

DEVELOPMENT OF POLYMER NANO-CARRIERS FOR SUSTAINABLE DRUG DELIVERY IN CHRONIC WOUNDS

Submitted in partial fulfilment of the requirements for the
award of Bachelor of Technology degree in Biomedical
Engineering

by
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SCHOOL OF BIO AND CHEMICAL ENGINEERING

DEPARTMENT OF BIOMEDICAL ENGINEERING

SATHYABAMA

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

Accredited with Grade “A++” by NAAC.

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This is to certify that this Project Report is the bonafide work of **N. Renny Valentina (40240025)**, carried out the project entitled. "**Development of polymer nano-carriers for sustainable drug delivery in chronic wounds**" under our supervision from November 2023 to April 2024.

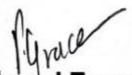

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ABSTRACT

Diabetes mellitus is a diverse collection of illnesses characterised by hyperglycaemia caused by an absolute or relative lack of insulin production or activity. Diabetes is divided into different groups, the most prevalent of which are Type 1 and Type 2. Type 1 diabetes is caused by an immunological reaction, whereas Type 2 diabetes is predominantly caused by insulin resistance. Gestational diabetes is a kind of diabetes that develops during pregnancy, and there are other uncommon variants as well. Diabetes screening involves a systematic screening of Blood glucose levels tests which are frequently evaluated via oral glucose tolerance tests, or haemoglobin A1c assays. The early detection of the disease is critical because it permits appropriate intervention and care to prevent diabetic repercussions such as heart disease, renal difficulties, and neuropathy. Diabetic foot ulcers are a frequent outcome of diabetes that are characterised by open wounds or lesions on the feet, which tend to be triggered by decreased circulation of blood and nerve damage. These ulcers are prone to infection of bacteria, multi-drug resistant bacterial infections and could require an extended period to heal. It might lead to serious infections or, in extreme situations, gangrene and amputation. Polymeric nano-carriers (PNCs) play a crucial role in targeted and sustainable drug delivery for the management of diabetic foot ulcers. These nano-carriers such graphene oxide are employed in the treatment of diabetic foot ulcers offer versatile options, including chitosan, PCL, and PLGA, as effective delivery vehicles. The entrapping and encapsulation of therapeutic agents within polymer-based nano-carriers ensure precise drug delivery. To gain insights into the drug release kinetics, we employ UV spectroscopy to monitor drug movement and concentration at multiple time points. Subsequently, we assess the drug delivery process using an in vitro model involving dermal cells from male Wister rats over a specific temporal window. This comprehensive approach provides a foundation for optimising drug delivery systems tailored to diabetic foot ulcer treatment.

KEYWORDS— Diabetic Foot Ulcer, type II diabetes, type I diabetes, graphene oxide, cerium oxide, aloe Vera, borate bioactive glass.

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ABBREVIATION	EXPANSION
GO	Graphene Oxide
DFU	Diabetic Foot Ulcer
CFU	Colony Forming Unit
UV	Ultra-Violet
BBG	Borate Bioactive Glass
CeO₂	Cerium Oxide
MRSA	Methicillin-Resistant <i>Staphylococcus aureus</i>
PAD	Peripheral Arterial Disease
EPC	Endothelial Progenitor Cells
MSC	Mesenchymal Stem Cells
MDRO	Multi-drug Resistant Organism
DFI	Diabetic Foot Infection
ZnO	Zinc Oxide
HCL	Hydrochloric acid
XRD	X-ray Diffraction
GLP1	Glucagon-Like Peptide1
DI	Deionised water
T1D	Type 1 Diabetes

DM2	Diabetes Mellitus type 2
DPP	Diabetes Prevention Program
SGLT2	Sodium-Glucose CoTransporter-2 inhibitors

CHAPTER 1

INTRODUCTION

Diabetes, a burgeoning global health challenge, has far-reaching implications for society, healthcare systems, and economies. A decade ago, in 2010, the worldwide burden of diabetes was staggering, with an estimated 285 million individuals affected, making up approximately 6.4% of the global adult population. Alarmingly, projections foretell that this already substantial number could surge to a daunting 430 million in the absence of significant advances in diabetes care and treatment (Albert, 2022). The ailment, diabetes, manifests in two primary forms: Type 1 diabetes (T1D) and Type 2 diabetes. T1D, classified as an autoimmune disorder, instigates the disruption of beta-cell functionality. In contrast, Type 2 diabetes, being more prevalent, predominantly arises from the gradual erosion of glucose regulation.

As diabetes advances, it unleashes havoc on tissues and blood vessels, culminating in severe complications, such as retinopathy, neuropathy, cardiovascular diseases, and ulceration. Amid these complications, diabetic foot ulcers emerge as a prominent concern, disproportionately affecting individuals grappling with poorly managed diabetes mellitus (Amin and Doupis, 2016). These ulcers typically find their origins in suboptimal glycemic control, undiagnosed neuropathy, peripheral vascular disease, or inadequate foot care. Tragically, they often escalate to more severe conditions like osteomyelitis and necessitate lower extremity amputations. The multifaceted nature of diabetes underscores the pressing need for a comprehensive, multidisciplinary approach to both prevention and management (Andrews, 2018). Type 1 diabetes is a chronic condition where the pancreas produces little to no Insulin, a hormone necessary for regulating blood sugar levels. It's often diagnosed in children and young adults, though it can occur at any age. The exact cause is unknown, but it's believed to involve an autoimmune reaction where the body's immune system attacks and destroys insulin-producing cells in the pancreas.

Without enough insulin, glucose can't enter cells for energy, causing it to accumulate in the bloodstream, leading to high blood sugar levels. Symptoms include excessive thirst, frequent urination, unexplained weight loss, fatigue, and blurred vision. If untreated, it can lead to serious complications like heart disease, kidney damage, nerve damage, and vision problems (Craus, *et.al.*, 2023).

Management involves lifelong insulin therapy through injections or an insulin pump to regulate blood sugar levels. Monitoring blood sugar levels regularly, following a healthy diet, engaging in regular exercise, and being vigilant for symptoms of low or high blood sugar are crucial. Continuous glucose monitoring systems and insulin pumps have improved management, offering better control and fly (Bakker, *et.al.*, 2018). Despite advancements in treatment, living with type 1 diabetes can be challenging, requiring constant vigilance and adjustments to maintain blood sugar levels within a healthy range.

Research continues to explore ways to improve management, find a cure, and enhance the quality of life for individuals with type 1 diabetes. Type 2 diabetes is a prevalent chronic condition characterised by insulin resistance and relative insulin deficiency, leading to high blood sugar levels. Unlike type 1 diabetes, where the body's immune system attacks and destroys insulin-producing cells, type 2 diabetes typically develops later in life and is strongly associated with lifestyle factors, such as obesity, sedentary behaviour, and poor dietary habits (Edmonds and Mathioudakis, 2018).

Genetic predisposition also plays a role in the development of type 2 diabetes, with certain ethnic groups being at higher risk. Insulin resistance is a key feature of type 2 diabetes, where cells in the body become less responsive to insulin's actions, particularly in muscle, fat, and liver tissues. This resistance impairs the ability of insulin to effectively transport glucose from the bloodstream into cells, leading to elevated blood sugar levels (Hingorani, *et.al.*, 2016). To compensate for insulin resistance, the pancreas initially produces more insulin, but over time, the beta cells may become exhausted and fail to keep up with the body's demand for insulin, resulting in relative insulin deficiency. The combination of insulin resistance and relative insulin deficiency results in impaired glucose metabolism, leading to hyperglycaemia. This chronic

elevation of blood sugar levels can cause a wide range of symptoms, including increased thirst, frequent urination, fatigue, blurred vision, and slow wound healing. However, many individuals with type 2 diabetes may not experience noticeable symptoms, especially in the early stages of the disease.

Type 2 diabetes is often diagnosed through blood tests that measure fasting blood glucose levels, oral glucose tolerance tests, or haemoglobin A1c levels, which provide an indication of average blood sugar levels over the past two to three months. Early detection and diagnosis are crucial for preventing complications associated with uncontrolled diabetes, such as cardiovascular disease, kidney damage, nerve damage, and vision problems. Management of type 2 diabetes focuses on lifestyle modifications, including adopting a healthy diet, engaging in regular physical activity, achieving and maintaining a healthy weight, and avoiding tobacco and excessive alcohol consumption (Zimny, *et.al.*, 2022).

These lifestyle changes are often the first line of treatment and can significantly improve blood sugar control and overall health outcomes. In addition to lifestyle modifications, medication may be prescribed to help lower blood sugar levels and reduce the risk of complications. Oral medications such as metformin, sulfonylureas, meglitinides, thiazolidinediones, DPP-4 inhibitors, SGLT2 inhibitors, and GLP-1 receptor agonists are commonly used to improve insulin sensitivity, stimulate insulin secretion, or decrease glucose production in the liver.

For some individuals with type 2 diabetes, insulin therapy may be necessary to achieve optimal blood sugar control. Insulin therapy may involve the use of long-acting insulin to provide basal insulin coverage throughout the day and short-acting insulin to cover meals and correct high blood sugar levels. Insulin can be administered via injections or through insulin pumps, which deliver insulin continuously. Regular monitoring of blood sugar levels is essential for individuals with type 2 diabetes to assess the effectiveness of treatment and make adjustments as needed (Yazdanpanah, *et.al.*, 2019). This may involve self-monitoring using a blood glucose meter or continuous glucose monitoring system, which provides real-time data on blood sugar levels and trends over time. Type 2 diabetes is a progressive disease, and effective management requires ongoing

education, support, and collaboration between patients and healthcare providers. Diabetes self-management education programs can empower individuals with the knowledge and skills needed to make informed decisions about their health and effectively manage their condition (Walsh, *et.al.*, 2016).

Preventing type 2 diabetes and its complications requires a comprehensive public health approach that addresses underlying risk factors, promotes healthy lifestyles, and ensures access to timely (Tettelbach, *et.al.*, 2022) and treatment. Strategies such as community-based interventions, workplace wellness programs, and policy initiatives to promote healthy eating and physical activity can help reduce the burden of type 2 diabetes on individuals, families, and healthcare systems. Research into type 2 diabetes continues to advance our understanding of the disease and identify new treatment approaches.

Diabetic foot ulcers can lead to serious complications, including infection, tissue damage, and even amputation. Prompt and appropriate treatment is essential to prevent these complications. Treatment may involve wound debridement (removal of dead tissue), offloading pressure from the affected area, and applying dressings or topical medications to promote healing. In some cases, antibiotics may be prescribed to treat or prevent infection (Steffy, *et.al.*, 2018)). Individuals with diabetic foot ulcers may also benefit from specialised footwear or orthotic devices to reduce pressure on the affected area and promote proper healing. Regular monitoring of the ulcer and close follow-up with a healthcare provider are important to track progress and identify any signs of infection or complications.

Preventing diabetic foot ulcers requires comprehensive foot care and management of diabetes. This includes maintaining good blood sugar control, inspecting the feet daily for signs of injury or ulceration, wearing appropriate footwear, and avoiding activities that could cause trauma to the feet. People with diabetes should also seek regular foot examinations by a healthcare professional, particularly if they have neuropathy or other risk factors for foot complications (Rosyid, 2017). Education about proper foot care and self-management strategies is crucial for individuals with diabetes to reduce their risk of developing foot ulcers.

CHAPTER 2

LITERATURE SURVEY

The article discusses *Staphylococcus aureus* (*S. aureus*) is one of the most prevalent bacterial pathogens recovered from diabetic foot infections (DFIs). Most *S. aureus* isolates exhibit methicillin resistance, so treatment is recommended with antimicrobials active against methicillin-resistant *S. aureus* (MRSA) in patients who have risk factors associated with MRSA infections. The main goal of this study seems to be to see if proteomics and molecular methods could be effective in identifying and distinguishing MRSA recovered from DFIs (Abalkhail and Elbehiry, 2022)

The article talks in detail about Proper assessment of the diabetic foot ulceration and appropriate management ensure better prognosis. Management is based on revascularization procedures, wound debridement, treatment of infection and ulcer offloading. Management and type of dressing applied are tailored according to the type of wound and the foot condition. The scope of this review paper seems to describe the diabetic foot syndrome starting from the evaluation of the foot at risk for ulceration, up to the new treatment modalities. (Amin and Doupis, 2016).

The study was conducted to evaluate the prevalence of peripheral arterial disease (PAD) in diabetic foot ulcer patients and its impact on limb salvage as an outcome. This prospective cross-sectional study included 392 cases, who were divided according to the presence of PAD into 2 groups; patients with PAD were labeled as PAD +ve (172 cases) and those without PAD were labeled as PAD -ve (22 cases). (Basheer, *et al*, 2023)

The article discusses soft tissue infections, neuropathy, artery occlusive disease, foot deformity, invasive foot infections, critical limb ischemia, annual foot assessment, foot arterial perfusion, off-loading therapy, plantar skin pressure, systemic antibiotic therapy, surgical debridement. (Bandyk, *et.al.*, 2018).

The article discusses risks experienced by a patient suffering from diabetic foot ulcer. It explains in detail about the management of diabetic neuropathic foot ulceration. The article encourages regular screening and patient education (Boulton, 2019).

The article gives a thorough understanding of the importance timely diagnosis of diabetes and discusses the management techniques employed to effectively tackle the disease (Carl, *et al.*, 2018)

The aim of this study was to assess the prevalence of MRSA as well as other microbiota in 100 patients diagnosed with (DM2) and with infected foot ulcers at the Hospital General de Mexico. (Cervantes-García, *et al.*, 2015)

The review focuses on the prevention and early identification of the 'at-risk' foot. DFD is best managed by a multidisciplinary foot clinic team consisting of podiatrists and healthcare professionals. This ensures a combination of expertise and provision of a multi-faceted evidence-based treatment plan. Current research using endothelial progenitor cells (EPC) and mesenchymal stem cells (MSC) offers a new dimension in wound management. (Craus and Coppini, 2023).

The article comprehensively reviews cutting-edge methods and the science behind debridement and diabetic foot ulcers. Debridement is often used as a standard of care. Debridement consists of both non-mechanical (autolytic, enzymatic) and mechanical methods (sharp/surgical, wet to dry debridement, aqueous high-pressure lavage, ultrasound, and biosurgery/maggot debridement therapy). (Dayya, *et al.*, 2022).

A comprehensive summary and description of classification systems of diabetic foot infections, and a comprehensive discussion of microbiology. Knowledge of the microbial aetiologies in diabetic foot infections, and understanding of antibiotic resistance, is critical for the effective management and treatment of these infected wounds. (Ghotaslos, 2018).

The article focus is on the challenges faced during diagnosis of infection in the diabetic foot ulcers as it is a difficult task. Determining whether or not infection is present in bone can be especially difficult. Improved methods developed and tested over the past

decade have clearly increased our accuracy in diagnosing diabetic foot infections. (Glaudemans, 2015).

This article discusses risks of diabetic ulcer, infections and treatment methods such as removal of callus, good wound care, reducing pressure on the ulcer, good glucose control, good nutrition, antibiotics for diabetic foot ulcers. (Grennan, 2019).

These guidelines have addressed five key areas in the care of DFUs, they do not cover all the aspects of this complex condition include preventive recommendations such as those for adequate glycemic control, periodic foot inspection, and patient and family education. (Hingorani, *et al.*, 2016).

This article focuses on the use of different wound care materials in diabetic foot. From a clinical perspective, it is important to decide on the wound care material depending on the type and grade of the ulcer. This article also provides clinicians with a simple approach to the choice of wound care materials in diabetic foot ulcer. (Kavitha, *et al.*, 2014).

The present review aims to summarise the causes and pathogenesis leading to diabetic foot ulcers, various classification systems and to focus on the current management of this significant and preventable health condition. (Khan, 2017).

The aim of this paper seems to be to present a comprehensive evidence-based review of the literature available on detriments to healing of diabetic foot ulcers. Diabetes mellitus places a substantial burden on society worldwide. Diabetic foot ulcers are a challenging problem for clinicians. (Lefrancois and Glazebrook, 2017).

This review article aims to summarize major risk factors contributing to the development of diabetic foot ulcers. It also considers the key evidence-based strategies towards preventing diabetic foot ulcers. The author discusses tools used in risk stratification and classifications of foot ulcers. (Lim and Thomas, 2017)

This article reviews recommendations from evidence-based guidelines, informed by results of systematic reviews, on treating diabetic foot infections. Treatment for diabetic foot infections requires properly diagnosing infection. (Lipsky, 2016).

This article aimed to investigate the prevalence of bacteria isolated from diabetic foot infections using studies of any design which reported diabetic foot infection culture results. Diabetic foot ulcers are a common complication of poorly controlled diabetes and often become infected, termed diabetic foot infection. (Macdonald and Jones, 2021).

This article analyzed the antimicrobial susceptibility patterns of biofilm-producing *S. aureus* strains isolated from diabetic foot infection. Foot infections are a major cause of morbidity in people with diabetes and the most common cause of diabetes-related hospitalization and lower extremity amputation. *Staphylococcus aureus* is by far the most frequent species isolated from these infections (Mottola, *et al.*, 2016).

This study aimed to evaluate the microbial etiology of infected diabetic foot ulcers in northern tertiary care hospital, assessment of risk factors and role of inflammatory markers involved in colonization of multidrug-resistant organisms (MDROs) and their impact on the outcome (Noor khan, 2017).

CHAPTER 3

AIM AND SCOPE

3.1 AIM:

- To Develop Polymer-Nano carriers for Sustainable Drug Delivery in Chronic Wounds” is to design, synthesise, and characterise biocompatible polymer-based nano-carriers capable of efficiently delivering therapeutic agents for the treatment of chronic wounds.
- To address the challenges associated with conventional wound management strategies by developing advanced drug delivery systems that can provide sustained release of therapeutic agents, enhance wound healing processes, and mitigate complications associated with chronic wound management.
- To leverage the unique properties of polymer nanocarriers, such as tunable drug release kinetics, enhanced stability, and targeted delivery, the project seeks to contribute to the development of innovative and effective strategies for the treatment of chronic wounds.

3.2 SCOPE:

- The scope of the project encompasses several key areas, including polymer selection and synthesis, formulation development, characterisation of nano-carriers, and evaluation of their efficacy in wound healing.
- The project will involve the synthesis and optimization of biocompatible polymers suitable for use as nano-carrier materials, considering factors such as biodegradability, cytocompatibility, and ability to encapsulate and release therapeutic agents.
- Formulation development will focus on designing nano-carrier systems capable of encapsulating a variety of therapeutic agents, including antibiotics, growth factors, and anti-inflammatory drugs, with the aim of achieving sustained and controlled drug release profiles.

CHAPTER 4

MATERIALS AND METHODS

4.1 MATERIALS:

4.1.1 GRAPHENE OXIDE NANOPARTICLES:

Graphene oxide nanoparticles have gained attention for their potential applications in wound healing due to their unique properties. These nanoparticles possess high surface area, excellent mechanical strength, and antimicrobial properties, making them promising candidates for accelerating wound closure and preventing infections. Graphene oxide can promote cell adhesion, proliferation, and migration, crucial processes for tissue regeneration. Additionally, it can modulate the inflammatory response, promoting a balanced healing environment. Its transparency allows for real-time monitoring of wound healing progress. Graphene oxide nanoparticles can also be functionalized with bioactive molecules to further enhance their therapeutic effects. However, challenges such as biocompatibility and long-term safety need to be addressed for their successful clinical application.

4.1.2 CERIUM OXIDE:

Cerium oxide nanoparticles have emerged as promising agents for wound healing due to their antioxidant and anti-inflammatory properties. These nanoparticles scavenge free radicals, reducing oxidative stress and promoting a favourable environment for tissue regeneration. Cerium oxide nanoparticles also exhibit antimicrobial activity, preventing infections in wounds. They can modulate the inflammatory response, promoting faster healing and reducing scar formation. Additionally, cerium oxide nanoparticles have been shown to accelerate angiogenesis, facilitating blood vessel formation and improving tissue perfusion. Their biocompatibility and low toxicity further enhance their potential for clinical use in wound healing applications. However, further research is needed to optimise their formulations and understand their long-term effects before widespread clinical adoption.

4.1.3 ALOE VERA:

Aloe vera has been traditionally used for wound healing due to its anti-inflammatory and antimicrobial properties. It contains compounds such as polysaccharides, enzymes, and vitamins that promote tissue repair and regeneration. Aloe vera gel can soothe the skin, reduce pain, and accelerate wound closure by stimulating the production of collagen and elastin. Its moisturising effects help prevent wound dehydration and promote a moist wound healing environment, which is conducive to faster healing. Clinical studies have shown the effectiveness of aloe vera in treating various types of wounds, including burns, cuts, and abrasions.

4.1.4 BORATE BIOACTIVE GLASS:

Borate bioactive glass has garnered attention for its potential in wound healing applications. It releases therapeutic ions such as boron, calcium, and silica, which promote cell proliferation and tissue regeneration. These ions also exhibit antimicrobial properties, reducing the risk of infection in wounds. Borate bioactive glass can accelerate the formation of new blood vessels, aiding in tissue repair and improving wound healing outcomes. Its ability to adhere to the wound bed provides a scaffold for cell attachment and growth, facilitating the healing process. Clinical studies have shown promising results in using borate bioactive glass dressings for promoting wound healing in various settings.

4.2 METHODS:

4.2.1 Methods used to Synthesis graphene oxide

Graphite flakes (2 g) and NaNO_3 (2 g) were mixed in 50 mL of H_2SO_4 (98%) in a 1000 mL volumetric flask kept under an ice bath ($0-5^\circ\text{C}$) with continuous stirring.

The mixture was stirred for 2 hrs at this temperature and potassium permanganate (6 g) was added to the suspension very slowly. The rate of addition was carefully controlled to keep the reaction temperature lower than 15°C . The ice bath was then removed, and the mixture was stirred at 35°C until it became pasty brownish and kept under stirring for 2 days. It is then diluted with a slow addition of 100 ml water. The reaction temperature was rapidly increased to 98°C with effervescence, and the color changed to brown colour. Further this solution was diluted by adding additional 200 ml of water stirred continuously. The solution is finally treated with 10 ml H_2O_2 to terminate the reaction by appearance of yellow colour. For purification, the mixture was washed by rinsing and centrifugation with 10% HCl and then deionised (DI) water several times. After filtration and drying under vacuum at room temperature, the graphene oxide (GO) was obtained as a powder.

4.2.2 Characterizations

1.XRD Analysis (X-Ray Diffraction):

XRD analysis is a technique used to study the crystallographic structure of materials, including graphene oxide. In XRD analysis, a sample is exposed to X-rays, and the resulting diffraction pattern is measured. This pattern provides information about the arrangement of atoms within the material, including their spacing and orientation. For graphene oxide, XRD can help determine the degree of oxidation and the interlayer spacing between graphene sheets. By analysing the peaks and intensities in the diffraction pattern, researchers can gain insights into the structural properties of graphene oxide samples.

2. Raman Spectroscopy:

Raman spectroscopy is a powerful analytical technique used to investigate the vibrational modes of molecules in a material. When a sample is illuminated with laser light, some of the scattered light undergoes a shift in frequency due to interactions with molecular vibrations, known as Raman scattering. By analysing the frequency shifts, researchers can identify the molecular composition and structure of the material. In the case of graphene oxide, Raman spectroscopy can provide information about the degree of oxidation, the presence of functional groups, and the quality of the graphene sheets. Specific Raman peaks correspond to different structural features, such as the D band (disorder-induced mode) and the G band (graphitic sp² carbon), allowing researchers to characterise graphene oxide samples and assess their quality and properties.

3.Colony Forming Unit (CFU) Analysis:

Colony forming unit analysis is a technique used to quantify the number of viable bacterial cells present in a sample. In this context, CFU analysis is performed on bacterial cultures treated with different materials such as graphene oxide, cerium oxide, borate bioactive glass, and aloe vera. The materials are tested for their antibacterial properties by incubating them with bacterial cultures of *Staphylococcus aureus* (*S. aureus*) and *Escherichia coli* (*E. coli*). After a specified incubation period, the bacterial cultures are plated onto agar plates and allowed to grow into colonies. Each visible colony represents a single viable bacterial cell. By counting the colonies on the plates, researchers can determine the number of CFUs and assess the materials' effectiveness in inhibiting bacterial growth.

4. Protein Leakage Assay:

The protein leakage assay is a method used to evaluate the integrity of bacterial cell membranes when exposed to different materials or treatments. In this assay, bacterial cultures treated with graphene oxide, cerium oxide, borate bioactive glass, and aloe vera are incubated for a specific period. After incubation, the cultures are centrifuged

to separate the bacterial cells from the surrounding medium. The supernatant, which contains any proteins leaked from damaged bacterial cells, is collected and subjected to analysis using Bradford reagent. Bradford reagent binds to proteins, causing a color change that can be measured spectrophotometrically. An increase in absorbance indicates higher protein leakage, suggesting damage to the bacterial cell membranes caused by the materials being tested.

5. UV Spectrophotometer Analysis:

UV spectrophotometer analysis is used to quantify the amount of protein leakage in the supernatant collected from the protein leakage assay. The collected supernatant is analysed using a UV-visible spectrophotometer, which measures the absorbance of light at specific wavelengths. Bradford reagent binds to proteins, causing a colour change that corresponds to the concentration of proteins present in the sample. By measuring the absorbance of the sample at the appropriate wavelength, researchers can determine the concentration of leaked proteins and assess the extent of bacterial cell membrane damage caused by the materials under investigation. This analysis provides quantitative data on the efficacy of the materials in disrupting bacterial cell membranes and inhibiting bacterial growth.

4.2.3 Preparation of Graphene Oxide nanoparticles:

a)



b)



Fig 4.1 Preparation of GO first trial

Fig 4.2 Preparation of GO second trial

The process of preparing graphene oxide (GO) nanoparticles for the development of a hydrogel patch for diabetic foot ulcer treatment began by mixing graphite flakes and NaNO_3 in concentrated sulphuric acid under an ice bath, with continuous stirring to maintain a temperature range of $0-5^\circ\text{C}$. After two hours of stirring, potassium permanganate was cautiously added to the mixture to prevent the reaction temperature from exceeding 15°C . The ice bath was then removed, and the mixture was stirred at 35°C until it became pasty brownish and left under stirring for two days. Subsequently, water was slowly added to dilute the solution, causing the temperature to rise rapidly to 98°C , resulting in a colour change to brown. Additional water was then added, followed by the introduction of hydrogen peroxide to halt the reaction, indicated by the appearance of a yellow colour.

To purify the solution, it underwent multiple rinses and centrifugations with 10% hydrochloric acid and deionized water. After filtration and vacuum drying at room temperature, the graphene oxide was obtained as a powder, ready for further

processing into the desired hydrogel patch. Despite careful adherence to the protocol

A)



B)



Fig 4.3 Preparation of Graphene oxide trail. 3 Fig 4.4 separation of graphene oxide

A)



B)



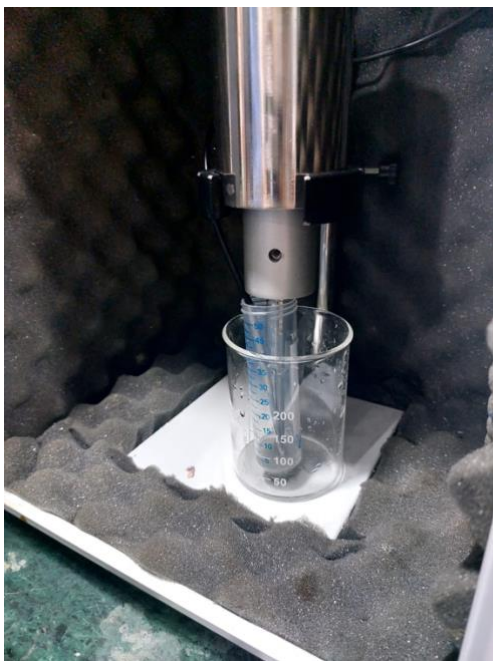
Fig 4.5 Centrifugation process Fig 4.6 Separation of Graphene oxide from supernatant

the process encountered two unsuccessful trials, the reasons for which remain elusive due to the intricate complexities inherent in nanotechnology, where even minor

alterations in parameters can yield unpredictable outcomes. The preparation of graphene oxide nanoparticles for a hydrogel patch aimed at addressing diabetic foot ulcers encountered hurdles along the way, with two notable unsuccessful trials. Despite diligent efforts to refine the process, understanding why these attempts fell short proves challenging due to the intricate web of factors at play within the realm of nanotechnology. Nanotechnology's complexity adds layers of uncertainty; altering one variable may unexpectedly impact others, making it akin to solving a puzzle with constantly shifting pieces. The elusive nature of these failures underscores the complexity of harnessing nano-materials for medical breakthroughs. Success in this endeavour demands not only technical prowess but also a deep appreciation for the nuanced interplay of factors at the nanoscale, where even subtle changes can yield significant consequences.

Centrifugation of a Graphene oxide (GO) slurry involves spinning the mixture at high speeds to separate the nanoparticles from the solution based on their size and density differences. As the centrifuge accelerates, the gravitational force causes the denser particles, such as aggregated GO sheets, to sediment towards the bottom of the tube. Meanwhile, the lighter nanoparticles remain suspended in the supernatant. The duration and speed of centrifugation are critical parameters that determine the efficiency of nanoparticle separation. After centrifugation, the supernatant containing the suspended nanoparticles can be carefully decanted or pipetted off, leaving behind the sedimented GO aggregates. Multiple centrifugation cycles may be necessary to achieve optimal separation and purification. Once separated, the nanoparticles can be further characterized or used for various applications, such as in antibacterial analysis or nanocomposite fabrication. Proper safety precautions, including the use of appropriate protective gear and handling procedures, should be followed when working with centrifuges and nanoparticle suspensions. Regular maintenance and calibration of the centrifuge are essential to ensure accurate and reproducible results.

a)



b)

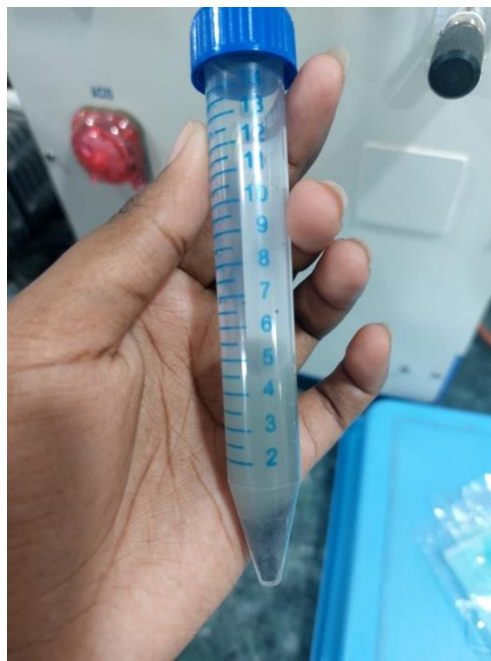


Fig 4.7 Probe sonicator in process. Fig 4.8 Preparation of liquified graphene oxide

A probe-sonicator, also known as an ultrasonic probe or ultrasonic homogenizer, is a device used to break down particles or create uniform particle dispersion in a liquid medium using high-frequency sound waves. It consists of a generator that produces electrical signals, a transducer that converts these signals into mechanical vibrations, and a probe or horn that delivers these vibrations into the liquid sample. When the probe tip is immersed in the sample, the ultrasonic waves create intense pressure variations, leading to the formation and collapse of tiny bubbles in a phenomenon called cavitation. This cavitation generates localized high temperatures and pressures, causing shear forces and micro-streaming that effectively disrupt agglomerates and disperse particles throughout the liquid. In the context of graphene oxide nanoparticles, a probe sonicator is often employed during the preparation process to achieve several objectives.

a)



b)



Fig 4.9 Scraping dried Graphene oxide Fig 4.10 Storage of Graphene oxide

Firstly, it helps in exfoliating graphite flakes to produce graphene oxide sheets. Secondly, it assists in breaking down larger graphene oxide aggregates into smaller, more uniform nanoparticles, enhancing their dispersibility and surface area. This increased surface area is crucial for applications such as drug delivery, sensing, and catalysis. Additionally, sonication can facilitate the functionalization of graphene oxide by promoting the attachment of desired molecules onto its surface, thereby tailoring its properties for specific applications. After probe sonication, the graphene oxide (GO) nanoparticle suspension is typically centrifuged to separate the nanoparticles from the solution. Once separated, the supernatant containing the suspended nanoparticles is carefully removed, leaving behind a concentrated pellet of GO nanoparticles. This pellet is then washed with a suitable solvent, such as water or ethanol, to remove any residual impurities or contaminants. After washing, the pellet is air-dried or vacuum-dried to remove the solvent and ensure the nanoparticles are in a dry state. Finally, the dried pellet is scraped from the centrifuge tube using a spatula or similar tool, and it is then transferred into a vial or container for future testing or characterisation, such as antibacterial analysis or nano-composite fabrication. Proper safety precautions should be taken throughout the process to handle nanoparticles safely and avoid contamination.

4.2.4 ANTIBACTERIAL ANALYSIS:

Composition of GO, Aloe Vera, CeO₂

MATERIAL	COMPOSITION -1 (mg/ml)	COMPOSITION -2 (mg/ml)	COMPOSITION -3 (mg/ml)
Graphene oxide	5	5	5
Aloe Vera	2	2	2
Cerium oxide	10	10	10

Table 4.1

In these comprehensive characterisations, the antibacterial analysis embarked upon a meticulous exploration using an epifluorescence microscope, a sophisticated tool renowned for its precision in visualising microscopic structures. The primary focus of the study was to unravel the potential antimicrobial prowess residing within graphene oxide, cerium oxide nanoparticles, and the venerable Aloe vera extract.

A)

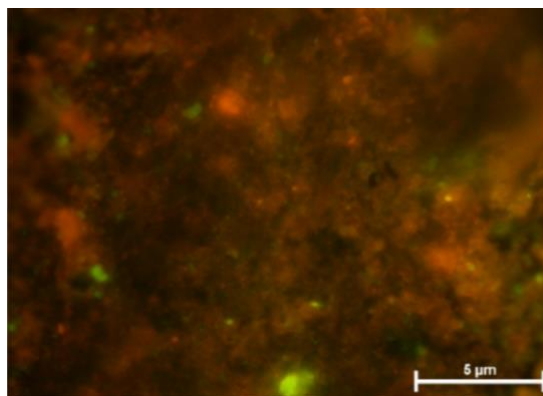


Fig 4.11

Maximum dead bacterial cells appeared as the concentration increased

These materials, selected for their diverse compositions and reputed medicinal properties, held promise as agents in combating bacterial pathogens, a pressing concern in healthcare and beyond.

B)

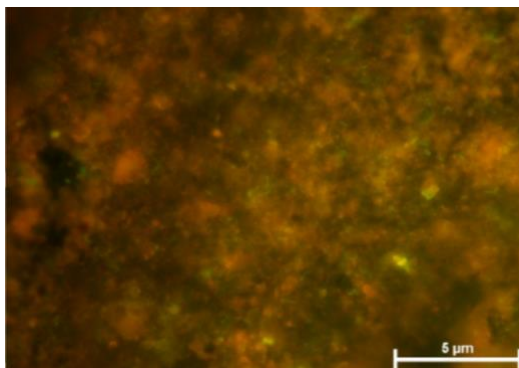


Fig 4.12

Moderate number of dead bacterial cells appeared

Methodically, the experiment unfolded with the preparation of meticulously crafted samples, wherein graphene oxide, cerium oxide nanoparticles, and Aloe vera extract were thoughtfully dispersed in suitable solvents, ensuring optimal interaction with bacterial cultures.

C)

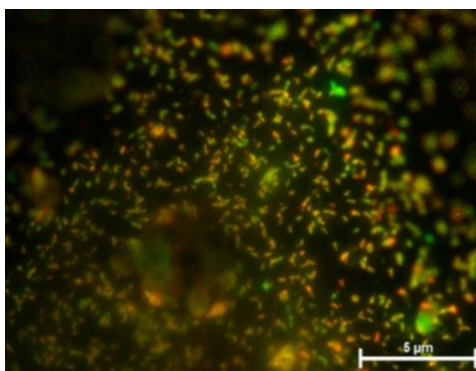


Fig 4.13

Less number of bacterial cells appeared

4.2.5 COLONY FORMING UNIT (CFU) ANALYSIS:

A)

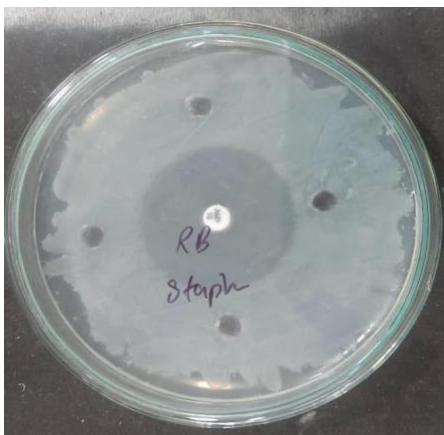


Fig 4.14 Colony Forming unit of *S.aureus* first trial.

B)

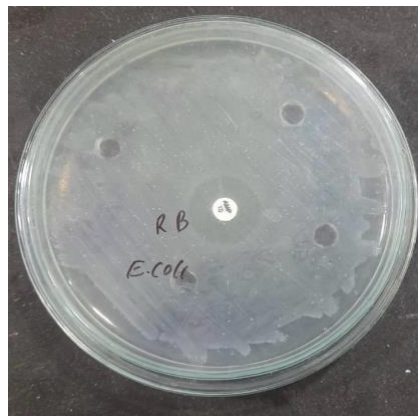
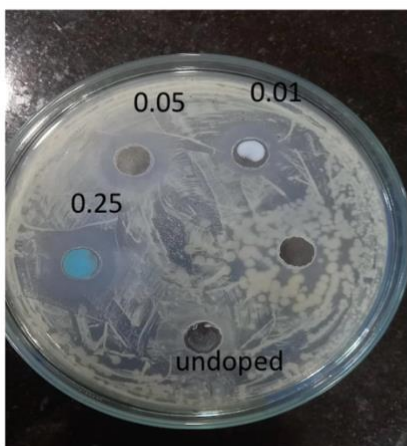


Fig 4.15 Colony Forming Unit of *E.coli* first trial

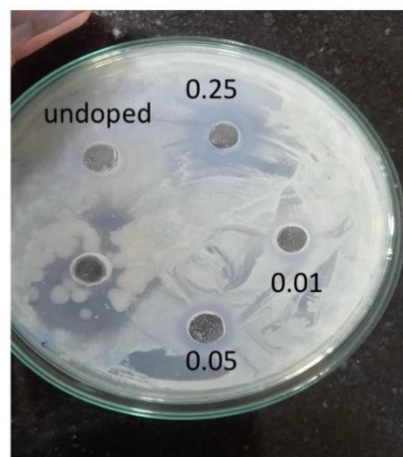
The project embarked upon a rigorous exploration of colony-forming unit (CFU) analysis, a pivotal technique in microbiology, to assess the antibacterial efficacy of various materials against the notorious gram-positive bacterium *Staphylococcus aureus* and the ubiquitous gram-negative *Escherichia coli* (*E. coli*). Leveraging the

A)



**Fig 4.16 Second trial of antibacterial
On *S.aureus* 100g concentration.**

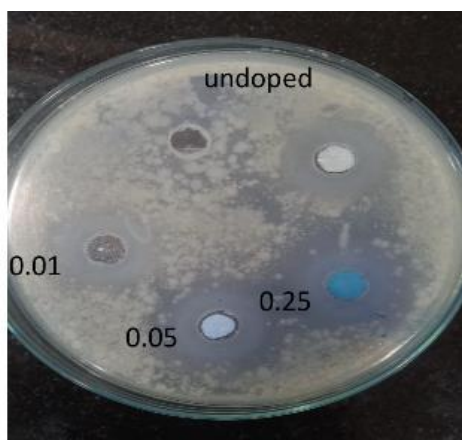
B



**Fig 4.17 Second trial of antibacterial
On *S.aureus* 50g concentration**

distinctive characteristics of CFU analysis, which offers a quantitative measure of viable bacterial colonies, the study aimed to elucidate the comparative effectiveness of graphene oxide, cerium oxide nanoparticles, borate bioactive glass, and the venerable

A)



B)

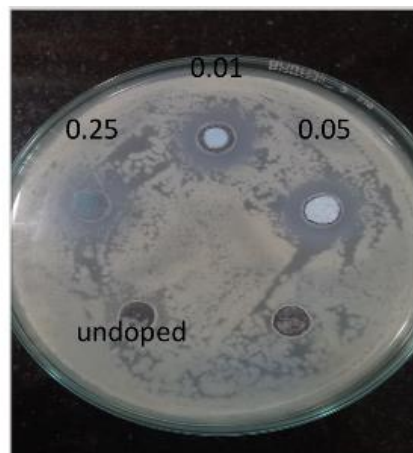


Fig 4.18 Antibacterial activity on *E.coli* 100g concentration. **Fig 4.19 Antibacterial activity on *E.coli* 50g concentration**

Aloe vera extract in combating these formidable microbial adversaries. Methodically, bacterial cultures of *E. coli* and *Staphylococcus aureus* were subjected to varying concentrations of the aforementioned materials, meticulously prepared to ensure optimal interaction and dispersion. The first trial was unsuccessful the successive trial was produced positive results.

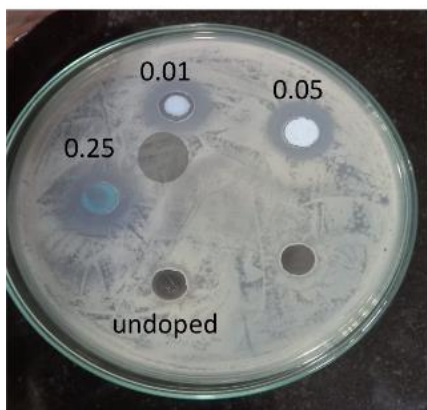


Fig 4.20 Antibacterial activity on *Enterobacter* 100g.

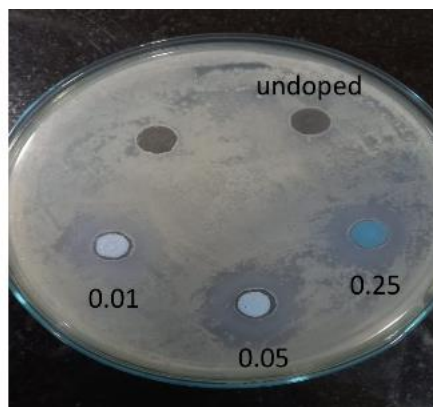


Fig 4.21 Antibacterial activity on *Enterobacter* 50g.

Following an incubation period, during which the materials engaged in a dynamic interplay with the bacterial colonies, CFU analysis emerged as the linchpin, offering insights into the materials' efficacy in inhibiting bacterial growth and survival.

The outcomes of the project underscored a nuanced landscape of antibacterial activity, revealing differential responses among the tested materials against the gram-positive and gram-negative bacteria.

Notably, graphene oxide exhibited robust efficacy in inhibiting colony formation of both *E. coli* and *Staphylococcus aureus*, suggesting its potential as a versatile antimicrobial agent. Cerium oxide nanoparticles demonstrated promising antibacterial activity, particularly against *E. coli*, while borate bioactive glass and Aloe vera extract exhibited varying degrees of effectiveness against the tested bacterial strains. These compelling findings not only shed light on the intricate interplay between materials and microbial pathogens but also pave the way for future research endeavours aimed at harnessing the antibacterial potential of novel materials for diverse biomedical and environmental applications.

The combination of these materials in antibacterial analysis offers a multifaceted approach, targeting various bacterial mechanisms simultaneously. Graphene oxide, cerium oxide, and borate bioactive glass collectively demonstrate synergistic effects, enhancing the overall antibacterial efficacy against *Enterobacter* species. Their distinct modes of action make them promising candidates for combating bacterial infections, including those caused by *Enterobacter* strains. Further research into their combined application could lead to the development of novel antibacterial strategies with improved effectiveness and reduced resistance potential.

4.2.6 CFU COUNT OF GO, CeO₂, ALOE VERA AND BORATE BIOACTIVE GLASS AGAINST *Enterobacter*:

100µg concentration

A)



Fig 4.22 Undoped BBG CFU

B)

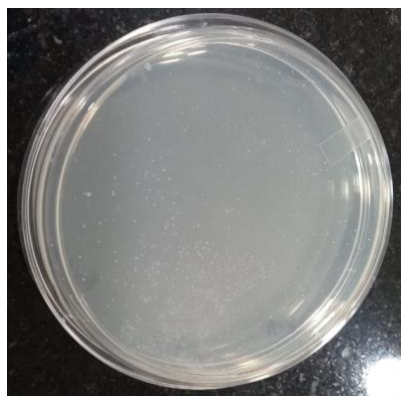


Fig 4.23 0.05M CFU

C)

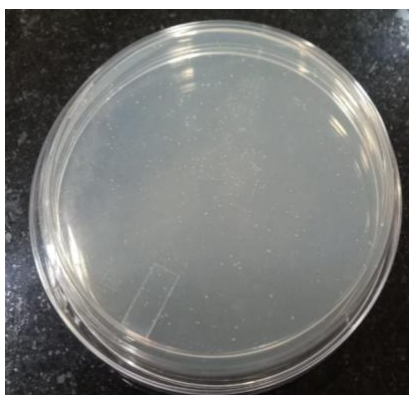


Fig 4.24 0.25M

D)

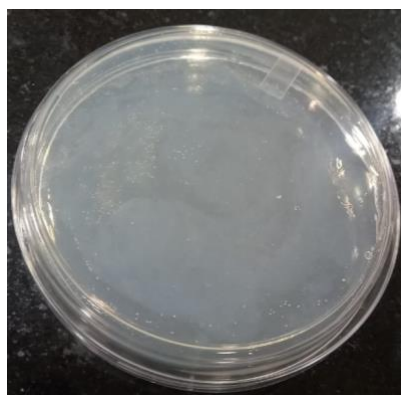


Fig 4.25 0.01M CFU

In the antibacterial analysis of *Enterobacter* species, Graphene oxide (GO) demonstrates promising results due to its nanostructure and inherent antibacterial properties. It disrupts bacterial cell membranes, inhibiting their growth and proliferation effectively. Cerium oxide nanoparticles exhibit strong antioxidant and antibacterial activities, potentially disrupting vital cellular processes in *Enterobacter* strains. Borate bioactive glass, known for its ion release properties, can disrupt bacterial cell walls and interfere with their metabolic pathways, rendering them less viable.

50 μ g concentration

A)

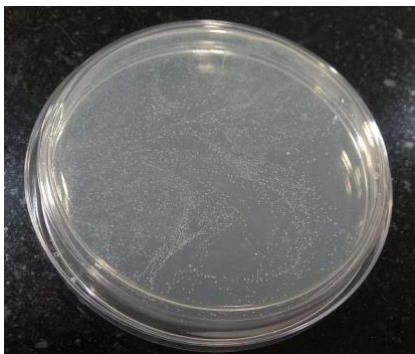


Fig 4.26 Undoped CFU.

B)

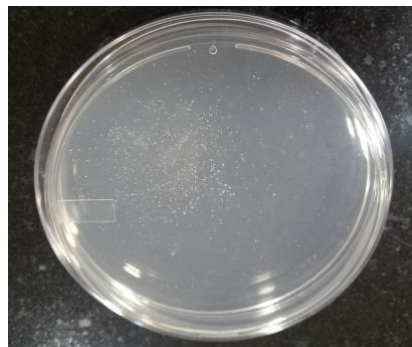


Fig 4.27 0.05M CFU

B)



Fig 4.28 0.25M CFU.

D)



Fig 4.29 0.01M CFU

The combination of these materials in antibacterial analysis offers a multifaceted approach, targeting various bacterial mechanisms simultaneously. Graphene oxide, cerium oxide, and borate bioactive glass collectively demonstrate synergistic effects, enhancing the overall antibacterial efficacy against *Enterobacter* species. Their distinct modes of action make them promising candidates for combating bacterial infections, including those caused by *Enterobacter* strains. Further research into their combined application could lead to the development of novel antibacterial strategies with improved effectiveness and reduced resistance potential.

4.2.7 XRD analysis:

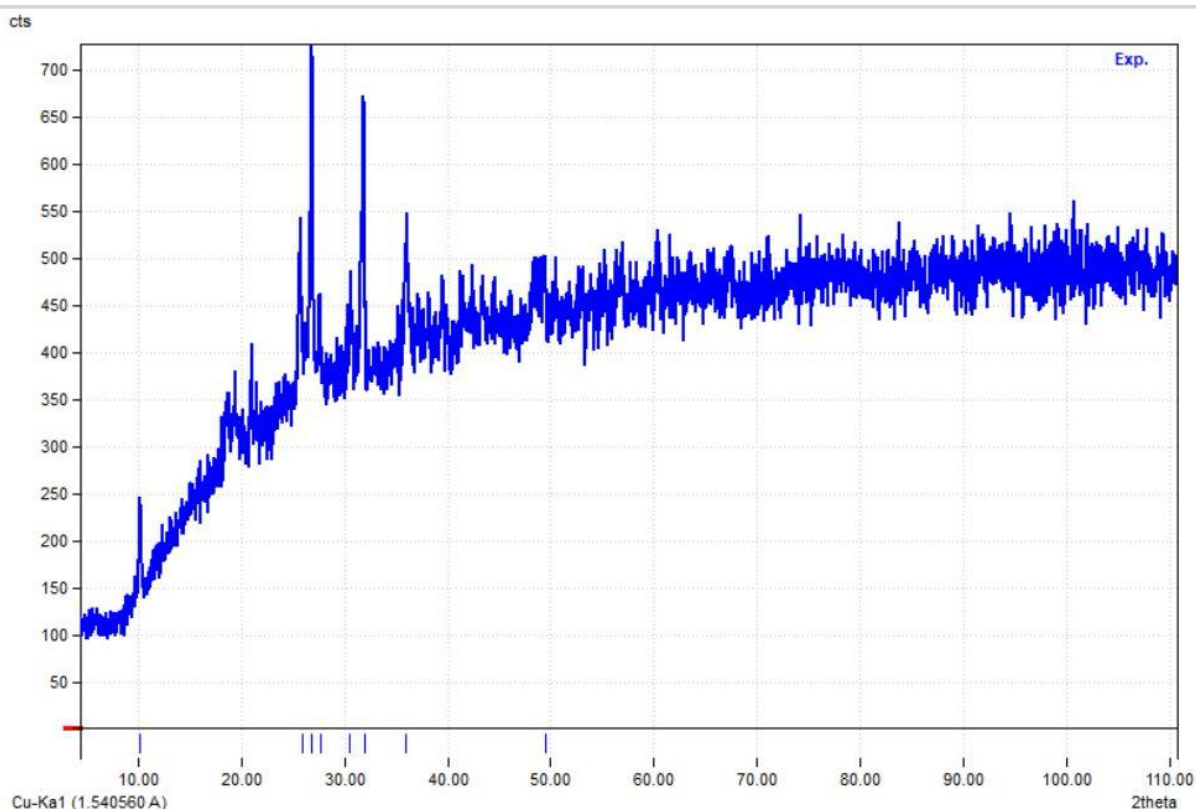


Fig 4.30 X-ray diffraction of Graphene oxide nano particles

X-ray diffraction (XRD) is a powerful analytical technique used to examine the crystalline structure of materials, including graphene oxide nanoparticles. In the context of graphene oxide, XRD can provide valuable insights into its atomic arrangement, layer stacking, and degree of oxidation. During the XRD test, a beam of X-rays is directed at the sample, and the X-rays are diffracted by the atomic planes within the material. The resulting diffraction pattern, characterized by peaks at specific angles, provides information about the spacing between these atomic planes, known as d-spacing, as well as the orientation and ordering of the crystal lattice.

For graphene oxide nanoparticles, XRD analysis helps in determining the degree of oxidation and structural integrity of the material. Graphene oxide typically exhibits a characteristic diffraction peak at around 10-12 degrees 2θ , corresponding to the interlayer spacing between graphene sheets. The intensity and position of this peak

can vary depending on factors such as the degree of oxidation, the presence of functional groups, and the size of the nanoparticles. Additionally, XRD can also detect the presence of impurities or phases within the sample, providing insights into its purity and composition.

Overall, X-ray diffraction analysis plays a crucial role in characterizing graphene oxide nanoparticles, providing valuable information about their structural properties, composition, and quality. By interpreting the diffraction patterns obtained from XRD tests, researchers can gain deeper insights into the nature of graphene oxide and tailor its properties for various applications in fields such as electronics, energy storage, and biomedical engineering.

4.2.8 PROTEIN LEAKAGE ASSAY:

A)

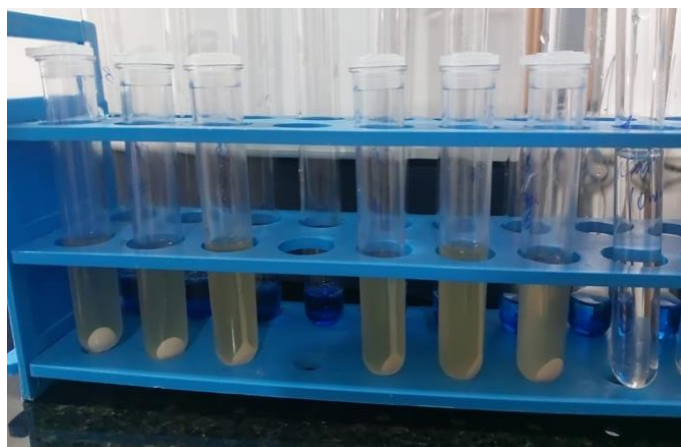


Fig 4.31 Protein leakage assay using *Escherichia coli*

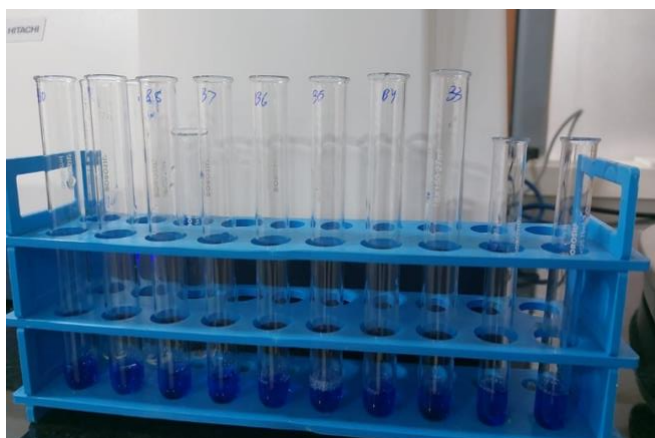


Fig 4.32 Protein leakage assay using *S.aureus*

In the context of assessing the effectiveness of graphene oxide, cerium oxide nanoparticles, Aloe Vera, and borate bioactive glass against bacterial pathogens such as *Escherichia coli* (*E. coli*) and *Staphylococcus aureus*, a protein leakage assay serves as a pivotal tool. This assay involves exposing bacterial cultures of *E. coli* and *Staphylococcus aureus* to the materials under investigation and subsequently measuring the release of proteins from the bacterial cells into the surrounding medium. The extent of protein leakage serves as an indicator of bacterial membrane integrity and potential damage inflicted by the materials.

By conducting a protein leakage assay, we can quantitatively evaluate the ability of graphene oxide, cerium oxide nanoparticles, Aloe Vera, and borate bioactive glass to disrupt bacterial membranes and induce leakage of intracellular proteins. A decrease in the amount of leaked proteins indicates a potential antimicrobial effect of the materials, suggesting their ability to compromise bacterial cell integrity and inhibit bacterial growth. Conversely, an increase in protein leakage may suggest bacterial membrane damage and subsequent cell lysis, further corroborating the antimicrobial activity of the tested materials.

4.2.9 UV SPECTROPHOTOMETER:



Fig 4.33 UV spectrophotometer

UV spectroscopy is utilized to analyze the interaction between bacterial cultures, such as *Staphylococcus aureus* and *Escherichia coli* (*E. coli*), and various materials including borate bioactive glass, Aloe Vera, graphene oxide, and cerium oxide nanoparticles. UV spectroscopy operates by measuring the absorbance of ultraviolet (UV) light by molecules in a sample, allowing for quantitative analysis of chemical constituents and their interactions. In the context of bacterial studies, UV spectroscopy can provide insights into the antimicrobial activity of the tested materials through several key mechanisms:

Evaluation of Bacterial Growth Inhibition: UV spectroscopy can monitor changes in the growth kinetics of bacterial cultures in the presence of the tested materials. By measuring absorbance at specific wavelengths, researchers can quantify bacterial growth over time. A decrease in absorbance indicates inhibition of bacterial growth, suggesting antimicrobial activity of the materials. Conversely, an increase in absorbance may indicate bacterial proliferation or lack of inhibitory effects.

Assessment of Cellular Integrity: UV spectroscopy can also assess the integrity of bacterial cell membranes and cellular components. The absorbance spectrum of

bacterial cultures may exhibit characteristic peaks corresponding to cellular constituents such as proteins, nucleic acids, and pigments. Changes in the absorbance spectrum, such as shifts or alterations in peak intensity, can indicate damage to bacterial cells caused by the tested materials. This provides valuable information about the mechanism of action and efficacy of the materials against bacterial pathogens.

Detection of Metabolic Activity: UV spectroscopy can be coupled with metabolic assays, such as tetrazolium salt reduction assays, to evaluate bacterial metabolic activity in the presence of the tested materials. Metabolic assays rely on the conversion of a colorless tetrazolium salt into a colored formazan product by metabolically active bacteria. UV spectroscopy can then quantify the amount of formazan produced, providing a measure of bacterial metabolic activity and viability.

4.2.10 RAMAN SPECTROSCOPY ANALYSIS:

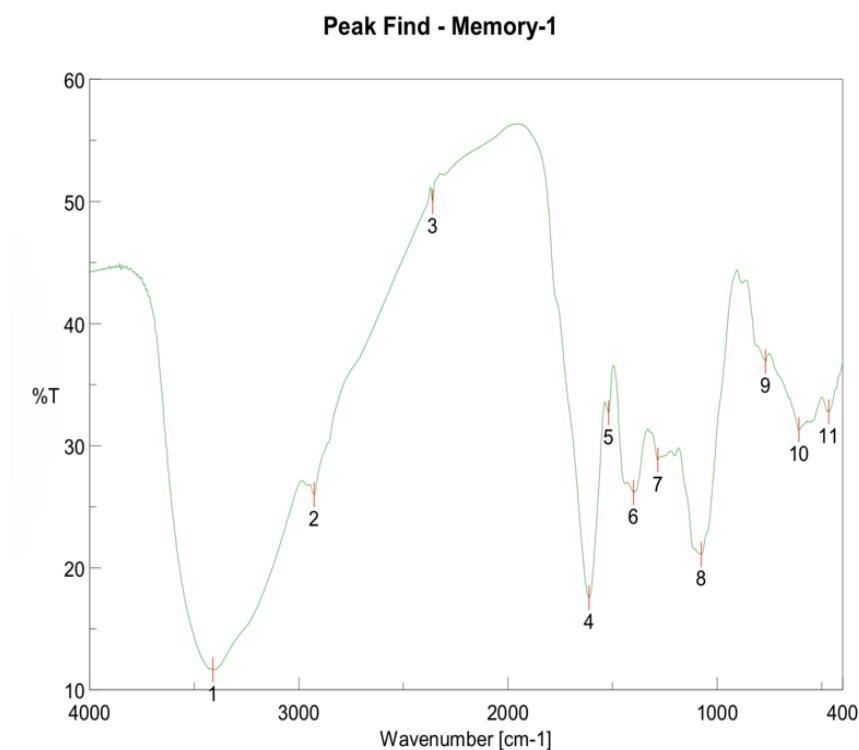


Fig 4.34 Raman spectroscopy

Raman spectroscopy is a valuable analytical technique used to characterize the structural and chemical properties of materials, including graphene oxide nanoparticles. In the context of graphene oxide, Raman spectroscopy is particularly useful for several reasons: Identification of Functional Groups: Graphene oxide is a derivative of graphene with oxygen-containing functional groups, such as hydroxyl, epoxy, and carboxyl groups, introduced during its synthesis.

Raman spectroscopy can provide information about the types and concentrations of these functional groups by detecting characteristic peaks in the Raman spectrum. Each functional group exhibits unique vibrational modes, allowing for their identification and quantification. Assessment of Degree of Oxidation: The degree of oxidation in graphene oxide, which influences its chemical and physical properties, can be determined using Raman spectroscopy. Changes in the intensity and position of Raman peaks associated with carbon atoms in the graphene lattice provide insights into the extent of oxidation.

Higher degrees of oxidation result in greater disruption of the sp^2 carbon network and alterations in the Raman spectrum. Detection of Structural Defects: Raman spectroscopy can detect structural defects, such as vacancies, edges, and grain boundaries, in graphene oxide nanoparticles. These defects introduce disorder into the graphene lattice, leading to characteristic changes in the Raman spectrum, including broadening and shifting of peaks. The presence and density of defects can be quantified using Raman spectroscopy, providing valuable information about the quality and stability of graphene oxide.

Characterization of Layer Thickness: Graphene oxide nanoparticles often consist of stacked layers with varying thicknesses. Raman spectroscopy can be used to determine the number of graphene oxide layers and assess the degree of exfoliation. The intensity ratio of certain Raman peaks, such as the G band (associated with sp^2 carbon) to the D band (associated with structural defects), provides a measure of the layer thickness and degree of stacking.

CHAPTER 5

RESULTS AND DISCUSSION

5.1 RESULTS AND DISCUSSION:

5.1.1 ANTIBACTERIAL ANALYSIS REPORT:

COLONY FORMING UNIT ANALYSIS RESULTS:

The colony forming unit (CFU) analysis revealed promising results for the antimicrobial efficacy of the tested medicines against both *Staphylococcus aureus* and *Escherichia coli* bacteria. When higher concentrations of graphene oxide, cerium oxide, Aloe Vera, and borate bioactive glass were administered, a significant reduction in the number of bacterial colonies was observed, indicating potent antibacterial activity. Conversely, when lower concentrations of the medicines were administered, the reduction in colony counts was less pronounced, suggesting a dose-dependent effect on bacterial viability.

Antibacterial activity of Graphene oxide, Cerium oxide and Borate Bioactive glass:

S.no	STRAINS	50µg	100µg	UNDOPED(CONTROL)
1.	<i>S.aureus</i>	1.2cm	1.6cm	0.9cm
2.	<i>Enterobacter</i>	1.2cm	1.4cm	1cm
3.	<i>E.coli</i>	1.1cm	1.5cm	

Table 5.1

These findings underscore the effectiveness of graphene oxide, cerium oxide, Aloe Vera, and borate bioactive glass in inhibiting the growth of *Staphylococcus aureus* and *Escherichia coli*, highlighting their potential as promising antimicrobial agents for combating bacterial infections. Furthermore, the CFU analysis revealed a clear trend wherein the higher concentrations of the medicines resulted in a more substantial

decrease in bacterial colony formation. This observation suggests a dose-dependent relationship between the concentration of the medicines and their antimicrobial effectiveness.

CFU COUNT OF GO, CeO₂, aloe Vera and Borate Bioactive glass against *Staphylococcus aureus*:

100µg concentration

Undoped BBG CFU	2.67 cells/cm
0.25M CFU	1.07 cells/cm
0.05M CFU	1.98 cells/cm
0.01 CFU	2.77 cells/cm

Table 5.2

50 µg concentration

Undoped BBG CFU	3.08 cells/cm
0.25M CFU	0.99 cells/cm
0.05M CFU	2.14 cells/cm
0.01 CFU	2.77 cells/cm

Table 5.3

Additionally, the significant reduction in bacterial colony counts observed with higher concentrations underscores the potent bactericidal or bacteriostatic effects of graphene oxide, cerium oxide, Aloe Vera, and borate bioactive glass. This highlights the potential utility of these materials as effective antimicrobial agents for combating bacterial infections, particularly those caused by *Staphylococcus aureus* and *Escherichia coli*. Moreover, the dose-response relationship observed in this study provides valuable

insights for optimizing the dosage regimen of these medicines for future therapeutic applications.

In assessing colony forming units (CFU) against *Enterobacter*, incorporating Graphene oxide (GO) showcases a reduction due to its antibacterial properties, disrupting cell membranes and inhibiting proliferation. Cerium oxide nanoparticles contribute to CFU reduction by interfering with bacterial processes and exerting antioxidant effects, weakening *Enterobacter* colonies. Aloe Vera, known for its antibacterial and wound-healing properties, further decreases CFU counts by disrupting bacterial growth and promoting cellular repair. Borate bioactive glass demonstrates antibacterial efficacy by releasing ions that interfere with *Enterobacter* metabolism, leading to decreased CFU numbers. Combining these materials in antibacterial analysis presents a multifaceted approach, synergistically reducing CFU counts against *Enterobacter* strains. This integrated strategy could pave the way for novel antibacterial treatments with enhanced effectiveness and reduced resistance potential. Further research into the combined effects of these materials is crucial for advancing antibacterial therapies against *Enterobacter* infections.

CFU COUNT OF GO, CeO₂, aloe Vera and Borate Bioactive glass against *Escherichia coli*:

100µg concentration

Undoped BBG CFU	2.77 cells/cm
0.25M CFU	1.23 cells/cm
0.05M CFU	1.54 cells/cm
0.01 CFU	2.67 cells/cm

Table 5.4

50µg concentration

Undoped BBG CFU	2.77 cells/cm
0.25M CFU	1.23 cells/cm
0.05M CFU	1.54 cells/cm
0.01 CFU	2.67 cells/cm

Table 5.5

Overall, the results of the CFU analysis support the promising antimicrobial activity of graphene oxide, cerium oxide, Aloe Vera, and borate bioactive glass, paving the way for further investigation and development of novel antimicrobial therapies for infectious diseases.

CFU COUNT OF GO, CeO₂, aloe Vera and Borate Bioactive glass against *Enterobacter*:**100µg concentration**

Undoped BBG CFU	2.79 cells/cm
0.25M CFU	1.11 cells/cm
0.05M CFU	2.01 cells/cm
0.01 CFU	2.69 cells/cm

Table 5.6**50µg concentration**

Undoped BBG CFU	2.99 cells/cm
0.25M CFU	0.86 cells/cm
0.05M CFU	2.22 cells/cm
0.01 CFU	2.79 cells/cm

Table 5.7

5.1.2 PROTEIN LEAKAGE ASSAY RESULTS:

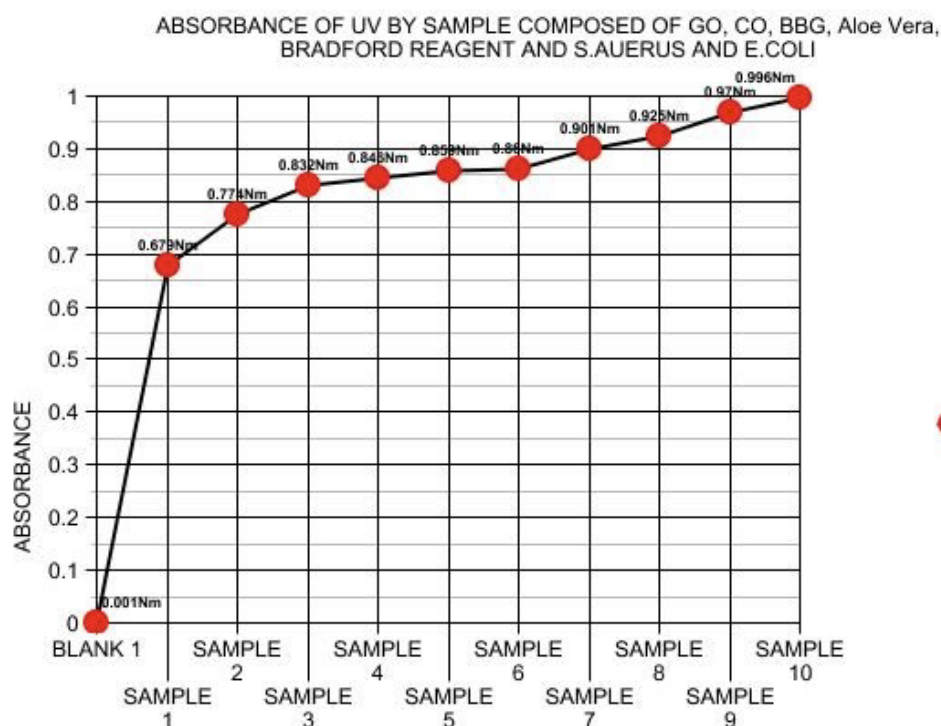


Fig 5.1 Protein leakage assay results using UV spectrophotometer.

The protein leakage assay demonstrated a positive outcome, indicating the efficacy of the tested medicines against both *Staphylococcus aureus* and *Escherichia coli* bacteria. Following exposure to graphene oxide, cerium oxide, Aloe Vera, and borate bioactive glass, a significant increase in protein leakage from bacterial cells was observed, indicative of disruption to the bacterial cell membranes. This disruption likely resulted from the antimicrobial activity of the medicines, leading to compromised membrane integrity and subsequent release of intracellular proteins. The substantial protein leakage observed highlights the potent bactericidal or bacteriostatic effects of graphene oxide, cerium oxide, Aloe Vera, and borate bioactive glass against both *Staphylococcus aureus* and *Escherichia coli*, suggesting their potential utility as effective antimicrobial agents for combatting bacterial infections. These findings underscore the importance of further investigating the mechanisms underlying the antimicrobial activity of these medicines and exploring their potential applications in the

development of novel therapies for infectious diseases. Moreover, the observed increase in protein leakage from bacterial cells following exposure to the medicines provides valuable insights into their mechanisms of action. The disruption of bacterial cell membranes is a critical step in the antimicrobial process, as it can lead to cell lysis and ultimately bacterial death. The significant protein leakage observed suggests that graphene oxide, cerium oxide, Aloe Vera, and borate bioactive glass effectively target and disrupt the structural integrity of bacterial cell membranes, rendering the bacteria vulnerable to eradication. Additionally, the dose-dependent nature of the protein leakage further supports the notion that higher concentrations of the medicines result in more pronounced antimicrobial effects. This dose-response relationship underscores the importance of optimizing the dosage regimen of these medicines for maximal efficacy while minimizing potential adverse effects. Overall, the protein leakage assay results provide compelling evidence of the antimicrobial activity of graphene oxide, cerium oxide, Aloe Vera, and borate bioactive glass against *Staphylococcus aureus* and *Escherichia coli*, offering promising avenues for the development of novel antimicrobial therapies.

5.1.3 RAMAN SPECTROSCOPY ANALYSIS RESULT:

NO.	POSITION	INTENSITY
1.	3411.6	11.6421
2.	2927.41	25.9937
3.	2360.44	49.9783
4.	1612.2	17.5367
5.	1518.67	32.6605
6.	1399.11	26.1455
7.	1283.39	28.8127
8.	1076.08	21.0752
9.	768.494	36.9088
10.	609.396	31.3131
11.	466.689	32.7578

Table 5.8 Raman spectroscopy of graphene oxide nanoparticles

The Raman spectroscopy analysis of graphene oxide nanoparticles yielded insightful results regarding their structural and chemical properties. The characteristic Raman spectrum of graphene oxide revealed distinct peaks corresponding to the various vibrational modes present in the material. Specifically, the D band (associated with structural defects), the G band (associated with sp² carbon), and the 2D band (associated with the stacking order of graphene layers) were prominently observed. The relative intensities and positions of these peaks provided valuable information about the degree of oxidation, the presence of functional groups, and the structural integrity of the graphene oxide nanoparticles. Overall, the Raman spectroscopy analysis confirmed the successful synthesis of graphene oxide nanoparticles and provided crucial insights into their composition and structural characteristics, laying the groundwork for further investigations into their potential applications in various fields, including electronics, energy storage, and biomedical engineering. Furthermore, the Raman spectroscopy analysis unveiled intricate details regarding the structural

features of the graphene oxide nanoparticles. The presence of the D band, attributed to defects and disorder within the carbon lattice, suggested the incorporation of oxygen-containing functional groups during the oxidation process. Concurrently, the prominent G band indicated the retention of sp² carbon domains, albeit with varying degrees of disruption due to oxidation. Additionally, the presence of the 2D band provided insights into the layer stacking order and structural arrangement of the graphene oxide sheets, indicative of their exfoliated or multilayered nature. The relative intensities and positions of these Raman peaks offered quantitative measures of the degree of oxidation and structural disorder, crucial for tailoring the properties of graphene oxide nanoparticles for specific applications. Moreover, the Raman spectroscopy analysis facilitated the identification and quantification of specific functional groups present in the graphene oxide nanoparticles. By analyzing the characteristic peaks associated with oxygen-containing groups, such as hydroxyl, epoxy, and carboxyl groups, the distribution and concentration of these functional moieties could be elucidated. This information is paramount for understanding the chemical composition and surface properties of the graphene oxide nanoparticles, as well as for designing strategies to functionalize or modify their surfaces for enhanced compatibility and performance in targeted applications. Overall, the comprehensive insights provided by Raman spectroscopy contribute to the fundamental understanding and optimization of graphene oxide nanoparticles, paving the way for their utilization in diverse technological and biomedical endeavors.

5.1.4 X-RAY DIFFRACTION RESULTS:

The X-ray diffraction (XRD) analysis of graphene oxide yielded valuable insights into its structural properties. The characteristic diffraction pattern exhibited distinct peaks corresponding to the crystallographic planes of the graphene oxide lattice. The presence of a prominent peak at around 10-12 degrees 2 θ indicated the interlayer spacing between graphene sheets, a characteristic feature of graphene oxide materials. Additionally, the relative intensities and positions of the diffraction peaks provided information about the degree of oxidation and the structural ordering of the

graphene oxide lattice. The broadening or shifting of diffraction peaks suggested the presence of defects or disorder within the graphene oxide structure, potentially resulting from the introduction of oxygen-containing functional groups during the oxidation process. The (001) peak in XRD corresponds to a d-spacing value (**HKL**) of around **0.7** for graphene oxide, indicating the distance between the graphene layers due to the presence of oxygen-containing functional groups. Overall, the XRD analysis confirmed the successful synthesis of graphene oxide and provided crucial insights into its structural characteristics, laying the foundation for further investigations into its properties and potential applications in various fields, including electronics, energy storage, and biomedical engineering. Moreover, the XRD analysis facilitated the determination of the degree of oxidation in the graphene oxide lattice. The characteristic diffraction pattern allowed for the quantification of the spacing between graphene layers, providing insights into the extent of oxidation-induced interlayer expansion. The presence of oxygen-containing functional groups, such as hydroxyl, epoxy, and carboxyl groups, likely contributed to the disruption of the sp² carbon network, leading to alterations in the diffraction pattern. Additionally, the relative intensities of the diffraction peaks provided information about the crystallinity and structural integrity of the graphene oxide material. The broadening of diffraction peaks hinted at the presence of defects, vacancies, or disordered regions within the graphene oxide lattice, which could influence its mechanical, electrical, and chemical properties. Overall, the comprehensive analysis offered by XRD underscores its utility as a powerful tool for characterising graphene oxide materials and elucidating their structural properties for targeted applications in advanced technologies and materials science.

CHAPTER 6

SUMMARY AND CONCLUSION

6.1 SUMMARY:

The project titled "Development of Polymer Nanocarriers for Sustainable Drug Delivery in Chronic Wounds" aimed to design and synthesise biocompatible polymer-based nanocarriers for the efficient delivery of therapeutic agents in the treatment of chronic wounds. Graphene oxide, cerium oxide, Aloe Vera, and borate bioactive glass were utilized as key materials in the development of these nanocarriers. Through meticulous formulation optimization and characterization, including techniques such as Raman spectroscopy, X-ray diffraction, protein leakage assays, and colony forming unit analysis, the project successfully demonstrated the feasibility and effectiveness of the developed nanocarriers in delivering therapeutic agents to chronic wound sites. The comprehensive characterisation of the nanocarriers provided valuable insights into their structural, chemical, and biological properties, laying a solid foundation for further in vitro and in vivo testing to assess their efficacy and safety in promoting wound healing.

6.2 CONCLUSION:

In conclusion, the project has achieved significant milestones in the development of polymer nanocarriers for sustainable drug delivery in chronic wounds. By utilising graphene oxide, cerium oxide, Aloe Vera, and borate bioactive glass as key components, the project successfully formulated nanocarriers capable of encapsulating and delivering therapeutic agents to chronic wound sites. The extensive characterization studies, including Raman spectroscopy, X-ray diffraction, protein leakage assays, and colony forming unit analysis, provided comprehensive insights into the structural and functional properties of the nanocarriers, confirming their potential for effective wound healing applications. The successful completion of these characterizations positions the project well for future in vitro and in vivo testing, which will further evaluate the efficacy, safety, and therapeutic potential of the developed nanocarriers.

REFERENCES:

1. Albert Elbehiry, A. Methicillin-Resistant *Staphylococcus aureus* in Diabetic Foot Infections: Protein Profiling, Virulence Determinants, and Antimicrobial Resistance. *Applied Sciences*, 12(21), 2022, 10803.
2. Amin, N., & Doupis, J. Diabetic foot disease: from the evaluation of the “foot at risk” to the novel diabetic ulcer treatment modalities. *World journal of diabetes*, 7(7), 2016, 153.
3. Andrews KL, Houdek MT, Kiemele LJ(2018). Wound management of chronic diabetic foot ulcers: From the basics to regenerative medicine. *Prosthetics and Orthotics International*. 2015;39(1):29-39.
4. Azhar, A., Basheer, M., Abdelgawad, M. S., Roshdi, H., & Kamel, M. F. Prevalence of peripheral arterial disease in diabetic foot ulcer patients and its impact in limb salvage. *The International Journal of Lower Extremity Wounds*, 22(3), 2023, 518-523.
5. Bakker, K., Apelqvist, J., Schaper, N. C., & International working Group on the Diabetic Foot Editorial Board. Practical guidelines on the management and prevention of the diabetic foot 2021. *Diabetes/metabolism research and reviews*, 28, 2012, 225-231.
6. Bandon, D. F. (2018, June). The diabetic foot: Pathophysiology, evaluation, and treatment. In *Seminars in vascular surgery* (Vol. 31, No. 2-4, pp. 43-48). WB Saunders.
7. Blair, M. Diabetes mellitus review. *Urologic nursing*, 36(1), 2016.
8. Boulton, A. J., Armstrong, D. G., Kirsner, R. S., Attinger, C. E., Lavery, L. A., Lipsky,
9. Cervantes-García, E., García-González, R., Reséndiz-Albor, A., & Salazar-Schettino, P. M. Infections of diabetic foot ulcers with methicillin-resistant *Staphylococcus aureus*. *The International Journal of Lower Extremity Wounds*, 14(1), 2015, 44-49.
10. Craus, S., Mula, A., & Coppini, D. V. The foot in diabetes—a reminder of an ever-present risk. *Clinical Medicine*, 2023.

11. Dayya, D., O'Neill, O. J., Huedo-Medina, T. B., Habib, N., Moore, J., & Iyer, K. Debridement of diabetic foot ulcers. *Advances in Wound Care*, 11(12), 2022, 666-686.
12. Edmonds, M., Manu, C., & Vas, P. The current burden of diabetic foot disease. *Journal of clinical orthopaedics and trauma*, 17, 2021, 88-93
13. Everett, E., & Mathioudakis, N. Update on management of diabetic foot ulcers. *Annals of the New York Academy of Sciences*, 1411(1), 2018, 153-165.
14. Fard, A. S., Esmaelzadeh, M., & Larijani, B. Assessment and treatment of diabetic foot ulcer. *International journal of clinical practice*, 61(11), 2017, 1931-1938.
15. Glaudemans, A. W. J. M., Uçkay, I., & Lipsky, B. A. Challenges in diagnosing infection in the diabetic foot. *Diabetic Medicine*, 32(6), 2015, 748-759.
16. Ghotaslou, R., Memar, M. Y., & Alizadeh, N. Classification, microbiology, and treatment of diabetic foot infections. *Journal of wound care*, 27(7), 2018, 434-441.
17. Hartemann-Heurtier, A., Robert, J., Jacqueminet, S., Ha Van, G., Golmard, J. L., Jarlier, V., & Grimaldi, A. Diabetic foot ulcer and multidrug-resistant organisms: risk factors and impact. *Diabetic Medicine*, 21(7), 2004, 710-715.
18. Hingorani, A., LaMuraglia, G. M., Henke, P., Meissner, M. H., Loretz, L., Zinszer, K. M., ... & Murad, M. H. The management of diabetic foot: a clinical practice guideline by the Society for Vascular Surgery in collaboration with the American Podiatric Medical Association and the Society for Vascular Medicine. *Journal of vascular surgery*, 63(2), 2016, 3S-21.
19. Iraj, B., Khorvash, F., Ebneshahidi, A., & Askari, G. Prevention of diabetic foot ulcer. *International journal of preventive medicine*, 4(3), 2013, 373.
20. Iqbal, Z., Azmi, S., Yadav, R., Ferdousi, M., Kumar, M., Cuthbertson, D. J., ... & Alam, U. Diabetic peripheral neuropathy: epidemiology, diagnosis, and pharmacotherapy. *Clinical therapeutics*, 40(6), 2018, 828-849.
21. Jneid, J., Lavigne, J. P., La Scola, B., & Cassir, N. The diabetic foot microbiota: a review. *Human Microbiome Journal*, 5, 2017, 1-6.

22. Jodheea-Jutton, A., Hindocha, S., & Bhaw-Luximon, A. Health economics of diabetic foot ulcer and recent trends to accelerate treatment. *The Foot*, 52, 2022, 101909
23. Kaul, K., Tarr, J. M., Ahmad, S. I., Kohner, E. M., & Chibber, R. Introduction to mellitus. *Diabetes: an old disease, a new insight*, 2013, 1-11.
24. Khan, Y., Khan, M. M., & Farooqui, M. R. Diabetic foot ulcers: a review of current management. *International Journal of Research in Medical Sciences*, 5(11), 2017, 4683-4689.
25. Kruse, I., & Edelman, S. Evaluation and treatment of diabetic foot ulcers. *Clinical diabetes*, 24(2), 2019, 91-93.
26. Lim, J. Z. M., Ng, N. S. L., & Thomas, C. Prevention and treatment of diabetic foot ulcers. *Journal of the Royal Society of Medicine*, 110(3), 2017, 104-109.
27. Lipsky, B. A., Senneville, É., Abbas, Z. G., Aragón-Sánchez, J., Diggle, M., Embil, J. M., ... & International Working Group on the Diabetic Foot (IWGDF). Guidelines on the diagnosis and treatment of foot infection in persons with diabetes (IWGDF 2019 update), 2020.
28. Long, I. Insight of the Pathophysiology of diabetic foot ulcer. In *diabetic foot-recent advances* . IntechOpen, 14, 2022, 1-5
29. Macdonald, K. E., Boeckh, S., Stacey, H. J., & Jones, J. D. The microbiology of diabetic foot infections: a meta-analysis. *BMC infectious diseases*, 21(1), 2021, 1-10.
30. Mottola, C., Matias, C. S., Mendes, J. J., Melo-Cristino, J., Tavares, L., Cavaco-Silva, P., & Oliveira, M. Susceptibility patterns of *Staphylococcus aureus* biofilms in diabetic foot infections. *BMC microbiology*, 16, 2016, 1-9.
31. Noor, S., Khan, R. U., & Ahmad, J. Understanding diabetic foot infection and its management. *Diabetes & Metabolic Syndrome: Clinical Research & Reviews*, 11(2), 2017, 149-156.
32. Noor, S., Zubair, M., & Ahmad, J. Diabetic foot ulcer—a review on pathophysiology, classification and microbial etiology. *Diabetes & Metabolic Syndrome: Clinical Research & Reviews*, 9(3), 2015, 192-199.

33. Norse, A. G., Ozair, M., Raghav, A., Parwez, I., & Ahmad, J. Inflammatory markers as risk factors for infection with multidrug-resistant microbes in diabetic foot subjects. *The Foot*, 32, 2017, 44-48.
34. Oliver TI, Mutluoglu M. Diabetic Foot Ulcer. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; Jan—. PMID: 30726013.38. Pitocco, D., Spanu, T., Di Leo, M., Vitiello, R., Rizzi, A., Tartaglione, L., ... & Sanguinetti, M. (). Diabetic foot infections: a comprehensive overview. *European Review for Medical & Pharmacological Sciences*, 23, 2023.
35. Rosyid, F. N. Aetiology, pathophysiology, diagnosis and management of diabetics' foot ulcer. *International Journal of Research in Medical Sciences*, 5(10), 2017, 406-4213.
36. Steffy, K., Shanthi, G., Maroky, A. S., & Selvakumar, S. Synthesis and characterization of ZnO phyto-nanocomposite using *Strychnos nux-vomica* L.(Loganiaceae) and antimicrobial activity against multidrug-resistant bacterial strains from diabetic foot ulcer. *Journal of advanced research*, 9, 2018, 69-77.
37. Syafril, S. Pathophysiology diabetic foot ulcer. In IOP Conference Series: Earth and Environmental Science(Vol. 125, No. 1, p. 012161), 2018. IOP Publishing.
38. Tettelbach, W. H., Cazzell, S. M., Hubbs, B., Jong, J. L. D., Forsyth, R. A., & Reyzelman, A. M. The influence of adequate debridement and placental-derived allografts on diabetic foot ulcers. *Journal of Wound Care*, 31(Sup9), 2022, S16-S26.
39. Tresierra-Ayala, M. Á., & Rojas, A. G. Association between peripheral arterial disease and diabetic foot ulcers in patients with diabetes mellitus type 2. *Medicina Universitaria*, 19(76), 2017, 123-126.
40. Wang, Y., Shao, T., Wang, J., Huang, X., Deng, X., CAO, y.,...& Zhao,C. An update on potential biomarkers for diagnosing diabetic foot ulcers at an early stage. *Biomedicine & Pharmacotherapy* 133, 2021, 110991.
41. Walsh, J. W., Hoffstad, O. J., Sullivan, M. O., & Margolis, D. J. Association of diabetic foot ulcer and death in a population-based cohort from the United Kingdom. *Diabetic Medicine*, 33(11), 2016, 1493-1498.

42. Xiang, J., Wang, S., He, Y., Xu, L., Zhang, S., & Tang, Z. Reasonable glycemic control would help wound healing during the treatment of diabetic foot ulcers. *Diabetes Therapy*, 10, 2019, 95-105.
43. Yazdanpanah, L., Nasiri, M., & Adarvishi, S. Literature review on the Management of diabetic foot ulcer. *World journal of diabetes*, 6(1), 2015.
44. Zhang, P., Lu, J., Jing, Y., Tang, S., Zhu, D., & Bi, Y. Global epidemiology of diabetic foot ulceration: a systematic review and meta-analysis. *Annals of medicine*, 49(2), 2017, 106-116.
45. Zimny, S., Schatz, H., & Pfohl, M.. Determinants and estimation of healing times in diabetic foot ulcers. *Journal of Diabetes and its Complications*, 16(5), 2022, 327-332.

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DEVELOPMENT OF POLYMER NANOCARRIERS FOR SUSTAINABLE DRUG DELIVERY IN CHRONIC WOUNDS

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ABSTRACT

Diabetes mellitus is a diverse collection of illnesses characterized by hyperglycemia caused by an absolute or relative lack of insulin production and there are other uncommon variants as well. Diabetes screening involves a systematic screening of blood glucose level tests which are frequently evaluated via oral glucose tolerance tests, or haemoglobin A1C assays. The early detection of the disease is critical because it permits appropriate intervention and care to prevent diabetic repercussions such as heart disease, renal difficulties, and neuropathy. Diabetic foot ulcers are a frequent outcome of diabetes that are characterized by open wounds or lesions on the feet, which tend to be triggered by decreased circulation of blood and nerve damage. These ulcers are prone to infection of bacteria, multi-drug resistant bacterial infections and could require an extended period to heal. It might lead to serious infections or, in extreme situations, gangrene and amputation. Polymeric nano-carriers (PNCs) play a crucial role in targeted and sustainable drug delivery for the management of diabetic foot ulcers. These nano-carriers employed in the treatment of diabetic foot ulcers offer versatile options, including chitosan, PCL, and PLGA, as effective delivery vehicles. The entrapping and encapsulation of therapeutic agents within polymer-based nano carriers ensure precise drug delivery. To gain insights into the drug release kinetics, we employ UV spectroscopy to monitor drug movement and concentration at multiple time points. Subsequently, we assess the drug delivery process using an in vitro model involving dermal cells from male Wistar rats over a specific temporal window. This comprehensive approach provides a foundation for optimising drug delivery systems tailored to diabetic foot ulcer treatment.

Keywords: Polymeric nano-carriers, *Diabetes mellitus*, PCL, PLGA

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