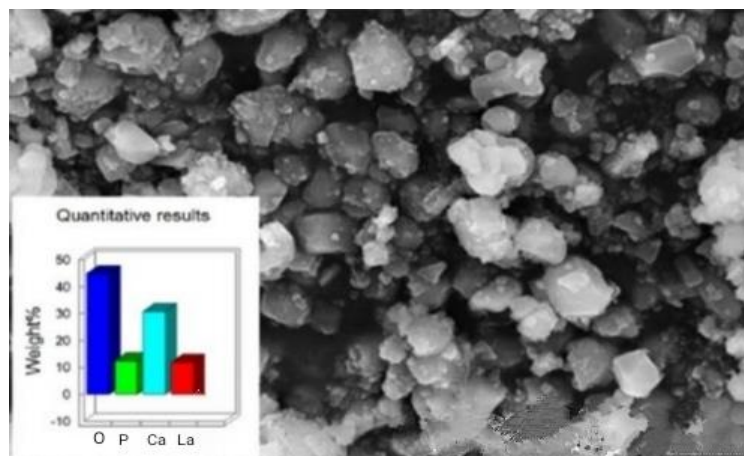


Fig.5.36: SEM analysis of (Mg: La - 80:20) diopside pellet

Table.5.15: Element composition of diopside pellets in SEM analysis

SAMPLE	Ca %	P%	Ce%	La%
Undoped	35.49	17.86	-	-
(Ca:Ce-20:80)	56.07	13.51	13.90	-
(Ca:Ce-50:50)	28.34	21.79	15.63	-
(Ca:Ce-80:20)	18.07	22.17	26.77	-
(Mg:La-20:80)	33.99	13.56	-	11.04
(Mg:La-50:50)	21.45	12.44	-	13.78
(Mg:La-80:20)	35.90	10.62	-	14.66

6.CONCLUSION

In this study the undoped, cerium nitrate doped and lanthanum nitrate doped diopside were prepared successfully by co-precipitation method. They were made into pellets of 10mm size using a pelletizer and calcinated in a heating furnace at 700°C for 2 hours. To test the bioactivity of the diopside pellets various invitro tests were conducted to determine its efficiency. The invitro biodegradability test determined the stability of the pellets in SBF and showed a slower degradation rate. The biomineralization test confirmed the deposition of the minerals which enhanced the hydroxy apatite formation on the surface of pellets. The protein adsorption test showed the maximum adsorption of proteins which enhanced the cell attachment. Likewise, the results from the antioxidant test proved that it can neutralize the free radical formation. The hemolysis test proved that these diopside pellets were non-haemolytic and does not damage the red blood cells. From the antimicrobial test, the zone of inhibition was clearly determined against *E.Coli* and *Staphylococcus* bacteria. The important cytotoxicity assay confirmed that these diopside pellets are non-toxic and does not cause major damage to the cells. The characterization test like FTIR, XRD and SEM were done to confirm the presence of functional group, bond vibration, crystalline structure and surface nature of diopside pellets. From this comparative study using the undoped, (Ca: Ce - 20:80), (Ca: Ce - 50:50), (Ca: Ce - 80:20), (Mg: La - 20:80), (Mg: La - 50:50), and (Mg: La - 80:20) diopside pellets, the (Ca: Ce - 20:80) cerium nitrate doped diopside showed maximum in vitro activity among other diopside pellets. Therefore, diopside pellets exhibit promising potential for enhancing bone regeneration, with their activity notably heightened when doped with elements such as cerium and lanthanum.

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ANNEXURE

MATERIALS TECHNOLOGY
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Current advancements in the development of bionic organs using regenerative medicine and 3D tissue engineering

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ABSTRACT

Bio-artificial organs, commonly referred to as bionic organs, are artificial structures that perform the same functions as natural organs. In regenerative medicine, damaged organs are repaired using biological components including growth factors and stem cells. The advancement of tissue engineering, which aims to harness the inherent regenerative potential of human body organs to rebuild normal biological function, has benefited greatly from the use of biomaterials. The rapidly expanding field of regenerative medicine has brought the usage of biomaterials and their functions in the production of new tissue. Organs-on-chips are seen as a concept performer in tissue engineering with significant potential for future 'clinical trials on a chip' and a step towards developing customized medicine. With the advancement of 3D printing technology, the manufacturing constraints of biomedical devices have been solved using cutting-edge biomimetic structures. This review covers the areas on bionic organs such as organ on a chip with tissue engineering, bio grafting, 3D bio-printing and the stem cell techniques that are employed in regenerative medicine.

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KEYWORDS

Bionic organs; 3D tissue engineering; regenerative medicine; biomaterials; biomimetic

Introduction

The human body is made up of intricate structures and material systems, current manufacturing techniques, which are constrained in their ability to choose and fabricate materials, are unable to produce biomedical devices that have intricate bio-inspired architectures and structures, which hinders the development of biomedical devices for a variety of applications. Due to their exceptional fabrication abilities, 3D printing techniques are widely used in many industries, including biomedical engineering, automotive, aviation, telecommunications, civil engineering, and sustainable energy [1,2]. By layer-by-layer depositing materials, such as metals, polymers, and ceramics, three-dimensional (3D) printing, sometimes referred to as additive manufacturing, is a technique that can create items with complicated architecture. Technology for 3D printing in the medical areas is a promising method for individualised care [3]. One of the most alluring applications of bioprinting and bio fabrication is the development of biological 3D tissue models, such as those for drug screening, disease models, tissues or organ on a

microchip, healthcare sensors, and biological actuators. To aid surgeons in their work and to better educate patients about their illnesses, 3D copies of a patient's damaged organ could be created. Scaffolds and implants are created using 3D printing processes for regenerative medicine. Stem cells for example, are routinely expanded ex vivo as part of the current tissue engineering approach before being transplanted into injured sites. Stem cells have considerable promise in tissue engineering and reconstructive therapeutics because of their distinct regeneration capacity and immune modulatory qualities. Tissue engineering depends heavily on biomaterials, but this does not mean that conventional, artificial biomaterials must be employed [4]. By using stem cells sown on artificial structures to replace damaged tissues, tissue engineering aims to regain the normal regulation of cell activity. The 3D surroundings inside the stem cell niche have an impact on stem cell therapy self-renewal and differentiation [5]. The scaffold approach has been abandoned, and attempts to create 3D tissue using cells and growth regulators are now regularly documented. Bio fabrication is the

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Review Article

Current advancements in the development of bionic organs using regenerative medicine and 3D tissue engineering

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KINETIC PARAMETER DETERMINATION USING VISUALISATION OF ENZYME KINETIC GRAPHS IN R AND PYTHON PROGRAMMING

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ABSTRACT

This study employs Python and R programming languages to analyse enzyme kinetics with kinetics plots such as the Michaelis-Menten Plot, Eadie-Hofstee plot, Lineweaver-Burk plot and Hanes-Woolf plot. Kinetic data, including, V_{max} , K_m , and $[S]$, is collected and processed. Graphical representations of the kinetic plots are obtained for the required enzymes. This study has various applications in the fields of biochemical, pharmaceutical, therapeutic, education and research. The required libraries are installed for both Python and the R programming language. The equations for every plot are applied to calculate reaction velocities and substrate concentration. The accurate inputs for the plots are given based on the programmed x and y axis respectively. The resulting enzyme kinetics data is saved in an Excel file. Python and R scripts generate various plots, and linear regression models extract kinetic parameters. Michaelis-Menten plots, Eadie-Hofstee plots, Hanes-Woolf plots, and Lineweaver-Burk plots are presented, demonstrating the visual representation of enzyme kinetics in both programming languages. The outputs obtained are stored separately for both languages in the Excel file. The results include the y-intercept, x-intercept, and slope which are interpreted to obtain the V_{max} and K_m values from respective plots. Kinetic parameters obtained from Python and R show close agreement. Python and R are effective tools for enzyme kinetics analysis, providing consistent and reliable results. The study showcases the applicability of programming languages in biochemical research of enzymes, streamlining data processing and analysis while reducing manual errors and labour.

Keywords: Enzyme kinetics, Python programming language, R programming language, Michaelis-Menten plot, Eadie-Hofstee plot, Lineweaver-Burk plot, Hanes-Woolf plot

EFFECT OF *Capparis decidua* - GHEE COMPOSITE IN FRACTURE BONE HEALING

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Abstract

Scaffolds are the substrate for cells which acts as the models in tissue regeneration. Biopolymer scaffolds have been well - studied and allowed many scientists to develop the fields of biomedicine and nanomedicine and be employed in a variety of tissue engineering applications. Different biomaterial combinations are available to construct biomaterials based scaffolds. Several scaffolds have been developed by combining natural biopolymers with synthetic polymers. Those scaffolds are employed in the tissue regeneration of bones, damaged blood vessels, ligaments, cartilage, urethral and neural. Thus, incorporating ethnomedicinal plant extracts in the existing biopolymer scaffolds hastens damaged tissue repair and regeneration. In the field of bone tissue engineering, the design of effective tissue - compatible plant - based composite scaffolds is essential due to their lesser toxicity effects, biocompatibility, cost - effectiveness, physical stability and biodegradability. From the traditional references and literature, *Capparis decidua* has many pharmacological features. The plant stems help to enhance the healing of fractured bone. Also, Ghee possesses some healing properties and promotes healing in other formulations. The purpose of the present work was to develop various herbal - based biopolymer scaffolds incorporating the medicinal plant *Capparis decidua* with Ghee as a carrying agent and establish the effectiveness of this *Capparis decidua* - Ghee biopolymer composite in fractured bone healing.

Keywords: Traditional medicine, tissue engineering, Bone regeneration, *Capparis decidua*, Ghee, Scaffolds

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