Innovation of Chemistry & Materials for Sustainability
Volume 2, Issue 1, Jan.- June **2025**
RESEARCH ARTICLE
ISSN (Print): 3049 – 2548
ISSN (Online): 3049 – 0146

Effects of Incorporation of Multicomponent Active Agents in Chitosan Biopolymer: A Simple Method for Sustainable Food Packaging

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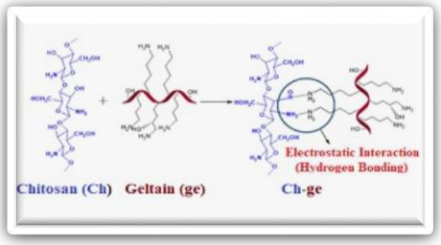
Abstract: Chitosan-gelatin (Ch-ge) based transparent films containing gallic-acid (GA) grafted starch nanoparticles (GA-SNPs) namely, Ch-ge-GA-SNP-1 and Ch-ge-GA-SNP-2 with different amounts of GA-SNPs have been synthesized by solution casting method and characterized. The incorporation of multicomponents showed effective properties of chitosan films. Scanning Electron Microscopy (SEM), XRD and FTIR analysis revealed a homogenous and transparent nature and uniform distribution of the newly synthesized films respectively. The antibacterial activity of the films against both gram-positive and gram-negative bacteria (*B. substilis* and *E. coli*) indicated that Ch-ge-GA-SNP-2 films had pronounced antibacterial activity. The antioxidant activity of Ch-ge-GA-SNP-2 films was determined by DPPH and ABTS methods and results corresponded to 80.9% of DPPH and 84.9% of ABTS free radicals scavenging activities. The results indicated that Ch-ge-GA-SNP-2 films present a definitive advantage in terms of their barrier properties, mechanical strength as well as antibacterial and anti-oxidant activity compared to pristine chitosan-gelatin films.



Keywords: *Gallic-acid loaded starch nanoparticles, chitosan-gelatin films, antioxidant activity, antibacterial activity*

1. Introduction

Sustainable bioactive food packaging systems of composite films or membranes and blends synthesized from natural polysaccharides in combination with other functional biomolecules such as proteins and plant extracts have extensively been studied in recent times as viable alternatives to unsustainable synthetic polymers.[1-4] At the forefront of this research, two naturally most abundant biopolymers chitosan, a deacetylated derivative of chitin, and starch-based biocomposite films have been found most promising in terms of their suitability for the intended purpose i.e., food packaging. The important attributes of these bio-based films and blends rely on their ease of access, biocompatibility, and biodegradability apart from exemplary moisture and oxygen barrier and antimicrobial properties which are very vital requirements in food safety and storage.[5-8] A profound challenge in fabricating these films lies in attaining the desired mechanical strength and plasticity which has so far precluded their mass production and use. This problem has, to a greater extent, been addressed by incorporating gelatin, a purified animal protein with exceptional film-forming capacity, into the chitosan and/or starch blends which compensates latter's shortcomings of brittleness, hydrophilicity, and inadequate tensile strength etc..[9-11] Biocomposites of chitosan-gelatin (Ch-ge) with proven antioxidant additives such as gallic-acid (GA, 3,4,5,-trihydroxy benzoic acid) represent a new class of 'green packaging system' having great future development potential.[12-16] The excellent biocompatibility of gelatin molecules results from the presence of biologically important functional groups such as carboxyl, hydroxyl and amino which help it blend with other natural bio-entities as potential film forming material. Films based on chitosan/gelatin have shown improved mechanical and barrier properties against water vapor and UV light[17] and veggies such as red bell pepper treated with the above edible chitosan/gelatin coatings showed a lowering in microbial decay and longer storability.[18]

Moreno et al. have reported the synthesis of starch/ gelatin films in order to promote polymer cross-linking between two bio-macromolecules through interactions between the starch carbonyl and gelatin amino groups which ultimately improved the desired properties (Schematic representation is shown in Supplementary).[19,20]

Conjugates combining chitosan and/or starch and gelatin remain little investigated in terms of the optimum macromolecular ratio of constituting biopolymers which can provide the best synergistic effect in improving these composites' mechanical and barrier properties.[21-24] Saldaña et al. have developed antimicrobial films based on chitosan, starch, and GA using subcritical water technology and evaluated the physicochemical and antimicrobial properties of the films whereas maximum elongation value of 100 percent and a decrease in water vapor permeability has been reported.[25]

It has been shown that nanoparticles of bioactive polysaccharides can be used as potential nanocarriers for the delivery of active ingredients to desired functional tasks.[26,27] Grafting of gallic-acid (GA) onto chitosan is reported to enhance antioxidant activities and favorably alter the rheological properties of the resulting conjugates. Starch nanoparticles (SNPs) score above all other natural alternatives as they can impart crucial physical attributes as inner reinforcement in polymer matrices.[28-30] Owing to the reactive nature of starch, SNP surface can be modified by grafting or cross-linking using potential bioactive compounds (e.g., flavonoids, vitamins, and gallic acid) rendering them more readily dispersible in the polymer matrix for their functional release as antioxidants or scavengers of free radicals.[31-33]

http://doi.org/10.63654/icms.2025.02083 *Innov. Chem. Mater. Sustain.* **2025**, *2*(1), 083-091
Received: 17 April 2025; Revised: 28 May 2025; Accepted: 31 May 2025; Published (online): 07 June 2025

Table 1: *The relative amounts of chitosan, gelatin and GA-SNPs in the resultant films with reaction molar ratio*

S. No.	Samples	Samples Designation	Reaction Ratio	Molar Ratio (Chitosan : Gelatin : GA-SNPs)
1.	Chitosan	Chitosan film	2 g Chitosan	0.124 M
2.	Chitosan: Gelatin	Ch-ge film	2 g Chitosan / 50 mg gelatin	0.124 : $1.67x10^{-6}$ M
3.	Chitosan: Gelatin: GA-SNPs	Ch-ge-GA-SNP-1	2 g Chitosan / 50 mg gelatin / 30 mg GA-SNPs	0.124 : $1.67x10^{-6}$: 0.0176 M
4.	Chitosan: Gelatin: GA-SNPs	Ch-ge-GA-SNP-2	2 g Chitosan / 50 mg gelatin / 50 mg GA-SNPs	0.124 : $1.67x10^{-6}$: 0.0294 M

In the above context to fulfil the gap by using the active agents, GA and SNP both on this study motivated us to investigate the effects of incorporation of multicomponent active agents in the form of gallic acid-loaded starch nanoparticles (GA-SNPs) in chitosan-gelatin (Ch-ge) biopolymer films for food packaging applications so that it becomes easy to understand the film qualities such as opacity, crystallinity, tensile strength, solubility, and water vapor permeability. SEM was used to examine the films microstructure. The antimicrobial and antioxidant properties of the synthesized composite films, hereafter named as Ch-ge-GA-SNP-1 and Ch-ge-GA-SNP-2, have also been evaluated.

2. Experimental Section

2.1 Materials

Gallic acid was purchased from CDH, India. Nutrient agar and nutrient broth were obtained from Himedia, Mumbai, India. Chitosan of higher molecular weight (>350 kDa and 79% deacetylated) was taken from CIFT, Cochin (Central Institute of Fisheries Technology). Glacial acetic acid and ethanol were obtained from Merck, India. Tween-80, starch and gelatin were purchased from CDH, India. The test strains *Escherichia coli* (gram -ve) and *Bacillus subtilis* (gram +ve) were purchased from IMTECH, Chandigarh, India. Milli-Q was used as solvent obtained from our laboratory during the research.

2.2 Methods

Preparation of chitosan-gelatin (Ch-ge) based films containing (GA-SNPs) gallic-acid loaded Starch nanoparticles

The modified chitosan-gelatin (Ch-ge) based films containing gallic-acid loaded starch nanoparticles (GA-SNPs) were prepared by solution casting method.[34] Table 1 presents the relative amounts of the chitosan, gelatin, and GA-SNPs in the resultant films with designation. The detailed preparation methods are shown in supplementary.

2.3 Evaluation of chitosan-gelatin-based films in aspects of physicochemical and biological properties

2.3.1 FTIR

FTIR was used to analysis of the structural interactions of chitosan-based films. To obtain the dehydrated chitosan film samples, placed in a desiccator containing silica gel at room temperature for 2 weeks. FTIR spectra were measured in the range of 400-4000 cm−1 at a resolution of 8 cm−1 using KBr pellets and a Nicolet 170 SXFT-IR spectrophotometer.

2.3.2 XRD

The XRD spectra of the films were analyzed in the range of 5°–80° and 4° min^{-1} on Rigaku Smart lab diffractometer. The film specimens were cut into rectangles and mounted on a slide of glass. Cu-Ka radiation with a nickel monochromator filtering wave at 40 kV and 30 mA has been used to record the spectra.

2.3.3 Scanning Electron Microscope (SEM) Technique

SEM technique used to examine the morphology of the synthesized films on instrument (Carl Zeiss EVO 50, Germany) in Materials Science and Technology Department, IIT Kanpur, Kanpur. Double-sided tape was used to stick the film samples to cylindrical aluminum stubs. The surface morphology of the stub supporting film was investigated with a thin layer of gold in an ion sputter coater and placed into a scanning electron microscope. The other characterization techniques as well as biological evaluation like light transmittance, solubility in water and swelling degree of the films, water vapor permeability (WVP) and mechanical properties, antibacterial activity and antioxidant activity by DPPH and $ABTS^{\cdot+}$ assay methods of chitosan based films are described.

3. Results and Discussion

3.1 FTIR spectra of films

The FTIR spectra of the GA-SNPs (gallic-acid loaded starch nanoparticles), chitosan, Ch-ge, Ch-ge-GA-SNP-1 and Ch-ge-GA-SNP-2 films shown in Fig. 1. From the FTIR spectra the major bands of GA-SNPs at 3296 cm^{-1} of O-H stretching and 2814 cm^{-1} is attributed to the N-H stretching.[35] And the bands at 1634 cm^{-1} is ascribed to C=O stretch of conjugated acid, 1341 cm^{-1} (O-H of the phenol alcohol) and 1006 cm^{-1} (C-O-H bending). The absorption bands around 1400-1650 cm^{-1} relates to the C=C stretching of gallic-acid.[36]

The characteristics peaks of pure chitosan film in the range of 3200-3300 cm^{-1} were ascribed to the O-H stretching related to the intermolecular H-bonding and the peak at 2900 cm^{-1} was attributed to the symmetric and antisymmetric $–CH_2$ stretching. The peaks at 1536 cm^{-1} and 1403 cm^{-1} were attributed to the N-H (amide II), and H-NC=O (amide III) stretching vibrations respectively and also the peaks around 1000-1100 cm^{-1} ascribed to the C-O-C stretching and C-O stretching which is easily view in the Fig. 1 (b).[37,38] In the Fig. 1, the band at 1544 cm^{-1} (amide-II) related to the combination band of the N-H bending vibrations and C–N stretching and band around 2905 cm^{-1} ascribed to the C-H stretching which

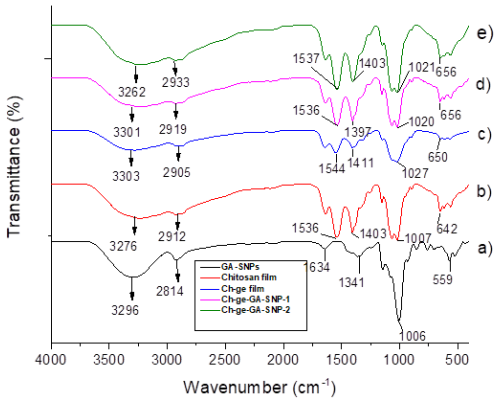


Figure 1: *FTIR spectra for (a) GA-SNPs (b) Chitosan film (c) Ch-ge film (d) Ch-ge-GA-SNP-1 (e) Ch-ge-GA-SNP-2*

Table 2: *Solubility in water, Absorbance and Opacity values of the chitosan based films*

Samples	Absorbance (A_{600})	Opacity (O) = A_{600} /X (X= film thickness in mm)	Solubility in water (%)
Chitosan film	0.059	0.4916 mm^{-1}	31.420 ± 0.437[a]
Ch-ge film	0.070	0.5998 mm^{-1}	30.480 ± 0.266[b]
Ch-ge-GA-SNP-1	0.089	0.7989 mm^{-1}	29.403 ± 0.140[c]
Ch-ge-GA-SNP-2	0.083	0.7255 mm^{-1}	27.320 ± 0.121[d]

a,b,c,d different letters in the same column indicate significant differences among formulations ($p < 0.05$).

suggested the presence of alkane. The peak around 3000 cm^{-1} was associated to the hydrogen-bonding of OH/NH stretching.[32,39] From FTIR spectra the results obtained of Ch-ge film and earlier reported papers are in good agreement.[40,41]

In the Fig. 1 (d) and (e) the addition of GA-SNPs into Ch-ge film, increased the peak intensity of Ch-ge-GA-SNP-1 and Ch-ge-GA-SNP-2 films which shown in figure. The FTIR spectra display a broad peak at around 3100-3300 cm^{-1} which could be ascribed to -NH_2 and -OH stretching. The peaks at 2900-2950 cm^{-1} represented C-H stretching, whereas the band at 1631 cm^{-1} could be attributed to C=O stretching. The peak at 1500-1550 cm^{-1} could be ascribed to NH_2 (Amide-II) and at 1400 cm^{-1} signifies C-N stretching and our results of FTIR spectra are in good agreement with the report of.[17] The interactions of gallic acid-starch nanoparticles (GA-SNPs) and gallic acid-starch nanoparticles (GA-SNPs) through Ch-Ge electrostatic interaction is shown in Supplementary which gives the clarity to the role of GA-SNPs in enhancing the film's properties.

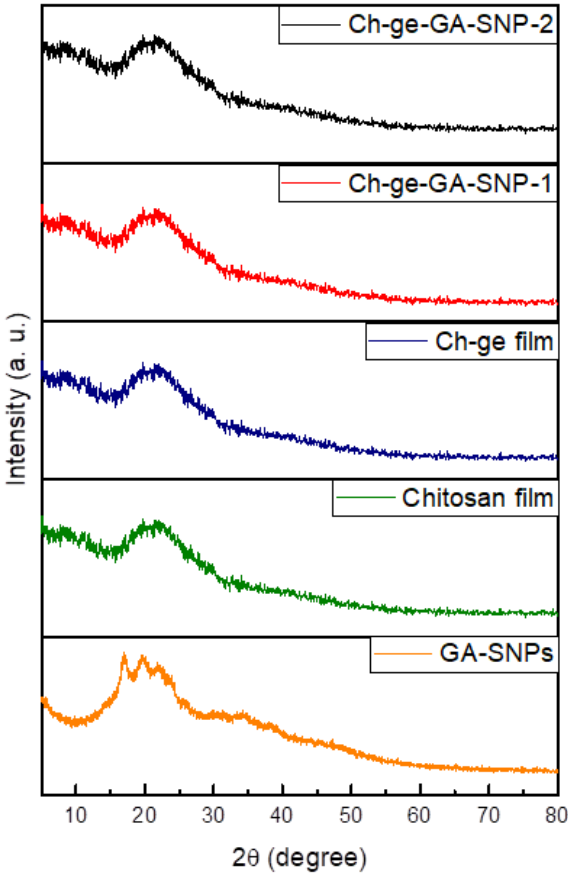


Figure 3: *XRD spectra for (a) GA-SNPs (b) Chitosan film (c) Ch-ge film (d) Ch-ge-GA-SNP-1 (e) Ch-ge-GA-SNP-2*

3.2 XRD

GA-SNPs peaks showed diffraction peaks at (2θ = 15.43°, 16.45°, 25.33°, 27.79° and 32.30°) which is appeared in its X-ray diffraction peaks and also indicates crystalline nature (in the Fig. 2). The crystallinity is the main important factor of the films to design specific application in the field of packaging industries. According to the previous study the XRD spectra of the pure chitosan films showing semi-crystalline state at 2θ =11.9° and 22.95°.[41] According to the literature Ahmed et al., [42], the chitosan and gelatin both biopolymers having lower compatibility i.e. in the blend films, each biopolymer having its own crystal region. But in diffraction pattern of the Ch-ge film, the spectrum of chitosan approximately disappears and become broader, exhibiting that the protein (gelatin) present in Ch-ge decreases the crystallinity of chitosan film. The Ch-ge film ascribed diffraction pattern at (2θ = 7.58° and 20.81°) and the results discussed in a line according to the literature.[40,43] In the XRD pattern of the Ch-ge films containing GA-SNPs the presence of GA-SNPs enhances the crystallinity of Ch-ge film and diffraction pattern at (2θ = 8.88°, 11.7° and 22.56°) which is shown in figure. The results of XRD spectra indicate the semi-crystalline state of Ch-ge-GA-SNP-1 and of Ch-ge-GA-SNP-2 film. These results of films suggested that the compatibility and interaction between different components in the films are good.

3.3 SEM

SEM images of chitosan, Ch-ge, Ch-ge-GA-SNP-1 and Ch-ge-GA-SNP-2 films shows in the Fig 3. The surface morphology of the only chitosan film shows a rough surface and compact texture, which is also explain in reported paper[44] and Ch-ge film shows a smooth and homogeneous surface in comparison of only chitosan film and when the incorporation of GA-SNPs to the Ch-ge film the roughness of films decreases and exhibits a smooth surface texture which is shown in figure. From the SEM analysis it is revealed that the surface of all biopolymer films apparent no presence of pores that might be effect on the WVP and tensile strength values. In general, we can say that the incorporation of GA-SNPs the morphology of the films improved.

3.4 Light transmission and transparency:

The transparency and light transmission of the newly synthesized films are shown in Table 2. Transparent film samples suggest that the films are homogeneous, i.e. there is no phase variation between the various components found in films. Because of light reflection or dispersion at the two-phase interface, if the compatibility between various constituents of the films is poor, the light transmission of the films is lower or the opacity of the films is higher, as reported.[40]

Since prepared chitosan-based films have strong UV barrier properties, chitosan/gelatin-based films containing GA-SNPs effectively blocked UV light, as according to these studies. This is due to the presence of protein (gelatin), which contains a high concentration of aromatic amino acids that absorb UV light and lead to improved UV barrier properties.[45]

3.5 Solubility in water and Swelling degree of the films

The Swelling Property of the films shows the material's hydrophilicity capability in the presence of appropriate water, showing that it is a water-retaining indicator. The swelling degree of the chitosan films largely depends upon the hydrophilic group presence in chitosan and biomacromolecules, in addition to the chemical interaction among the molecules.[46] Pure chitosan films without adding GA-SNPs it was found that highly hydrophilic in nature and more swelled to their original weight, similar results have been reported by Jafari.[47] However, the addition of gelatin and GA-SNPs into the chitosan films was found to extensively increment the film's hydrophobicity character with a significant decrease in the swelling property of the film, and also when increased the concentration of GA-SNPs the swelling property decreased, which is shown in Fig. 5 (A). This may be caused by the interaction between GA-SNPs, gelatin, and chitosan, as

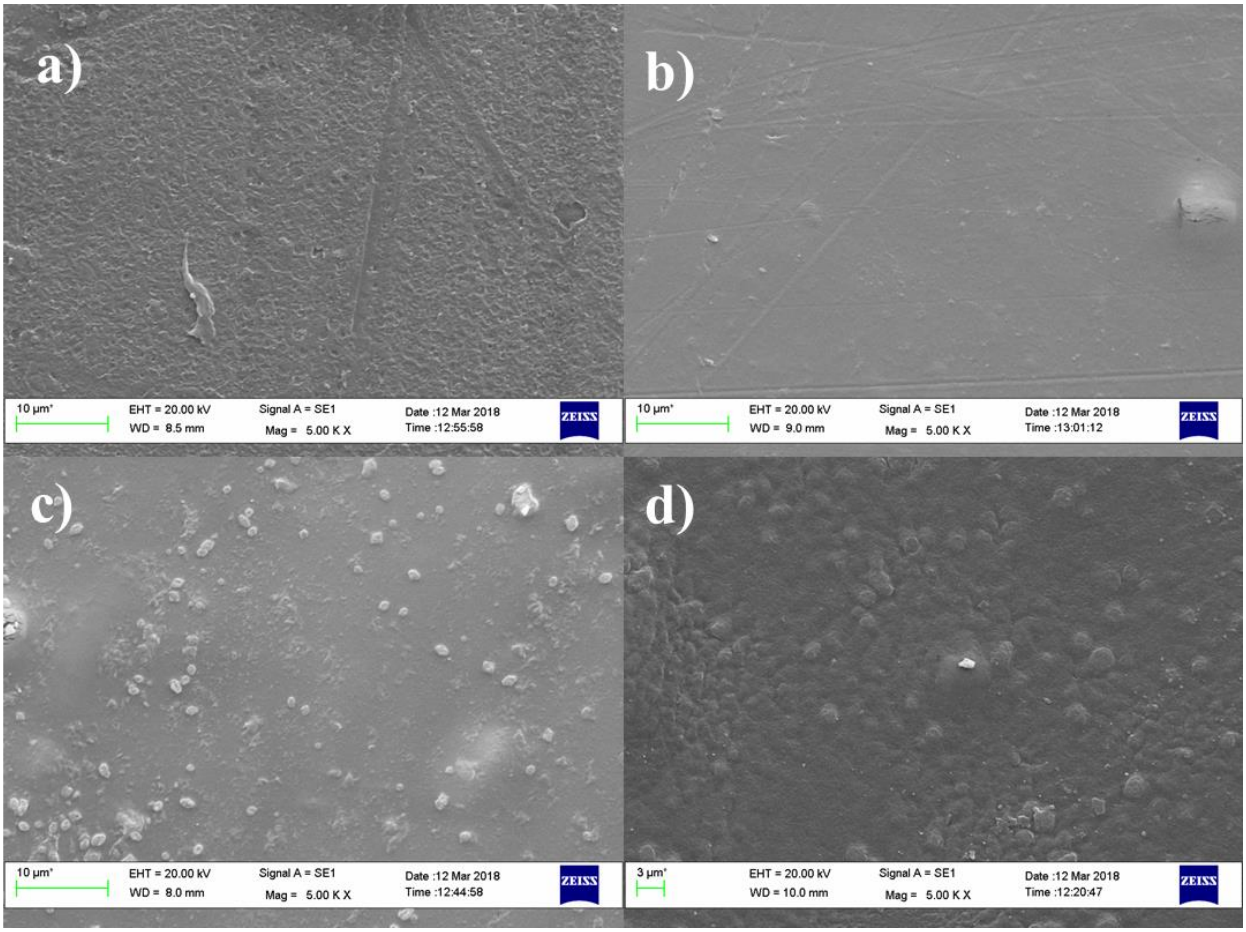


Figure 3: *SEM images for (a) Chitosan film (b) Ch-ge film (c) Ch-ge-GA-SNP-1 (d) Ch-ge-GA-SNP-2*

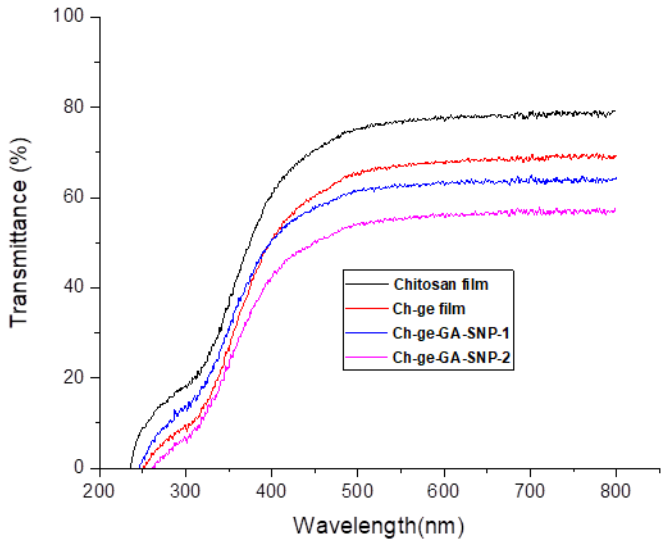


Figure 4: *Transmittance for (a) Chitosan film (b) Ch-ge film (c) Ch-ge-GA-SNP-1 (d) Ch-ge-GA-SNP-2*

well as the strong intermolecular hydrogen bonding interaction, which limited the amount of groups available in the network capable of forming hydrogen bonds with water molecules, decreasing the swelling property.[48] Furthermore, water solubility was chosen as an indicator for evaluating water resistance and film stability because it reflected resistance to external moisture. At room temperature, the solubility of prepared films in water was examined as a percentage, which was showing in Table 2 and less solubility in water of chitosan films was found with a value of 31.420 ± 0.437 % approx, similar result was also described by Jara and team.[49] However, when gelatin and GA-SNPs were added to the chitosan films, the solubility in water was decreased by 29.403 ± 0.140 % and 27.320 ± 0.121 % of Ch-ge-GA-SNP-1 and Ch-ge-GA-SNP-2 respectively, mainly because gelatin and GA-SNPs also effectively responded to the chitosan matrix, making it very difficult for water molecules to penetrate the matrix and reducing the number of hydrophilic groups. Due to this reason, when increased the concentration of GA-SNPs into Ch-ge film, more hydrophilic groups were reduced and decreased the water solubility of the films. This explanation is supported by the reported paper on olive oil by Akyuz and co-worker.[50]

In conclusion, more hydrophobic molecules in GA-SNPs were found to inhibit with a water attack the destruction of hydrogen bonding between molecules of films, which was useful for waterproofing and producing integrity, and these findings were described in previously published reports.[51,52]

3.6 Mechanical Properties

The mechanical properties of the films indicate the lastingness and also their capability to sustain food integrity during handling, sailing and storage.[53] The mechanical properties of chitosan, Ch-ge and Ch-ge containing GA-SNPs films were measured by Tinius Olsen Testing Machine and summarized in Table 3. In this study, Tensile strength (TS) is defined as the form of maximum stress which is imposed against a film that it can be subjected to before break, while elongation at break (EAB) is a measure of capacity of a film to stress. Wu et al. reported the enhancement in tensile strength of the films were ascribed to compliant physical cross-linking due to the hydrogen bonding between the chitosan chain and gallic-acid which influence the film structure.[54] The concentration of polyphenols and type of chitosan had positive effects on the results of tensile strength; explicitly, when the concentration of polyphenols increased the resulted in tensile strength values due to this reason the value of the tensile strength of Ch-ge-GA-SNP-2 is more than Ch-ge-GA-SNP-1. The lowest value swelling index of Ch-ge-GA-SNP-2 can be ascribed to the interaction between polyphenols and biopolymers that the inner gaps, decreased the water sorption, this type of interaction can be responsible for the significant enhancement in tensile strength of the films which is reported in the paper and also indicated that different type of cross-linking amid the cassava starch and chitosan, improvement in the tensile strength but decreased EAB, and also a contribution to the decrease of the film solubility in water and WVP.[25]

Table 3: *Mechanical Properties and WVP of the chitosan-based films*

Samples	Thickness	Tensile Strength	Elongation at break (EAB) (%)	WVP X 10^{-10} (g m^{-1} s^{-1} Pa^{-1})
Chitosan film	0.121 ± 0.002[a]	9.187 ± 0.127[a]	28.600 ± 0.304[a]	7.290 ± 0.171[a]
Ch-ge film	0.113 ± 0.003[b]	12.460 ± 0.105[b]	21.397 ± 0.468[b]	3.164 ± 0.125[b]
Ch-ge-GA-SNP-1	0.122 ± 0.002[a]	18.343 ± 0.262[c]	19.437 ± 0.273[c]	2.085 ± 0.109[c]
Ch-ge-GA-SNP-2	0.115 ± 0.003[b]	23.737 ± 0.231[d]	22.453 ± 0.236[d]	1.540 ± 0.024[d]

a, b, c, d different letters in the same column indicate significant differences among formulations ($p < 0.05$).

3.7 WVP

The WVP is an essential factor for food packaging films because its primary purpose is to prevent moisture of transferring from the prepared film to the external environment. In compared with the only chitosan film, the resultant films had lower WVP values. The Table 3 shows the WVP of the resulting films. The WVP values of chitosan films containing GA-SNPs are lower in comparison to chitosan films. As shown in Table 3, the WVP values decrease after the addition of GA-SNPs, and also higher WVP value of Ch-ge-GA-SNP-1 in comparison to the Ch-ge-GA-SNP-2 ranged between 2.085 ± 0.109 X 10^{-10} g m^{-1} s^{-1} Pa^{-1} and 1.540 ± 0.024 X 10^{-10} g m^{-1} s^{-1} Pa^{-1}. Similar results of WVP were reported by working on wheat starch-chitosan edible films[55] and chitosan-tapioca starch.[56] As usual, the chitosan and Ch-ge films both showed the higher the WVP value and the addition of GA-SNPs into the Ch-ge films significantly WVP value reduced. The lowest WVP value for the Ch-ge-GA-SNP-2 film was obtained. However, introducing polyphenols to the synthesized films improved their barrier properties. As previously stated that the presence of polyphenols in biopolymer edible films can form hydrogen bonds and hydrophobic interactions with the polar groups in the biopolymers, restricting the number of free OH groups that can interact with water.[57] The presence of higher concentration values of GA-SNPs into the structural matrix of the Ch-ge film

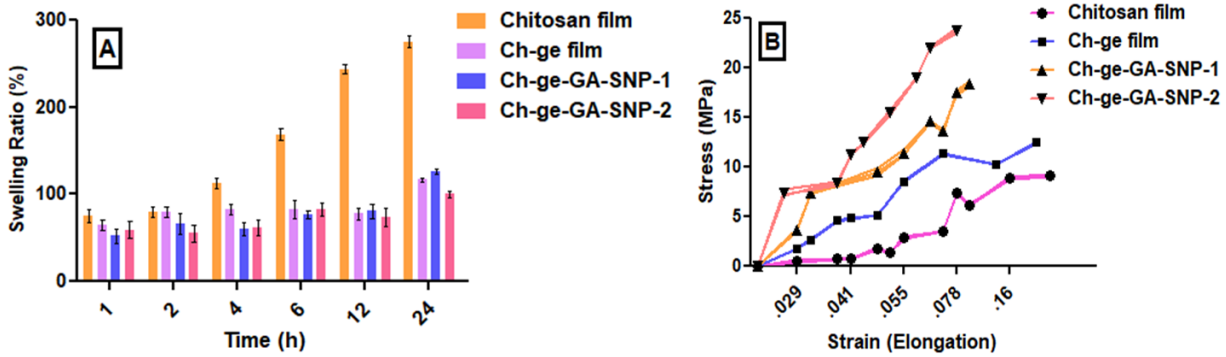


Figure 5: *(A) Swelling property for chitosan based films (B) Stress-strain curve of chitosan based films.*

Table 4: *Table for Inhibition zone of chitosan, Ch-ge Ch-ge-GA-SNP-1 and Ch-ge-GA-SNP-2 films*

Test Cultures	Chitosan film (mm)	Ch-ge film (mm)	Ch-ge-GA-SNP-1 (mm)	Ch-ge-GA-SNP-2 (mm)
Gram Positive *(B. subtilis)*	20±2	22±2	25±2	29±2
Gram Negative *(E. coli)*	22±2	26±2	27±2	32±2

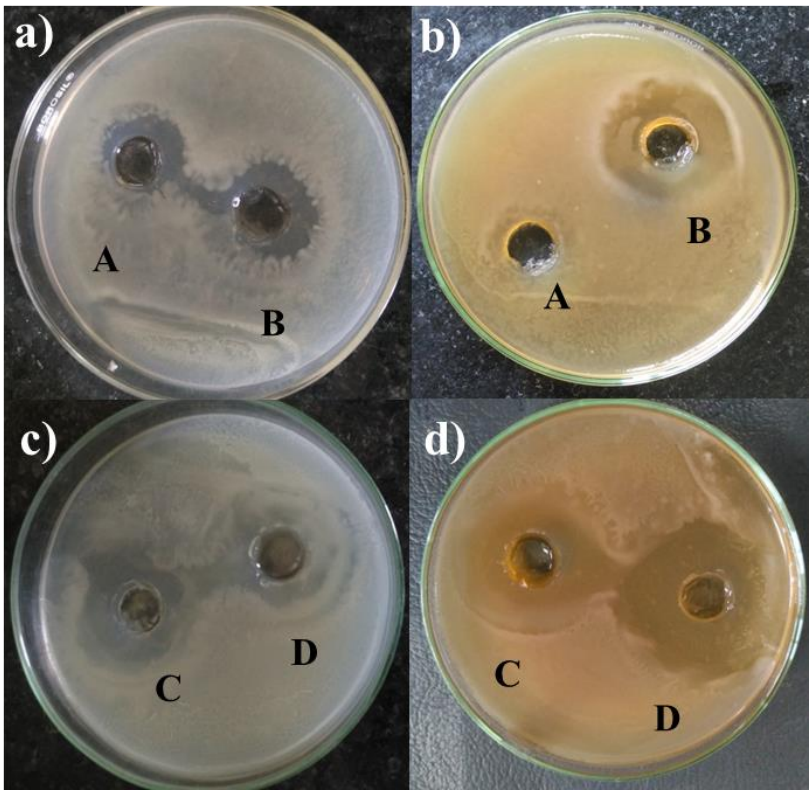


Figure 6: *Inhibition zone of (A) Chitosan film (B) Ch-ge film (C) Ch-ge-GA-SNP-1 (D) Ch-ge-GA-SNP-2 films against (a, c) B. subtilis and (b, d) E. coli*

stimulates internally rearrangement and restricts water permeability; this may be because the form of chitosan and concentration of polyphenols had a reliable impact on the WVP value. Similar results have also been reported by Liu and co-worker.[58] The films with higher concentration values of GA-SNPs have strong mechanical strength and lower water vapor permeability, according to the different studies of the crosslinked films.

3.8 Antibacterial activity

The result of the antibacterial effect of chitosan-based films was evaluated against bacterial strain *B. subtilis* (Gram-positive bacteria) and *E. coli.* (Gram-negative bacteria) using agar well diffusion method and the concentration of films were 20 mg/mL in 1% acetic acid. A significant increment in the zone of inhibition (ZOI) of the synthesized Ch-ge-GA-SNP-1 and Ch-ge-GA-SNP-2 films as compared to that of chitosan and Ch-ge films due to the presence of GA-SNPs. As reported papers related gallic-acid and SNPs to have significant antibacterial properties.[20] In Table 4, the ZOI (mm) of chitosan-based films against bacterial strain are presented (Fig. 6).

3.9 The result of antioxidant property of chitosan, Ch-ge, Ch-ge-GA-SNPs-1 and Ch-ge-GA-SNPs-2 films

Two methods for determining antioxidant activity were used in this study: the DPPH assay and the ABTS assay.

(A). DPPH assay:

The DPPH (2,2-diphenyl-1-picrylhydrazyl) assay was used to analyze the scavenging activities of Ch-ge films and Ch-ge films containing GA-SNPs in our study. The absorbance value at wavelength 517 nm was used to evaluate the reducing potential of all samples. The resultant absorbance value decreases as the sample concentration increases. The % scavenging activity of DPPH radical as seen in Fig. 8(A). At a concentration of 1 mg/mL, the DPPH radical scavenging activity of Ch-ge-GA-SNP-1 and Ch-ge-GA-SNP-2 films are 73.34% and 80.9% respectively.[14] In comparison to chitosan and Ch-ge films, the antioxidant ability of Ch-ge-GA-SNP-1 and Ch-ge-GA-SNP-2 films increased after GA-SNPs were added to the film.

(B) $ABTS^{\cdot+}$ assay

A blue-green compound of ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) is used to evaluate antioxidant activity in the food industry. The stabilization of unstable free radicals in the ABTS assay was found to be slightly faster than in the DPPH assay in this report. At a concentration of 1 mg/mL the result of radical scavenging activity of Ch-ge-GA-SNP-1 and Ch-ge-GA-SNP-2 films was estimated to be 79.3% and 84.9% respectively, as shown in Fig. 8(B). The antioxidant activities of Ch-ge-GA-SNP-1 and Ch-ge-GA-SNP-2 films improved after GA-SNPs were added to the film, compared to chitosan and Ch-ge films, indicating that the antioxidant ability of Ch-ge-GA-SNP-2 film was improved. The antioxidant ability of the ABTS assay was

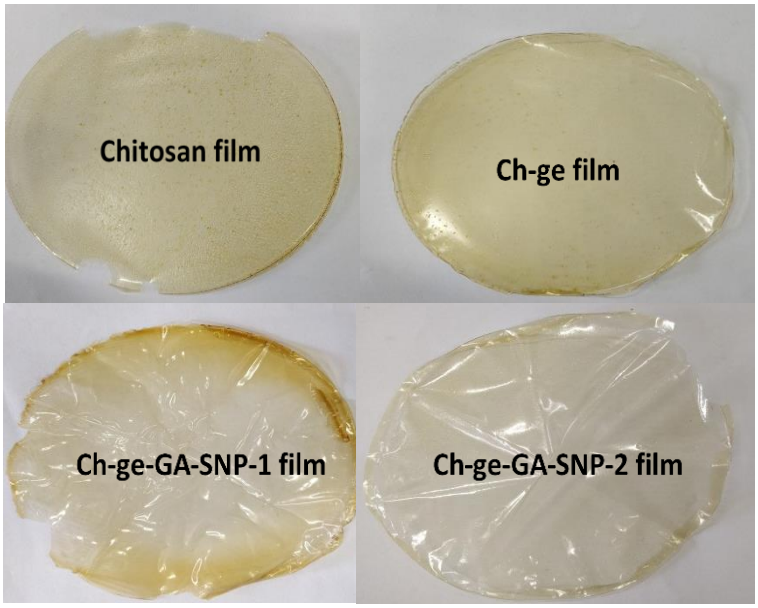


Figure 7: *Photographs of synthesized chitosan films*

shown to be faster than that of the DPPH assay, which can be seen in Table 5.

4. Conclusion

In the present study, gallic-acid grafted starch nanoparticles (GA-SNPs) have been synthesized by a modified facile method. Their effect on the physicochemical and biological properties of chitosan-gelatin (Ch-ge) bioactive film has been studied in the GA-SNPs incorporated nano biocomposite Ch-ge-GA-SNPs films produced by simple solution casting technique. The newly fabricated films have been characterized by UV-vis, XRD, FTIR, and morphology is evaluated by SEM techniques. The results indicate that non-covalent interactions and hydrogen bonding between chitosan-gelatin and GA-SNPs which intensifies with increasing starch nanoparticles' concentration might have contributed in decreased WVP and swelling properties of the films. The Ch-ge-GA-SNP-2 films have also shown considerable enhancement in other properties such as water solubility, transparency, tensile strength, and resistance to UV absorption as well as antioxidant and antibacterial properties. Although the results are promising, several challenges remain to be addressed in the future. These include determining the optimal amount of nanoparticles to maintain both the strength and bioactivity of the film, understanding the durability and biodegradability of the films under different food storage conditions, and ensuring their compatibility with various types of food. Additionally, large-scale production and obtaining regulatory approval are essential steps for their use in food packaging.

Overall, the incorporation of multicomponent active agents into chitosan biopolymer matrices presents a promising and sustainable alternative to conventional plastic packaging, with significant potential to improve food safety, extend shelf life, and promote environmental sustainability

Author Contribution Declaration

The authors have no conflicts of interest regarding this investigation. Sristi conceptualized the idea of the study, data

Table 5: *Antioxidant activity of chitosan, Ch-ge, Ch-ge-GA-SNP-1 and Ch-ge-GA-SNP-2 films*

Test Cultures	Chitosan film	Ch-ge film	Ch-ge-GA-SNP-1	Ch-ge-GA-SNP-2
DPPH* scavenging activity (%) (A)	40.6	47.7	74.0	80.9
ABTS*+ scavenging activity (%) (B)	43.9	51.7	79.3	84.9

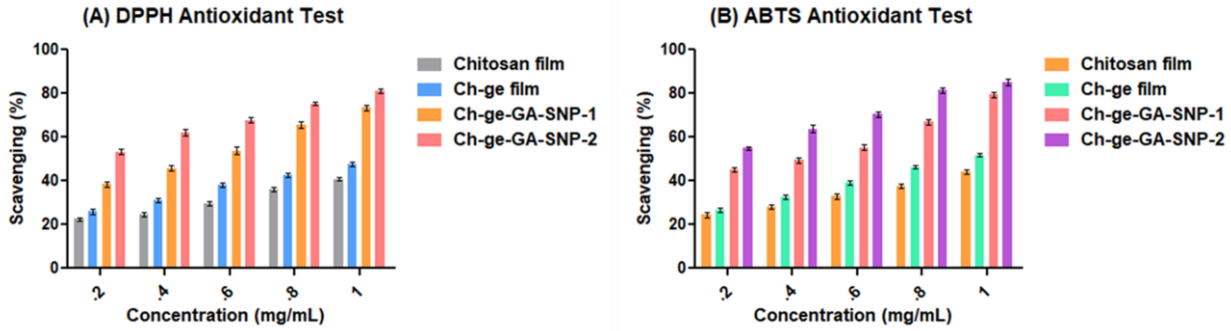


Figure 8: *(A) DPPH radical scavenging activity of chitosan based films (B) ABTS radical scavenging activity of chitosan based films*

analysis, manuscript writing and reviewing. Mehrotra confirmed the manuscript writing and reviewing. Dutta involved in data validation and manuscript reviewing and acquired funding.

Attention: The authors have no financial conflicts of interest to disclose.

Funding sources

No funding source.

Data Availability Declaration

The newly generated and analysed data are available within the article and its supporting information.

Supporting Information

The details preparation of chitosan-gelatin (Ch-ge) based films containing (GA-SNPs) gallic-acid loaded starch nanoparticles are given in the supporting information.

Acknowledgements

The authors are thankful to Central Instrumentation Facility, Indian Institute of Technology Kanpur for SEM analysis and Material Research Centre, MNIT Jaipur for recording of FTIR spectra. One of us (SY) is grateful to Director, MNNIT Allahabad, for providing a senior research fellowship.

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