MAJOR PROJECT

FABRICATION OF XANTHAN GUM AND HYDROXYPROPYLGUAR GUM- BASED BIOACTIVE FILM AS A FOOD PACKAGING MATERIAL

A DISSERTATION

SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS

FOR THE AWARD OF THE DEGREE

OF

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IN

CHEMISTRY

[Organic specialization]

Submitted by-

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STUDENT DECLARATION

I, **Ishita,** hereby certify that the work which is being submitted in this major project report entitled, **FABRICATION OF XANTHAN GUM AND HYDROXYPROPYLGUAR GUM-BASED BIOACTIVE FILM AS A FOOD PACKAGING MATERIAL,** in the partial fulfilment for the award of the degree of Master of Science in Chemistry (Organic specialization) at **Delhi Technological University** is an authentic record of my own work carried out by me under the supervision of Prof. Rajinder K. Gupta (Department of Applied Chemistry).

I, further declare that the project report has not been submitted to any other Institute/University for the award of any degree or diploma or any other purpose whatsoever. Also, it has not been directly copied from any source without giving its proper reference. This work has not previously formed the basis for the award of any Degree, Diploma Associateship, Fellowship or other similar title or any recognition.

Ishita

[2K21/MSCCHE/56]

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CERTIFICATE

I, hereby certify that the project dissertation titled, **FABRICATION OF XANTHAN GUM AND HYDROXYPROPYLGUAR GUM-BASED BIOACTIVE FILM AS A FOOD PACKAGING MATERIAL,** which is submitted by, **ISHITA [2K21/MSCCHE/56],** 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.

ACKNOWLEDGEMENT

Abstract

The packaging industry faces the upcoming challenge of fabricating packaging materials that possess specific qualities such as mechanical strength, durability, biocompatibility, and biodegradability. In this regard, hydrogels present new opportunities for the development of effective packaging materials. Since most biopolymer-based hydrogels are designed to naturally degrade, they serve as a sustainable and environmentally friendly alternative for green packaging. This study focuses on synthesizing a hydrogel specifically for food packaging applications. A hydrogel was synthesized by incorporating ZnO nanoparticles (ZnO) and Curcumin (Cur) into Xanthan gum (XG) and Hydroxypropyl guar gum (HPGG) using Citric acid (CA) as a crosslinker. The hydrogel's structural characteristics were analyzed using techniques such as FTIR and XRD. Thermal analysis was conducted through TGA, while morphological examination was done using SEM. Rheological analysis helped determine the viscoelastic behavior of the hydrogel. Furthermore, anti-microbial, anti-oxidant, and cytotoxicity assays were performed to evaluate the hydrogel's antibacterial, scavenging, and cytotoxicity activities. The resulting XG-HPGG-CA-ZnO-Cur film demonstrates excellent potential as an edible food packaging material.

Keywords Xanthan gum, Hydroxypropyl guar gum, Citric acid, ZnO, Curcumin, Food packaging

Abbreviations

XG   Xanthan gum

HPGG   Hydroxypropyl guar gum

CA   Citric acid

Cur   Curcumin

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CHAPTER-1

INTRODUCTION

Food packaging materials ensure that the quality of food is maintained throughout its shelf life. Food packaging materials play a crucial role in maintaining the desired quality of food throughout its storage period. Incorporating hydrogel as part of food packaging systems presents an effective approach, particularly in managing water content, a factor that frequently affects food longevity [1]. The primary purpose is to prolong the shelf life of processed food items and preserve their freshness even after undergoing complex and lengthy storage procedures [2]. These exceptional materials consist of a network of hydrophilic polymers capable of absorbing and retaining water.

Their main purpose is to extend the shelf life of processed foods and preserve their freshness following rigorous and time-consuming storage processes. Plastic polymers have been a practical and economical option for packaging due to their low cost and resistance to water accessibility. However, plastic films are not fully recyclable or biodegradable, leading environmentalists to look for materials derived from renewable resources [3].

Notwithstanding these advantages, environmentalists have emphasized the need to replace plastics with materials sourced from renewable resources. This is because plastic films are neither completely recyclable nor biodegradable, potentially leading to substantial challenges in waste management and disposal. Researchers have developed ecologically friendly packaging options, such as biopolymers, as a promising replacement for non-biodegradable plastics in food packaging [4]. To improve polymer performance, superabsorbent hydrogels, capable of absorbing up to 100% of their dry weight in water and other water-compatible fluids, are being explored [5].

There is a rising need for bio-based raw materials to solve the waste disposal issues to a great extent. In this context, biopolymers-especially those derived from organic resources that are renewable, have been viewed as promising environmentally friendly alternatives to non-biodegradable plastics. Given the health risks associated with synthetic polymers and the surge in plastic waste, active packaging materials made from natural antimicrobial

ingredients are gaining interest. Proteins, polysaccharides, and lipids are some diverse food-grade polymers being investigated for this purpose.

These active packages are made using a variety of natural antimicrobial active ingredients. Antimicrobial packaging made from biobased materials can prevent undesired changes in food quality and ecological waste. They can serve as a carrier of nutrients as well. These ingestible biodegradable polymers can be applied directly to the surface of the food product or used as films. Biobased antimicrobial packaging can prevent undesired changes in food quality and ecological waste while carrying nutrients. These biodegradable polymers can be used as films or applied directly to the food [6].

XG is a high molecular weight acidic biopolymer obtained from (*Xanthomonas campestris)* during aerobic fermentation using corn or sugar cane derivatives. Its primary function is to thicken food and cosmetics due to its molar ratio of d-glucosyl, d-mannosyl, and d-glucuronyl acid residues in a 2:2:1 ratio along with varying amounts of pyruvyl groups [7]. About half of the terminal mannose residues are connected by a ketal linkage through a pyruvic acid moiety. The side chain of the molecule contains both glucuronic acid and pyruvic acid groups, which contribute to its anionic property. This unique structure and functionality have garnered significant attention for this hydrocolloid, particularly due to its effectiveness in challenging conditions such as acidity, high salt content, and high shear stress. XG exhibits hydrocolloid nature, making it highly functional in difficult settings such as high shear stress, high salt and acidic conditions. Furthermore, the ability of this hydrocolloid to form conjugates with other polymers, proteins, peptides, and non-peptides presents potential applications as a drug carrier. These conjugates exhibit desirable properties such as stability against enzyme degradation, inertness, biocompatibility, and effective solubility. Additionally, XG (referring to the hydrocolloid) can be employed in relatively small quantities to achieve zero-order release kinetics and delay drug release in vitro. XG can also be used in small amounts to achieve zero-order release kinetics and delay drug release in vitro [8].

Another biopolymer with significant characteristics in food packaging is guar gum (GG), which is a high molecular weight polysaccharide obtained from *Cyanopsis tetragonoloba*. GG is composed of a polysaccharide chain consisting of mannose as the linear backbone and has a side chain made up of a [2:1] ratio of mannose to galactose [9]. Hydroxypropyl guar gum (HPGG), a derivative of GG, is created by reacting GG with propylene oxide. HPGG

produces highly viscous and biodegradable solutions. Compared to GG, HPGG is more thermally stable and soluble, making it a more efficient choice for food packaging applications [10].

Combining two biopolymers to synthesize hydrogels, increases their stability and usefulness [11]. Crosslinkers can enhance the mechanical properties of biopolymers, making them suitable for food packaging. Chemical crosslinking is the most effective method, particularly for biopolymers. Various crosslinking agents, such as Ca^{2+} ions, citric acid, glutaraldehyde and boric acid can be used [4]. Covalent bonds, hydrogen bonds, ionic interactions, hydrophobic and dipole-dipole interactions can all crosslink polymer chains, resulting in hydrogels with various shapes such as films, coatings and sheets. [11].

ZnO nanoparticles have been added to various food packaging materials, such as coatings and films, to improve their functionality and enhance food quality and safety. The Food and Drug Administration has deemed ZnO a generally recognized as safe (GRAS) substance, making it a safe food additive. [12]

Along with ZnO, Cur has also been added to the hydrogel solution to enhance its antimicrobial properties. It has gained increasing attention in recent years due to its numerous health benefits, such as anti-inflammatory, antioxidant, and anticancer properties. Curcumin has also been found to have potential applications in food packaging as an antimicrobial and antioxidant agent. [13]

The goal of this study is to develop a novel hydrogel based on XG and HPGG cross-linked with CA for food packaging applications. Moreover, thermal analysis (TGA), structural characteristics (FTIR and XRD), morphology (SEM), rheological measurement (viscosity, amplitude and frequency sweep) mechanical strength, fruit preservation, anti-microbial activity, anti-oxidant activity and cytotoxicity assay were analysed for the films.

CHAPTER-2

LITERATURE REVIEW

Hydrogels have attracted significant attention in the field of biomedical applications due to their unique characteristics, including high water content and biocompatibility. XG-CA hydrogel, among various types of hydrogels, has shown immense potential owing to its adjustable properties and ability to form crosslinks. However, despite extensive research on hydrogels, there is a lack of studies specifically focusing on crosslinked hydrogels using HPGG. Additionally, there is currently no existing research exploring the integration of (Cur) and (ZnO) into XG-HPGG-CA hydrogels. This literature review aims to address the research gap in this area and highlight the importance of investigating XG-HPGG-CA hydrogels incorporating Cur and ZnO.

Through an extensive review of the available literature, it is evident that there is a significant research gap in the area of HPGG-CA crosslinked hydrogels. Despite the potential demonstrated by HPGG-CA hydrogel systems in diverse fields like drug delivery, tissue engineering, and wound healing, there has been limited exploration of their crosslinked variations in the food packaging industry. This gap in research presents a valuable opportunity to investigate the synthesis, characterization, and potential applications of HPGG-CA crosslinked hydrogels.

The existing literature reveals a notable disparity in research between CMGG with CA and HPGG with CA hydrogels. CMGG with CA hydrogels have received more attention and investigation compared to HPGG with CA. Studies focusing on CMGG with CA hydrogels have explored various applications, including drug delivery, tissue engineering, and wound healing, among others. [14] In light of this, we have employed films based on HPGG with CA hydrogels in our food packaging applications. By leveraging the unique properties of HPGG with CA hydrogels, such as biocompatibility, tunability, and water content, we aim to enhance the functionality and safety of food packaging materials. Through this research, we seek to contribute to the development of innovative and sustainable solutions for food packaging that align with the growing demand for environmentally friendly and efficient packaging materials.

Curcumin possesses remarkable attributes as a food packaging material, particularly its antimicrobial and antioxidant properties against various pathogenic microorganisms, including Bacillus subtilis, E. coli, and S. aureus. [15] These properties play a critical role in food packaging by preventing food spoilage and reducing the risk of foodborne illnesses. Integrating curcumin into packaging materials such as films, coatings, and plastics can effectively enhance their antimicrobial capabilities. Moreover, curcumin serves as a natural preservative, reducing the reliance on synthetic preservatives that may have adverse effects on human health. By scavenging free radicals and inhibiting the formation of reactive oxygen species, curcumin prevents food oxidation. This feature is advantageous for prolonging the shelf life of packaged foods and minimizing food waste.

The utilization of zinc oxide (ZnO) nanoparticles in food packaging has gained significant popularity due to their antimicrobial properties, UV light-blocking capabilities, and gas barrier functions.[16] These nanoparticles effectively impede the growth of bacteria and fungi, thereby extending the shelf life of food products and mitigating the risk of contamination. Furthermore, upon ingestion, ZnO breaks down into Zn^{2+} ions, which can offer potential benefits to the human body. In fact, studies have demonstrated that wheat proteins fortified with ZnO exhibit favourable zinc absorption properties.[17]

Additionally, despite the extensive use of Cur and ZnO in the biomedical field, no studies have reported their incorporation into HPGG-CA hydrogels. Both Cur and ZnO possess unique properties that make them attractive candidates for various biomedical applications. Cur, a natural compound derived from turmeric, exhibits antioxidant, anti-inflammatory, and anticancer properties. ZnO nanoparticles, on the other hand, possess antimicrobial and wound healing properties. Incorporating these bioactive components into HPGG-CA hydrogels could provide synergistic effects and potentially enhance the hydrogel's therapeutic potential.

Significance and future prospects

The absence of research on HPGG-CA crosslinked hydrogels and the lack of studies incorporating Cur and ZnO present an exciting avenue for future exploration. Investigating the synthesis and characterization of HPGG-CA crosslinked hydrogels and their potential incorporation with Cur and ZnO could lead to the development of advanced biomaterials with enhanced properties for various biomedical applications. The potential benefits include improved drug delivery systems, wound healing dressings, and tissue engineering scaffolds.

Research gap

In conclusion, the current literature review highlights the research gap regarding HPGG-CA crosslinked hydrogels and the absence of studies incorporating Cur and ZnO. The scarcity of research in these areas emphasizes the need for further investigations to explore the synthesis, characterization, and application potential of HPGG-CA crosslinked hydrogels incorporating Cur and ZnO. Such studies have the potential to contribute to the development of advanced biomaterials with enhanced properties and broaden the scope of biomedical applications.

CHAPTER-3

SYNTHESIS AND CHARACTERIZATION

EXPERIMENTAL SECTION

MATERIALS

XG was obtained from a local market, while HPGG was procured from Hindustan Pvt. Ltd. CA was purchased from CDH in New Delhi, Curcumin (Cur) was obtained from SD Fine Chemical in Mumbai, and ZnO was purchased from Sigma Aldrich in the USA. Glycerol was obtained from Fisher Chemicals in the UK. Milli Q water was utilized for all experimental procedures.

METHODS

PREPARATION OF XG-HPGG-CA FILM
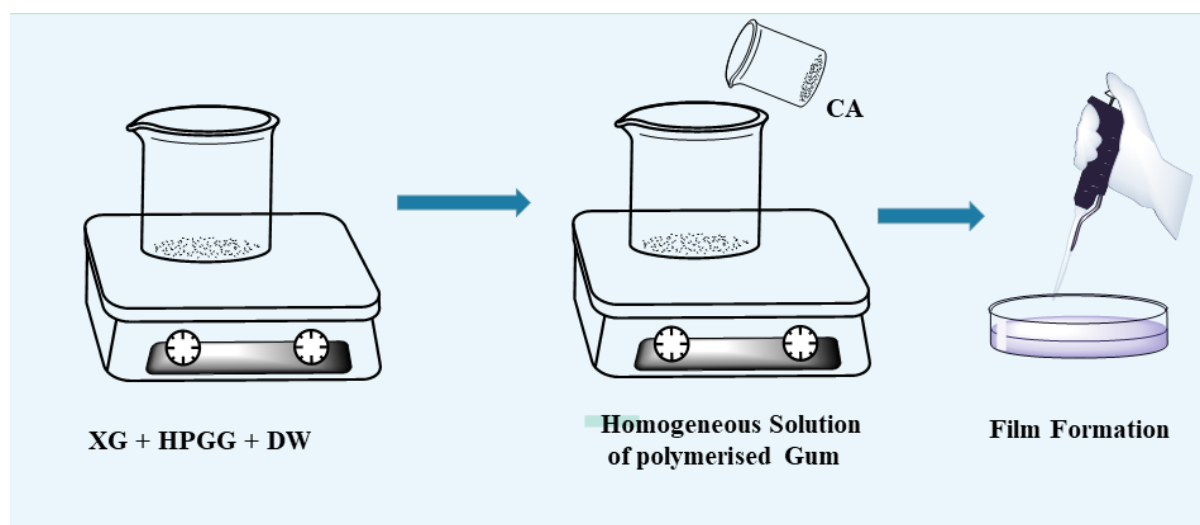
Fig.1 Synthesis of XG-HPGG-CA Film

Packaging films were fabricated using food-grade XG and HPGG, which are hydrophilic polymers known for their easy solubility in water. The ratio of gums to CA was maintained at 1:1.5. The required amount of XG and HPGG was dissolved in distilled water at regular intervals. To create a homogenous solution, CA was added as a cross-linker to the mixture of XG and HPGG. Glycerol was included as a plasticizer in the homogenized solution. A

consistent mixture of ZnO and Cur was added to the hydrogel preparation and stirred continuously for 3 hours. To eliminate trapped air, sonication was performed before pouring the solution into a Petri dish. The dish was then placed in an oven at 45°C for 24 hours to allow for drying. Finally, the dried solution was used to create films for subsequent characterization analysis.

Fig.2 XG-HPGG-CA Film

Fig.3 XG-HPGG-CA Powder

CHARACTERIZATION OF XG, HPGG, XG-HPGG-CA AND XG-HPGG-CA-ZNO-CUR FILM

FT-IR Spectroscopy

Functional group analysis of XG, HPGG, XG-HPGG-CA, and XG-HPGG-CA-ZnO-Cur was performed using the Perkin-Elmer model 2000 FT-IR. The data was collected in the range of 4000-400 cm^{-1} with 32 scans at a resolution of 4 cm^{-1}.

X-ray diffraction analysis

For X-ray diffraction analysis, a wide-angle X-ray diffractometer (Bruker D8 Advance) was utilized. Cu-Kα radiations of λ = 1.5406 A^{o} were used, with a scanning range of 2θ (5°-80°) and a scan rate of 1°/min. The measurements were conducted at 20 kV and a current of 10 mA.

SEM

The surface morphology of the samples was examined using the SEM (JEOL JSM-6610LV) at magnifications of 500, 1000, and 2500 under an accelerating voltage of 20 kV.

TGA

Thermal stability analysis was conducted using TGA (TGA 4000). The samples were heated from 25°C to 800°C at a heating rate of 10°C/min in an N_2 atmosphere.

Rheology

The rheological properties of XG-HPGG-CA and XG-HPGG-CA-ZnO-Cur solutions were determined using the Anton Paar Modular Compact Rheometer 302 (MCR) at room temperature. The tests were performed using a 40mm parallel plate configuration.

Mechanical properties of the film

The mechanical properties of the XG-HPGG-CA-Zno-Cur film were assessed using an Instron RP01 5982 Universal Testing Machine. The load range was set from 1N to 20N, with an extension range of 1000mm and a gauge length of 25mm. The test and approach speed were maintained at 500mm/min, with no preload. For mechanical testing, a strip measuring 15mm in width and 150mm in length was cut from each film. Four readings were taken for each sample under air conditions, and the average result was used to evaluate the mechanical properties of the film.

In-vitro antimicrobial activity of films

Disk Diffusion Method

The antimicrobial activity of the films was evaluated against Escherichia coli and Staphylococcus aureus, representing Gram-negative and Gram-positive bacteria, respectively, using the agar disk diffusion method. Bacterial cultures were prepared in nutrient broth and inoculated onto agar plates. Disks cut from each film were placed on the plates, and after incubating the plates at 37°C for 14-16 hours, the fully formed zones (ffz) were measured using a digital calliper. The films used in the experiment had a diameter of 0.7cm.

Food Sample Testing

To conduct a comparative study on the rate of food spoilage, fresh strawberries were selected as food samples. One strawberry was coated with the synthesized hydrogel, while the other strawberry was left uncoated and stored in glass petri plates. Regular testing intervals of 24 hours were conducted over a span of 1 week. All studies were carried out under natural lighting conditions at a constant temperature of 25ºC.

Antioxidant Activity

The UV-vis spectrophotometer was used to evaluate the antioxidant activity of hydrogel solution by DPPH free-radical scavenging [18]. A modified method based on a previous study was used to determine the photochemical stability, in which varying amounts of hydrogel solution (3mg, 5mg, 7mg, 20mg, and 50mg) of XG-HPGG-CA-ZnO-Cur were dissolved in 50mL ethanol solution at 37°C [17]. Eq.-1 was used to determine the percentage of DPPH scavenging activity after mixing 1 mL of the solution with 3 mL of ethanol solution

containing DPPH (1mg/20mL) and incubating it in the dark at room temperature for 30 minutes. The absorbance was measured at 517nm.

$$\text{Scavenging Activity of DPPH (\%)} = \left(1 - \frac{A_{sample}}{A_{control}}\right) \times 100 \qquad \text{--Equation-1}$$

The value of $A_{control}$ indicates the absorbance of 1mL ethanol and 3mL ethanol solution of DPPH, while A_{sample} refers to the absorbance of 1mL XG-HPGG-CA-ZnO-Cur solution and 3mL DPPH solution, as described in a previous study [19].

Cytotoxicity Evaluation by MTT Assay

The cytotoxicity of the films on the Hep-G2 cell line was determined using the SRB assay [20]. Initially, the cells were cultured in a 96-well plate with DMEM medium supplemented with 10% FBS and 1% antibiotic solution at 37°C with 5% CO2, with a density of 8000 cells/well. After a 24-hour incubation period, the cells were exposed to varying concentrations of dosage (ranging from 0.05-1.25%), which were prepared in an incomplete medium. Following another 24 hours of incubation, each well plate received 100 µL of 10% Tri Chloro Acetic Acid (TCA) and was incubated for 1 hour. Subsequently, the plate was washed with DM water and allowed to air dry at room temperature. To proceed, a final concentration of 0.04% SRB solution was added to each well plate and incubated for 1 hour. After the incubation, the plate was washed with 1% (v/v) acetic acid to remove any unbound dye and allowed to air dry at room temperature. A Tris base solution (pH=10.5) was then added to each well, and the plate was shaken on an orbital shaker for 10 minutes to solubilize the protein-bound dye. Finally, the plate was read at 510 nm using an ELISA plate reader (iMark, Biorad, USA).

CHAPTER-4

RESULTS & DISCUSSIONS

STRUCTURAL CHARACTERIZATION

4.1 FT-IR Spectroscopy
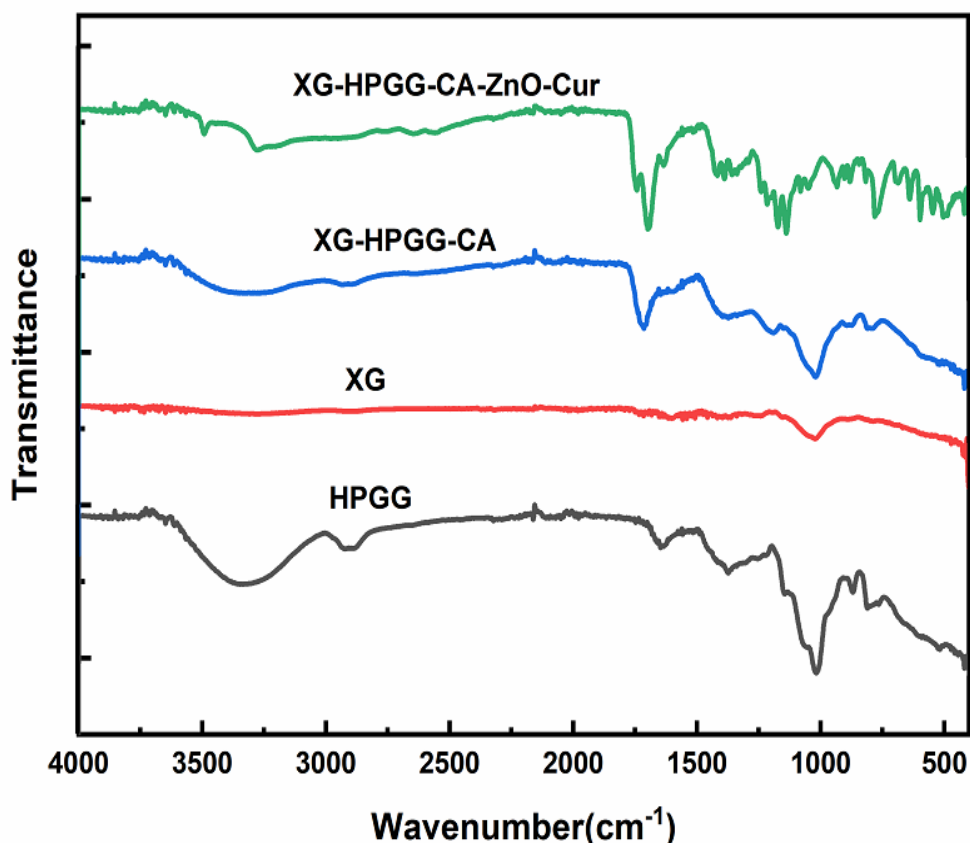
Fig.4 FTIR Spectra of HPGG, XG, XG-HPGG-CA and XG-HPGG-CA-ZnO-Cur Hydrogel

The functional groups of XG, HPGG, XG-HPGG-CA, and XG-HPGG-CA-ZnO-Cur were analysed through FTIR Spectra analysis in the range of 4000-400 cm^{-1}. HPGG exhibited characteristic peaks at specific wavenumbers, including 2912 cm^{-1} for C-H stretching, 3342 cm^{-1} for N-H stretching, and 1635 cm^{-1} for C=O stretching vibrations. [21]. XG showed characteristic peaks at 3325cm^{-1} for O-H stretching and 1608cm^{-1} for C=C stretching vibrations [22]. In the FTIR spectra of XG-HPGG-CA (Film), noticeable peak shifts were observed compared to XG and HPGG. These shifts occurred at 3360 cm^{-1}, 2921 cm^{-1}, and 1722 cm^{-1}, indicating the influence of crosslinking with CA. Similarly, in the FTIR spectra of XG-HPGG-CA-ZnO-Cur (Film), a prominent peak was observed at 3273 cm^{-1},

corresponding to -OH stretching vibrations, and at 1697 cm⁻¹, indicating carbonyl (C=O) stretching vibrations.[23].   The observed peak shifts are primarily attributed to the esterification process with CA, as well as the inclusion of ZnO and Cur. These modifications are of significant importance in a range of applications, particularly in the field of food packaging.

4.2 X-ray diffraction analysis
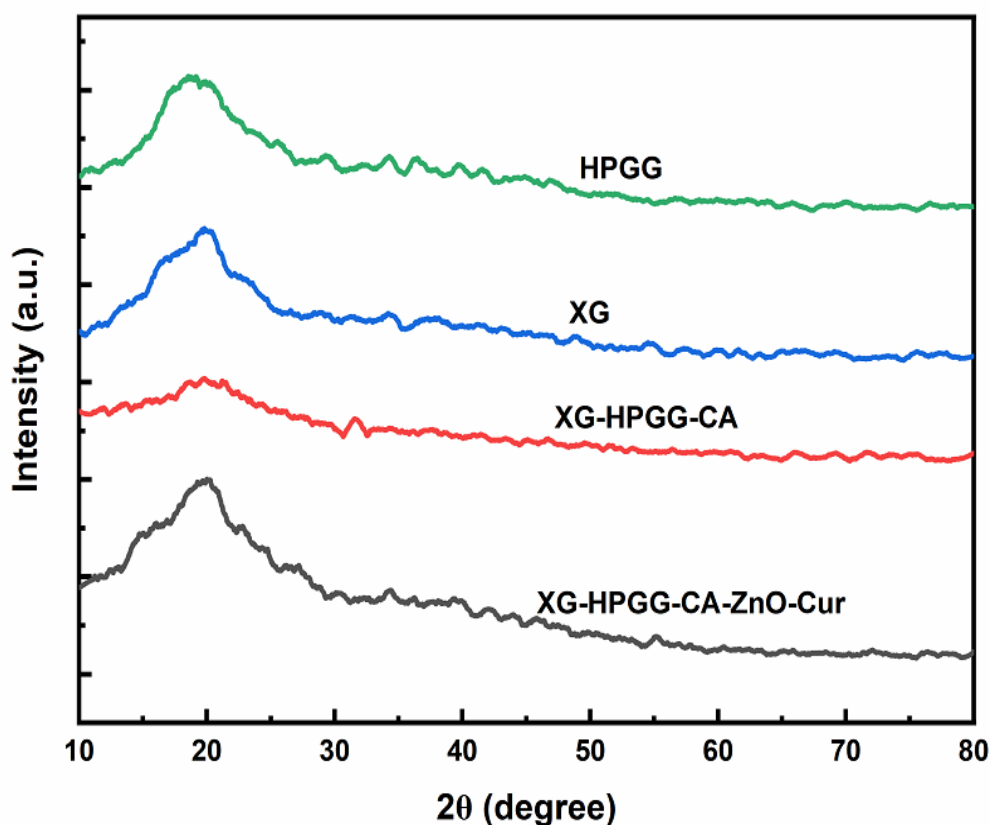
Fig.5 XRD Spectra of HPGG, XG, XG-HPGG-CA Film and
XG-HPGG-CA-ZnO-Cur Film

Fig.5 shows the X-ray diffractograms of HPGG, XG, XG-HPGG-CA Film, and XG-HPGG-CA-Cur-ZnO Film. In HPGG, a broad peak was observed at 18.86º, while XG exhibited a similar peak at 19.96º. XG-HPGG-CA (Film) displayed a peak at 19.92º, which can be attributed to the cross-linking of the two gums with CA. The X-ray diffractogram of XG-HPGG-CA-Cur-ZnO (Film) showed a peak shift to 18.64º, indicating esterification. The XRD patterns of XG, HPGG, XG-HPGG-CA, and XG-HPGG-CA-ZnO-Cur indicated an amorphous structure with relatively low overall crystallinity. The presence of crystalline regions in XG and HPGG, observed at 19.96º and 18.86º respectively, can be attributed to the hydroxyl groups. The hydrogen bonds in XG and HPGG played a role in maintaining their stability, and a decrease in crystallinity was observed in XG-HPGG-CA and XG-HPGG-CA-ZnO-Cur when these hydrogen bonds were disrupted. [24]–[27].

4.3 Texture and morphology of the biopolymer (SEM)
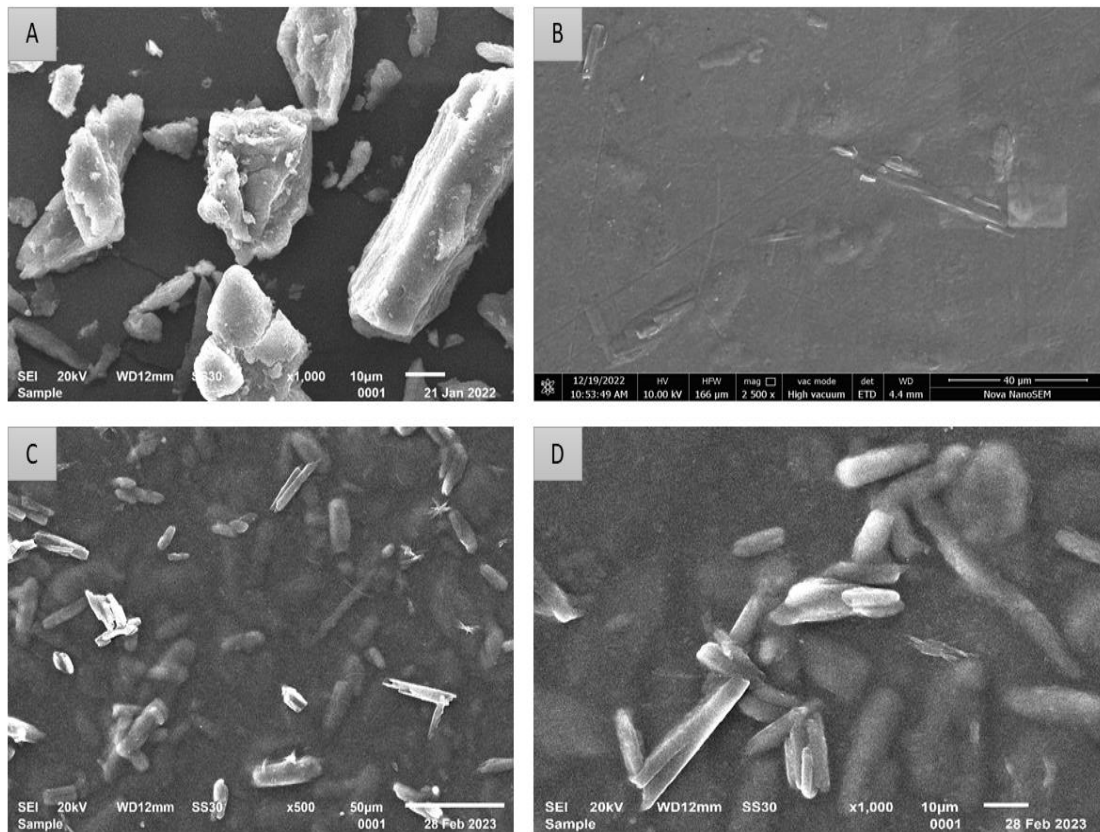
Fig.6 Scanning Electron Micrographs (SEMs) at different magnifications of (A) XG x1000 (B) XG-HPGG-CA Film x2500 (C), (D) XG-HPGG-CA-ZnO-Cur Film at 500 and 1000 magnifications respectively.

SEM images of XG, XG-HPGG-CA Film and XG-HPGG-CA-ZnO-Cur hydrogel taken at different magnifications are in Fig.6. SEM has been demonstrated to be quite useful for determining and verifying morphological traits[28][29]. The interior structure of the hydrogel films was heterogeneous and interlinked, which can result in significant swelling and permeability as well as facilitate cellular growth.Fig.6(A) images showed fibrous network structure and appearance of larger granules at higher magnification for XG. Crosslinking of XG and HPGG with CA was responsible for homogeneous covering of XG-HPGG-CA hydrogels in Fig.6(B).

On adding ZnO and Cur to the XG-HPGG-CA Film the morphology of film changed from smooth surface to rough and hollow-follicular, as shown in Fig.6 (C, D). The shift in structure from homogeneous to heterogeneous nature after cross linking is due to presence of

ZnO and Cur constituents and the irregular pore network structure in XG-HPGG-CA-ZnO-Cur is responsible for the heterogeneity in the polymeric hydrogel[30].

These structural characteristics provide additional evidence that cross-linked hydrogel networks were formed during the polymerization phase[31]. The SEM images of XG polymer showed granular structure with rough surface morphology. These structures offer more channels for water to escape from specific areas of the polymer matrix [19].

The specific pore volumes, pore diameters, pore size distributions, and specific surface areas of the polymer matrix are all usefully provided by the surface morphology of polymers[19]. This structure of XG-HPGG-CA-ZnO-Cur Film suggests the suitability of this biopolymer for biomedical applications, including serving as a food packaging material [32].

4.4 TGA
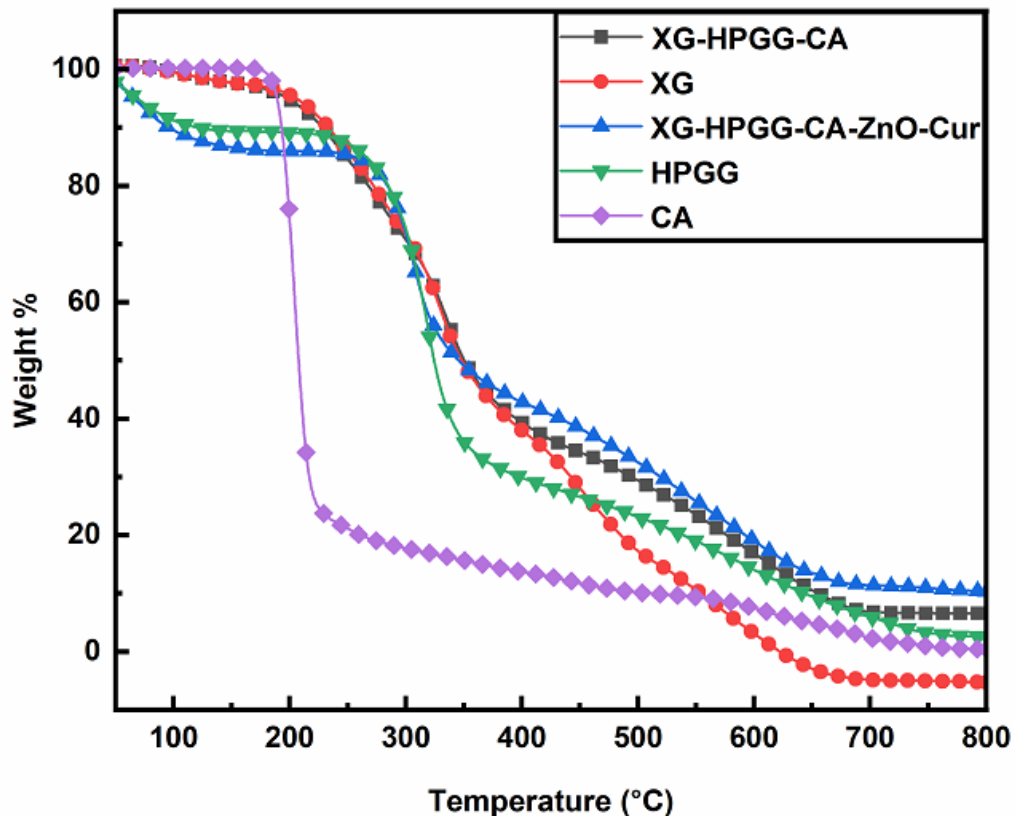
Fig.7 Thermogravimetric analysis of CA, HPGG, XG, XG-HPGG-CA Film and XG-HPGG-CA-ZnO-Cur Film at a heating rate of 10°C/min in N$_2$ environment.

The TGA (Thermogravimetric Analysis) results for XG, HPGG, CA, XG-HPGG-CA film, and XG-HPGG-CA-ZnO-Cur film are depicted in Fig.7. To account for weight loss attributed to trapped moisture, the initial decomposition temperature (IDT) was utilized for each sample. In the case of XG, an IDT of 13.66% was observed within the temperature range of 29°C to 222.27°C. Similarly, HPGG exhibited an IDT of 8.94% in the temperature range of 45.26°C to 183.92°C. CA, on the other hand, demonstrated an IDT of 83.772% within the temperature range of 163.68°C to 332.63°C. The crosslinked polymers, namely XG-HPGG-CA film and XG-HPGG-CA-ZnO-Cur film, displayed weight losses of 3.239% and 2.268%, respectively, within nearly the same temperature range. This indicates that the enhanced stability observed in XG-HPGG-CA-ZnO-Cur film can be attributed to the presence of ZnO and Cur.

The initial weight loss can be attributed to the loss of absorbed moisture in the polymer matrix. T_{max}, which refers to the temperature corresponding to the maximum rate of mass loss [1], was found to be (222.27°C to 418.32°C), (183.92°C to 392.92°C), (17.089°C to 388.9°C), and (151.2°C to 461.7°C) for XG, HPGG, XG-HPGG-CA film, and XG-HPGG-CA-ZnO-Cur film, respectively. These findings indicate that the thermal stability of gums, such as XG and HPGG, decreases during the decomposition stages. However, the polymerization and film formation processes of XG-HPGG-CA and XG-HPGG-CA-ZnO-Cur exhibit an increased level of stability, as evidenced by the lower percentage of weight loss compared to XG and HPGG. [33]

4.5 Rheology
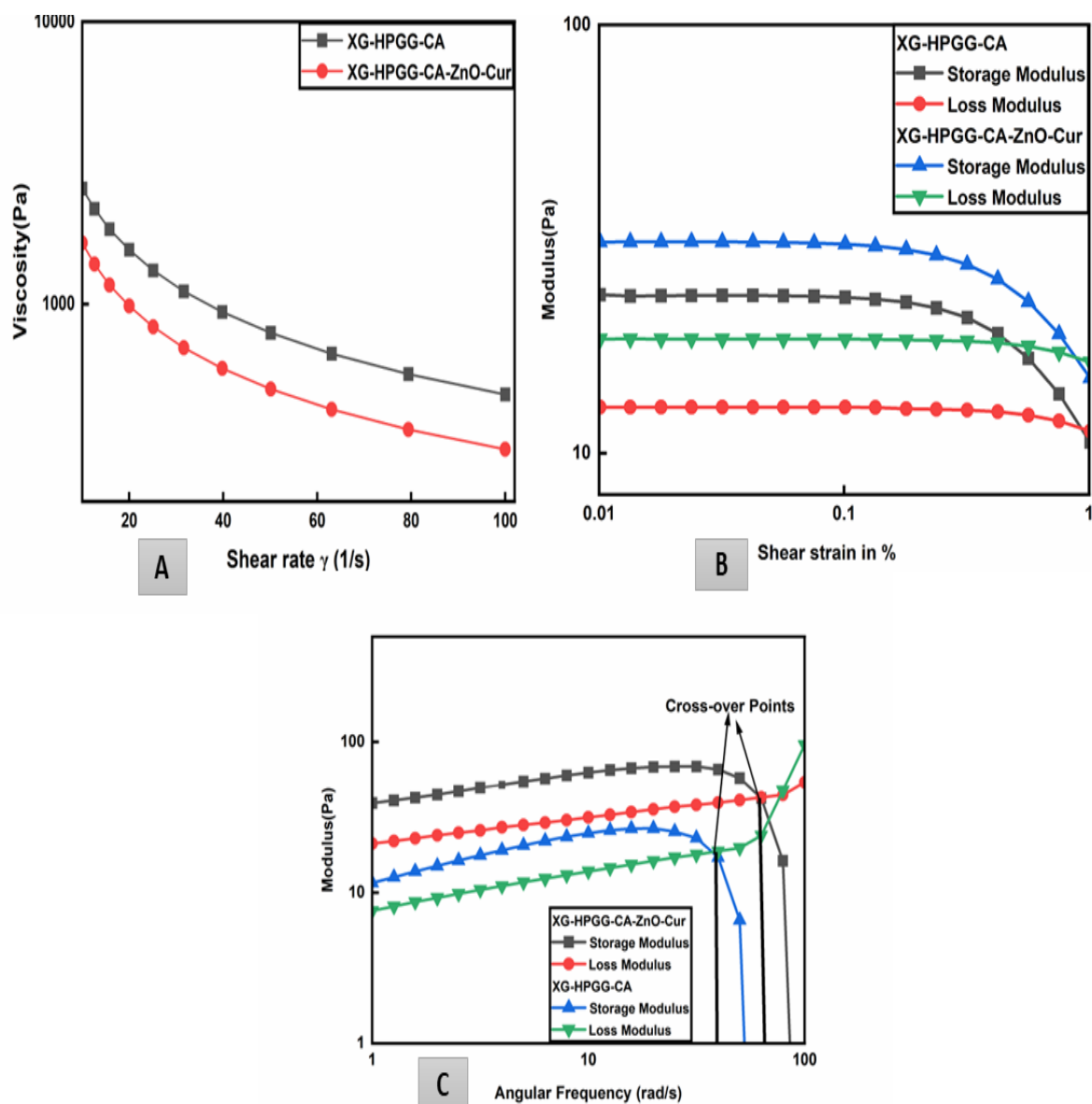
Fig. 8. Rheological properties of XG-HPGG-CA and XG-HPGG-CA-ZnO-Cur solutions: (A) Shear viscosity profile of XG-HPGG-CA and XG-HPGG-CA-ZnO-Cur solutions with varying shear rate from $10s^{-1}$ to $102s^{-1}$ (B) Amplitude sweep of XG-HPGG-CA and XG-HPGG-CA-ZnO-Cur solutions (C) Frequency sweep of XG-HPGG-CA and XG-HPGG-CA-ZnO-Cur solutions.

Viscosity

Rheology is the scientific field that investigates the behaviour of materials under deformation and flow, exploring the relationship between force, deformation, and time. It involves experimental methods used to analyse and evaluate the rheological properties of various substances. [34] Shear rheological testing was conducted by varying the shear rate from $10s^{-1}$ to $102s^{-1}$.

The results indicated that XG-HPGG-CA-ZnO-Cur solutions exhibited a more pronounced shear thinning behaviour compared to XG-HPGG-CA solutions, as depicted in Fig.8. The enhanced shear-thinning characteristics were attributed to the inclusion of ZnO and Cur in the composition of XG-HPGG-CA-ZnO-Cur. In both cases, a significant decrease in viscosity was observed due to the alignment of XG-HPGG-CA and XG-HPGG-CA-ZnO-Cur induced by shear in the direction of flow. [35]

Amplitude sweep

An amplitude sweep test was performed to evaluate the linear viscoelastic (LVE) range and assess the structural and mechanical stability of the hydrogel. This test helped determine the upper and lower limits of the LVE range [36]. A plot of the storage modulus (G') and loss modulus (G") against shear strain percentage was generated, as depicted in Figure 5. The results revealed that within the linear viscoelastic (LVE) range, the storage modulus (G') remained constant at low deformation, indicating the structural integrity of the sample. [37]

At lower strains, both XG-HPGG-CA-ZnO-Cur and XG-HPGG-CA exhibited viscoelastic solid behaviour, while at higher strains, they demonstrated viscoelastic fluid behaviour due to the disruption of their structure, indicating the end of the linear viscoelastic (LVE) region. The plateau value of G' within the LVE region represents the sample's inherent rigidity. For XG-HPGG-CA-ZnO-Cur, the LVE range was characterized by G' = 29.8 Pa, while for XG-HPGG-CA, G' = 22.9 Pa, which can be attributed to the incorporation of Cur and ZnO in the hydrogel composition.

The shear modulus was found to reach approx 80% of its starting value when the LVR was determined to end, which is often the shortest when the sample is at its most solid state [38].

The solution with ZnO and Cur showed a lower critical value (γc) of 18.6% compared to the solution with XG-HPGG-CA (γc) of 13%.

Frequency sweep

The frequency sweep test is used to assess the viscoelastic properties of a material by comparing the values of (Storage Modulus) G' and (Loss Modulus) G" over a range of frequencies [39]. Ajovalasit et al conducted a study to investigate the effects on storage and loss moduli within a frequency range by adding additives and concluded that all hydrogels showed viscoelastic nature and found that both G' and G" showed positive slopes with the loss modulus increasing more rapidly compared to the storage modulus [40].

In the frequency sweep test, the shear strain percentage was maintained constant within the linear viscoelastic (LVE) region while the frequency was varied from 1 to 100 rad/s. The results depicted in Fig.8 reveal that for both XG-HPGG-CA and XG-HPGG-CA-ZnO-Cur solutions, the loss modulus (G") dominates at high frequencies, whereas the storage modulus (G') dominates at low frequencies, indicating viscoelastic solid behaviour. Furthermore, the moduli of XG-HPGG-CA-ZnO-Cur are consistently higher than those of XG-HPGG-CA across all frequencies, providing additional evidence of enhanced stability attributed to the presence of ZnO and Cur.

4.6 Mechanical Properties

Table 1 presents the findings pertaining to the tensile properties evaluation of the XG-HPGG-CA-ZnO-Cur film, including measurements of thickness (mm), width (mm), maximum load (N), elongation at break (%), and tensile strength (MPa).

Table.1 The tensile properties of XG-HPGG-CA-ZnO-Cur

	Thickness (mm)	Width (mm)	Maximum Load (N)	Elongation at break (%)	Tensile Strength (MPa)
1	0.05000	15.00000	4.38273	49.41076	5.84364
Mean	0.05000	15.00000	4.38273	49.41076	5.84364
Minimum	0.05000	15.00000	4.38273	49.41076	5.84364
Maximum	0.05000	15.00000	4.38273	49.41076	5.84364

The XG-HPGG-CA-ZnO-Cur Film showed an elongation at break % of 49.41076 which indicated its ability to withstand stretching and deformation before breaking. It has a high tensile strength of 5.84364 MPa which was indicative of its ability to withstand tension and resist breaking under force. These properties have made this hydrogel suitable for biomedical applications such as food packaging material.

4.7 In Vitro Test

4.7.1 Disk Diffusion Method
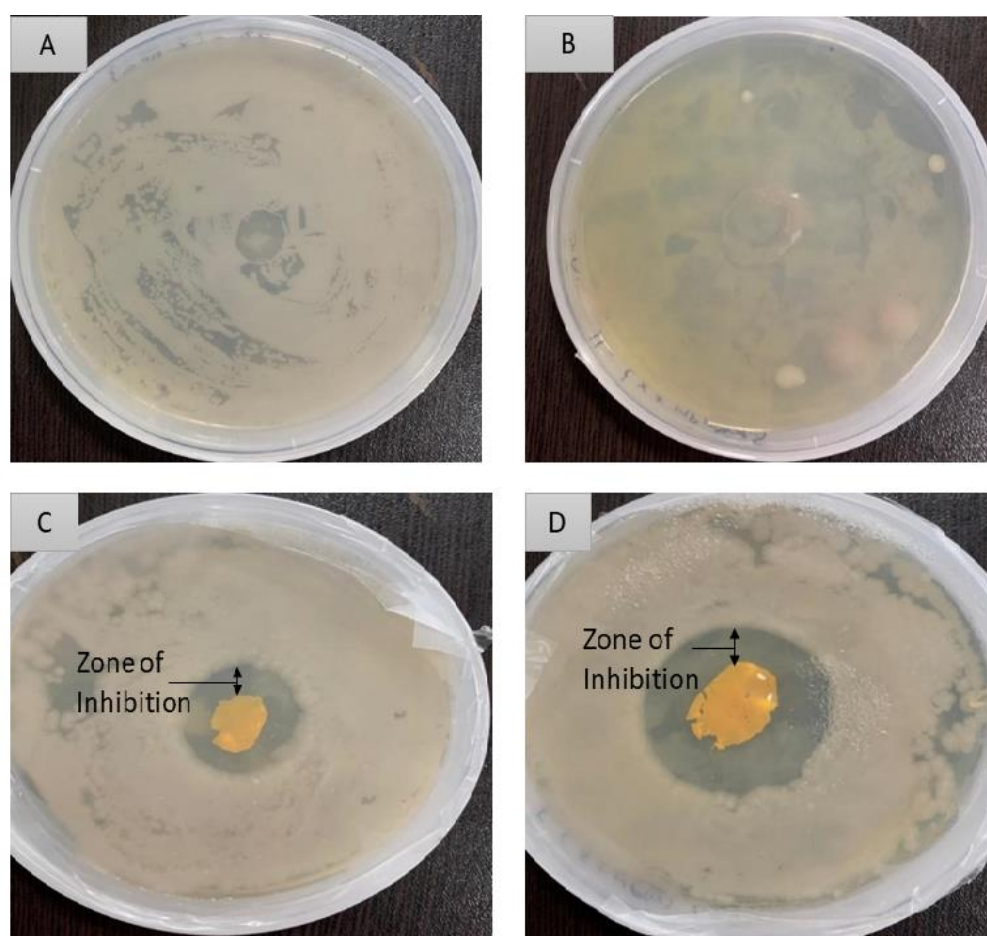


Fig.9 Illustrates the disk diffusion test conducted at 37°C to determine the antimicrobial activity of (A, B) XG-HPGG-CA Film and (C, D) XG-HPGG-CA-ZnO-Cur Film against *Escherichia coli* and *Staphylococcus aureus*.

The adoption of active food packaging technology, which involves the integration or immobilization of antimicrobial agents within the packaging material to prevent microbial

growth and enhance the shelf life of food products, is increasingly becoming popular.[41] The antimicrobial activity of XG-HPGG-CA Film and XG-HPGG-CA-ZnO-Cur Film composites against *S. aureus* and *E. coli* bacterial strains was evaluated using the disk diffusion method, as presented in Fig.9. XG-HPGG-CA Film exhibited no significant antibacterial activity against either S. aureus or E. coli, as indicated in Fig.9. It was unable to penetrate the bacterial cell. However, XG-HPGG-CA-ZnO-Cur Film composites (Fig.9 C, D) displayed a distinct inhibition zone measuring 87mm and 102mm, respectively, against both *S. aureus* and *E. coli.* These results indicate that XG-HPGG-CA-ZnO-Cur film possesses strong antibacterial properties against both gram-negative and gram-positive bacteria, suggesting its potential for broad-spectrum antibacterial activity even at low concentrations.

The presence of an inhibition zone in the XG-HPGG-CA-ZnO-Cur nanocomposite suggests that the biocidal action of ZnO NPs involves disrupting the membrane by generating surface oxygen species rapidly, ultimately leading to the death of pathogens [42]. This demonstrates the potential of XG-HPGG-CA-ZnO-Cur Film composites as an effective active food packaging technology for inhibiting microbial growth and extending the shelf life of food products.

4.7.2 Food sampling test
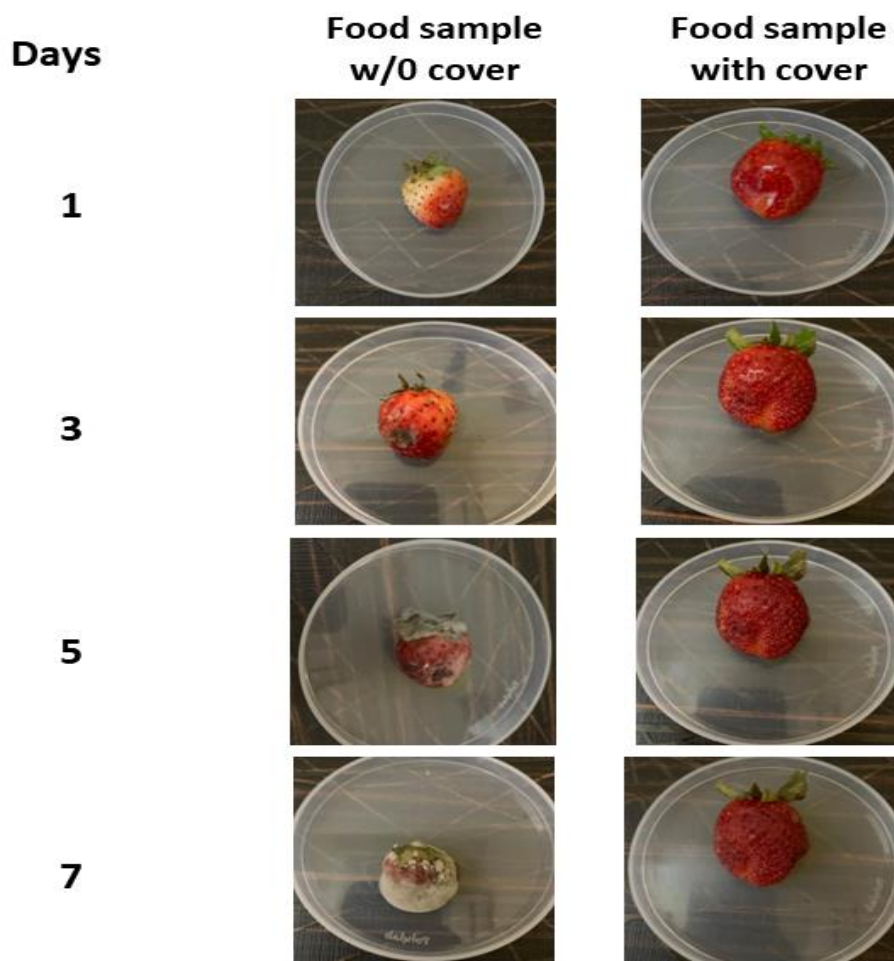
Fig.10 Food sample testing over strawberry sample at 35°C (from left to right) food sample without coating and food sample with coating of XG-HPGG-CA-ZnO-Cur solution.

Following the antimicrobial testing using the disk diffusion method, the films were assessed as potential food packaging materials for coated and non-coated food samples. In order to provide a protective layer, the strawberries were immersed in the XG-HPGG-CA-ZnO-Cur solution and allowed to air dry, creating a barrier on the surface [43]. Despite the significant antioxidant properties of strawberries, their susceptibility to mechanical damage can result in the development of infectious symptoms. Additionally, their postharvest shelf life is typically limited to approximately five days when stored at 4°C. [44]. The study aimed to enhance the shelf life of strawberries by coating them with an effective hydrogel.

In Fig. 10, one strawberry is depicted with a coating of XG-HPGG-CA-ZnO-Cur film, while the other remains uncoated. The coated strawberry demonstrated slower deterioration compared to the uncoated sample. On Day 1, no microbial growth was observed on either

fruit sample. By Day 3, microbial growth was observed on the non-coated fruit sample, and by Day 7, the fruit had deteriorated. In contrast, the strawberries coated with XG-HPGG-CA-ZnO-Cur film showed no microbial growth until Day 7. These results confirm the potential of XG-HPGG-ZnO-Cur film as a food packaging material with antimicrobial properties, with Cur and ZnO proving to be effective ingredients in active packaging films. Additionally, biopolymer sheets with water permeability properties can be used for packaging fruits and vegetables with high respiration rates [45].

4.8 Antioxidant Activity
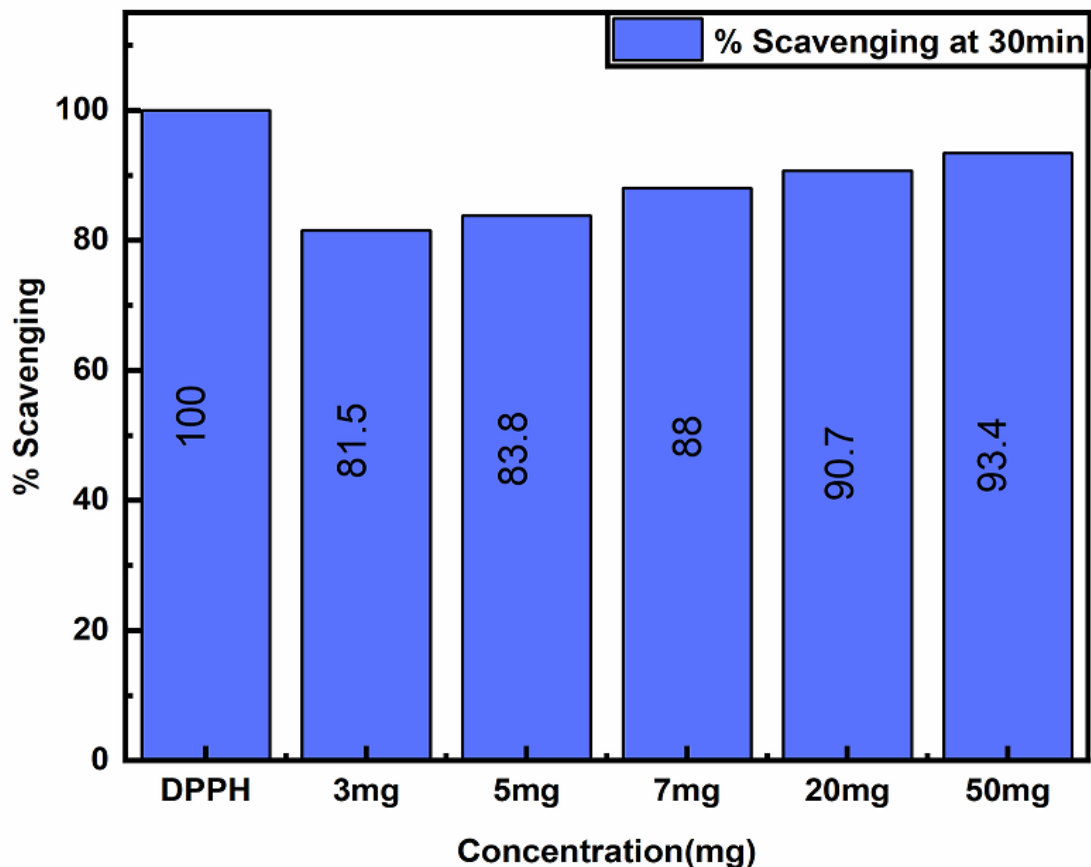
Fig.11 Scavenging of DPPH radical by XG-HPGG-CA-ZnO-Cur

While the primary source of activity in Cur is attributed to its conjugated diene moiety, its antioxidant properties are also influenced by the adjacent phenolic groups (shown in Fig. 11). The outcomes of the DPPH Assay indicated that Cur facilitated the transfer of a hydrogen atom, resulting in the conversion of the stable radical diphenyl-2-picryl hydrazyl into a non-radical yellow diphenyl-picryl hydrazine diamagnetic molecule. A notable increase in antioxidant activity was observed in the assay. The incorporation of ZnO nanoparticles with Cur resulted in a remarkable enhancement of antioxidant activity against DPPH free radicals [47]. The antioxidant activity was assessed by treating solutions with different concentrations (3mg, 5mg, 7mg, 20mg, 50mg) with DPPH solution for 30 minutes. This led to a gradual increase in antioxidant activity, with percentages of 81.5%, 83.8%, 88%, 90.7%, and 93.4%, respectively. The incorporation of antioxidant activity in food packaging films can play a beneficial role in regulating the generation of reactive oxygen species during the packaging process [48].

4.9 Cytotoxicity evaluation by MTT Essay
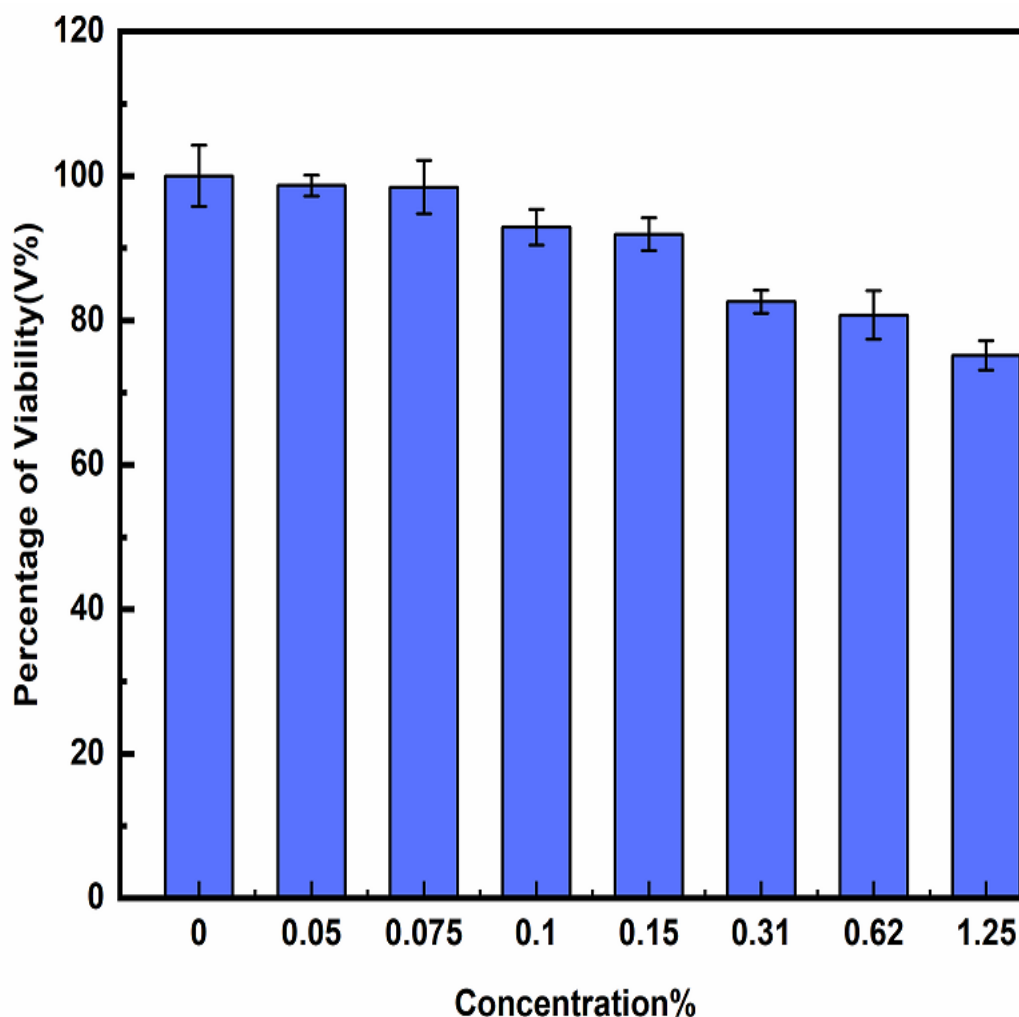
Fig.12 The viability percentage of Hep-G2 cells determined by subjecting them to different concentrations of XG-HPGG-CA-ZnO-Cur for 24 hours.

The biocompatibility of XG-HPGG-CA-ZnO-Cur films was evaluated using human hepatic cells (Hep-G2) as representative cell lines. The findings of the cytotoxicity assay for various concentrations are displayed in Fig.12. The study involving Hep-G2 cell culture revealed that the cells were capable of adhering to the film surfaces, undergoing proliferation, and spreading, as evidenced by Fig. 12 [20]. Following the incubation period, the cells cultured on XG-HPGG-CA-ZnO-Cur films displayed regular morphology. The viability percentages for all film samples at various concentrations, as depicted in Fig.12, exceeded 80%, indicating the biocompatibility of the films with Hep-G2 cells. These findings provided further evidence of the cytocompatibility of the bioactive film, as the cytotoxicity of XG-HPGG-CA-ZnO-Cur films, as assessed in the assay, decreased.

Conclusions

In summary, this investigation successfully developed XG-HPGG-CA-ZnO-Cur films with remarkable antimicrobial properties and a notable zone of inhibition, making them well-suited for application as edible food packaging material. The incorporation of glycerol also enhanced their thermal and mechanical stability compared to XG-HPGG-CA-ZnO-Cur films. Through the utilization of various analytical techniques such as FTIR, XRD, SEM, TGA, rheology, and in-vitro tests, the characterization of the samples revealed that XG-HPGG-CA-ZnO-Cur films facilitated cell growth and differentiation, underscoring their potential as a food packaging material. These findings indicate the potential reduction of the use of harmful plastics in food packaging by adopting these edible and cost-effective alternatives. Overall, XG-HPGG-CA-ZnO-Cur films exhibit promising prospects as sustainable and biocompatible substitutes for food packaging applications.

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