VITAMIN-C TEMPLATED BIOACTIVE GLASS NANOPARTICLES AND THEIR INTEGRATION INTO METHYL CELLULOSE-PECTIN FILMS FOR BIOMEDICAL APPLICATIONS

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DECLARATION

We, **FARHEEN AND SHAILY**, hereby declare that the work which is being submitted in this dissertation entitled "**VITAMIN-C TEMPLATED BIOACTIVE GLASS NANOPARTICLES AND THEIR INTEGRATION INTO METHYL CELLULOSE-PECTIN FILMS FOR BIOMEDICAL APPLICATIONS"** in the partial fulfilment for the award of the degree of **Master of Science** in the **Department of Applied Chemistry**, Delhi Technological University is an authentic record of our own work carried out during the period from **August, 2025 to April, 2026** under the supervision of **Dr. Deenan Santhiya (Associate Professor, Department of Applied Chemistry, Delhi Technological University)**.

We further declare that the dissertation has not been submitted by us for the award of any other degree of this or any other Institute.

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CERTIFICATE

This is to certify that the Project report entitled "**VITAMIN-C TEMPLATED BIOACTIVE GLASS NANOPARTICLES AND THEIR INTEGRATION INTO METHYL CELLULOSE-PECTIN FILMS FOR BIOMEDICAL APPLICATIONS"** which is submitted by **FARHEEN FATMA (2K24/MSCCHE/30)** and **SHAILY GOYAL (2K24/MSCCHE/63)**, Department of Applied Chemistry, Delhi Technological University, Delhi in partial fulfilment of the requirement for the award of the degree of **Master of Science**, is a record of the project work carried out by the students under my supervision. To the best of my knowledge, this work has not been submitted in part or full for any Degree or Diploma to this University or elsewhere.

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ABSTRACT

Bioactive glass has a phenomenal ability to interact when in contact with biological tissues and promote healing through controlled ion release. In this study, bioactive glass nanoparticles were synthesized using Vitamin C which acts as a natural template. The NPs were characterized by UV-Visible Spectroscopy to confirm the formation of glass network, Zetasizer analysis for the size and surface charge of the particle, Fourier Transform infrared spectroscopy to analyse its structural properties. Further, BGNPs were incorporated into films consisting of Methyl Cellulose and Pectin. In this film, glycerol was used as a plasticizing agent to reduce brittleness and boost the flexibility of the film. Methyl Cellulose provided mechanical strength to the film and enhanced its transparency while pectin provided gelling and antioxidant potential. The film was prepared with an aim to develop platform suitable for biomedical applications.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

- BGNPs Bioactive glass Nanoparticles
- BG Bioactive glass
- NBG Nano Bioactive glass
- MCPF Methyl Cellulose Pectin Film
- PVA Poly Vinyl Alcohol
- ECM Extracellular matrix
- SEM Scanning Electron Microscopy

CHAPTER 1: INTRODUCTION

Dermal wound healing is a complex and precisely coordinated physiological mechanism involving four interconnected stages: hemostasis, inflammation, proliferation, and tissue remodeling. Among these, hemostasis occurs first. Additionally, the formed clots provide a viable and interactive framework for the migration of inflammatory cells and fibroblasts that contribute to the advanced stage of wound healing. Thus, it is crucial to design a multifunctional nanosystem that can fulfill the needs of different phases of wound repair. Bioactive glass nanostructures represent a novel group of biocompatible materials with significant potential in dermal tissue repair as they accelerate re-epithelialization, enhance vascular formation, and modulate inflammation [1].

It has been more than 50 years since the term "bioactivity" was first used to describe one of the functions of ceramics and glasses. Bioactive Glass is a glass material that has gained significant attention in the medical field. BG has emerged as a promising class of biomaterial for tissue engineering and bone regeneration [2] by interacting with the biological system to promote osteogenesis, angiogenesis, and antibacterial activities through the release of ionic products that stimulate osteoinductivity, depending on the type of ion released and its concentration, specific properties can be achieved.

Bioactive glass possesses the unique ability to bond directly with living bone through the formation of a hydroxy carbonate apatite (HCA) layer on its surface. The controlled release of therapeutic ions such as Si^{4+}, Ca^{2+}, P^{5+} plays a crucial role in stimulating osteoblast proliferation and extracellular matrix mineralization.

Several techniques have been reported for the fabrication of BGNPs, such as microemulsion frame spray, laser spinning, or mostly synthesized by solgel method using organic solvents. In

contrast, this work focuses only on bioinspired method at ambient condition was developed by drawing inspiration from naturally occurring nanostructured materials.

Herein, we describe the synthesis of bioactive glass articles utilizing vitamin C as a template following bio inspired route.

Out of many bioactive molecules, vitamin C has proven to be a promising contender due to its substantial antioxidative and antibacterial capabilities, along with its essential role in human health maintenance.

BGNP has also been reported to be bioresorbable during tissue repair. In the present study, we are fabricating a BG-incorporated film using methyl cellulose and pectin as biopolymer blends. The combination of pectin and cellulose enhances the overall performance of wound dressing by improving mechanical strength, enhancing moisture retention capacity, and promoting tissue regeneration. Thus, numerous studies have explored methods to reduce the brittleness of edible films by incorporating plasticizers into the film matrix.

Generally, a plasticizer is used to improve the flexibility of the film. Glycerol is a common plasticizer used to reduce the brittleness of the film due to its lower molecular weight, which makes it easily insert into the polymer chain [3]. Furthermore, we investigate the effect of methyl cellulose and pectin films on wound healing with those using glycerol as a plasticizer.

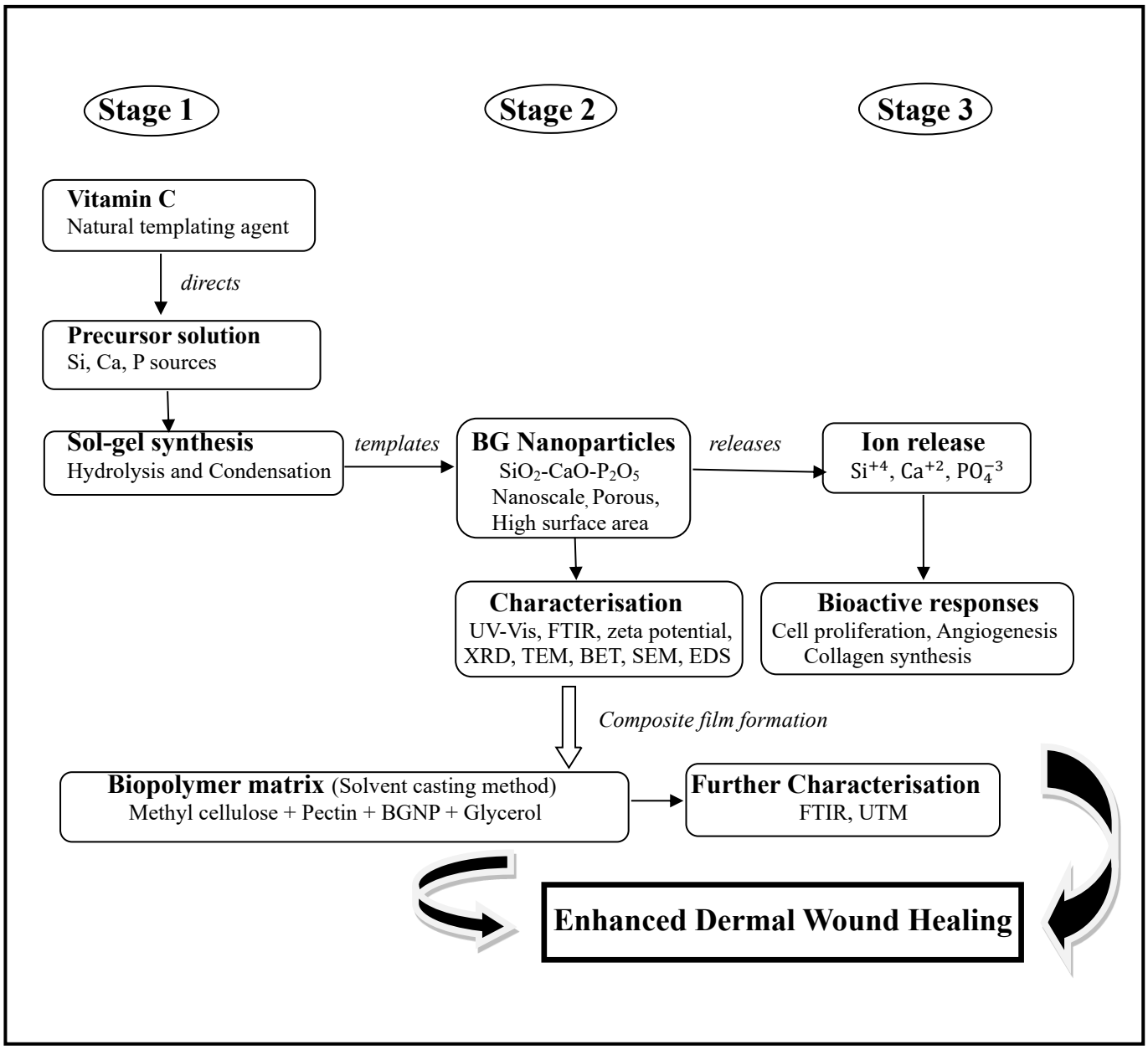


Figure1: Schematic representation of Vitamin C-templated BGNPs synthesis and integration into composite film for enhanced dermal wound healing

CHAPTER 2: LITERATURE REVIEW

2.1. Bioactive Glass: (A Brief Historical Perspective)

Few developments in modern materials science have remained as influential as bioactive glass. In the early 1970s, Larry Hench demonstrated that a silicate-baseiold ceramic could do more than simply exist beside living bone it could chemically bond with it. This breakthrough

established a lasting connection between chemistry and bogy that continues to shape research

today. The material, later known worldwide as 45S5 Bio-glass, consisted of a simple combination of silicon dioxide, sodium oxide, calcium oxide, and phosphorus pentoxide in carefully balanced proportions.

In the years that followed, the field of Bioactive glass advanced significantly. As growing with the improved compositions, it is founded that the replaced conventional high-temperature melting techniques with the more controlled sol-gel method, and began exploring broader possibilities for these materials. It focuses on the shifted from simply by achieving bone bond by this the Research has made that these shown that these goals are achievable in the given amount. Today we had seen the Bioactive Glasses are produced in particle form.

Common linked feature which have the following applications in the fundamental concept first recognized by Larry Hench. As it said that the Bioactive Glass does not remain stable by a biological environment in it. This is been approached because of the Bioactivity, arises from the controlled loss of the glass structure which causes the ions that promote for the further, cellular growth, and tissue development.

2.2. Making Bioactive Glass Smaller: The Nanoparticle Advantage

2.2.1 Why Particle Size Matters

There is something which we can talk about idea by making a material small as a result of which makes it highly reactive. As by the research we know that the powder which we called as the fine particles It will dissolves faster and a thinner membrane responds more quickly to its environment, and a nanoparticle have more surface area per unit mass than any macroscopic piece of the same material ever could. Basically the bioactive glass in this intuition translates into a measurable biological advantage. People had showed that Bioglass particles with diameters of 35 to 40 nanometres exhibited a specific surface area of around 79 square metre/gram compared to 2.7 square metre/per gram for small particles of its comparable composition. That is nearly a thirty across the difference and also have the real consequences. More surface mean the faster Ion release more nucleation sites for separate crystallisation and have greater density of binding opportunities for the proteins that mediate cell adhesion.

The cell culture experiment have shown this picture consistently. The studies were comparing on its nanoscale and the microscale bioactive glass under of the identical condition has repeatedly shown that the nanoparticulate form promote the apatite formation in simulated body fluid as we found the greater adhesion of osteoblast like cells which have more pronounced expression of Osteogenic marker gene including Alkaline phosphatase, RUNX2 and collagen type I. The thing is particularly striking at the advantages are not simply a consequence of obtaining the larger surface in the Nano Format appear to be engage intracellular signalling pathways in ways that the microscale material does not. Thus we have been shown as the following trigger more sustained activation as of the extracellular regulated as the kinase so that we can push cell by the cell cycle from the G_0/G_1 phase into active proliferation more efficiently.

2.2.2 Bioactive Glass Nanoparticles Making

Its synthesis will involve the strategy for the dominate mainly of the literature on BGNP production and thus it will signify that that the logic is set of trade off. By this flame spray pyrolysis it will tell to know about that the technique which we know it will include its in the burning of the liquid precursor mixture of the temperature by exceeding upto the thousand degree [4]. As the organic components combust to their metallic species and their formation in the gas phase for the formation of the nanoparticle with diameter typically between 20 to 80 nm. The method is fast and scalable but it require good working equipment to produce such type of the particles.

As the formation of the microemulsion synthesis it is a very different approach by spreading the tiny water droplets which have size of the 5 to 20 nm in diameter suspended in an oil phase and it is stabilised by the other surfactant molecules. These droplets is been acting as the nano scale reaction vessels on confining the reagent and by producing particles of relatively uniform. It has been founded that the drawback is yield. As it has been mentioned that the method is slow, produces small quantities and consumes large volume of oil and surfactant.

The Sol-Gel route is been found as the emerged of its workhorse of laboratory in scale for the BGNP synthesis. Starting from the liquid precursors it is typically consists of the silicon Alkoxide such as TOSH with the calcium and phosphorus sources. This method builds up the Silica Network by a sequence of the hydrolysis followed by the condensation reaction at Room temperature or its slightly above. This will leads to the Resulting in the Gel is been Dried and then calcined at 600 to 700 C for the stabilization of the glass structure and after it will lead to the burn off from any residual which have its own organic material. This will help the beauty of this approach lies in its formation as it will have small particle size, porosity, surface area, and chemical composition which can adjust through changing the pH, solvent ratios, or in the order of precursor addition through the type of concentration of any surfactant used. Thus the

Sol-Gel route for the accommodation of their incorporation of the organic tempting through the agents like during synthesis.

2.3. Vitamin C found as Natural Agent

The concept made up of using organic molecules by taking as the direct in the structure of Sol-Gel Derived Material is well established in their synthesis of their Mesoporous in the silicas and related materials. The surfactant, block co polymers and biological macro molecules will have all been used for the structure in the directing agents like by the interacting through the developing of their inorganic network and leaves behind their characteristic pore architecture when removed by Calcination. By their present work it has founded that the Vitamin C and Ascorbic Acid conducting as the naturally derived from the Agent uses for BGNP synthesis for their broader movement toward greener, bio-inspired synthesis in their methodology.

The ascorbic acid is found to be the intriguing the candidate for this purpose. It will carrying the multiple OH groups which is coordinated by the metal ions in the solution to form hydrogen bond with the silanol species, by the influencing of their developing silica network. Its mild behaviour in the Reducing Character adds another dimension, particularly through the relevant if transition metal dopant are found in their glass. Therefore the use of a biologically benign vitamin as in the template will found as the rather then by using the synthetic surfactant in the organic solvent as it will reduce the potential for their residual toxicity by their final material as in their of the important consideration of the given the intended biomedical application. To the best of the authors knowledge its been specific approach for the BGNP synthesis which had not previously been reported to the literature and making it as the novel and potentially significant.

After we know that the synthesis of the Vitamin C is been founded as the template is removed during Calcination leaving behind BGNP whose size is being the morphology of the and the

surface properties will have been founded by the shaped by the template by making of the matrix interaction. By using the characterization the particles using UV-Visible spectroscopy by applying in the FTIR spectroscopy is been carried out to understand these properties and to the evaluation in the whether for the vitamin C as this approach is been used for the produces particles with the characteristics for the favourable in the biological application.

2.4. Characterizing

By the material study we know that the lives or dies by the quality of its characterization. Through the synthesis we been given as a powder or a film as it characterisation which tell us whether that we made why it behaves the way it does and what might be improved in the future. The characterization toolkit deployed in their present study spans optical spectroscopy, particle sizing and the surface charge measurement and Vibrational spectroscopy by its a combination that provide a reasonably complete picture of the synthesis nanoparticle and the composite film into which they were incorporated.

UV Visible spectra will offered various optical properties for the nanoparticle for its system and it is particularly useful for the monitoring the fate of the species having this during synthesis. In the context of this vitamin C templated for the BGNPs this method can track the changes in the optical signature for the Ascorbic acid as it interacts with the developing glass network, providing the indirect evidence of template-matrix interaction for the completeness of template removal after by the given calcination.

Analysis of the two complementary measurements such as the 1.Dynamic light scattering for the hydrodynamic particle size, electrophoretic for the scattering of the zeta potential. The hydrodynamic diameter is been measured by their dynamic light scattering and it will reflects the particle core so that the together we can make up with the surface for the adsorbed layer in their shell of the associated solvent molecules and for the making it more relevant metric for

predicting colloidal behaviour to a geometric diameter alone. Zeta potential is mainly used for the stability indicator in which the particles of an absolute values greater than roughly by the estimation measures to the 30 mv which tend to be repel from one another so that the sufficiently to remain dispersed with the lower value as it would for a tendency to getting it forward [2]. For BGNPs in the incorporation for the polymer films have the good colloidal stability which would facilitate the homogeneous mixing and it will lead to the helping as the to avoid the formation of large film to geeting the integrity and uniformity of biological performance.

FTIR spectroscopy is been found to be the most informative single technique which is available for the characterising bioactive glass as we have seen. The vib fingerprint of the Si-O-Si appear to be as the strong asymmetric stretching which will ocuuer near at band of 1080 cm-1 while a seeing as the symmetric stretching of the band near at the $800cm^{-1}$. Silanol groups is to be founded for the Reactive surface species responsible for initiating apatite nucleation which will give a characteristic signal at around $950cm^{-1}$. That will leads to the phosphate into the glass is confirmed by bands near 1000 to 1220 wave numbers and a doublet will appear at approximately 569 and $603cm^{-1}$. Together these features will provide a confirmation for the glass network has formed a intended which had the key for the functional groups necessary for bioactivity are present at the surface.

Techniques which will include such as the X-Ray diffraction which had the confirm the amorphous nature of the glass as an important since it will leads to the crystallisation during Calcination can reduce the activity while in the scanning of the electron microscopy reveal particle morphology and the distribution of particle through the composite film. Energy dispersive X-ray spectroscopy will allow to extend the SEM imaging for the providing the localised for the elemental information confirming that calcium, silicon, and phosphorus are all present in the expected in their proportions.

2.5. Pectin

2.5.1 Chemistry and Gelation

Pectin occupies a curious position in the scientific literature-it has been known and used in food science for over a century, yet its potential as a functional biomaterial has only been seriously explored in the past two or three decades. The polymer is extracted commercially from citrus peel and apple pomace and consists primarily of galacturonic acid units linked in a linear chain, with carboxylic acid groups that may be methylated to varying degrees. This degree of esterification is the key structural parameter that controls how pectin behaves in solution and in the solid state

Pectin is a versatile natural polymer whose properties depend largely on its degree of methylation. High-methoxyl pectin, which contains more than 50% methylated carboxyl groups, forms gels under acidic conditions and in the presence of high sugar concentrations through hydrogen bonding and hydrophobic interactions. In contrast, low-methoxyl pectin contains fewer methyl groups and more free carboxyl groups, allowing it to form gels in the presence of divalent ions such as calcium through ionic crosslinking, commonly explained by the egg-box model. Because of these different gelation mechanisms, pectin can be used in a wide range of applications, including encapsulation systems, films, and tissue engineering scaffolds.

Apart from its structural properties, pectin also offers several biological benefits that make it attractive for biomedical applications. It is resistant to digestion in the upper gastrointestinal tract but can be broken down by colonic microorganisms, making it useful for colon-targeted drug delivery systems [5]. In wound-healing applications, one of its most important characteristics is its antioxidant activity. Pectin can help neutralize reactive oxygen species that accumulate at wound sites and may otherwise slow down tissue repair and cell growth.

This natural antioxidant property adds further value to pectin-based composite films and supports their potential use in biomedical and wound-healing applications [6].

High methoxyl pectins, carrying more than fifty percent methylation of their carboxylate groups, gel under acidic conditions and in the presence of high co-solute concentrations through hydrogen bonding and hydrophobic interactions. Low methoxyl pectins, with fewer methyl groups, expose a greater density of free carboxylate charges and gel rapidly in the presence of divalent cations such as calcium through an ionic crosslinking mechanism elegantly described by the egg-box model. This versatility in gelation chemistry makes pectin a highly adaptable platform for encapsulation, film formation, and scaffold fabrication across a wide range of conditions.[6]

2.5.2 Pectin in Film-Based Biomedical Applications

The development of the pectin-based film for the pharmaceutical and the biomedical use had accelerated market over the past fifteen year as a result it is been driven by the growing of natural polysaccharide film which will offer for the biocompatibility, tunablility, degradation and functional versatility which will lead for the synthetic polymer film as it will often cannot match [7]. Chitosan pectin films has been demonstrated for the two polymer which will be combined to produce the material along with the mechanical properties superior to the either polymer alone with their alone with electrostatic interaction between positively charged chitosan and negatively charged pectin.

Researchers had took the concept for the developing of the pectin PLGA composite for tissue regeneration. Their design is been made for the mechanical robustness of the hydrophobic PLGA network with their biological Signalling the capacity of their hydrophilic pectin phase, along their crosslinking of the calcium ion. Osteoblast culture on these composite which has

been confirmed that the pectin component facilitated cell adhesion and proliferation this will illustrates that their biological character of the formation of the polysaccharide can actively direct cellular for their behaviour within the composite.

The relevance of all this will leads to the present study is straight forward for the things. Pectin brings a set of different functional property like the gel forming ability, antioxidant activity, cell-interactive potential Thus that the complement is been founded duplicate the contribution of the bioactive glass nanoparticles. Together, the two components like as the address of the different aspect of their biological environment in which their composite will film perform better with the polymer phase providing the structural strength.

2.6. Methyl Cellulose: The Structural Backbone of the Film

Pectin will contribute for the gelling ability for the biological activity to the composite film system, methyl cellulose contributes structural integrity and optical clarity. As it is been derived from the cellulose nature thus the most abundant biopolymer on earth will been made by the following by the partial methylation of the OH- group is founded as the backbone of their chain, and the methyl cellulose is been dissolve and readily found in the cold water and then it will foem the gell upon heating through a complex mechanism which is been found in the inverse of most polymer gelation processes. In film form it will leads to the products like as the coherent sheets that have long been use in pharmaceutical formulation as controlled release matrices and tablet coating.

The contribution of the methyl cellulose for the composite of the polysaccharide films is well eastblished. Its Semi crystalline for the chain architecture provide the of stiffnes which will have the pure pectin film for the typically lack then for the reducing brittlenes and improving the ease with which the film can be handled cut and applied. Optical transparency for the

another attribute worth noting in their dressing application the ability to visually inspect the wound through the dressing by the disturbing it is clinically valuable.

While adding the glycerol addeding the plasticising agent in the present formulation work by the inserting the itself between the polymer chain of both pectin and methyl cellulose disrupting the Intermolecular hydrogen bond will lead to the would and otherwise give the film a brittle, glassy character. This will result in the reduction as the effective glass transition temperature and have the corresponding increase in film flexibility and elongation at break.

2.7. Literature About the Polymer-BG Composite

2.7.1 PVA and Chitosan Hybrid Systems

Mansur and Costa have produced some of the most systematically characterised polymer bioactive glass hybrid scaffolds [8] in their early literature and working with PVA and chitosan in combination with a 3degree SiO_2-CaO-P_2O_5 glass. As it will approach toward the combining PVA solutions with a sol-gel glass precursor mixture and a surfactant generated highly porous three-dimensional scaffolds with interconnected macro pores in the range of 10 to 500 mm. A pore architecture well suited to its bone in growth. FTIR and the small angle X-ray scattering characterisation demonstrated as the polymer chain interacted with the glass network through H-bonding between −OH group of PVA and silanol groups on the glass surface, and that the degree of hydrolysis of the PVA had influenced the both the kinetic of gelation and the final mechanical properties of the scaffolds.

The MTT cell has show that neither the glass component nor the polymer phase introduced measurable cytotoxicity by finding that it is independent of the PVA/glass ratio. This is the important validation for concept. The elastic model has measured for these scaffolds and the

ranging from the approximately 2.6 to 6.0 Mega Pa for the fell within the range considered appropriate for cancellous bone replacement, demonstrating for the mechanical design and biological safety could be achieved simultaneously.

2.7.2 Natural Polymer Composites: Alginate, Chitin, Gelatin

The incorporation of BGNP into natural polymer has created a rich body of literature [9] by the spanning a wide variety of the polymer chemistry and it's structural format [10,11]. Although the polysaccharide has obtained from brown seaweed and then its is combined with BGNP by Srinivasan to produce the composite scaffolds for periodontal tissue regeneration [12]. The BGNP contributed improved biomineralisation and for the greater protein adsorption, enhanced cell attachment with the ALP activity in the human periodontal ligament for the fibroblast showing a characteristic peak at seven days that the author attributed to the culmination of osteoblastic differentiation stimulated by the ionic dissolution products of the glass.

Chitin BGNP composite which is been reported by researchers and showed that the glass particles is dispersed for the homogeneously with the polysaccharide As it would show that the freeze drying process and that their presence increased swelling, degradation rate and apatite forming ability compare by the pure chitin scaffolds. Critically kwon as the human primary osteoblast cell cultured on these composites formed the mineralised bone nodule in the absence of the supplementary osteogenic factor which Deal with the compelling demonstration that the material environment itself is being sufficient to direct cell differentiation.

As the Gelatin based BGNP composite provide another example [13]. As the researchers examined that the influence of BGNP size on the properties for their gelatin based nanocomposite scaffold and found that the smaller particle had been produced for the stronger

and stiffer material as well found the greater bioactivity It will creates the double benefit as it will leads to the increased surface area which has enhanced the particle.

2.7.3 Silk Fibroin Films: A Particularly Instructive Comparison

The work on the silk fibroin or the nano bioactive glass composite film [14]. It will deserve particular attention here not only because it represents one of the more thorough characterisation of the natural polymer BGNP film system as it will consists because it is in the experimental design and then closely parallel for the approach to adopted in their present study. Silk fibre is being chosen as the polymer part because of its excellent bio compatibility and for the film forming properties as it shares with many natural polymer the limitation of being biologically inert. As it is to promote the formation of a mineral interface with bone tissue.

Composite film is fabricate by solvent casting and characterise by their FTIR As we all know that the water contact angle for the measurement or in immersion for 1.5x SBF. By the FTIR analysis it is been confirmed that the Si-O-Si and Si-O-Ca characteristic the band of the glass are identifiable for the composite spectra alongside the amide band of the silk fibre and importantly, that the secondary structure of the protein which have the balance between random coil and betasheet conformation. As it was not significantly disrupted by their presence of the glass particle. This is a meaningful finding because the mechanical properties of silk fibre.

As the hydrophilicity measurement shown that the NBG incorporation had the progressively improved for the wettability of the composite films and with the statistically significant difference becoming the apparent for the loading of 10 wt.%. As the immersion in SBF increases for the seven day to produced the conspicuous deposits of the hydroxy carbonate apatite on the composite film with the glass loading of 4 wt.% and above As we know that the pure silk fibre and composites with only 1 wt.% NBG showed no observation on the point. For the threshold loading below the density to exposed for the glass particle is insufficient to

nucleate for the apatite effectively. Osteoblast have the culture study which have shows progressive improvement in both proliferation and ALP activity with increasing the glass content for the 10 wt% composite significantly for the outperforming the pure polymer.

2.8. Bioactive Glass for the Soft Tissue and for the Wound Healing

As we have seen that the bioactive glass is an exclusively a bone material. Then for the same ionic dissolution of the product which can we stimulate their osteogenes part which will also influence their behaviour of fibro blasts, endothelial cells, and and their keratinocytes in the cell of their types which is mostly directly involved in their wound healing [15]. Researcher had found the bioactive glass particle stimulated to their release of their vascular endothelial growth factor from the cultured rat fibroblasts which is been pointing towards a pro-angiogenic mechanism that could accelerate the neovascularisation of the healing wounds [16]. Then the subsequent work confirmed that the silicon and calcium ions is being release from the glass to activate the signalling pathways, which result in the tissues exposed from the bioactive glass dissolution for the products tend to the develop richer vascular network more quickly than from its control [17].

The electrospun poly (epsilon-caprolactone) composite membranes which have the silver-cobalt doped BGNPs specifically used for the wound healing purposes [18]. The rationale for doping was well considered and the silver ions are among the most effective natural antibacterials and been founded to be the disrupting the bacterial cell membranes which will include the RNA and DNA replication and blocking the cellular respiration across the broad spectrum of the pathogens. Cobalt, meanwhile the hypoxic signalling in which the body uses to trigger angiogenesis it activates the hypoxia-inducible factor which in turn regulates the production of the erythropoietin and various growth factors.

The important observation from the study is that the cobalt-doped BGNP not produce the

hydroxyapatite deposits upon SBF immersion. Then it might be appear to be the limitation from their bone tissue of their engineering perspective, it is actually desirable for soft tissue wound for the dressings where the mineralisation of the dressing would be inappropriate and potentially harmful. This will lead to the example illustrates from the broader principle in composite biomaterial design. Thus the response must be matched to the target tissue and the composition of the glass.

The composite film system is been developed at the present work and it takes about the somewhat different approach to the wound relevant for the functionality [19] and then on relying at the antioxidant properties of pectin and the controlled ion release of the BGNP rather than metal ion doping. This represents a complementary strategy By using of the inherent biological activity of the polymer alongside the bioactivity of the glass rather than engineering all biological functionality into the inorganic component.

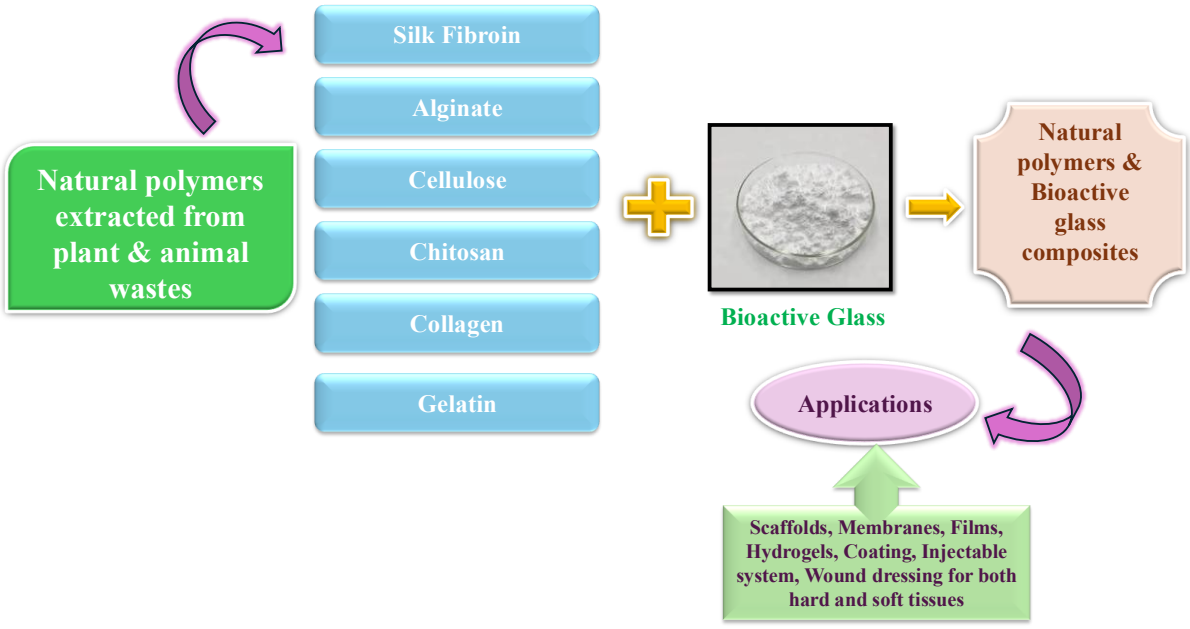


Figure 2: Diagrammatic illustrations of natural polymer with BG to create Composites

2.9. In Vitro Bioactivity Testing for the Simulated Body Fluid

Kokubo had published their protocol for preparing the simulated body fluid in the early 1990s and they provided the biomaterial field with one of the most widely adopted screening tools [20]. SBF is a solution in which the ionic composition is being broadly consists of the human blood plasma and the logic of the test is straightforward If a material can induce the formation of a hydroxyapatite layer on surface when immersed in SBF. So it is likely to do the same when implanted in the body and bone. This simplicity of the test and the relatively is found to be modest in the equipment requirements and the accumulated body of comparative data in the literature have made SBF testing a near-universal first-line characterisation for any material intended for hard tissue applications.

The polymer BGNP composite films is made up of the SBF testing as it will reveal the important information beyond the merely confirming bioactivity. The thickness and the density of the apatite layer it will forms the timescale over which it appears and its distribution across the film on its surface it all reflect the accessibility of the glass particles within the polymer and their capacity to nucleate mineral deposition. As the result showed in their silk fibre NBG study, there is the minimum glass loading below which apatite formation is not observed, because too many of the particles are found within the polymer bulk and cannot interact with the fluid.

Thus the mechanism which will lead by their bioactive glass which induces the apatite formation is well characterised. Thus the silicon ions released from the glass surface they are been deposited on their polymer chains thus where the Si-OH groups will act as nucleation sites for calcium phosphate crystallisation. Simultaneously, calcium ions released from the glass increase the ionic activity product of the surrounding fluid with respect to apatite, lowering the supersaturation threshold for nucleation. Together, these effects accelerate apatite formation well beyond what would occur spontaneously in SBF at body temperature. In composite systems containing pectin, the carboxylate groups of the polysaccharide may

provide additional nucleation sites, further enhancing the mineralisation potential of the material- a synergy that has been noted in other natural polymer-glass composite systems.

2.10. Mechanical and Physical Properties of Composite Film

A composite film which is promising for the mechanically making of the limited clinical value. Thus the mechanical requirement for the biomedical film will vary widely depending on the application for a wound dressing which must be flexible enough to get the conform on the wound surface it will resist for the tearing during application and removal while a tissue engineering scaffold which will must provide the adequate structural support for the cell during the regeneration process. Therefore the mechanical properties of a composite film which is therefore takes the prerequisite for assessing fitness for purpose.

Thus performing the tensile testing it is found the standard approach for the characterizing the behaviour of thin film thus the measuring on the Young's modulus therfore the ultimate tensile strength and elongation is at the break. Thus the addition of the rigid nanoparticle to a polymer which is been typically increases the stiffness but it will reduces the extensibility as the particle to restrict the chain mobility and if it is present at the sufficient densityit will act as the stress concentrators as it will take that the initiate fracture to lower strains. Thus the magnitude of these will effect and it will depend upon the critically on the quality of their particle dispersion. It is well distributed about the nanoparticle. As while it is been made the cluster concentrate stress and can reduce strength than enhance it. By achieving the homogeneous dispersion as it is found the important and processing the objective of the achieving adequate.

Studies had provided the complementary information about their the film it will behave in a wet biological environment. Although the most natural polysaccharide will leads to the film absorb water readily and monitoring the kinetics of swelling and mass loss in physiological buffer gives a useful indication of the material lifetime as it will lead to the increase at the rate

of which it will release the incorporated bioactive agents. The presence of BGNP will lead to the modulate their kinetics and in a obvious way. Thus therfore the ionic dissolution of their glass creates the local alkaline of the micro environment that can be buffer for the acidic degradation of their product of some polymer and their potentially to be extending their material lifetime.

2.11. Biocompatibility: Cell Studies

No matter how is the elegant is the chemistry is for the low impressive thus the mechanical properties had make a material intended for the biomedical application which must ultimately can demonstrate the it will does not harm the living cells. Cytotoxicity will leads to the assessment that is why therefore a non-negotiable component is been responsible for the biomaterial study and the approach and then it will used in the literature which will reflect the diversity of the biological questions. Thus the most commonly used for the tool of the assessing the cell is been founded as the viability and their metabolic activity as it will contact for the with to test the material. This will assay exploit the ability of their metabolically active cells to reduce the given yellow tetrazolium salt of the purple formazan product, with their intensity of the colour being proportional to the number of viable active cells. Thus the critically it will result in the consistent were came to the across the range of composition therefore it would been suggesting that the biocompatibility of the composite was robust as it will rather than narrowly optimised.

More and more the detailed and the biological assessment are been made and been move for the beyond simple it is found the viability to examine how cell is been interact with the material and how they adhere it strongly spread effectively, and it will proliferate over the extended period, and ultimately causes for the differentiate into the specialised cell type for the relevant to the targeted tissue. Researchers has been demonstrated that level of rigour in their silk fibre

in NBG study showing that not only that osteoblasts remained viable on the composite film as it proliferated more actively and been expressed for the higher level of alkaline phosphatase in the glass content increased. Thus the morphological characterisation is of the cell by SEM imaging at day three showed the filopodia extending between adjacent cells, indicating active intercellular communication and engagement.

For the pectin methyl cellulose of the BGNP composite film of the present study cell which studies will be the ultimate arbiter of the material's biomedical potential. The combination of their pectin which carries the biological pedigree as in the ECM mimicking the polysaccharide with the pro cellular ionic micro environment created by the dissolving BGNP suggest that the material design been is likely to be cell compatible. Whether it actively promotes the cellular responses relevant to its intended application wound healing soft tissue engineering or a more generalised the biomedical platform remains to be established by this experiment

Figure 3: Various Applications of BGNPs

CHAPTER 3: MATERIALS AND METHODOLOGY

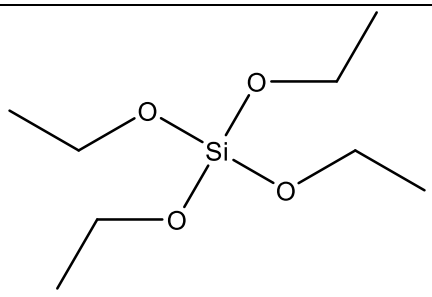
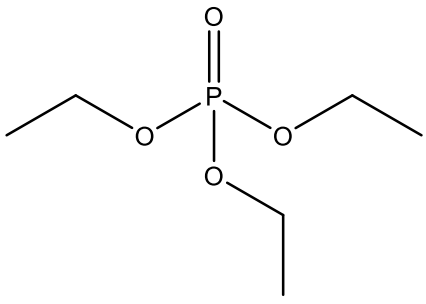
3.1 Materials

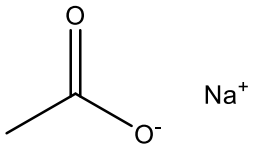
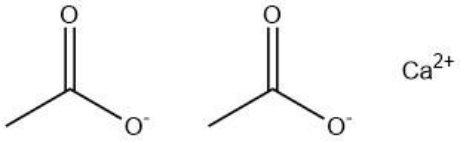
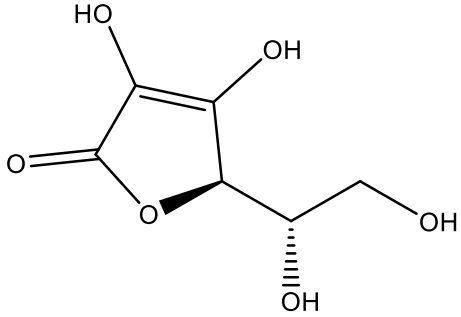
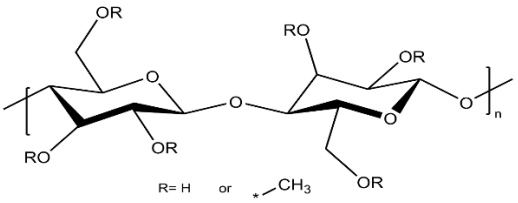
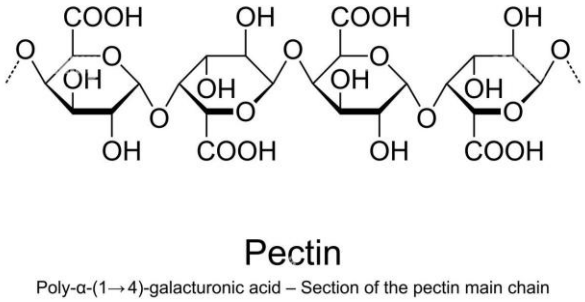
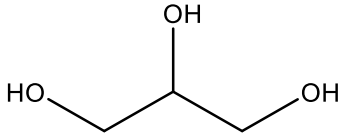
BG nanoparticles were synthesised using precursors, namely tetraethyl orthosilicate (TEOS) (C.N. 86578), triethyl phosphate (TEP) (C.N. 821141), sodium acetate (NaAc) (C.N. S2889), calcium acetate (CaAc) (C.N. 1086334), and natural template Vitamin C (MW= 176.12, purity 99%) are procured from Sigma-Aldrich Company.

Other chemicals required for film preparation, such as Methyl Cellulose, Pectin, and glycerol, were purchased from Sigma-Aldrich. All chemicals were of analytical reagent grade and used as received without further purification.

Milli-Q water was used throughout all experiments. All other reagents used for experiments were of analytical reagent (AR) grade. The chemical structure of chemicals/biochemicals, along with the manufacturing company used in this study, is listed in Table 1.

Table 1: Chemical structure of chemicals/biochemicals used in the study

S.No.	Name of the chemicals/Biochemicals	Chemical Structure
1.	Tetraethyl orthosilicate (TEOS)	
2.	Triethyl phosphate (TEP) (C.N. 821141)	

3.	**Sodium acetate (NaAc) (C.N. S2889)**	
4.	**Calcium acetate (CaAc) (C.N. 1086334)**	
5.	**Vitamin C (MW= 176.12, purity 99%)**	
6.	**Methyl Cellulose**	
7.	**Pectin**	
8.	**Glycerol**	

3.2. Methodology

3.2.1. Preparation of Bioactive glass nanoparticles (BGNPs)

BG NPs were synthesized using a previously reported bio-inspired technique with Vitamin-C serving as a biocompatible and green templating agent to regulate the nanoparticle formation and surface properties.

Briefly, the vitamin-c template (1mg/ml) was dissolved in 10mM TRIZMA buffer at pH 8 under continuous stirring at physiological temperature, followed by the addition of BG precursors, namely TEOS(92.9g/l), TEP(10g/l), NaAc(63.6g/l), and CaAc(42.1g/l), each at an interval of 30 min to allow controlled hydrolysis and condensation in the order as mentioned above. After that, the reaction mixture was maintained in a water bath for the next 24 hours at 37 °C to allow complete nanoparticle formation. Then the sample was centrifuged (10,000 rpm, for 15 min) to obtain deposits and the pellet was washed with 70% v/v ethanol to remove unreacted precursors and residues. The washed nanoparticles were dried in an oven and stored in a desiccator until further use.

3.2.2. Preparation of Methyl Cellulose-Pectin Composite films

Biopolymer films incorporating BG NPs were synthesized using a solvent casting method. To prepare MCPF (functional film), Methyl Cellulose (0.75g,2.5%w/v), Pectin (0.756g, 2.5%w/v) were each dissolved in 30ml Milli-Q water at an interval of 30 min with continuous stirring to ensure homogeneous distribution of nanoparticles throughout the matrix. Then, BGNPs were dispersed in the above solution at a concentration of 1 mg/mL (0.03 g in 30 mL) for 30 min with continuous stirring. Glycerol (0.25 g) was added as a plasticizer to improve film flexibility and reduce brittleness.

The final film-forming solution was poured into a glass petri dish and allowed to dry in an oven for 24-48 hours. The dried films were carefully peeled off after complete drying and stored in a desiccator for further characterization.

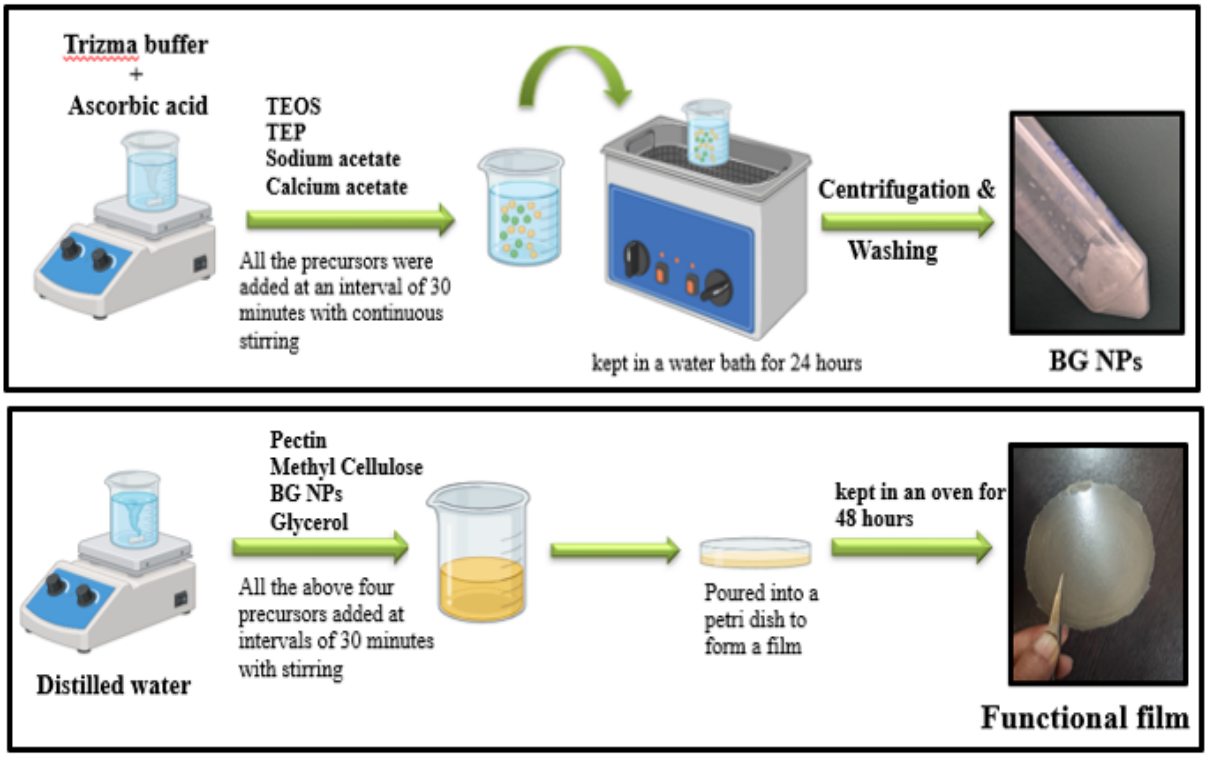


Figure 4: Experimental Procedure

CHAPTER 4: RESULTS AND DISCUSSION

4.1 Characterisation of Bioactive Glass Nanoparticles (BGNPs)

The BGNPs were synthesized using a bio-inspired aqueous method at a physiological temperature (37 °C). As most lab syntheses push much higher temperatures, here we maintained physiological temperature for the BGNPs synthesis. Vitamin C was used as a natural templating agent. The precursors TEOS, TEP, sodium acetate, and calcium acetate were added sequentially at an interval of 30 minutes into a 10mM TRIZMA Buffer at pH 8 under continuous stirring. This gradual addition was important to allow controlled hydrolysis and condensation of the silicate network without everything crashing out at once in the solution. The prepared solution was placed in a water bath, and after 24-48 hours, the suspension was centrifuged, washed with ethanol, and then dried. The washing step was one of the key steps that we found out in further analysis.

To analyze the synthesized BGNPs, several characterization techniques, such as UV-Visible spectroscopy, FTIR spectroscopy, Zeta potential, and UTM for film characterization, were used.

UV-Visible spectroscopy: UV-Visible spectra were recorded for BGNPs. All the samples were finely crushed and dispersed in a suitable solvent at an appropriate concentration to ensure sufficient transmittance. Baseline correction was performed using a suitable solvent before sample analysis. The suspension was then transferred to quartz cuvettes of path length 1cm, and analysis was done at a resolution of 1nm, within the range of 200-600 nm. The absorption spectra were recorded to identify significant absorption peaks associated with electronic transitions of the constituent molecules and functional groups present in the sample.

FTIR recording: To evaluate the FTIR data, a Perkin-Elmer FT-IR instrument was used in the ATR mode. The data was acquired with a 4cm 1 resolution with 8 scans per run. For scanning, neat samples were implemented on the scanning window.

Zeta Potential Measurements: Using a Zetasizer Nano ZS based on the light scattering principle (Malvern Instruments, UK) instrument, the Zeta-potential of BGNPs was monitored in trizma buffer pH 8. Before the measurements, the BGNPs were dispersed using ethanol as a dispersing medium. A minimum of three readings was recorded for the given sample.

Tensile Testing: To evaluate the elastic modulus, elongation at break (EB), and tensile strength (TS), for MCPFs, a universal testing machine (ZwickRoell, Z010, USA) with a force range of 10 kN, following ASTM D882-10. MCPFs were cut into 50 mm × 5 mm dimensions, and the moving speed of the grip was adjusted to 12.5 mm/min. The maximum tensile force (W_{max}(N)) and the strain (%) values were recorded.

4.1.1 UV-Visible Spectroscopic Analysis of BGNPs

The UV-Vis absorption spectrum was recorded in the range of 190-600 nm. The most prominent feature was the sharp peak at 203 nm, with an absorbance value of 2.0. Then the absorption sharply declined, and at 240 nm it reached 0.4 and stayed at about 0.08 to 600 nm. In general, it looked like a clean spectrum.

That sharp peak at 203 nm corresponds to the electronic transitions in the Si−O−Si network that formed during TEOS condensation. These are $\pi \rightarrow \pi^*$ and $n \rightarrow \sigma^*$ transitions, perhaps with some changes from the phosphate groups in TEP. The rapid decrease in absorbance instantly after the peak, and the lack of any other features between 250 and 600 nm, is very reassuring. Washing with ethanol significantly removed the unreacted organics and any metal traces.

Since there was no absorption peak at 261 nm, directly pointing that there was no free vitamin C, specifically from its lactone ring chromophore. This means free, unbound ascorbic acid was removed during washing. What remains must be physically or chemically entrapped within the glass network, not merely on its surface [21,22].

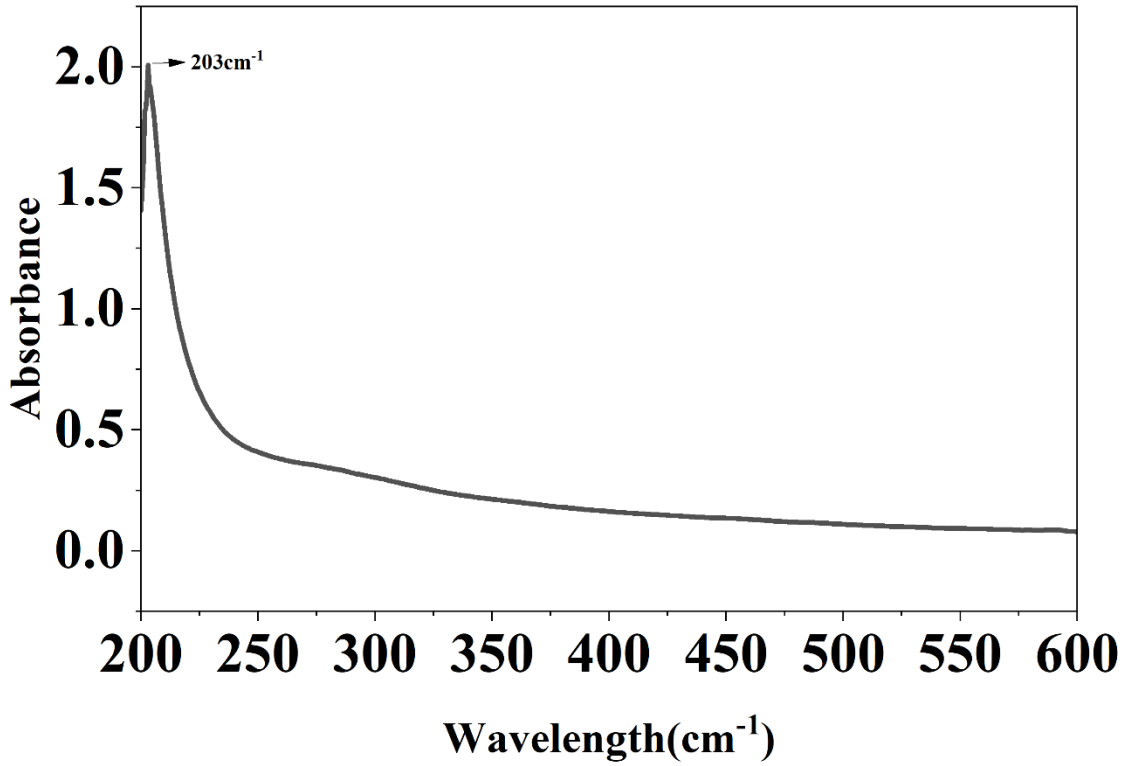


Figure 5: UV-Visible absorption spectrum of BGNPs

4.1.2 FTIR Spectroscopic Analysis of BGNPs

FTIR had also given us a far more detailed picture about the structure and molecular composition of our synthesised material. The spectrum was observed over a range of 4000-400 cm^{-1} . Initially, the peaks confirms two things that we were looking for i.e., the formation of silicate glass network and confirms preservation of vitamin C into the structure of our material.

A wide band was observed around 3482 cm^{-1} is visible when examining the higher wavenumbers initially. Water that has been adsorbed on the surface and surface silanol (Si-OH) groups are the sources of this band, which is caused by O-H stretching. Its breadth fits our aqueous production process and is typical for amorphous silica solids. Critically, when these −OH groups were added to simulate body fluid, helping to initiate the deposition of hydroxycarbonate apatite (HCA). This is essential in demonstrating the bioactivity of bioglass material [23].

The C-H stretching was observed to be a weak band around 2982cm-1 which is reassuring as it shows that the Vitamin C is still present even after washing it with ethanol. It wasn't just stuck on the surface but got trapped inside the silicate matrix. That's how it is supposed to work in the bio-inspired templating process, as stated. [22].

Asymmetric and symmetric COO^{-} stretching was observed around bands at 1553 and 1403 cm^{-} as the fingerprint region. These are from acetate-derived species. As we have used sodium acetate and calcium acetate as precursors in our synthesis. Moreover, the band near 1342 cm^{-1} suggests some C−O interaction between the inorganic matrix and retained template.

The most significant peak was observed with a strong broad band around 1021 cm^{-1}. This is due to the Si−O−Si asymmetric stretching, the fingerprint region of the silicate network. The bending peaks around 637 and 461 cm^{-1} further confirms that a proper glass network was synthesised successfully.

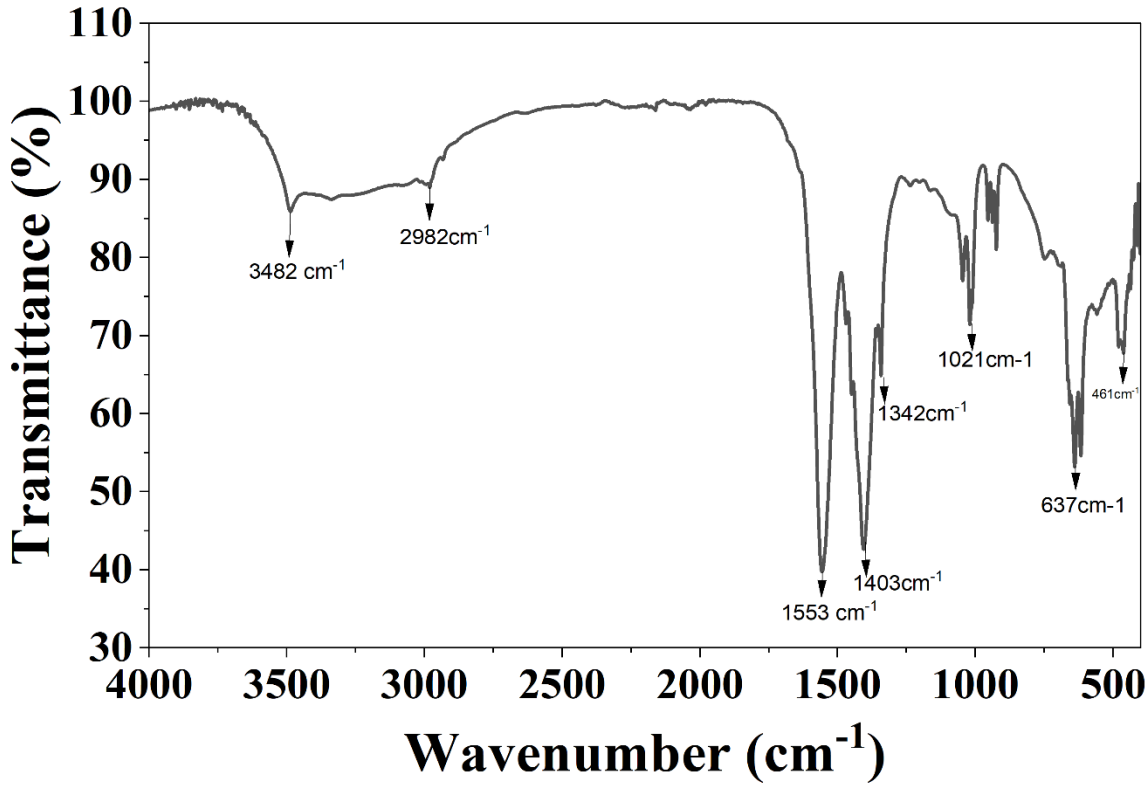


Figure 6: FTIR spectrum of BGNPs

4.2 Characterisation of Methyl Cellulose-Pectin Composite Film (MCPF)

4.2.1 FTIR Spectroscopic Analysis of MCPF

The Composite film (MCPF) was prepared using the solvent casting method. Methyl cellulose (2.5% w/v) and pectin (2.5% w/v) were dissolved sequentially in Milli-Q water with continuous stirring; BGNPs were incorporated at 1 mg/mL, and glycerol was added as a plasticiser, and the mixture was poured onto the glass Petri dishes and dried in an oven. Once dried, the film was carefully peeled off from the Petri dish using forceps.

The FTIR spectrum of the MCPF was recorded over the same range from 4000-400 cm^{-1}. Instantly, the spectrum looked very different from the BGNP spectrum, which is expected because this was predominantly organic and polysaccharide-based material.

The most distinguished feature is a deep, broad absorption band near 3336 cm^{-1}, with the transmittance dropping to 65%. This is the region of O–H stretching, and the sheer breadth and depth of this band point is extensive to intermolecular hydrogen bonding between the −OH groups of carboxyl, hydroxyl groups of pectin and methyl cellulose. In practical terms, that level of polymer-polymer interaction is exactly what we were looking for in film cohesion [24].

Peak around 2902 cm^{-1}, signifies a C−H symmetric stretch from the methyl groups on the methyl cellulose backbone, which confirms that the component is present and that it was accounted for. The band around 1638 cm^{-1} is assigned for C = O stretching from the galacturonic acid units present in pectin, though it could also include some O−H bending bounded by water retained in the hygroscopic matrix after drying the sample.

The bands around 1377 and 1231 cm^{-1} reflects the C−H bending and asymmetric C−O−C stretching, attached to methyl group deformations in MC and the ether linkages running through both side chains of polysaccharides. The band at 1046 cm^{-1} appears to be the strongest peak in the fingerprint region, dropping to a transmittance of 30% for the glycosidic C−O−C stretch. Representing the fundamental repeating bond in both pectin and methyl cellulose, so its intensity at equal polymer concentrations makes sense. Further deformation modes around 496 cm^{-1} conclude the skeletal features [25,26].

One thing that needs to be observed is the Si−O−Si peaks from the BGNPs (expected at 1021 and 461 cm^{-1}) aren't clearly visible in the film spectrum. This occurs due to the loading issue at 1 mg/mL; the BGNP contribution is quite small relative to the dominant polysaccharide matrix, and the polymer absorptions in that region simply overlaps and mask up the glass peaks. It doesn't mean the BGNPs are not present, as it just means that at this loading, FTIR alone isn't sensitive enough to detect them.

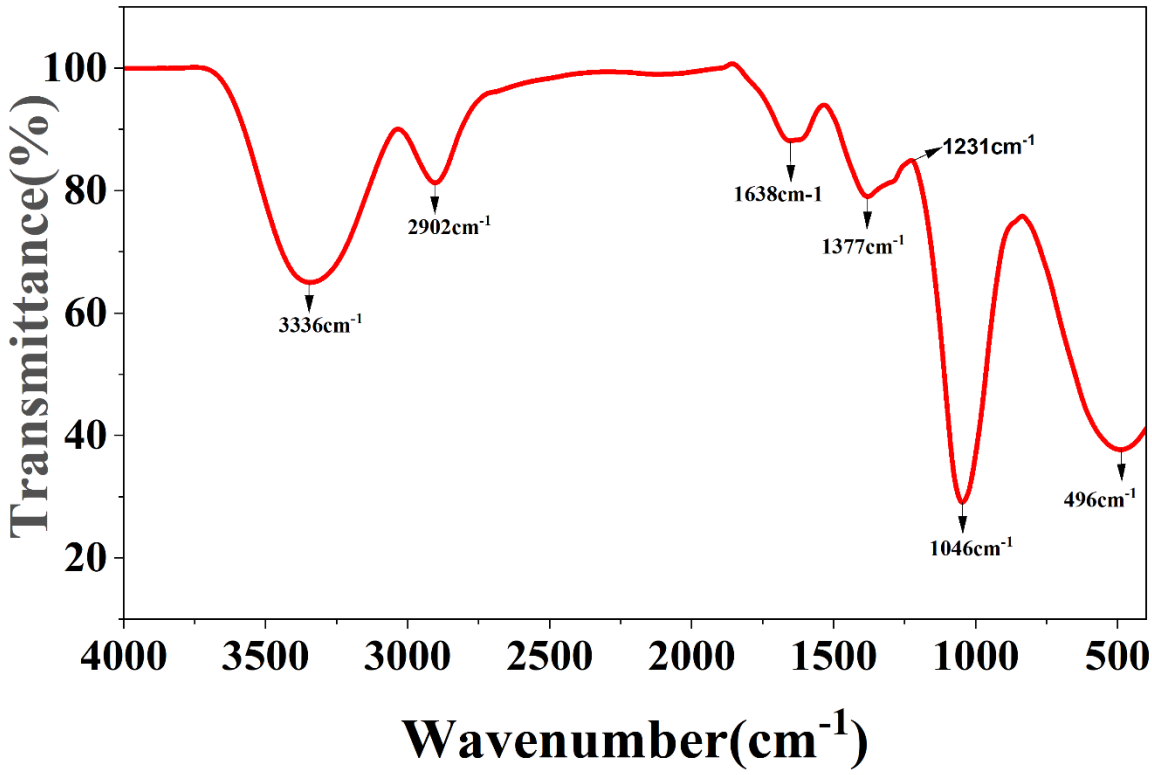


Figure 7: FTIR spectrum of MCPF

4.3 Mechanical Properties of MCPF: Tensile Testing

Universal Testing Machine (UTM) was used to evaluate how the film would actually hold up to physical and tensile strength testing following ASTM D882-10 standards. Film strips (50 mm × 5 mm) were pulled at a crosshead speed of 12.5 mm/min. The resulting stress-strain curve were shown in Figure 6.

Stress-Strain Behaviour and Tensile Strength

As the curve is rising steeply and non-linearly from the origin. There is no separate yield point, just stress going up and up. The film reached a maximum tensile strength of approximately 23.9 N/mm² (MPa) at about 4.4% strain and then suddenly failed. The stress vertically dropped almost +to zero. No gradual yielding, no necking, just done.

That fracture behaviour confirms the film fails in a relatively brittle manner, without significant plastic deformation beforehand.

Interpretation of Mechanical Performance

The tensile strength of about 23.9 MPa is quite a remarkable one considering that this is from a biopolymer composite film. There are two factors responsible for this phenomenon. The first is that there is substantial intermolecular hydrogen bonding between methyl cellulose and pectin, as explained by the FTIR data; this gives cohesion in the matrix itself. The other factor is that BGNPs act as the reinforcing agent themselves, preventing the mobility of chains during stress application [25,27].

The 4.4% elongation at break reflects the intrinsic rigidity of both MC and pectin as polysaccharides-neither is particularly flexible once film-cast, and the nanoparticles only add to that stiffness. The initial steep slope tells us that the elastic modulus is high and the film resists deformation well under normal handling conditions. This kind of profile-high strength, limited elongation, brittle fracture is typical behaviour of biopolymer films loaded with bioactive glass nanoparticles, as stated in the literature [25,26].

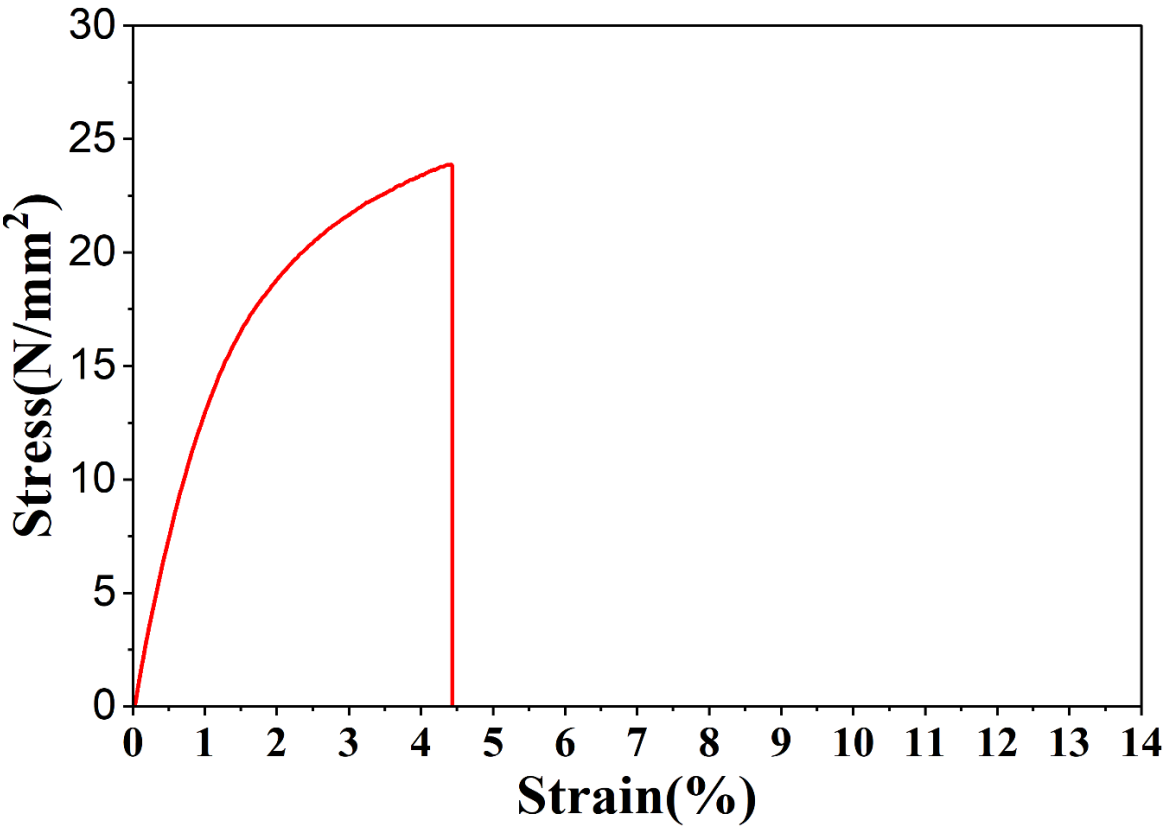


Figure 8: Stress-strain curve obtained from tensile testing for MCPF

CHAPTER 5: CONCLUSIONS AND FUTURE RECOMMENDATIONS

5.1 Overall Summary

This project started with our main aim: To find out whether we could make bioactive glass nanoparticles (BGNPs) using a greener, more gentle approach and then to use them so we can improve simple polysaccharide film. Looking back, I think the work had achieved that aim fairly well, even though along the way there were plenty of moments of uncertainty, especially when interpreting the spectroscopic data for the first time.

The idea of using Vitamin C is used as a template during the sol-gel synthesis of bioactive glass was inspired by the previously reported work which showed that sugar based agents could guide the formation of silicate nanoparticle under mild, near physiological conditions. Their fructose liked system demonstrated by the organic molecules with multiple OH^- groups could interact with the condensing with the silica network during gelation, producing nanoparticles with high surface area and retained organic content. Based on that concept, Vitamin C *(ascorbic acid)* was selected for this study because it is readily available, non-toxic, and carries both lactone and diol functional group that could be potentially participate in hydrogen bonding or coordinate weakly with the silicate frameworkmuch in the way fructose was proposed to function in the earlier work.

The synthesis was carried out at 37 °C in a TRIZMA buffer at pH 8 conditions that were chosen purposely to keep the process as biocompatible and environmentally friendly as possible. The precursors used TEOS for silicon, TEP for phosphorus, sodium acetate, and calcium acetate for the sodium and calcium components, are all relatively mild reagents compared to the harsher acid or alkali catalysts commonly used in conventional sol-gel bioactive glass synthesis. This kind of room-temperature, aqueous sol gel route has been advocated with the Bioactive glass

literature as a way of producing nanoparticles with better compositional homogeneity and more reactive surfaces than melt-derived glasses [2,28].

Once the nanoparticles were made, they were incorporated into a methyl cellulose- pectin composite film (MCPF) by a simple solvent casting method. Films based on polysaccharide blends have been of interest for biomedical and food packaging applications because both methyl cellulose and pectin are biocompatible, edible, and capable of forming reasonably strong films on their own. The idea of adding inorganic nanoparticles like BGNP to reinforce or functionalise the polysaccharide matrices is not entirely new similar approaches we have been explored with hydroxyapatite, silica, and zinc oxide nanoparticles, but the specific combination of a Vitamin C templated bioactive glass with the methyl cellulose-pectin matrix does not appear to have been reported before, which gave this study some novelty.

Characterisation of the BGNPs was carried out using UV-Visible spectroscopy and FTIR spectroscopy, and the film was evaluated by FTIR and tensile testing on a Universal Testing Machine (UTM). These techniques were chosen because they were available in the laboratory, appropriate for the sample type, and collectively sufficient to answer the main questions about whether the synthesis had worked and whether the film had reasonable mechanical properties. Further, a complete picture of the nanoparticle system would ideally include particle sizing by DLS, morphology by TEM, and confirmation of amorphous structure by XRD, but those are left as recommendations for future work, which is discussed later.

Overall, this study successfully demonstrated the synthesis of Vitamin C-templated bioactive glass nanoparticles and their incorporation into a methyl cellulose-pectin composite film. UV-visible and FTIR analyses confirmed the formation of the bioactive glass and showed that the characteristic functional groups of both polymers remained intact after film preparation. The composite film also exhibited a tensile strength of about 23.9 MPa, indicating good mechanical stability and reinforcement from the dispersed nanoparticles.

Each component of the film plays an important role. The bioactive glass nanoparticles provide controlled ion release and potential bioactivity, while pectin contributes antioxidant and gel-forming properties that may support wound healing. Methyl cellulose improves the structural strength and transparency of the film, and glycerol enhances flexibility by reducing brittleness.

Although the results are promising, further studies are needed to evaluate bioactivity in simulated body fluid and to assess cytotoxicity using relevant cell lines. Additional optimization of nanoparticle and glycerol content may further improve the balance between strength and flexibility.

In conclusion, the developed composite film represents a promising and environmentally friendly biomaterial with potential applications in wound dressing and other biomedical fields.

CHAPTER 6: REFERENCES

1. Gurtner, G. C., Werner, S., Barrandon, Y., & Longaker, M. T. (2008). Wound repair and regeneration. *Nature*, *453*(7193), 314-321. https://doi.org/10.1038/nature07039
2. Julian R. Jones, Review of bioactive glass: From Hench to hybrids, Acta Biomaterialia, Volume 9, Issue 1,2013, Pages 4457-4486. https://doi.org/10.1016/j.actbio.2012.08.023
3. Vieira, M. G. A., Da Silva, M. A., Dos Santos, L. O., & Beppu, M. M. (2011). Natural-based plasticizers and biopolymer films: A review. *European Polymer Journal*, *47*(3), 254-263. https://doi.org/10.1016/j.eurpolymj.2010.12.011
4. Brunner, T. J., Grass, R. N., & Stark, W. J. (2006). Glass and bioglass nanopowders by flame synthesis. *Chemical Communications*, (13), 1384-1386. https://doi.org/10.1039/B517501A
5. Gautam, M., & Santhiya, D. (2019). Pectin/PEG food grade hydrogel blend for the targeted oral co-delivery of nutrients. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, *577*, 637-644. https://doi.org/10.1016/j.colsurfa.2019.06.027
6. Mishra, R. K., Banthia, A. K., & Majeed, A. B. A. (2012). Pectin based formulations for biomedical applications: a review. *Asian J. Pharm. Clin. Res*, *5*(4), 1-7.
7. Munarin, F. A. B. I. O. L. A., Tanzi, M. C., & Petrini, P. A. O. L. A. (2012). Advances in biomedical applications of pectin gels. *International journal of biological macromolecules*, *51*(4), 681-689. https://doi.org/10.1016/j.ijbiomac.2012.07.002
8. Herman S. Mansur, Hermes S. Costa, Nanostructured poly(vinyl alcohol)/bioactive glass and poly(vinyl alcohol)/chitosan/bioactive glass hybrid scaffolds for biomedical applications, Chemical Engineering Journal, Volume 137, Issue 1,2008, Pages 72-83. https://doi.org/10.1016/j.cej.2007.09.036
9. Leite, Á. J., & Mano, J. F. (2017). Biomedical applications of natural-based polymers combined with bioactive glass nanoparticles. *Journal of materials chemistry B*, *5*(24), 4555-4568. http://dx.doi.org/10.1039/C7TB00404D
10. Boccaccini, A. R., Erol, M., Stark, W. J., Mohn, D., Hong, Z., & Mano, J. F. (2010). Polymer/bioactive glass nanocomposites for biomedical applications: a review. *Composites science and technology*, *70*(13), 1764-1776. https://doi.org/10.1016/j.compscitech.2010.06.002

11. Sergi, R., Bellucci, D., & Cannillo, V. (2020). A review of bioactive glass/natural polymer composites: State of the art. *Materials*, *13*(23), 5560. https://doi.org/10.3390/ma13235560
12. Srinivasan, S., Jayasree, R., Chennazhi, K. P., Nair, S. V., & Jayakumar, R. (2012). Biocompatible alginate/nano bioactive glass ceramic composite scaffolds for periodontal tissue regeneration. *Carbohydrate Polymers*, *87*(1), 274-283. https://doi.org/10.1016/j.carbpol.2011.07.058
13. Barreto, M. E., Medeiros, R. P., Shearer, A., Fook, M. V., Montazerian, M., & Mauro, J. C. (2022). Gelatin and bioactive glass composites for tissue engineering: a review. Journal of functional biomaterials, 14(1), 23. https://doi.org/10.3390/jfb14010023
14. Zhu, H., Liu, N., Feng, X., & Chen, J. (2012). Fabrication and characterization of silk fibroin/bioactive glass composite films. *Materials Science and Engineering: C*, *32*(4), 822-829. https://doi.org/10.1016/j.msec.2012.01.033
15. Negut, I., & Ristoscu, C. (2023). Bioactive glasses for soft and hard tissue healing applications-a short review. *Applied sciences*, *13*(10), 6151. https://doi.org/10.3390/app13106151
16. Day, R. M. (2005). Bioactive glass stimulates the secretion of angiogenic growth factors and angiogenesis in vitro. *Tissue engineering*, *11*(5-6), 768-777. https://doi.org/10.1089/ten.2005.11.768
17. Hench, L. L., & Polak, J. M. (2002). Third-generation biomedical materials. *Science*, *295*(5557), 1014-1017. https://doi.org/10.1126/science.1067404
18. Nandhakumar, M., Thangaian, D. T., Sundaram, S., Roy, A., & Subramanian, B. (2022). An enduring in vitro wound healing phase recipient by bioactive glass-graphene oxide nanocomposites. *Scientific reports*, *12*(1), 16162. https://doi.org/10.1038/s41598-022-20575-z
19. Moura, D., Souza, M. T., Liverani, L., Rella, G., Luz, G. M., Mano, J. F., & Boccaccini, A. R. (2017). Development of a bioactive glass-polymer composite for wound healing applications. *Materials Science and Engineering: C*, *76*, 224-232. https://doi.org/10.1016/j.msec.2017.03.037
20. Kokubo, T., & Takadama, H. (2006). How useful is SBF in predicting in vivo bone bioactivity?. *Biomaterials*, *27*(15),2907-2915. https://doi.org/10.1016/j.biomaterials.2006.01.017

21. Goel, H., & Santhiya, D. (2022). Role of Trigonella foenum-graecum leaf extract in tailoring the synthesis and properties of bioactive glass nanoparticles. *Sustainable Materials and Technologies*, *33*, e00485. https://doi.org/10.1016/j.susmat.2022.e00485
22. Dey, N., Mohny, F. P., Reshma, G. B., Rao, D., Ganguli, M., & Santhiya, D. (2023). Bioinspired synthesis of bioactive glass nanocomposites for hyaluronic acid delivery to bone and skin. *International Journal of Biological Macromolecules*, *253*, 127262. https://doi.org/10.1016/j.ijbiomac.2023.127262
23. Mehrabi, T., Mesgar, A. S., & Mohammadi, Z. (2020). Bioactive glasses: a promising therapeutic ion release strategy for enhancing wound healing. *ACS biomaterials science & engineering*, *6*(10), 5399-5430. https://doi.org/10.1021/acsbiomaterials.0c00528
24. Coban, S. N., Polatoglu, I., & Eroglu, E. (2024). Methyl cellulose/okra mucilage composite films, functionalized with Hypericum perforatum oil and gentamicin, as a potential wound dressing. *International Journal of Biological Macromolecules*, *254*, 127757. https://doi.org/10.1016/j.ijbiomac.2023.127757
25. Kaur, M., & Santhiya, D. (2022). Fabrication of soy film with in-situ mineralized bioactive glass as a functional food for bone health. *Food Bioscience*, *47*, 101767. https://doi.org/10.1016/j.fbio.2022.101767
26. Khatreja, K., & Santhiya, D. (2024). Physicochemical characterization of novel okra mucilage/hyaluronic acid-based oral disintegrating films for functional food applications. *International Journal of Biological Macromolecules*, *278*, 134633. https://doi.org/10.1016/j.ijbiomac.2024.134633
27. Gupta, N., & Santhiya, D. (2017). In situ mineralization of bioactive glass in gelatin matrix. *Materials Letters*, *188*, 127-129. https://doi.org/10.1016/j.matlet.2016.11.045
28. Rahaman, M. N., Day, D. E., Bal, B. S., Fu, Q., Jung, S. B., Bonewald, L. F., & Tomsia, A. P. (2011). Bioactive glass in tissue engineering. *Acta biomaterialia*, *7*(6), 2355-2373. https://doi.org/10.1016/j.actbio.2011.03.016