*IOSR Journal Of Pharmacywww.iosrphr.org*
*(e)-ISSN: 2250-3013, (p)-ISSN: 2319-4219*
*PP. 32-42*

# Synthesis, Characterization And Applications of Chitosan-O-Vanillin Schiff Bases/Polypropylene Glycol Blend

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***ABSTRACT:*** *The present work deals with the synthesis and characterization of chitosan-o-vanillin schiff base/polypropylene glycol blend. The characterization of the prepared chitosan schiff base/polypropylene glycol blend has been carried out by advanced analytical techniques such as FT-IR, XRD, TGA and SEM studies. The obtained FT-IR results clearly indicate 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 change in crystallinity and the increased thermal behavior was elucidated from XRD and TGA analysis respectively. The scanning electron microscopic (SEM) studies of the prepared chitosan schiff base/polypropylene glycol blend showed the microporous structure with rough surface morphology. The antimicrobial potential of the synthesized chitosan-o-vanillin schiff base/polypropylene glycol blend against three bacterial species namely Bascillus subtilis, E. coli and pseudomonas and against three fungal species namely Aspergillus flavus, penicillium notatum and Rhizopus were tested and evaluated. The obtained bactericidal and fungicidal action of chitosan-o-vanillin schiff base/polypropylene glycol blend reveals that the prepared chitosan-o-vanillin schiff base/polypropylene glycol blend has the greater potential to kill the microorganisms to a greater extent and hence this chitosan-o-vanillin schiff base/polypropylene glycol blend was found to be suggested as the promising material for biomedical applications.*

***Keywords:*** *chitosan, schiff base, o-vanillin, polypropylene glycol, antibacterial, antifungal.*

## I. INTRODUCTION

Schiff bases are the compounds with an imine group (–RC=N–) usually synthesized from the condensation of primary amines and active carbonyl groups [1][2]. In the year 1864, a scientist namely Hugo Schiff reported about the first schiff base formation [3] and these schiff bases were widely used in especially the medical and pharmaceutical fields [4]. Because of their wide spectrum of biological activities including antifungal, antidiabetic, antitumor, antiproliferative, anticancer, herbicidal and anti-inflammatory activities [5] several chitosan schiff bases was prepared by coupling various types of aldehyde with free amine groups of chitosan in last few years by many researchers and these chitosan Schiff bases have been pointed to as promising antibacterial agents [6]. The chitosan Schiff-bases were reported to be more potent antimicrobial agents than chitosan[7][8][9].

Chitosan is actually a copolymer of ß (1→4)-2-acetamido-2-deoxy-d-glucopyranose and ß-(1→4)- 2-amino-2-deoxy-d-glucopyranose with deacetylation greater than 60% obtained from the biopolymer chitin. Due to the presence of highly reactive functional groups such as NH and OH, the biopolymer chitosan can undergo immense structural possibilities for chemical and mechanical modifications to generate novel properties, functions and applications[10]. Recently several derivatives of chitosan were prepared recently including carboxylation [11], schiff base formation [12][13] and methylation [14]. Novel aminated chitosan-aromatic aldehyde Schiff bases have been synthesized, characterized and finally their antimicrobial activities were evaluated by El-Refaie Kenawy and his coworkers [15]. Reported results indicate that both 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.

Raj K Singh and his coworkers report the use of an acylated chitosan schiff base as an ecofriendly multifunctional biolubricant additive [16]. Leonhardt and his coworkers synthesized Schiff bases of chitosan from salicylaldehyde and 2-pyridinecarboxaldehyde. They obtained palladium complexes of these Schiff bases and investigated their catalytic activity for Suzuki and Heck reactions [17]. In recent years, the chitosan is modified by means of blending process and this was found to be an attractive method for providing new desirable characteristics to chitosan [18]. This is mainly due to its simplicity, availability of a wide range of synthetic and natural polymers for blending and effectiveness for practical utilization. Supriya Prasad and her coworkers reported about the use of chitosan schiff base (CSB)/polyethylene glycol (PEG) blend as antimicrobial agents [19]. Hence based on the above literature survey, in the present research work, the chitosan schiff base was

initially formed by the condensation of chitosan with the o-vanillin and to improve the properties of chitosan Schiff base another polymer polypropylene glycol was added to form chitosan Schiff base/polypropylene glycol (CSB/PPG) blend. The prepared blend was then characterized for its formation and its suitability for biomedical applications has been tested.

## II. MATERIALS AND METHODS

### 2.1Materials

Chitosan was purchased from India sea foods, Cochin, Kerala which is 92% deacetylated. The aldehyde o-Vanillin and polypropylene glycol was procured from Thermo Fisher Scientific India Private Ltd, Mumbai. The solvents such as the glacial acetic acid and ethanol were purchased from Sisco Research laboratories Private Ltd. Mumbai and SD fine chemicals private Ltd. Mumbai.

### 2.2Preparation of Chitosan schiff bases

About 1gram of chitosan dissolved in 50ml of 2% acetic acid was stirred well effectively for over a period 30 minutes to get a homogeneous viscous gel. To the above prepared homogeneous viscous chitosan gel, a required amount of o-vanillin (1 ml) dissolved in 10ml of ethanol was then added. The above solution mixture was then mixed well and allowed to stirr well effectively using a magnetic stirrer for over a period of 30 minutes until a white viscous gel of chitosan-o-vanillin schiff base is formed.

### 2.3Preparation of Chitosan Schiff base / Polypropylene glycol blend

The above prepared chitosan o-vanillin schiff base (1ml) was then mixed with the prepared polypropylene glycol (PPG) solution (1 ml of PPG in 10 ml ethanol).This mixture was then stirred using magnetic stirrer for over a period 30 minutes. Further in the sequence after the stirring process is over, the solution mixture were poured into plastic petridish and allowed to dry at room temperature and this will result in the formation of CSB/PPG blend. The photographical representation of the binary chitosan schiff bases/poly propylene glycol blend prepared using o-vanillin was represented below

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

**Figure-(A):** Photograph of chitosan –o-vanillin schiff base / polypropylene glycol blend

## III. CHARACTERIZATION

### 3.1Fourier Transform Infra red spectroscopy (FTIR)

Fourier transform infrared (FTIR) spectra of the prepared samples (pure chitosan, chitosan schiff bases, chitosan schiff base/poly propylene glycol blend) were performed with a Perkin Elmer 200 FT-IR spectrometer using Potassium bromide pellets. The FT-IR spectrum was obtained in the wave number range from 4000 to 350 $cm^{-1}$ with a resolution of $4cm^{-1}$.

### 3.2X ray diffraction (XRD)

X-ray diffraction patterns of prepared samples were tested by an X-ray scattering SCHIMADZU diffractometer using Ni filter Cu kα radiation source (λ=0.15nm), set at scan rate of 10°C using voltage of 40kV and a current of 30 mill amperes.

### 3.3Thermogravimetric Analysis (TGA)

Thermo gravimetric analysis of the prepared samples was conducted to measure the thermal weight loss of the sample on a TGA Q500 V20.10 build 36 instrument at a heating rate of 20°C per minute under nitrogen atmosphere.

**3.4Scanning electron microscope (SEM)**

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

**3.5Antimicrobial studies**

The effect of various compounds on the several bacterial strains and fungal strains were assayed by Ager well diffusion method.The two bacterial strains and two fungal strains were used. The bacteria used were Escherichia coli and Bacillus Subtilis species. The fungal strains used were Aspergillus flavous and Rhizopus species. The agar well diffusion method used was adapted from the punch plate assay for inhibitory substance present in microbiology standard method manual.

**3.5.1Muller Hinton Agar Medium**

The medium was prepared by dissolving 38.0 g of the commercially available Muller Hinton Agar (procured from Himedia) in 1000 ml of distilled water. To the above MHA medium, about 10g of Agar Agar was added in order to get solidified. The dissolved medium was then mixed well and poured onto100 mm petriplates (25-30ml / plates) while still molten.

**3.5.2Nutrient Broth for Bacterial Strain**

One litre of Nutrient broth was prepared by dissolving 13g of commercially available nutrient medium (Himedia) in 1000ml distilled water and boiled to dissolve the medium completely. The medium was dispersed as desired and sterilized by autoclaving at 15 lbs pressure (121°C for 15 minutes).

**3.5.3SDS nutrient broth for fungal strain**

One litre of SDS nutrient broth was prepared by dissolving 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. The pH was maintained at 6.

**3.5.4Inoculum preparation-preparation of bacterial pathogens**

The overnight cultures (0.2ml) of each bacterium was dispersed into 20ml of sterile nutrient broth and incubated for about 3-5 hours to standardize the culture. A loopful of the standard cultures was used for the antibacterial assay.

**3.5.5Inoculum preparation – preparation of fungal pathogens**

The fungi was isolated from soil and maintained on potato dextrose Agar slants and sub cultured for every 15 days. 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.

**3.5.6Plate preparation of Antibacterial assay**

Initially the chitosan-o-vanillin/polypropylene glycol (PPG) blend (2mg/ml) were prepared in the form of solution using water as a solvent. Control was run for each bacterial strain and fungal strain and it was then inoculated into the well. In order to prepare the plates for antibacterial assay initially the above prepared Muller Hinton Agar medium was sterilized. About 20ml of media was then poured in petriplates and allowed for solidification. The bacterial lawn culture (pathogen) prepared in the above manner was then placed over the medium using sterile cotton swap and labeled. The wells were made in the media with the help of a metallic borer with centers at least 24mm. A minimum amount of the above prepared binary blend sample (chitosan schiff base/polypropylene glycol blend) diluted in water was then introduced in the respective wells. Also in addition the other well is supplemented with reference antibacterial drug (ampicilin). After application of samples and standards, the plates were incubated on individual racks which not stacked on top of one another, for 24 hours at 37°C for antibacterial studies.

The complete procedure of the plate preparation was done in a laminar air flow to maintain strict sterile and aseptic condition. The antibacterial activity was then determined by measuring the diameter of zone grown around the samples showing complete inhibition (mm) using a ruler. It was then compared with the diameter of zone of inhibhition grown around the ampicillin (standard).

**3.5.7Plate preparation for Antifungal assay**

The antifungal activity of binary blend sample was studied against two fungal cultures, Aspergillus flavous and Rhizopus species. Sabouraud dextrose (SDS) agar was prepared, sterilized and the culture plates were prepared similarly as that of Muller Hinton Agar. After solidification of media, respective fungal spore suspensions were transferred to petriplates. The wells were made in the media with the help of a sterile metallic boror with centers at least 24 mm.Recommended concentration of the above prepared CSB/PPG blend sample (2mg/ml) dissolved in water was introduced into the wells. The plates were then placed in fume hood for 36

hrs. After this process is over, the plates were incubated for 72 hours. The results were recorded as zones of inhibition which was then compared with polymycin B Sulphate (standard).

## IV. RESULTS AND DISCUSSION

### 4.1Fourier transform infrared spectroscopy

Fourier transform infrared (FTIR) spectroscopy was used to identify the chemical structure of the blended films and possible interactions between their components [20]. FTIR is mainly used to determine of funtional groups in frequency range from 400cm$^{-1}$ to 4000cm$^{-1}$. The presence of a peak at a specific wave number would indicate the presence of a specific chemical bond. This analysis had eventually confirmed the functional groups in relation to absorption occurred and to change in chemical composition. The FTIR spectral details of pure chitosan, chitosan-o-vanillin schiff base and chitosan-o-vanillin schiff base/propylene glycol blend prepared in ratio (1:1) were represented in Fig (1)-(3).

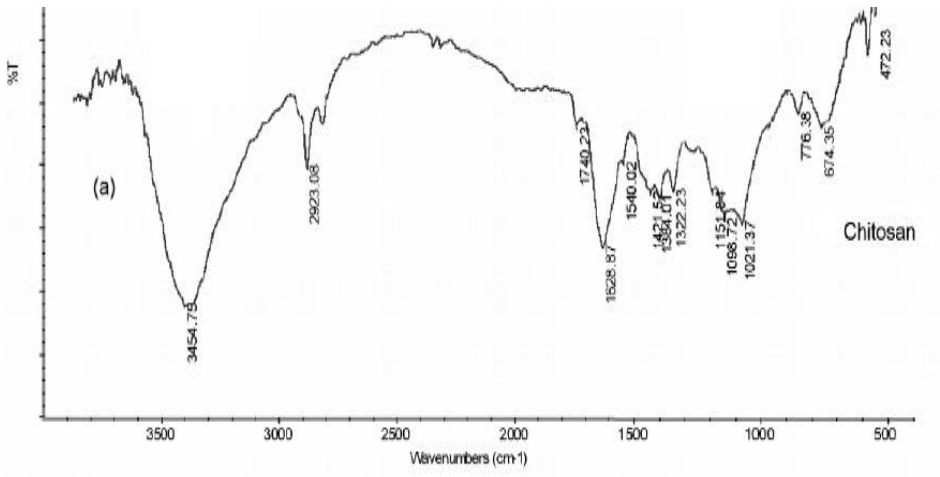



**Fig-(1): FT-IR spectrum of pure chitosan**

Fig-(1) shows the FT-IR spectral details of pure chitosan. The prominent peak obtained at 3454.75 cm$^{-1}$ was attributed to the presence of O-H, N-H symmetrical stretching vibrations. Strong peaks which were observed at 1628.87cm$^{-1}$, 1540.02 cm$^{-1}$ and 1421.52 cm$^{-1}$ was assigned to the presence of C=O stretching (amide-I band)[21],N-H bending and C-H deformation. Absorption bands obtained at 1384.01 cm$^{-1}$,1322.23 cm$^{-1}$,1151.84cm$^{-1}$, 1098.72 cm$^{-1}$ and 1021.37 cm$^{-1}$ were due to the presence of OH in plane bending in alcohols, twisting and wagging in $CH_2$ group, C-O stretching in secondary alcohols, stretching of C-O-C bridge, C-C stretching and skeletal vibrations involving the C-O stretching respectively. Also, in addition certain small peaks were appeared at around 674.35 cm$^{-1}$ and 472.23 cm$^{-1}$ possibly as a result of NH wagging and C-C bending vibrations.

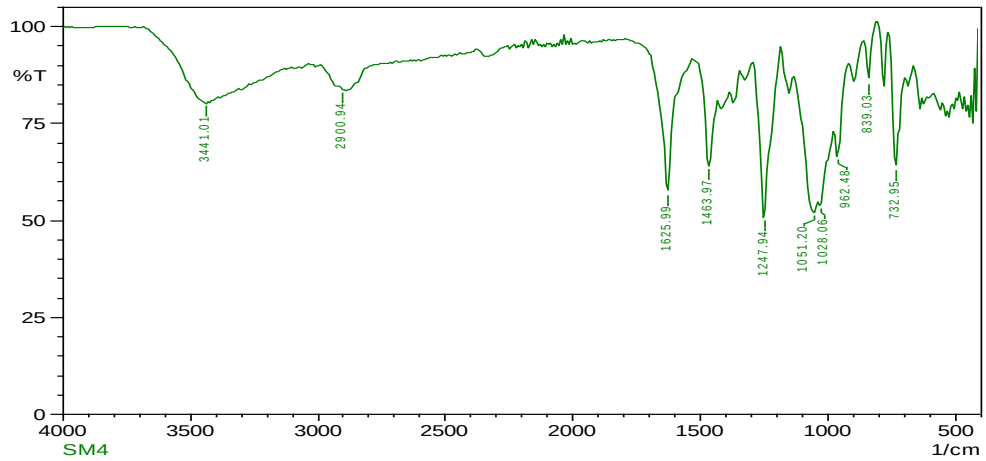



**Fig-(2):** FT-IR spectrum of chitosan- o-Vanillin schiff base

The FT-IR spectral details of chitosan-o-vanillin schiff base was represented in Fig-(2). The FT-IR spectrum of chitosan-o-vanillin schiff base shows the prominent peaks at 3441.01 $cm^{-1}$ for hydroxyl stretching and 2900.94 $cm^{-1}$ for asymmetrical CH stretching in methylenic group. The absorption bands obtained at 1625.99 $cm^{-1}$ and 1463.97 $cm^{-1}$ are characteristic of C=N stretching vibration [22][23] and C=C stretching due to aromatic ring respectively. The distinctive absorption bands appearing at 1247.94 $cm^{-1}$, 1051.20 $cm^{-1}$, 1028.06 $cm^{-1}$, 839.03 $cm^{-1}$ and 732.95 $cm^{-1}$ was assigned to the C-O stretching in phenol, C-O-C linkage, alcoholic C-O stretching, C-H deformation and C-H out of plane bending in aromatic compound respectively.

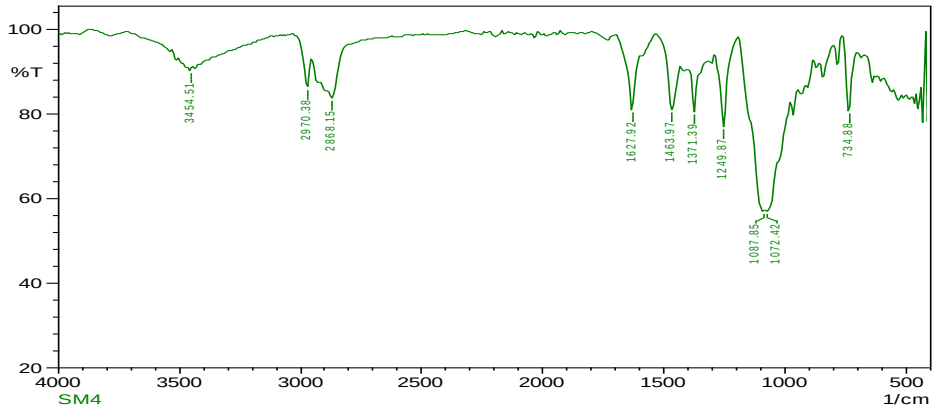



**Fig – (3)**: FT-IR spectrum of chitosan-o-vanillin schiff base/ polypropylene glycol blend

Fig-(3) shows the FT-IR spectral details of the chitosan-o-vanillin schiff base / polypropylene glycol blend prepared in 1:1 ratio. The broad peak which was obtained at 3454.51$cm^{-1}$ indicates the presence of intermolecular H bonded OH group. The peaks which were observed at 2970.38 $cm^{-1}$ and 2868.15 $cm^{-1}$ proves the presence of asymmetric C-H stretching in $CH_3$ group and aldehydic C-H stretching. A strong absorption band appearing at 1627.92 $cm^{-1}$ was assigned to the C=N stretching. In addition, several strong sharp bands at 1463.97 $cm^{-1}$, 1371.39 $cm^{-1}$, 1249.87 $cm^{-1}$, 1072.42 $cm^{-1}$ , 734.88 $cm^{-1}$ due to aromatic C=C stretching vibration, C-O stretching in phenols, C-O-C linkage and out of plane bending in substituted aromatic compound have also been obtained.

On comparing the FT-IR spectral details of chitosa-o-vanillin schiff base and chitosa-o-vanillin schiff base /polypropylene glycol blend with pure chitosan, it was observed that in case of chitosan –o-vanillin schiff base a new strong peak was appeared at around 1600 $cm^{-1}$ corresponding to the presence of C=N stretching and this obtained result concludes that the chitosan gets interacted effectively with the aldehydes leading to the formation of schiff bases. In addition to this the appearance of certain new peaks for methyl group (2970.38 $cm^{-1}$), shift in the peak positions and the observed reduction in the intensity of peaks it was suggested that the polypropylene glycol gets blended effectively with the chitosan-o-vanillin schiff base.

### 1.1. X-Ray diffraction

The X-ray diffraction (XRD) is the accurate, user friendly analytical technique which was found to be a useful for determining the structure and crystallization of polymer matrices [24]. X-ray diffraction patterns were measured to investigate the change of crystalline nature of biopolymeric material after modification process. This has been proved to be a useful tool to study crystal lattice arrangements and yields very useful information on degree of sample crystallinity. The degree of crystallinity of a sample measures the ratio of the crystalline part to the amorphous part when two semi crystalline polymers were mixed. The degree of crystallinity can be expressed as follows

$$Xc(\%) = \frac{Ac}{Ac + Aa}\ X\ 100$$

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

TABLE-1 and Fig- (4)-(5) shows the X-ray diffractogram details of pure chitosan and the binary blend of propylene glycol with chitosan-o-vanillin schiff base derivative.

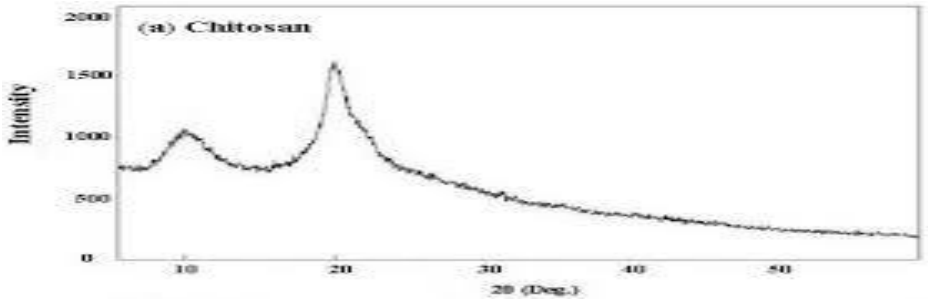



**Fig-(4):** X-ray diffrractogram of pure chitosan

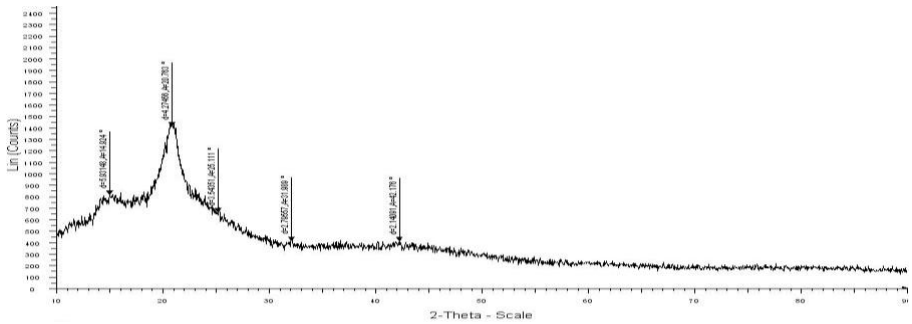



**Fig-(5**): X-ray diffrractogram of chitosan – o-vanillin schiff base / Polypropylene glycol blend

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

| **Samples** | $2\theta$ | **Degree of crystallinity (%)** |
|---|---|---|
| Pure Chitosan | $11^o$,$20^o$ | 12.7 |
| Chitosan-o-Vanillin schiff base / Polypropylene glycol blend | $14^o$,$20^o$ | 3.28 |

From the X-ray diffractograms of pure chitosan and chitosan-o-vanillin schiff base/PPG blend, it was observed that when compared to pure chitosan the appearance of characteristic peak at 2Ɵ=20° in case of CSB/PPG blend prepared was found to be much wider. From the obtained broad nature of the peak and from the lower percentage degree of crystallinity values, it was concluded that the binary blend of polypropylene glycol with the schiff base prepared using o-vanillin has highly amorphous nature when compared to the pure chitosan. This was mainly attributed to the deformation of the strong hydrogen bonds in the chitosan backbone with the substitution of aldehyde groups on the NH groups of pure chitosan biopolymeric molecule.In addition, the intensity change and peak shift from the pure chitosan in the case of XRD of chitosan-o-vanillin schiff base/polypropylene glycol blend showed that the good interaction has taken place effectively between chitosan schiff base with the poly propylene glycol during blending. Hence from the observed results it was concluded that when compared to the pure chitosan, the chitosan schiff bases prepared using different aldehydes, the chitosan-o-vanillin schiff base /polypropylene glycol blend prepared in 1:1 ratio was found to be having the highly amorphous nature.

**1.2. Thermo gravimetric analysis**

Thermo gravimetric Analysis (TGA) measures the amount and rate of change in the weight of a material as a function of temperature or time in a controlled atmosphere. TGA is a useful technique to assess the thermal stability of polymer and its blends. These 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-o-vanillin schiff base.

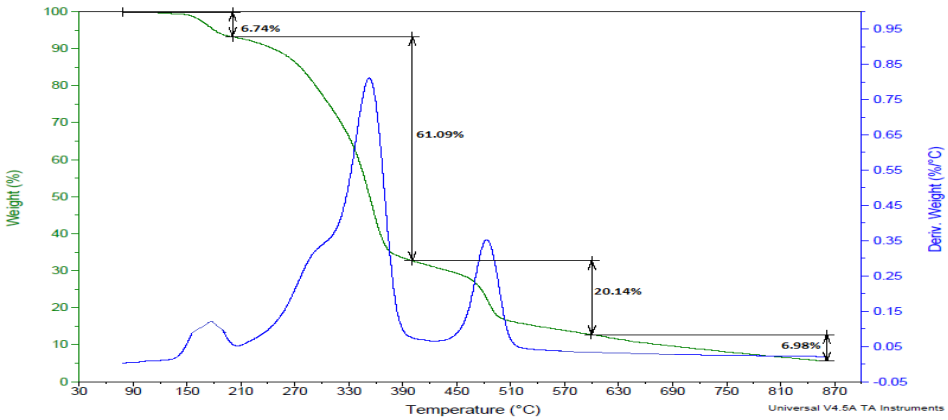



**Fig-(6):** TGA thermo gram of chitosan-o-vanillin/polypropylene glycol blend

Fig-(6) represents the TGA thermogram details of chitosan-o-vanillin / propylene glycol blend. The first weight loss observed in the temperature range of 80 $^{o}$C to 220 $^{o}$C was mainly due to the loss of water molecule. The second stage of weight loss starts at 220 $^{o}$C and ends at 400 $^{o}$C corresponds to the decomposition (thermal and oxidative) of chitosan, vaporization and elimination of volatile products. The result shown in Fig-6, indicates that there's increase in water content of the biopolymeric chitosan molecule after modification by aldehydes followed by further treatment with polypropylene glycol resulting in the blend formation. This result confirms increase of compound hydrophilicity and also it shows that the modified chitosan schiff base exhibited higher thermal stability.From the above figure, it was evident that the blended polymer is stable to a certain extent, which shows that 80% of the blend is disintegrated within 690$^{o}$C. Maximum weight loss occurs at the temperature range of 210$^{o}$C to 400$^{o}$C. At the end of the experiment i.e., at 900$^{o}$C, 5.05% of the blend remained as a residue. From 600$^{o}$C there is only linear shallow decrease in weight with increase in temperature. The observed TGA results indicate that the chitosan-o-vanillin schiff base/polypropylene glycol blend was found to be highly thermally stable and this was conformed from the obtained higher initial decomposition temperature (280°C).

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

Scanning electron microscopy is an extremely useful method for visual confirmation of surface morphology and the physical state of the surface. The surface morphology and cross sectional morphology of binary blend of polypropylene glycol with chitosan-o-vanillin schiff base characterized by SEM studies was represented in Fig-(7).

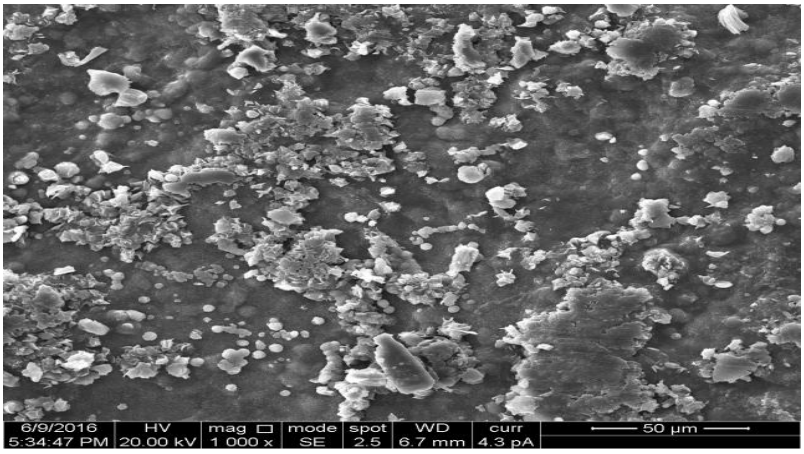



**Fig-(7):** SEM image of chitosan-o-Vanillin schiff base/ Polypropylene glycol blend

The SEM images of binary blend of polypropylene glycol with chitosan-o-vanillin schiff base showed a microporous structure with rough surface morphology. The expected interactions between chitosan matrix and aldehyde was well exposed through hydrogen bonding and the formation of imine linkage which make the schiff base derivatives stable with many hydrophilic sites at the surface. The cross sectional morphology of the same

showed the fine interaction with pores and micro voids. The pores are effective in increasing the functional surface in the Schiff bases which enabled the same to be used promising material and from fig-(7) it was also suggested that a very good interfacial adhesion was evidenced between chitosan-schiff bases and polypropylene glycol.

## V. ANTIMICROBIAL STUDIES

### 5.1Antibacterial activity

Due to easy availability, non toxicity and cost-effectiveness, chitosan and its derivatives make it as a suitable antimicrobial agent against many disease causing bacteria, fungi and other microorganisms. The antimicrobial activity of chitosan will depend on several factors such as the kind of chitosan (deacetylation degree, molecular weight) used, the pH of the medium and the temperature. The presence of different functional groups along chitosan backbone (i.e.; hydroxyl and amine groups) simplifies its chemical modifications.

Schiff's bases and their metal complexes show very good antibacterial activities against E. coli and B. subtilis. It was also reported that the activity of the chitosan Schiff bases were stronger than that of chitosan. It was observed that the antibacterial activity of chitosan is increased 2-3 fold in the corresponding chitosan Schiff bases. Schiff's bases of various compounds are reported to possess Anticonvulsant [25] , antiproliferative [26], antifungal, cytotoxic [27], anticancer [28] and anti HIV activities [29].

Because of the presence of π bond and lone pair of electrons present on the nitrogen atoms, the chitosan-o-vanillin schiff base shows variety of biological activities. By utilizing the well diffusion method, the antibacterial activity of the binary blend of polyropylene glycol with chitosan –o-vanillin schiff base was tested against gram-positive bacteria Bacillus subtilis species and gram-negative bacterias namely pseudomonas and E-coli. The drug ampicillin which is an effective antibacterial agent towards the selected three bacteria was used as a reference antibacterial agent in order to compare the results. Likewise, the drug Polymyxin B sulphate which is an effective antifungal agent towards the fungal species selected was used 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-(i).**

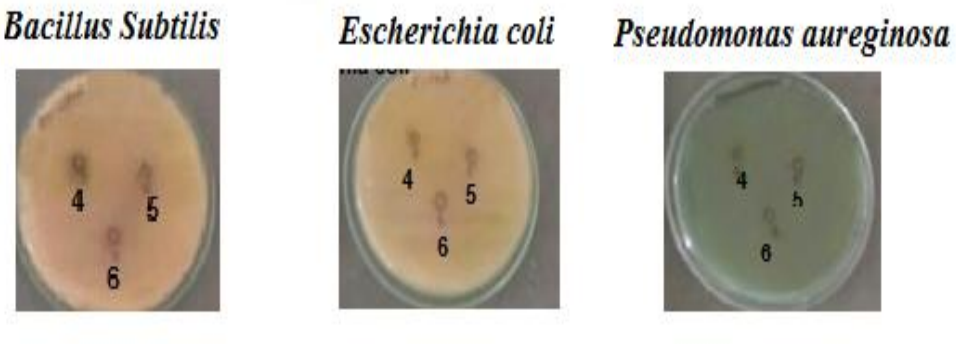
**Table-(2):** Antibacterial activity of chitosan-o-vanillin schiff base

| **Organism (bacteria)** | **Zone of inhibition(mm)** | **Ampicillin (standard)** |
|---|---|---|
| Bacillus | 15 | 17 |
| E.coli | 12 | 15 |
| Pseudomonous | 6 | 21 |

**Table-(3):** Antibacterial activity of chitosan schiff bases /poly propylene glycol blend

| **Organism (bacteria)** | **Zone of inhibition(mm)** | **Ampicillin (standard)** |
|---|---|---|
| Bacillus | 14 | 17 |
| E.coli | 22 | 21 |
| Pseudomonous | 9 | 25 |

The photograph of the antibacterial activities of the binary blend of polypropylene glycol with chitosan –o-vanillin schiff base was represented below (Fig-(i))




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

**Fig-(i):** Antibacterial activity of binary blend of polypropylene glycol with chitosan –o-vanillin schiff base

From the observed results presented in the TABLE-(2)-(3) and Fig-(i), it was evident that the chitosan-o-vanillin schiff base/polypropylene glycol blend showed the maximum antibacterial activity against Bacillus subtilis, E.coli than pseudomonas. The difference may be attributed to their different cell walls [30]. In Bacillus subtilis, a typical Gram-positive bacterium, its cell wall is fully composed of the peptide polyglycogen, which has plenty of pores which allow foreign molecules to come into the cell without difficulty. But the cell wall of E.coli and Pseudomonas a gram–negative bacterium has a bilayer structure which acts as a barrier against foreign molecules.

The mechanism behind the inhibition of bacterial growth is due to the combination of cationically charged amino-group of chitosan with the anionic components on the cell surface leading to the increased permeability of the membranes and leakage of cell material from tissues resulting in the death of microbial cells. The bacterial growth was suppressed mainly due to the positive charge of chitosan by impairing the exchanges with the medium, chelating transition metal ions and by the inhibition of various enzymes [31][32].Results reveals that the prepared chitosan-o-vanillin schiff base/polypropylene glycol blend shows higher antibacterial activity when compared to the chitosan-o-vanillin schiff base.

**5.2Antifungal activity**

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

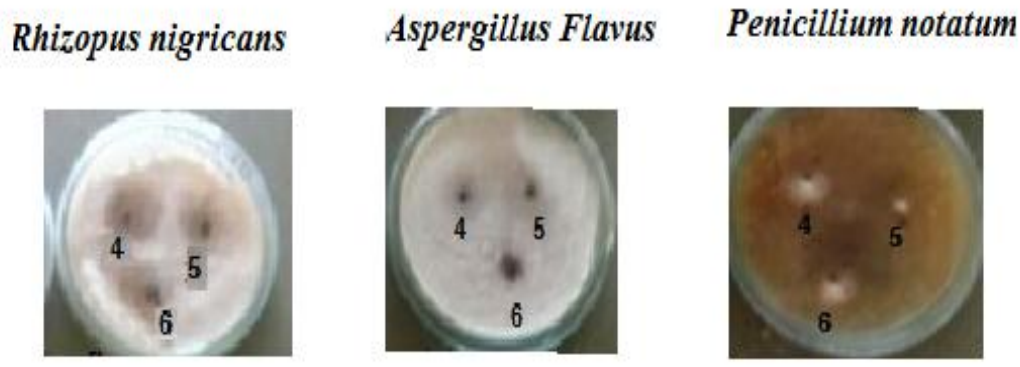
**Table-(4):** Antifungal activity of chitosan-o-vanillin schiff base

| Organism (fungi) | Zone of inhibition(mm) | Polymyxin B sulphate (standard) |
|---|---|---|
| Aspergillus Flavus | 14 | 11 |
| Rhizobus | 15 | 11 |
| Pencillum | 18 | 11 |

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

| Organism (bacteria) | Zone of inhibition(mm) | Polymyxin B sulphate (standard) |
|---|---|---|
| Aspergillus Flavus | 19 | 11 |
| Rhizobus | 24 | 11 |
| Pencillum | 16 | 11 |

The photograph of the antifungal activities of the binary blend of polypropylene glycol prepared using chitosan-o-vanillin schiff bases prepared was represented below (Fig-(ii))




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

**Fig-(ii) :** Antifungal activity of chitosano-vanillin schiff bases/Polypropylene glycol blends prepared using different aldehydes

The results presented in the TABLE-(4)-(5) and Fig-(ii) indicate that the chitosan-o-vanillin schiff bases/polypropylene glycol blend shows higher antifungal activity against all Aspergillus flavus, Penicillium

and Rhizopus species when compared to the chitosan-o-vanillin schiff base. The results of the antimicrobial investigations indicate that the chitosan-o vanillin schiff base/polypropylene glycol blend shows higher antifungal activity when compared to chitosan-o-vanillin Schiff bases. The overall results conclude that the prepared chitosan-o-vanillin schiff base/polypropylene glycol blends exhibit a very good antibacterial and antifungal and hence it can be used in near future for further applications with expected success on a very large scale.

## VI. CONCLUSION

The prepared chitosan-o-vanillin schiff bases /polypropylene glycol blend characterized by FT-IR spectral studies reveals that the strong band obtained at around 1600 $cm^{-1}$ corresponding to the presence of C=N imine bond stretching. This concludes the formation of schiff bases by the interaction between the aldehydic group (-CHO) and the amine group ($NH_2$) present in o-vanillin 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 thermal stability and crystallinity behaviour studied by TGA and XRD measurements indicate that the chitosan-o-vanillin schiff base/polypropylene glycol blend was found to be thermally stable and has highly amorphous nature. The surface morphology of chitosan-o-vanillin schiff base/polypropylene glycol blend identified from the scanning electron microscopic (SEM) indicate that the chitosan-o-vanillin schiff base/ polypropylene glycol blend showed a microporous structure with rough surface morphology. The antibacterial and antifungal activities of the Chitosan-o-vanillin schiff bases/polypropylene glycol blend shows that it exhibit a very good antibacterial and antifugal activities and hence it can be used in near future for further applications with expected success on a very large scale.

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