

## BIOMEDICAL APPLICATIONS OF CHITOSAN-SALICYLALDEHYDE SCHIFF BASE/ POLYPROPYLENE GLYCOL BLEND

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### ABSTRACT

The present research work deals with the synthesis and characterization of chitosan-salicylaldehyde Schiff base/polypropylene glycol blend. Advanced analytical techniques such as FT-IR, XRD, TGA and SEM studies were employed to characterize the prepared samples. The obtained FT-IR results clearly shows the formation of Schiff base (C=N) linkage in chitosan and in addition the appearance of new peaks also suggested that the polypropylene glycol gets effectively binded with the chitosan schiff base. The XRD and TGA studies indicate the amorphous nature and the enhanced thermal behavior of blended Schiff base sample. The scanning electron

microscopic (SEM) studies of the prepared chitosan Schiff base/polypropylene glycol blend showed the microporous structure with rough surface morphology. Evaluation was done to test the antimicrobial potential of the synthesized chitosan salicylaldehyde Schiff base/polypropylene glycol blend against three bacterial species namely *Bacillus subtilis*, *E. coli* and *pseudomonas* and against three fungal species namely *Aspergillus flavus*, *penicillium notatum* and *Rhizopus nigricans* respectively. From the obtained higher zone of inhibition values, it was evident that the chitosan-salicylaldehyde Schiff base/polypropylene glycol blend has the very good antimicrobial behavior and hence this chitosan-salicylaldehyde Schiff base/polypropylene glycol blend was found to be suggested as the promising material for biomedical applications.

**KEYWORDS:** Chitosan, salicylaldehyde, Schiff base, polypropylene glycol, antibacterial, antifungal.

## INTRODUCTION

Chitosan biopolymer is a linear abundant cationic aminopolysaccharide widely distributed in nature which is consisting of 2-deoxy-D-glucosamine and 2-deoxyN-acetyl-D-glucosamine units linked by glycosidic  $\beta$ -(1 $\rightarrow$ 4) bonds obtained by the deacetylation of chitin.<sup>[1][2]</sup> This chitosan biopolymer possesses certain interesting biological activities such as antitumour, antifungal, antiviral, antibacterial activities and as well as it possesses important physiological properties like biocompatibility, biodegradability and nonallergenic qualities.<sup>[3][4]</sup> The presence of different functional groups along chitosan backbone (i.e.; hydroxyl and amine groups) simplifies its chemical modifications and in order to enhance its biological activities, recent researchers reported that the chitosan biopolymer can be chemical modified in various ways such as cyanoethylation, phosphorylation, carboxymethylation and Schiff base condensation.<sup>[5][6][7]</sup>

The Schiff bases are the compounds with an imine group ( $-\text{C}=\text{N}-$ ) usually synthesized from the condensation of primary amines and active carbonyl groups.<sup>[8][9]</sup> Presence of amine groups in the chitosan polymeric chain leads to the possibility of a several chemical modifications like the preparation of Schiff bases ( $-\text{RC}=\text{N}-$ ) by reaction with aldehydes and ketones.<sup>[10][11]</sup> The production of corresponding schiff bases by the reaction of chitosan with aromatic aldehydes in acetic acid has been described by Tirkistani.<sup>[12]</sup>

Arulmurugan and his coworkers reported that because of their wide spectrum of biological activities such as antibacterial, antifungal and antitumor activities, the Schiff bases synthesized by the condensation of primary amines with carbonyl groups were of great importance in medicinal and pharmaceutical fields.<sup>[13]</sup> The Schiff base's azomethine linkage ( $-\text{C}=\text{N}$ ) is an essential structural requirement which is mainly responsible for biological activities. The mechanism behind the antimicrobial activity of chitosan is the penetration of chitosan into the cells and subsequently binding to the DNA and partial inhibition of RNA and protein synthesis.<sup>[14]</sup>

Xiaoxiao Jin and his coworkers synthesized the Citral chitosan Schiff base and its antimicrobial agent has been tested.<sup>[15]</sup> El-Refaie Kenawy and his coworkers synthesized novel aminated chitosan-aromatic aldehyde Schiff bases and its antimicrobial activity has been evaluated.<sup>[16]</sup> Results reveals that the aminated chitosan modified with p-hydroxy benzaldehyde and vanillin showed small inhibit effect against fungi species, however they shows a higher inhibitory effect against a wide variety of Gram-positive bacteria and Gram-

negative bacteria. Salicylaldehyde is a common highly functionalized arene that has often been exploited as a key precursor to a variety of chelating agents.<sup>[17]</sup> Gou and his coworkers synthesized Salicylaldehyde Schiff base as novel, easily available colorimetric and fluorescent double-sensor. Reported results indicate that the sensor exhibits highly selective and sensitive recognition toward  $\text{Cu}^{+2}$  in aqueous solution via a naked eye color change from colorless to yellow and toward  $\text{Al}^{+3}$  via a significant fluorescent enhancement in ethanol over a wide range of tested metal ions.<sup>[18]</sup>

Recently, different approaches such as blending and grafting methods were investigated to develop the chitosan with new desirable characteristics.<sup>[19]</sup> The main aim and the ultimate goal of polymer blending is a practical one to achieve commercially viable polymers with either unique properties or lower cost than some other means might provide.<sup>[20]</sup> Polypropylene glycol (PPG) is the commonly used simplest propylene oxide based polyol polymer utilized for various applications since it finds applications as a hydraulic fluid, rubber lubricant, antifoam agent, etc. Supriya Prasad and her coworkers reported about the use of chitosan schiff base (CSB)/polyethylene glycol (PEG) blend as antimicrobial agents.<sup>[21]</sup> Hence based on the above literature survey, this study was performed to evaluate antimicrobial activity of the chitosan Schiff base/polypropylene glycol (CSB/PPG) blend and also the prepared blend was characterized for its formation and tested its suitability for biomedical applications.

## MATERIALS AND METHODS

### Materials

Salicylaldehyde and polypropylene glycol was procured from Thermo Fisher Scientific India Private Ltd, Mumbai. The chitosan biopolymer utilized in this study was purchased from India sea foods, Cochin, Kerala which is 92% deacetylated. Certain solvents namely the glacial acetic acid and ethanol were purchased from Sisco Research laboratories Private Ltd. Mumbai and SD fine chemicals private Ltd. Mumbai. All the reagents used in the present research work were of analytical grade.

### Preparation of Chitosan schiff bases

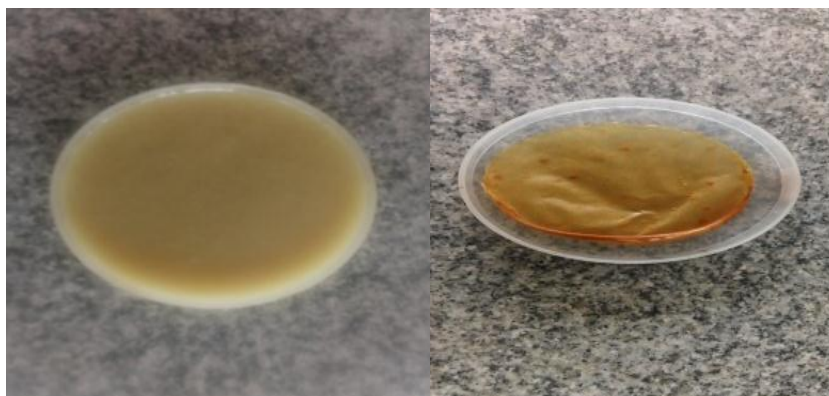
Initially a homogeneous viscous chitosan gel was prepared by dissolving about 1 gram of chitosan in 50 ml of 2% acetic acid through the complete effective stirring process for over a period 30 minutes. A required amount of salicylaldehyde (1 ml) dissolved in 10 ml of ethanol was then added to the above prepared homogeneous viscous chitosan gel. This

solution mixture was then stirred well effectively using a magnetic stirrer for 30 minutes until a white viscous gel of chitosan-salicylaldehyde schiff base is obtained.

#### **Preparation of Chitosan-salicylaldehyde Schiff base / Polypropylene glycol blend**

About 1ml of the above prepared chitosan salicylaldehyde schiff base was then mixed with the prepared polypropylene glycol (PPG) solution (1 ml of PPG in 10 ml ethanol). Followed by this addition, the complete stirring of this solution mixture was carried out for a period of 30 minutes and further in the sequence, this stirred solution mixture were poured into plastic petridish and allowed to dry at room temperature resulting in the formation of CSB/PPG blend. The photographical representation of the binary chitosan schiff bases/poly propylene glycol blend prepared using salicylaldehyde was represented below (Fig.A).

#### **Wet sample dry sample**



**Fig. A: Photograph of chitosan salicylaldehyde schiff base / polypropylene glycol blend Characterization.**

#### **Fourier Transform Infra red spectroscopy (FTIR)**

The FT-IR spectrum of the prepared samples (pure chitosan, chitosan schiff bases, chitosan schiff base/poly propylene glycol blend) were recorded with a Perkin Elmer 200 FT-IR spectrometer in the wave number range from 4000 to 350  $\text{cm}^{-1}$  with a resolution of 2  $\text{cm}^{-1}$  using Potassium bromide pellets.

#### **X ray diffraction (XRD)**

X-ray scattering SHIMADZU diffractometer using Ni filter Cu  $\text{K}\alpha$  radiation source ( $\lambda=0.15\text{nm}$ ), set at scan rate of  $10^\circ\text{C}$  using voltage of 40kV and a current of 30 mill amperes was utilized in this present research work to record the X-ray diffractogram patterns of prepared samples.

**Thermogravimetric Analysis (TGA)**

The change in the weight loss of prepared samples as a function of temperature was measured using TGA Q500 V20.10 build 36 instrument at a heating rate of 20°C per minute under nitrogen atmosphere.

**Scanning electron microscope (SEM)**

In order to understand the surface morphology of the binary blend of polypropylene glycol with chitosan schiff bases prepared using salicylaldehyde, initially the prepared samples were cut into pieces of various sizes, wiped with filter paper and then coated with a thin gold-palladium layer by a sputter water unit (VG – Micro tech, VCK yield). The surface and cross section topography was analysed with a Cambridge stereo scan 440 scanning electron microscope (SEM Leica Cambridge UK) operated at an acceleration voltage of 20kV.

**Antimicrobial studies**

Three bacterial strains namely *Escherichia coli* and *Bacillus Subtilis* and *pseudomonas aureginosa* species and three fungal strains namely *Aspergillus flavus*, *Rhizopus* and *penicillium notatum* species were used in this study to analyze the potentiality of chitosan – salicylaldehyde Schiff base/ polypropylene glycol blend in killing microbial species by utilizing Agar well diffusion method. As per the microbiology standard method manual, the agar well diffusion method used was adapted from the punch plate assay for inhibitory substance.

**Muller Hinton Agar Medium**

The MHA medium was synthesized by dissolving about 38.0 g of the commercially available Muller Hinton Agar (procured from Himedia) in 1000 ml of distilled water and in order to get solidified, about 10g of Agar Agar was added to the above prepared MHA medium. Followed by this process, the dissolved MHA medium was then mixed well and poured onto 100 mm petriplates (25-30ml / plates) while still molten.

**Nutrient Broth for Bacterial Strain**

Dissolve about 13g of commercially available nutrient medium (Himedia) in 1000ml distilled water and it was then boiled well to dissolve the medium completely. As desired, this nutrient medium was dispersed and sterilized by autoclaving at 15 lbs pressure (121°C for 15 minutes).

**SDS nutrient broth for fungal strain**

Dissolve 10g of commercially available peptone type1, bacteriological (HiMedia) and 40g of dextrose (Reachem Lab Chem Pvt,Ltd) in 1000ml distilled water and boiled to dissolve the medium completely.

**Inoculum preparation-preparation of bacterial pathogens**

In order to standardize the culture, the overnight cultures (0.2ml) of each bacterium was dispersed into 20ml of sterile nutrient broth and incubated for about 3-5 hours and was then used for studying the antibacterial assay.

**Inoculum preparation – preparation of fungal pathogens**

For every 15 days, the fungi was isolated from soil and maintained on potato dextrose Agar slants and sub cultured. The inoculums was prepared by adding sterile distilled water to the culture slants and disperses the spores by using sterile loop and inoculated into the medium.

**Plate preparation of Antibacterial assay**

The above prepared Muller Hinton Agar medium was sterilized well to prepare the plates for antibacterial assay. About 20ml of MHA medium was poured in petriplates and it is allowed for solidification process. Using sterile cotton swap, the bacterial lawn culture (pathogen) prepared in the above manner was placed over the MHA medium and followed by this it is labeled. Further in the sequence, with the help of a metallic borer, the wells were made in the media in centers with at least 24mm. After this process is over, a minimum amount of the binary blend sample (chitosan schiff base/polypropylene glycol blend) prepared in the form of solution (2mg/ml water) was then introduced in the respective wells and in addition to this, the other well is supplemented with reference antibacterial drug (ampicilin). For antibacterial studies, after the application of samples and standards is over, the plates were incubated on individual racks which not stacked on top of one another, for 24 hours at 37°C. In order to maintain strict sterile and aseptic condition, the complete procedure of the plate preparation was done in a laminar air flow. Finally the antibacterial activity was evaluated by measuring the diameter of zone of inhibition grown around the samples measured in mm using a ruler and compared with the diameter of zone of inhibition grown around the ampicillin (standard).



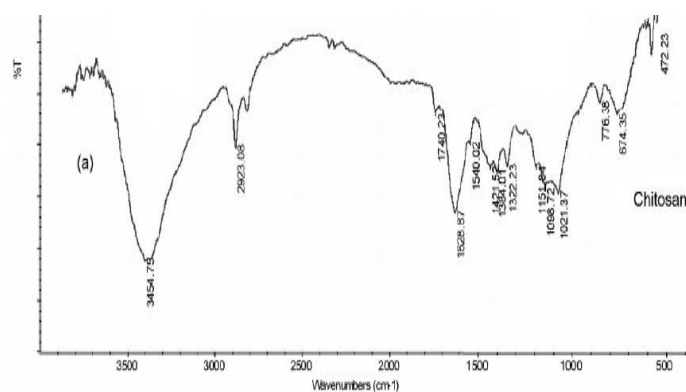
### Plate preparation for Antifungal assay

Similar to that of Muller Hinton Agar medium, the sabouraud dextrose (SDS) agar was prepared, sterilized and the culture plates were prepared to study the antifungal activity of binary blend sample against three fungal cultures, *Aspergillus flavus*, *Rhizopus* and *penicillium notatum* species. Respective fungal spore suspensions were transferred to petriplates and after the solidification process of sabouraud dextrose (SDS) agar medium, with centers at least 24 mm, the wells were cut in the media with the help of a sterile metallic boror. Followed by this construction of wells, the recommended concentration of the above prepared CSB/PPG blend sample (2mg/ml) dissolved in water was then introduced into the wells. These samples included plates were then placed in fume hood for 36 hrs and after this process is over, the plates were incubated for 72 hours. Finally the results were recorded as zones of inhibition which was then compared with polymycin B Sulphate (standard).

## RESULTS AND DISCUSSION

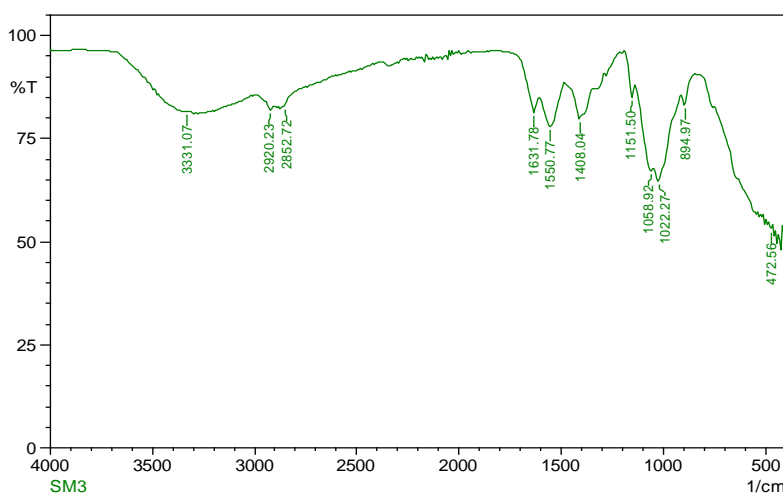
### Fourier transform infrared spectroscopy

Fourier transform infrared (FTIR) spectroscopy is mainly considered as a useful tool for analysing the chemical composition. Due to its high sensitivity, this FT-IR spectrum is helpful for detecting changes in the functional groups.<sup>[22]</sup> FTIR works on the basis of functional groups and provide information in the form of peaks.<sup>[23]</sup> The chemical structure of the blended samples and the possible interactions between their components was identified from the FT-IR studies.<sup>[24]</sup> In relation to absorption occurred and to change in chemical composition, this FT-IR analysis had eventually confirmed the functional groups. Fig.(1)-(3) represents the FTIR spectral details of pure chitosan, chitosan-salicylaldehyde schiff base and chitosan-salicylaldehyde schiff base/propylene glycol blend prepared in 1:1 ratio.



**Fig.1: FT-IR spectrum of pure chitosan.**

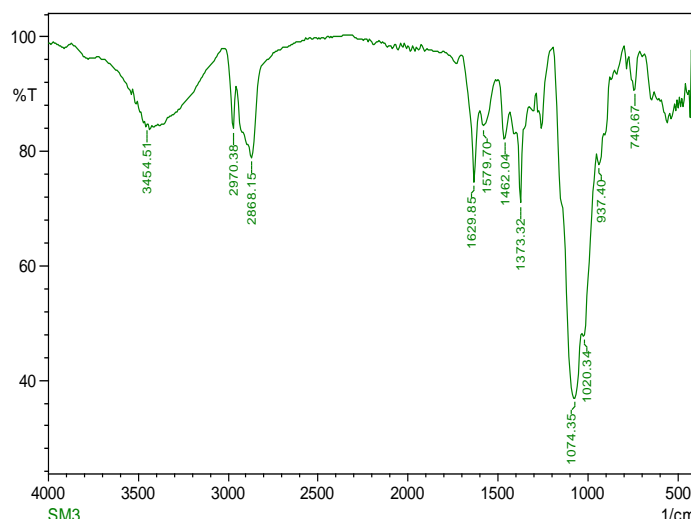
The FT-IR spectral details of pure chitosan presented in Fig.1 shows the prominent peak at  $3454.75\text{ cm}^{-1}$  corresponding to the presence of O-H, N-H symmetrical stretching vibrations. The presence of C=O stretching (amide-I band), N-H bending and C-H deformation was concluded from the appearance of strong peaks at various wavenumbers such as  $1628.87\text{ cm}^{-1}$ ,  $1540.02\text{ cm}^{-1}$  and  $1421.52\text{ cm}^{-1}$ . Certain absorption bands obtained at  $1384.01\text{ cm}^{-1}$ ,  $1322.23\text{ cm}^{-1}$ ,  $1151.84\text{ cm}^{-1}$ ,  $1098.72\text{ cm}^{-1}$  and  $1021.37\text{ cm}^{-1}$  was indicative of the presence of OH in plane bending in alcohols,  $\text{CH}_2$  bending (twisting and wagging), secondary alcoholic C-O stretching, stretching of C-O-C linkage, C-C stretching and skeletal vibrations involving the C-O stretching respectively. In addition certain small peaks appeared at around  $674.35\text{ cm}^{-1}$  and  $472.23\text{ cm}^{-1}$  was assigned to the NH wagging and C-C bending vibrations.



**Fig. 2: FT-IR spectrum of chitosan-salicylaldehyde schiff base**

The FT-IR spectral details of chitosan-salicylaldehyde schiff base was represented in Fig.2. The broad peak which was obtained at  $3331.07\text{ cm}^{-1}$  was attributed the presence of OH hydroxyl and NH stretching vibration.<sup>[25]</sup> The peaks which were observed at  $2920.23\text{ cm}^{-1}$  and  $2852.72\text{ cm}^{-1}$  proves the presence of asymmetrical C-H stretching in  $\text{CH}_2$  group and aldehydic C-H stretching vibration. A strong absorption band which was obtained at  $1631.78\text{ cm}^{-1}$  concludes the presence of C=N stretching (Schiff base) formed due to chitosan-salicylaldehyde interactions. Strong peak appeared at  $1550.77\text{ cm}^{-1}$  indicate the presence of aromatic C=C stretching and a peak at  $1408.04\text{ cm}^{-1}$  corresponds to N-H bending. In addition, the presence of functional groups such as C-O stretching in alcohol<sup>[26]</sup>, C-O-C linkage, C-C stretching, C-H out of plane deformation and C-C bending in chitosan- salicylaldehyde schiff base was confirmed by the appearance of peaks at  $1151.50\text{ cm}^{-1}$ ,  $1058.92\text{ cm}^{-1}$ ,  $1022.27\text{ cm}^{-1}$ ,  $894.97\text{ cm}^{-1}$  and  $472.56\text{ cm}^{-1}$  respectively.





**Fig. 3: FT-IR spectrum of chitosan-salicylaldehyde schiff base/ polypropylene glycol blend.**

The FT-IR spectral details of the chitosan-salicylaldehyde schiff base / polypropylene glycol blend (Fig.3) prepared in 1:1 ratio showed a broad band in the range of  $3454.51\text{ cm}^{-1}$ , assignable to intra-molecular hydrogen bonded  $\text{-OH}$  groups. Further, the spectrum of chitosan-salicylaldehyde / polypropylene glycol blend shows the medium intensity band at  $2970.38\text{ cm}^{-1}$ ,  $2868.15\text{ cm}^{-1}$  which can be assigned to C-H stretching in methylenic group, aldehydic group and the strong band observed in the range of  $1629.85\text{ cm}^{-1}$  region was attributed to  $\text{-C=N-}$  stretching vibration. A peak at  $1579.70\text{ cm}^{-1}$  corresponds to C-H deformation and a peak at  $1462.04\text{ cm}^{-1}$  corresponds to C=C stretching in aromatic ring. Certain peaks appeared at  $1373.32\text{ cm}^{-1}$ ,  $1020.34\text{ cm}^{-1}$ ,  $937.40\text{ cm}^{-1}$  and  $740.87\text{ cm}^{-1}$  was assigned to OH bending in alcohol, C-O-C linkage, C-O stretching in alcohol, C-H out of plane bending and C-H out of plane bending in aromatic compound respectively.

The comparison of FT-IR spectral details of chitosan-salicylaldehyde schiff base and chitosan-salicylaldehyde schiff base /polypropylene glycol blend with pure chitosan, reveals that a new strong peak which was appeared at around  $1600\text{ cm}^{-1}$  corresponding to the presence of C=N stretching in case of chitosan salicylaldehyde schiff base and this obtained result concludes that the chitosan gets interacted effectively with the salicylaldehyde leading to the formation of schiff bases. Also in addition, from the observed shift in the peak positions due to OH group and the reduction in the intensity of peaks it was suggested that the polypropylene glycol gets blended effectively with the chitosan-salicylaldehyde schiff base.

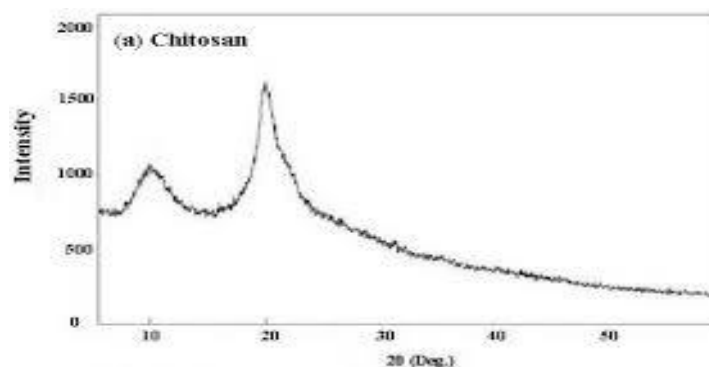
### X-Ray diffraction

X-ray diffraction technique is used as one of the most potential characterization tools and a non destructive technique which is mainly used for measuring phase identification, quantitative analysis and to determine structure imperfections of samples from various disciplines such as geology, polymeric, environmental, pharmaceutical, and forensic sciences.<sup>[27]</sup> This XRD technique was utilized to characterize the organic, inorganic crystallite materials and polymeric materials. When two semi crystalline polymers were mixed, the ratio of the crystalline part to the amorphous part gives rise to the degree of crystallinity. The degree of crystallinity can be expressed as follows:

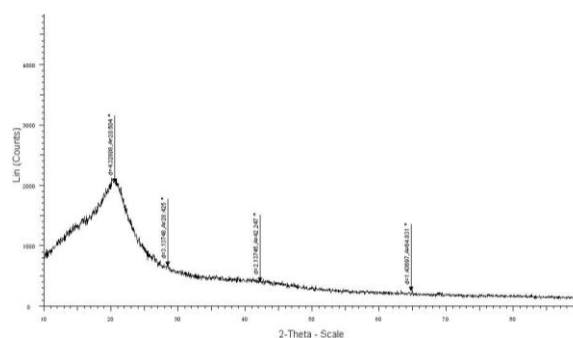
$$X_c(\%) = \frac{A_c}{A_c + A_a} \times 100$$

where  $X_c$ =Degree of crystallinity;  $A_c$ =Crystalline area on the X-ray diffraction and  $A_a$ =Amorphous area on the X-ray diffraction.

The X-ray diffractogram details of pure chitosan and the binary blend of propylene glycol with chitosan-salicylaldehyde schiff base was presented in Table-1 and Fig. (4)-(5).



**Fig. 4: X-ray diffractogram of pure chitosan.**



**Fig. 5: X-ray diffractogram of chitosan – salicylaldehyde schiff base / Polypropylene glycol blend**

**Table (1): XRD details of pure chitosan and chitosan schiff bases/polypropylene glycol blend.**

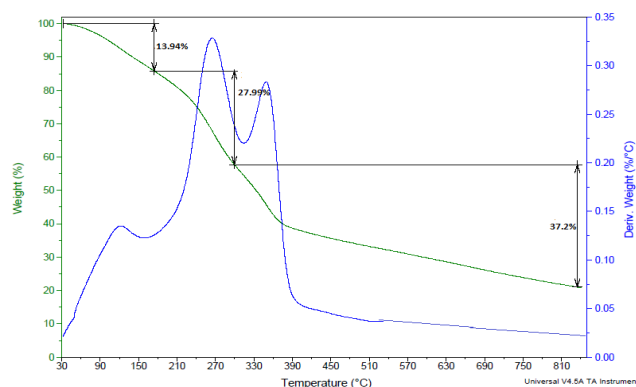
Samples	$2\theta$	Degree of crystallinity (%)
Pure Chitosan	$11^\circ, 20^\circ$	12.7
Chitosan-salicylaldehyde schiff base / Polypropylene glycol blend	$20^\circ$	3.03

From the X-ray diffractograms of pure chitosan and chitosan-salicylaldehyde schiff base/PPG blend, it was observed that when compared to pure chitosan, the broad characteristic peak were observed at  $2\theta=20^\circ$  in case of prepared CSB/PPG blend and from the obtained broad nature of the peak, it was evident that the binary blend of polypropylene glycol with the schiff base prepared using salicylaldehyde has highly amorphous nature when compared to the pure chitosan. In addition to this, from the observed lower percentage degree of crystallinity values also, it was suggested that the chitosan-salicylaldehyde Schiff base/polypropylene glycol blend has more amorphous nature.

The reason behind the enhanced amorphous behavior in case of binary blend might be due to the deformation of the strong hydrogen bonds in the chitosan backbone by the substitution of aldehyde groups on the NH groups of pure chitosan biopolymeric molecule. Also from the intensity change and peak shift from the pure chitosan in the case of XRD of chitosan-salicylaldehyde schiff base/polypropylene glycol blend it was identified that the good interaction has taken place effectively between chitosan schiff base with the poly propylene glycol during blending.

### Thermo gravimetric analysis

Thermogravimetric analysis, is a procedure which helps to determine a number of different properties of a particular material including the amount of weight lost during heating and cooling, endothermic properties, exothermic properties and more. With the help of TGA results, it is possible to note the temperature upto which the material does not loss weight and it is also possible to know the temperature at which material starts decomposing.<sup>[28][29]</sup> This TGA studies help to reveal the molecular structure such as the sequence and arrangement of repeating units and side groups in the polymers as well as the nature of the chain ends and of the crosslink's between chains. Fig.6 represents the TGA thermo gram details of binary blend of polypropylene glycol with chitosan-salicylaldehyde schiff base.

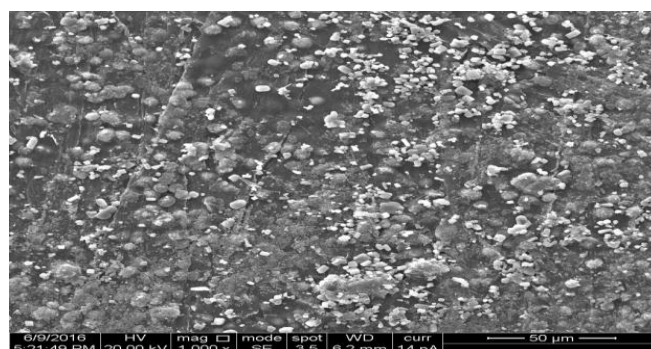


**Fig. 6: TGA thermo gram of chitosan-salicylaldehyde/polypropylene glycol blend.**

The TGA thermogram details of chitosan-salicylaldehyde schiff base/polypropylene glycol blend indicate that three stages of weight loss had taken place. The possible reason for appearance of this first stage weight loss step is the release of typical strong hydrogen-bonded water (40° to 180°C). On the other hand, very higher (second stage) mass loss was observed in the temperature range from 180° to 300°C which could be attributed to the loss of cleavage of glycoside linkage in the chitosan backbone present in schiff base. The III stage weight loss occurred from 300° to 420°C, provided evidence mass loss of other low temperature volatile species and also the degradation of the chitosan schiff base polymer blend. At the end of the experiment i.e., at 840°C only 20.87% of the blend remained as a residue. From 510°C there is only linear shallow decrease in weight with increase in temperature.

### 1.1. Scanning electron Microscopic (SEM) studies

SEM analysis allows us to examine and characterize particles and nanoparticles, fracture surfaces, surface morphologies, composites and their constituents and microstructures of prepared cross-sections. The surface morphology and cross sectional morphology of chitosan-salicylaldehyde schiff base/polypropylene glycol blend characterized by SEM studies was represented in Fig.7.



**Fig-7: SEM image of chitosan-salicylaldehyde schiff base/ Polypropylene glycol blend.**

The SEM images of chitosan-salicylaldehyde schiff base/polypropylene glycol blend showed a microporous structure with rough surface morphology and the cross sectional morphology of the same showed the fine interaction with pores and micro voids. A very good interfacial adhesion evidenced between chitosan-schiff bases and polypropylene glycol (Fig.7) was identified from the pores which are effective in increasing the functional surface in the Schiff bases which enabled the same to be used promising material for various applications.

## Antimicrobial studies

### Antibacterial activity

In the present research work, the antibacterial activity of the binary blend of polypropylene glycol with chitosan salicylaldehyde schiff base was tested against gram-positive bacteria *Bacillus subtilis* species and gram-negative bacterias namely *pseudomonas* and *E-coli* using well diffusion method. The comparison of zone of inhibition values were carried out with the help of the drug ampicillin as an effective reference antibacterial agent towards the selected three bacteria. Likewise, for studying the antifungal activity, the drug Polymyxin B sulphate which is an effective antifungal agent towards the fungal species was selected as a reference antifungal agent. The zone of inhibition values grown around the prepared sample against the growth of the selected bacteria measured in mm using ampicillin was used as standard was shown in Table-(2)-(3) and Fig.8.

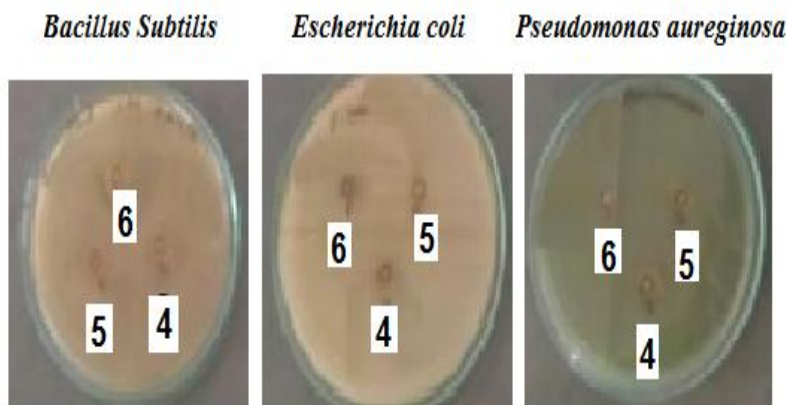
**Table. (2): Antibacterial activity of chitosan-salicylaldehyde schiff base.**

Organism (bacteria)	Zone of inhibition(mm)	Ampicillin (standard)
<i>Bacillus</i>	11	17
<i>E.coli</i>	20	15
<i>Pseudomonous</i>	15	21

**Table. (3): Antibacterial activity of chitosan salicylaldehyde schiff base /poly propylene glycol blend.**

Organism (bacteria)	Zone of inhibition(mm)	Ampicillin (standard)
<i>Bacillus</i>	20	17
<i>E.coli</i>	19	21
<i>Pseudomonous</i>	22	25

The photograph of the antibacterial activities of the binary blend of polypropylene glycol with chitosan –salicylaldehyde base was represented below (Fig.8).



where number 4 denotes- chitosan-salicylaldehyde schiff base/polypropylene glycol blend

**Fig.8: Antibacterial activity of binary blend of polypropylene glycol with chitosan – salicylaldehyde schiff base.**

From the observed results presented in the Table-(2)-(3) and Fig.8, it was evident that the chitosan-salicylaldehyde schiff base/polypropylene glycol blend showed the greater antibacterial activity against all the three bacterial species (*Bacillus subtilis*, *E.coli* than *pseudomonas*).

Combination of cationically charged amino-group of chitosan with the anionic components on the cell surface leading to the increased permeability of the membranes is the mechanism behind the inhibition of bacterial growth. Due to this combination, the death of microbial cells takes place. By impairing the exchange of positive charge of chitosan with the medium and by the inhibition of various enzymes, the bacterial growth has been suppressed.<sup>[30][31]</sup>

### Antifungal activity

The antifungal activities of the binary blend of polypropylene glycol with chitosan-salicylaldehyde Schiff base was tested against *Aspergillus flavus*, *Rhizopus* and *Pencillum* by well diffusion method. The zone of inhibition values of the chitosan-salicylaldehyde Schiff base and chitosan-salicylaldehyde Schiff base/polypropylene glycol blend were measured in mm and the results of screening of antifungal activities of the chitosan-salicylaldehyde Schiff base and chitosan-salicylaldehyde schiff base /polypropylene glycol blend is listed in the Table-(4) –(5) and Fig.9.



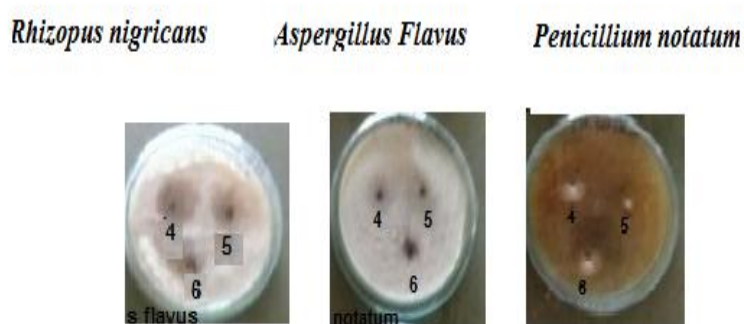
**Table. (4): Antifungal activity of chitosan-salicylaldehyde schiff base.**

Organism (fungi)	Zone of inhibition(mm)	Polymyxin B sulphate (standard)
<i>Aspergillus Flavus</i>	10	11
<i>Rhizobus</i>	16	11
<i>Pencillum</i>	13	11

**Table. (5): Antifungal activity of chitosan-salicylaldehyde schiff base /polypropylene glycol blend.**

Organism (bacteria)	Zone of inhibition(mm)	Polymyxin B sulphate (standard)
<i>Aspergillus Flavus</i>	18	11
<i>Rhizobus</i>	20	11
<i>Pencillum</i>	15	11

The photograph of the antifungal activities of the binary blend of polypropylene glycol prepared using chitosan-salicylaldehyde schiff bases prepared was represented below (Fig.9).



where number 4 denotes- chitosan-salicylaldehyde schiff base/polypropylene glycol blend

**Fig. 9: Antifungal activity of chitosan-salicylaldehyde schiff bases/Polypropylene glycol blend prepared using different aldehydes.**

The results presented in the Table-(4)-(5) and Fig.9 indicate that the chitosan-salicylaldehyde schiff bases/polypropylene glycol blend shows higher antifungal activity against all *Aspergillus flavus*, *Penicillium* and *Rhizopus* species. The overall results conclude that the prepared chitosan-salicylaldehyde schiff base/polypropylene glycol blends exhibit a very good antibacterial and antifungal activities.

## CONCLUSION

The present study mainly aims to synthesize, characterize and evaluate the antibacterial activity of chitosan-salicylaldehyde Schiff base /polypropylene glycol blend. The prepared

chitosan-salicylaldehyde Schiff base /polypropylene glycol blend characterized by FT-IR spectral studies indicate that the strong band obtained at around  $1600\text{ cm}^{-1}$  corresponding to the presence of C=N imine bond stretching. The appearance of this new peak concludes the formation of schiff bases by the interaction between the aldehydic group (-CHO) and the amine group ( $\text{NH}_2$ ) present in salicylaldehyde and chitosan. Also in addition the blend formation of chitosan schiff base with polypropylene glycol (PPG) was confirmed by presence of broad peak due to hydrogen bonding. The crystallinity behaviour and the thermal stability studied by XRD and TGA measurements reveals that the chitosan-salicylaldehyde schiff base/polypropylene glycol blend was found to possess highly amorphous nature and thermally stable behaviour. SEM studies of chitosan-salicylaldehyde schiff base/polypropylene glycol blend showed a microporous structure with rough surface morphology. From the observed higher zone of inhibition values, it was evident that the Chitosan-salicylaldehyde schiff bases/polypropylene glycol blend exhibit a very good antibacterial and antifungal activities and hence it can be used on a large scale in near future for further applications with expected success.

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