International Journal of Pharmacy and Biological Sciences
ISSN: 2321-3272 (Print), ISSN: 2230-7605 (Online)
IJPBS | Volume 9 | Special Issue 2 | 2019 | 150-167
| Research Article | Biological Sciences |Open Access |MCI Approved|
|UGC Approved Journal|

Evaluation of The Antimicrobial Activity of Nanochitosan/Polyurethane/Polypropylene Glycol Ternary Blends

M. Saranya, T. Gomathi and P.N. Sudha*
Biomaterials Research Lab, Department of Chemistry, DKM College for Women (Autonomous), Vellore, India

Received: 30 Jan 2019 / Accepted: 20 Feb 2019 / Published online: 01 Apr 2019
Corresponding Author Email: drparsu8@gmail.com

Abstract

In the present study Nanochitosan /Polyurethane /Polypropylene glycol NCS/PU/PPG ternary blends in different ratios (1:1:1, 1:2:1 and 1:1:2) in the presence and absence of cross-linking agent Glutaraldehyde were prepared and expected to possess for its activity against a wide range of microorganisms, One gram positive bacteria (*Bascillus subtilis)* and one gram negative bacteria (*Escherichia Coli) and* antifungal activities against *Aspergillus niger, Aspergillus flavus* and *Penicillium notatum* by well diffusion method were used as test organisms in the present research work. The characterization of the prepared sample was carried out using FTIR, XRD, studies. From the FTIR results, the shift in peaks to higher wavenumber confirms the formation of blend. X-ray diffraction studies (XRD) has been carried out to get information on the amorphous or crystalline nature of the blend. The data indicates that the effectiveness of ternary blends of NCS/PU/PPG varies and is dependent on species of target microorganisms.

Keywords
Microorganisms, Characterization, Ternary blends, Cross-linking agent

*****

INTRODUCTION

Recently one of the major issues and concern for governments around the globe were the antimicrobial resistance inside an extensive variety of infectious agents.[1] It is a public wellbeing menace that influences nations and various fields which, in turn, undermine to the achievements of modern medicine.[2] Chitosan is a positively charged molecule and the target of its antimicrobial action is the negatively charged cell wall of bacteria, where it binds and disrupts the normal functions of the membrane, e.g., by promoting the leakage of intracellular components and also by inhibiting the transport of nutrients into the cells.[3,4,5] Nanoparticles have unique physicochemical properties attributed to their high surface area to volume ratio, size, shape and surface structure. They have a wide range of potential biomedical and pharmaceutical applications including drug and gene delivery [6] and tissue engineering. [7] Chitosan is converted to nanosize by ionotropic gelation technique. [8]

Polyurethane foam is excellent for separation and preconcentration of trace metal ions in environmental samples with inexpensive method. Polyurethane is a good sorbent for some organic and inorganic pollutants in wastewater. [9] Blending of polymers improve their physical and chemical properties.[10] Blending occurs due to the formation of intermolecular hydrogen bonding and molecular close chain packing.[11]

Polypropylene glycol is produced by anionic ring-opening polymerization of propylene oxide. Most of the researchers used glutaraldheyde as crosslinkers because it forms, uniform crosslinking among the polymers. The blends of NCS was found to act a good sorbent for the remediation of even industrial effluent. Similar blends have shown good biocompatibility and found useful in antimicrobial application.

The biocompatible and biodegradable polymer chitosan was used as reducing and stabilizing agent for the synthesis of gold nanoparticles embedded in it. Here in, for the first time, two different chitosan grades varying in the average molecular weight and deacetylation degree (DD) were used with an optimized gold precursor concentration. Several factors were analyzed in order to obtain antimicrobial but not cytotoxic nanocomposite materials. Films based on chitosan with medium molecular weight and the highest DD exhibited the highest antibacterial activity against biofilm forming strains of *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The resulting nanocomposites did not show any cytotoxicity against mammalian somatic and tumoral cells. They produced a disruptive effect on the bacteria wall while their internalization was hindered on the eukaryotic cells. This selectivity and safety make them potentially applicable as antimicrobial coatings in the biomedical field. [12]

Helander et al. studied the effects of chitosan treatment on the cell membranes of Gram-negative bacteria and found evidence for extensive cell surface alterations, marked by thickening and formation of vesicular structures on the outer membranes of both *Escherichia coli* and *Salmonella typhimurium*. They reasoned that chitosan binds to the outer membrane of Gram-negative bacteria, there by affecting its barrier properties, probably through complex formation with various lipopolysaccharides. Highly cationic mutants of *S. typhimurium* were also found to be more resistant to chitosan than the parent strains. [13] Morimoto et al. reported the specific binding of a chitosan derivative with a receptor on the cell surface of *Pseudomonas aeruginosa.*[14] Hence, in the present study NCS/PU/PPG ternary blends were prepared and expected to possess antimicrobial therapy.

MATERIALS AND METHODS

MATERIALS

The material used for this study mainly includes Chitosan (92% deacetylated) which was purchased from India Sea Foods, Cochin, Kerala. Polyurethane foam and polypropylene glycol were obtained from Star foams, Ranipet and Nice chemicals Pvt Limited respectively. Also, the crosslinking agents such as sodium tripolyphosphate, glutaraldehyde and the solvent glacial acetic acid were proccured from Finar Chemicals, Ahmedabad and Thomas Bakers chemicals Pvt Ltd, Mumbai respectively. All the above chemicals used in the present research work were of analytical grade.

PREPARATION OF NANOCHITOSAN

As per the ionotropic gelation method, the nanochitosan was prepared from chitosan molecule upon contact with sodium tripolyphosphate as ionic crosslinking agent (TPP).[15] Due to nontoxic nature of these components and ease of operation, the gel ionization technique is adopted for the synthesis of nanochitosan. Initially the homogeneous viscous chitosan gel was prepared by dissolving 1 g of chitosan in 200 ml of 2% acetic acid solution under magnetic stirring. About 0.8 g of sodium tripolyphosphate (TPP) dissolved in 107 ml of conductivity water was then added drop wise with continuous stirring to the above prepared chitosan gel. This solution mixture was then allowed to react for 30 minutes at room temperature. A milky coloured emulsion like appearance of nanochitosan obtained by the effective reaction between chitosan and TPP ions was then allowed to settle as suspension by adding conductivity water in excess for 24 hours. After this process is over, the supersaturated solution was decanted and then the thick emulsion (isolated nanochitosan) was kept in freezer until further use.

PREPARATION OF NANOCHITOSAN / POLYURETHANE/ POLYPROPYLENEGLYCOL (1:1:1) BLEND.

Ternary biopolymeric blend was prepared by mixing 1.0 g of nanochitosan sonicated in 20 ml distilled water to 1.0 g of polyurethane (dissolved in 200 ml 1:1 formic acid) and 1.0 ml of polypropyleneglycol (dissolved in 10 ml of water). The crosslinking process has been achieved by the addition of 6 ml glutalraldehyde to the above prepared solution mixture. This solution mixture was then stirred well effectively for a period of 30 minutes using the electrical stirrer. After this process is over, the solution mixture was dried in vacuum and used for further analysis.

CHARACTERIZATION

The FT-IR spectra of prepared nanochitosan and nanochitosan / polyurethane/ polypropylene glycol of various ratios blend samples were recorded in the wave number range of 400 – 4000 cm^{-1} by Perkin Elmer 200 FTIR spectrophotometer with the resolution of 2 cm^{-1}. By utilizing Shimadzu XD-DI Diffractometer with Ni filter Cu Kα radiation source (λ=0.154nm), set at scan rate = 10˚/min, using a voltage of 40kV and a current of 30 mA the XRD patterns were tested.

ANTIMICROBIAL ACTIVITY

The antibacterial activity of the NCS/PU/PPG films prepared in various ratios were tested against the two bacterial strains by disc diffusion method using Muller Hinton Agar (MHA) medium. One-gram positive bacteria (*Bascillus Subtilis)* and one-gram negative bacteria (*Escherichia Coli) a*nd antifungal activities against *Aspergillus niger, Aspergillus flavus* and *Penicillium notatum* were used as test organisms in the present research work.

In order to study the antibacterial activity of the prepared samples, initially the microorganisms *Escherichia coli* and *Bascillus Subtilis were* inoculate on Muller Hinton Agar (MHA) medium and spread uniformly using sterile spreader in Petri plates. After solidification of the MHA medium, a small amount of the NCS/PU/PPG films prepared in various ratios were then placed on different cultured agar plates. The plates were allowed to stand for 1 h at room temperature for the diffusion of the substances and before the growth of organism commenced, the plates were incubated on individual racks for 24 h at 37°C. The antibacterial activities of the prepared NCS/PU/PPG films prepared in various ratios were then evaluated by measuring the diameter of zone of inhibition grown around the discs against the test microorganisms using the ruler.

TEST MICROORGANISMS

The *in vitro* biological screening effect of the prepared blends were tested agains the two bacterial strains and three fungal strains. The gram-positive bacteria such as *Bascillus Subtilis*, gram negative bacteria such as *Escherichia coli* and fungi *Aspergillus niger, Aspergillus flavus* and *Penicillium notatum* were used as test microorganism.

The antimicrobial used to study were allowed to diffuse out into the medium and interact in a plate freshly seeded with the test organisms. The resulting zones of inhibition are made uniformly circular for a confluent lawn of growth. The diameters of zone of inhibition are measured in millimeters.

RESULTS AND DISCUSSION

FOURIER TRANSFORM INFRARED (FTIR) SPECTROSCOPY

FTIR spectroscopy is an excellent method used extensively for the qualitative analysis in which the chemical nature of a substance such as chemical bonds, molecular orientations, molecular energy levels and molecular interactions which can be assessed by identifying specific absorption peaks for particular groups.[16,17] Hence, each blend has been analyzed using FT-IR and the possible interactions between the functional groups in a polymer blend were studied.

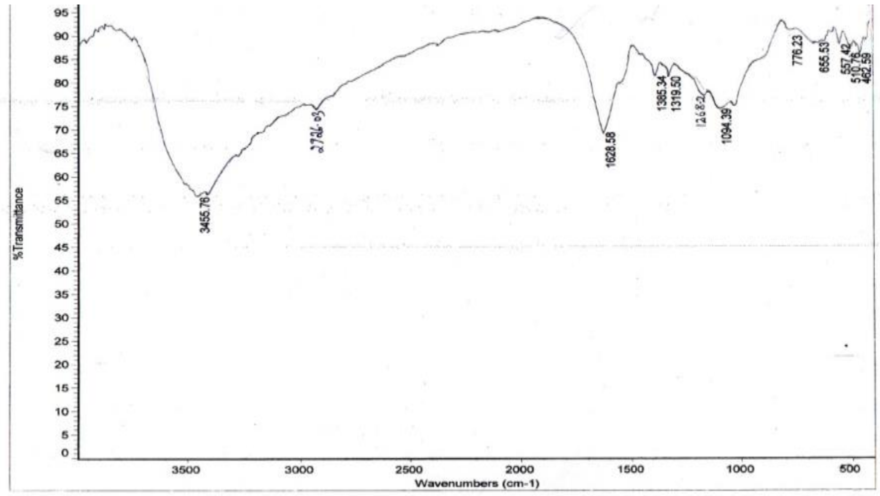

Figure 1: FTIR Spectra of nanochitosan (NCS)

The FTIR spectrum for nanochitosan (NCS) (Fig-1) represents the formation of nanochitosan using ionotropic gelation method. The absorption band obtained at 3385.92 cm^{-1} attributed to –NH group in chitosan, which gets broadened by the physical interactions with TPP, while the shoulder appeared at 2920.57 and 1635.20 cm^{-1} may be due to CH stretching, amide at same position after crosslinking with TPP indicating the interaction of chitosan amide with added polyions.[18]

Strong bands obtained at 2920.57 cm^{-1}, 2908.57 cm^{-1}, 1510.05 cm^{-1}, 1376.80 cm^{-1} and 1219.00 cm^{-1} indicate the presence of asymmetrical and symmetrical stretching in CH_2 group, NH_3^+ stretching, NH bending, OH in plane bending in alcohols and P=O stretching.[19] The band obtained at 1163.38 cm^{-1} indicated the overlapping peak of C–O stretching in polysaccharide and formation of chitosan nanoparticles due to the interaction of ammonium ion and phosphate ion in chitosan nanoparticle molecules.[20] Absorption bands obtained around 1113.24 cm^{-1} and 1038.87 cm^{-1} were assigned to C-O-C linkage and P-O stretching and C-C stretching respectively.

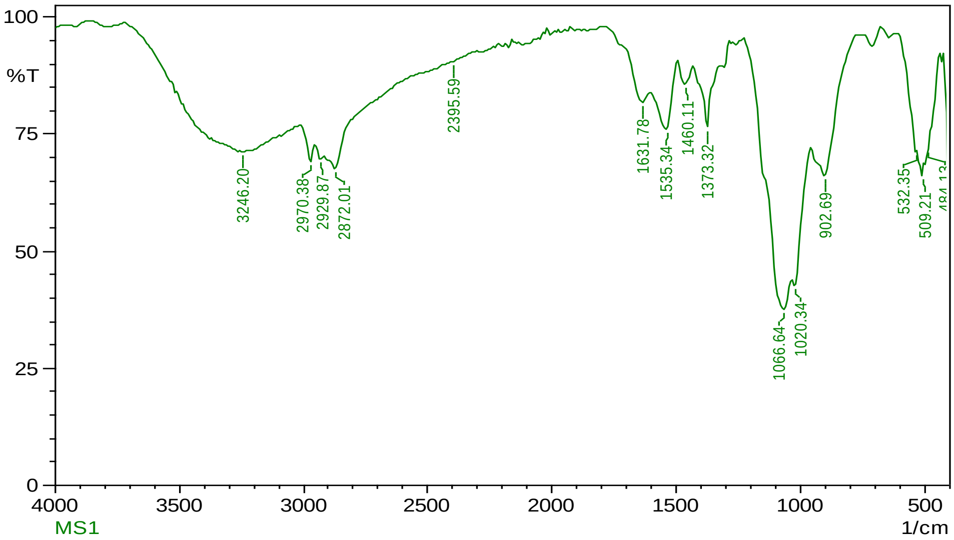

Figure-2: FTIR Spectrum of NCS/PU/PPG-(1:1:1) ternary blend

(Fig-2) represents the FTIR spectrum of Nanochitosan/ Polyurethane/Polypropylene glycol - (1:1:1) blend film. A broad band is obtained at 3246.20 cm^{-1} corresponds to intermolecular hydrogen bonded O-H stretching and NH stretching. [21]

The bands obtained at around from 2970.38 to 2872. 01 cm^{-1}, corresponds to asymmetrical and symmetrical C- H stretching vibrations and a band obtained at 1631.78 cm^{-1} is due to C=O stretching. The prominent bands obtained at 1536.34 cm^{-1}, 1460.11 cm^{-1} and 1373.32 cm^{-1} indicates the presence of N-H bending (amide II), CH_2 bending vibrations (amide III) and C-O asymmetric stretching vibration respectively. Bands are also observed at 1066.64 cm^{-1}, 1099.43 cm^{-1} and 1020.34 cm^{-1} may be due to the presence of indicates P-O stretching in TPP and C-O-C linkage respectively.

On comparing the FTIR spectrum of Nanochitosan/ Polyurethane/Polypropylene glycol - (1:1:1) blend film with bare nanochitosan, the band which is responsible for OH and NH stretching vibrations in the nanochitosan (3385 cm^{-1}) is shifted to the lower region of 3246 cm^{-1}. This confirms the participation of OH and NH functional group of nanochitosan in blend formation. Also, the band for C=O and NH bending vibration of the ternary blend is shifted to the higher wavenumber region compared with nanochitosan, thereby indicating the inception of PU and PPG in the nanochitosan matrix. Thus, the observed FTIR spectrum proves the effective blending of the three polymers, nanochitosan, polyurethane and Polypropylene glycol.

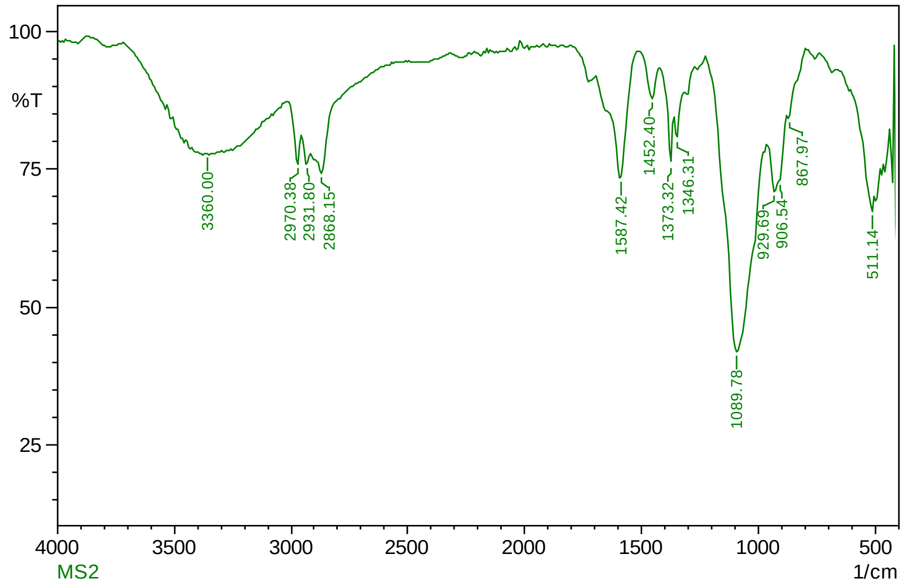

Figure-3: FTIR spectrum of NCS/PU/PPG-glu (1:1:1) ternary blend

The FTIR spectral details of ternary blend (NCS/PU/PPG) in the presence of glutaraldehyde (1:1:1-glu) is depicted in (Fig-3). The prominent bands observed at 3360, 2970, 2931 and 2868 cm^{-1} may be due to intermolecular hydrogen bonding O-H stretching, N-H stretching, asymmetrical and symmetrical C-H stretching vibrations.[22]

The characteristic bands observed at 1587, 1452, 1373 and 1346 cm^{-1} indicates the presence of C=N stretching, N-H bending stretching, C-O asymmetrical stretching, CH_2 bending (amide III). The band at 1089 cm^{-1} indicates P-O stretching in TPP, C-O-C linkage. The bands observed at 929, 906, 867 and 511 cm^{-1} belongs to N-H bending, O-H bending, CH out of plane deformation, C-C bending vibrations respectively.

The addition of crosslinker is well seen the group frequencies and the finger print region of NCS/PU/PPG 1:1:1 blend prepared in the presence of crosslinking agent. The bands for OH and NH functional groups were shifted to the higher region when compared with the NCS/PU/PPG 1:1:1 blend prepared in the absence of crosslinking agent. Also, the inclusion of dialdehyde was confirmed by the splitting of C-H bands in its region and there is the formation of C=N imine linkage. These observed shifts in the frequencies and the appearance of new peak confirms that NCS/PU/PPG were effectively blended and crosslinked with glutaraldeyde.

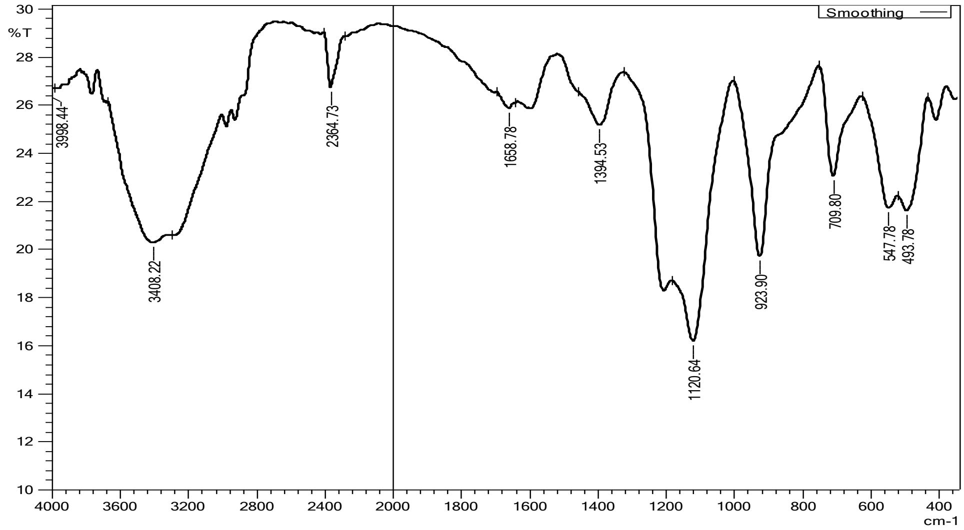

Figure-4: FTIR spectrum of NCS/PU/PPG-(1:2:1) ternary blend

(Fig-4) represents the FTIR spectral details of NCS/PU/PPG-(1:1:2) ternary blend. A broad band obtained at 3408.22 cm^{-1} corresponds to intermolecular hydrogen bonded O-H and NH stretching vibrations. A band obtained at 1668.78 cm^{-1} indicates C=O stretching modes of vibration and a band at 1394.53cm^{-1} indicating the presence of C-O asymmetrical stretching. The band at around 1120.04 cm^{-1} corresponds to the presence of P-O stretching in TPP, C-O asymmetric and symmetric stretching and C-O-C stretching vibration. The observed bands at 923.90 and 709.80 cm^{-1} indicates N-H bending, O-H bending and 547.78 cm^{-1} corresponds to C-C bending respectively.

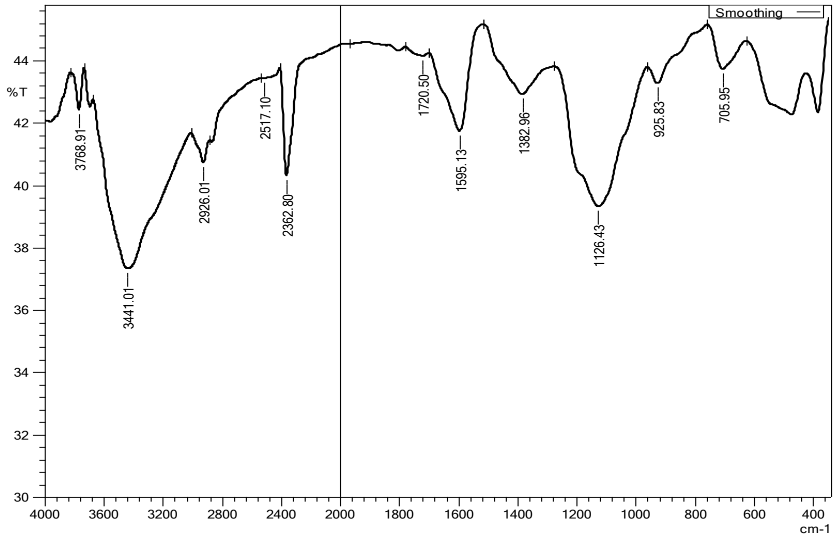

Figure-5: FTIR spectrum of NCS/PU/PPG-glu (1:2:1) ternary blend

The FTIR spectrum of NCS/PU/PPG 1:2:1-glu ternary blend is depicted in (Fig-5). The prominent peaks observed at 3441.01, 2926.01, 1720.50, 1595.13 and 1362.96 cm^{-1} are due to interamolecular hydrogen bonding O-H stretching, N-H stretching, asymmetrical and symmetrical C- H stretching, C=O stretching, C=N stretching, N-H bending respectively.[23] The band at 1120.04 cm^{-1} indicates P-O stretching in TPP. The bands at 925.83 and 705.96 cm^{-1} belongs to N-H bending, O-H bending, C-C bending vibrations respectively. On comparing with bare chitosan nanoparticles and with 1:1:1 ternary blend, some of the bands get shifted, confirms that NCS/PU/PPG glu (1:2:1) ternary blend were effectively blended.

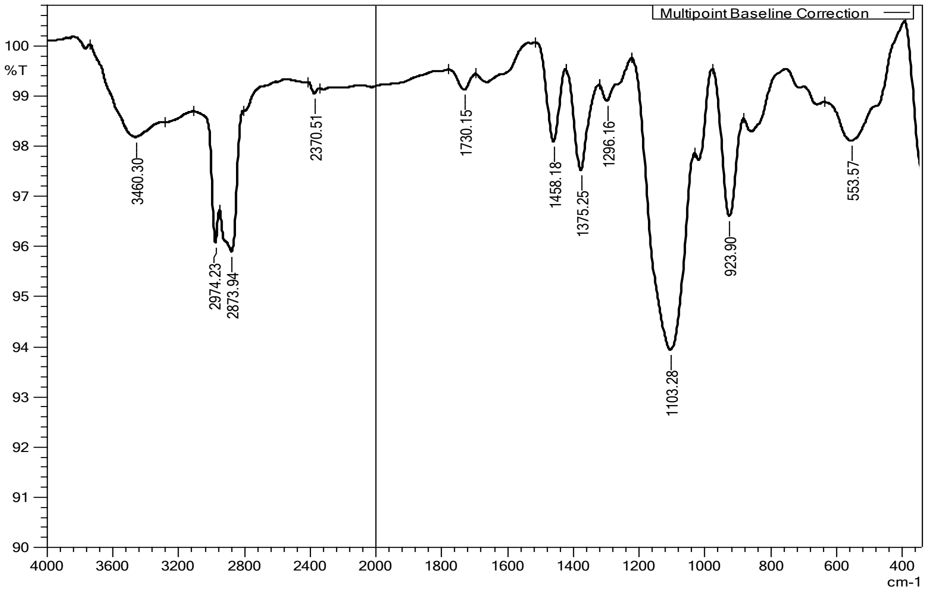

Figure-6: FTIR spectrum of NCS/PU/PPG-(1:1:2) ternary blend

(Fig-6) represents the FTIR spectral details of NCS/PU/PPG-(1:1:2) ternary blends. A broad band obtained at 3460.30 cm^{-1} corresponds to intermolecular hydrogen bonded O-H and NH stretching.[24] The bands obtained at 2974.23 and 2873.94 cm^{-1} are due to asymmetrical and symmetrical C-H stretching. The bands obtained at 1730.15, 1458.18, 1375.25, 1298.16 and 1103.28 cm^{-1} indicates the presence of C=O stretching, CH bending, C-O asymmetric stretching, P=O stretching and P-O stretching vibrational modes respectively. A band obtained at 923.90 cm^{-1} indicates N-H bending and at 563.57 cm^{-1} corresponds to C-C bending respectively. [25]

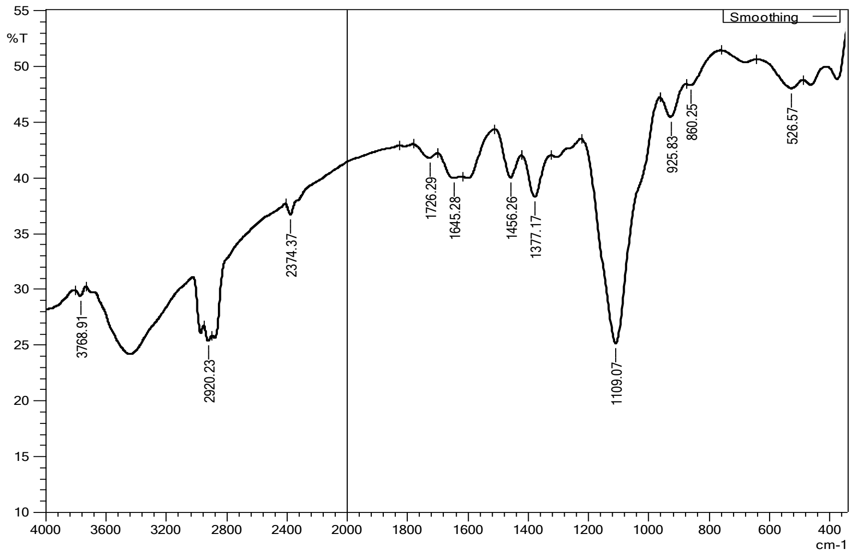

Figure-7: FTIR spectrum of NCS/PU/PPG-glu (1:1:2) ternary blend

(Fig-7) **s**hows the FTIR spectral details of ternary blend (NCS/PU/PPG) in the presence of glutaraldehyde (1:1:2-glu)**.** The prominent peaks observed at 3446.00 and 2920 cm^{-1} are due to interamolecular hydrogen bonded O-H stretching and N-H stretching, asymmetrical and symmetrical C- H stretching respectively. [26]

The characteristic bands observed at 1726.29 cm^{-1} is for C=O stretching, 1645.28 cm^{-1} denotes C=N stretching, 1456.26 and 1377.17 cm^{-1} indicates the presence of C- H bending and C-O asymmetric stretching. The band of 1109 cm^{-1} indicates P-O stretching in TPP and C-O-C linkage.

The characteristic band for C=N stretching vibration at around 1640 cm^{-1} was in agreement with the bands obtained by Graham and George (1981) and Freedman (1961) where it was at 1630 cm^{-1}. The band at around 1570 cm^{-1} corresponds to free amino groups. The obtained evidences suggest that certain interactions had taken place effectively between the functional groups present in nanochitosan, Polyurethane and Polypropyleneglycol components. There is no significant differences between FT-IR spectra for (NCS/PU/PPG) ternary blend prepared in various ratios. Only certain shifts of the various absorption bands to lower wave numbers (band attributed to the carboxylation, and C-O stretching in alcohols) were evidenced.

FTIR spectra of all the prepared nanochitosan derivatives showed the characteristic bands. It can be seen that the characteristic absorption bands were seen between the wavenumbers 1590 cm^{-1} and 1648 cm^{-1}, confirming the formation of imine linkage during crosslinking. The characteristic band at 1660 cm^{-1} almost disappeared representing a decrease in $-NH_2$ group content which indicates that the amino groups in chitosan reacted with the aldehyde to form Schiff bases.[27]

It is well accepted that spectral shifts of small magnitude provide a useful criterion for miscibility and also give the information about the nature of the specific interaction in a variety of blends.[28] In the nanochitosan blends, the C=O stretching band were shifted to lower wave number, also during mixing the polymers, the inter molecular hydrogen bonds, which formed was confirmed by the shift of OH vibration band from around 3434 to 3299 cm^{-1}, therefore some specific interactions could be expected to be formed between the molecules.

Moreover, on comparing with FTIR spectra of pure nanochitosan, with the absorption bands of NCS/PU/PPG blends in the presence of glutaraldehyde and absence of crosslinker, the peaks get broadened due to hydrogen bonding. Also, the intensity of CH_2 stretching and C-O stretching gets increases when compared with the blends in the absence of glutaraldehyde. This confirms the incorporation of crosslinker in the biocomposites for improving the properties of NCS/PU/PPG blends.

X-RAY DIFFRACTION STUDIES

XRD is a nondestructive technique. It is widely applied to identify crystalline phases and orientation, and even measure thickness of thin films and multi-layers. In polymers, this information is very useful to determine the percent crystallinity of the polymer or the effect of crystalline materials on an amorphous polymer.

This technique is the best and accurate analytical method which is mainly used to understand the skeleton structures in semi crystalline polymers like thermoplastics, thermoplastic elastomers and liquid crystalline polymers.[29] The X-ray diffraction analysis is used to determine the structure, complexation and crystallization of the polymer matrix.[30,31] XRD analysis were carried out to determine the nature of materials wheather the material is amorphous or crystalline.[32]

CRYSTALLINITY

The degree of crystallinity of a Sample measures the ratio of the crystalline part and the amorphous part.[33]

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

Xc = Degree of crystallinity.
Ac = Crystalline area on the X-ray diffraction peak.
Aa = Amorphous area on the X-ray diffraction peak.

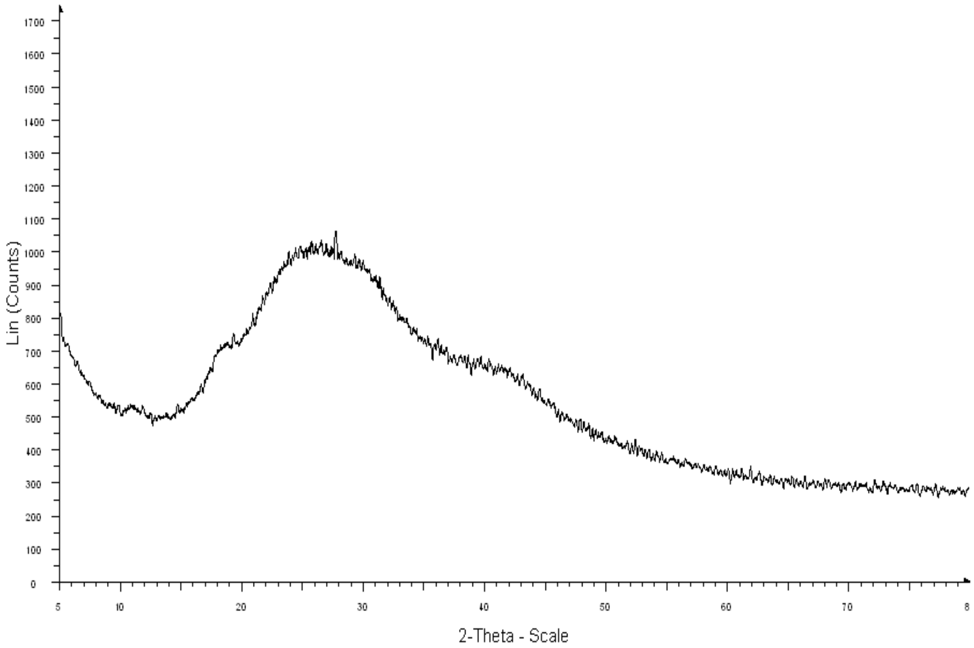

Figure-8: X Ray Diffractogram pattern of nanochitosan

The XRD pattern of nanochitosan obtained using ionic gelation technique is shown in (Fig –8). The X-ray diffractogram of nanochitosan shows a broad peak at around 2θ=28º, showing that the crystal structure of chitosan was decreased after crosslinking with sodium tripolyphosphate. The broadening of the peaks is due to the deformation of the crystalline regions by the increased packing of chitosan chains by ionic crosslinking.[34] Chitosan nanoparticles are comprised of a dense network structure of interpenetrating polymer chains crosslinked to each other by TPP counter ions was confirmed using this XRD results. [35]

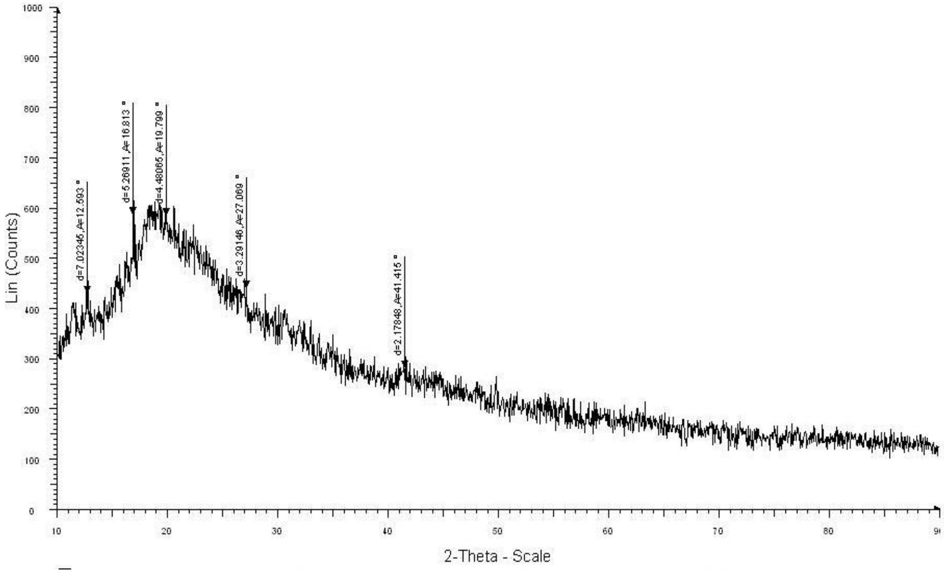

Figure - 9: X Ray Diffractogram pattern of NCS/PU/PPG-(1:1:1) ternary blend

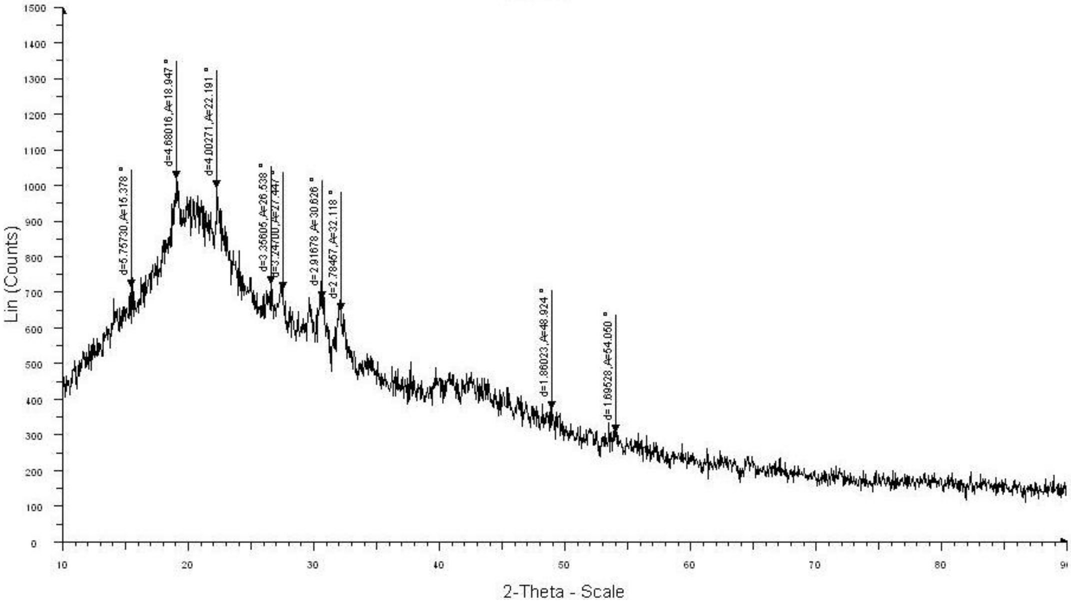

Figure - 10: X Ray Diffractogram pattern of NCS/PU/PPG-glu(1:1:1) ternary blend

(Fig – 9 and 10) shows the XRD pattern of NCS/PU/PPG ternary blend prepared in 1:1:1 ratio in the absence and presence of glutaraldehyde. The X-ray diffractogram shows peaks at various 2θ values such as 19°, 20° and 42° respectively, showing number of crystalline forms of the blend. The characteristic peak of NCS/PU/PPG ternary blend prepared in 1:1:1 ratio show variation in intensity suggesting that ordering of polymer crystallinity was disturbed due to the interactions between the functional groups present in Nanochitosan (NCS)/ Polyurethane (PU)/ Polypropylene glycol (PPG). Also, on comparing the % degree of crystalanity values, the degree of crystalanity is less for the ternary blend prepared in the presence of glutaraldehyde.

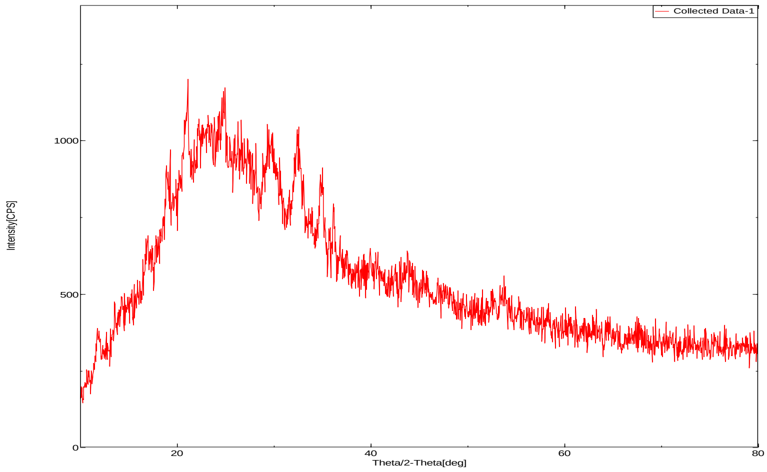

Figure 11: X Ray Diffractogram pattern of NCS/PU/PPG - (1:2:1) ternary blend

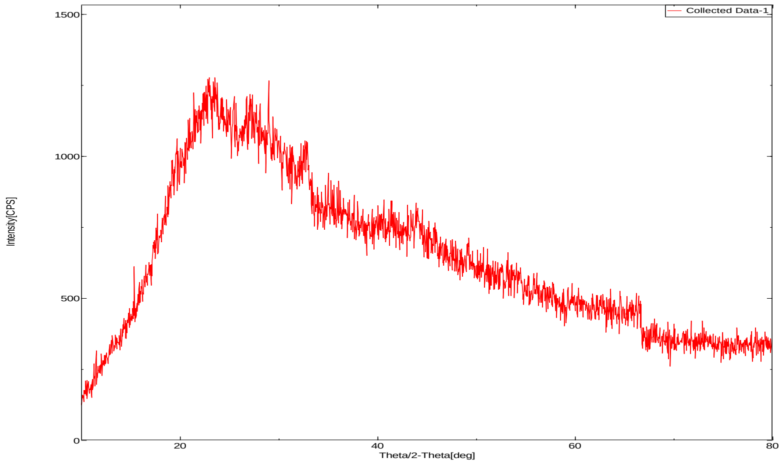

Figure 12: X Ray Diffractogram pattern of NCS/PU/PPG-glu (1:2:1) ternary blend

The XRD pattern of NCS/PU/PPG ternary blend prepared in 1:2:1 ratio the absence and presence of glutaraldehyde is shown in (Fig – 11 and 12). The XRD pattern shows respective 2θ values at 22°, 30° and 32°. These peaks indicate that new crystalline forms were introduced during blend formation compared with nanochitosan. The diffractogram of the NCS/PU/PPG (1:2:1) blend showed certain peaks with diminished intensities indicating that it might have undergone amorphization during the preparation.[36]

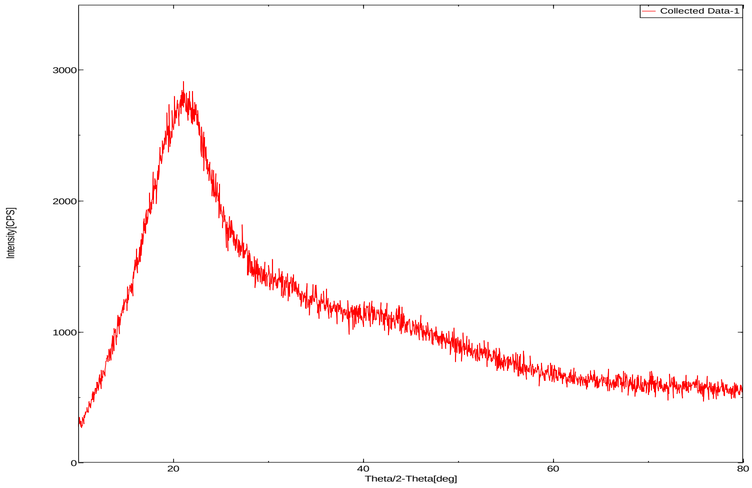

Figure - 13: X Ray Diffractogram pattern of NCS/PU/PPG-(1:1:2) ternary blend

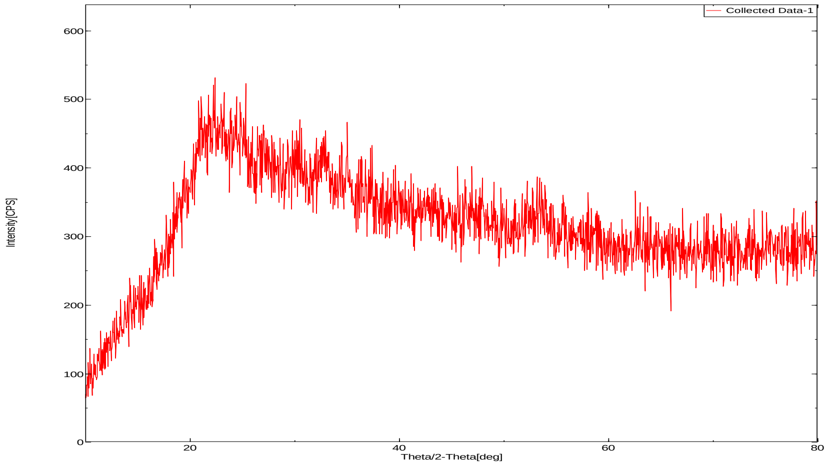

Figure – 14: X Ray Diffractogram pattern of NCS/PU/PPG-glu(1:1:2) ternary blends

The XRD pattern of NCS/PU/PPG ternary blend prepared in 1:1:2 ratio in the absence and presence of glutaraldehyde is shown in (Fig – 13 and 14). The peak at 2θ= 22º for NCS/PU/PPG ternary blend prepared in the presence of glutaraldehyde, conclude that more poor crystalline state or amorphous forms were introduced when compared to the uncross linked NCS/PU/PPG blend. However, not all diffraction planes were clearly identified due to the initial peak overlap and hybridization resulting due to molecular interactions between Nanochitosan / polyurethane / Polypropylene glycol.

The above obtained results indicated that there was an effective crosslinking, molecular miscibility and also certain interaction takes place between the polymers and crosslinking agent.[37]

Table 1: X Ray diffractogram details of NCS/PU/PPG ternary blends

Samples	2θ	% of crystallinity
Nanochitosan	28°	4.64
Nanochitosan/polyurethane/Polypropylene glycol (1:1:1)	19°	7.32
Nanochitosan/polyurethane/Polypropylene glycol (1:1:1)-glu	20° and 24°	4.11
Nanochitosan/polyurethane/Polypropyleneglycol (1:2:1)	20°, 30° and 32°	6.92
Nanochitosan/polyurethane/Polypropylene glycol (1:2:1)-glu	22° and 30°	5.79
Nanochitosan/polyurethane/Polypropylene glycol (1:1:2)	22°	6.81
Nanochitosan/polyurethane/Polypropylene glycol (1:1:2)-glu	22°	5.26

On comparing the XRD pattern of pure chitosan nanoparticles with various ratios of ternary blends (Table-1 and Fig-8 – 14) the XRD pattern of NCS/PU/PPG ternary blend shows shift in 2θ values. Thus, decreasing the crystallinity results in an improvement in metal ion sorption properties. [38]

Major peaks, appearing at various 2θ values in NCS/PU/PPG blend gave less intense diffraction pattern suggesting that it was less crystalline. The less intense peak and less crystalline entity (more amorphous) obtained in NCS/PU/PPG film might be due to the destruction of the packing of the polymers by the strong interaction which occurred between the Nanochitosan(NCS)/ Polyurethane(PU)/ Polypropylene glycol(PPG).[39]

The crystallinity of prepared ternary blend is a key-parameter in the accessibility to internal sites for both metal ions and water region as a result of the loss of crystallinity due to the loss of hydrogen bonding,[40] which demonstrated that the conjugation suppressed the crystallization to some extent. The intermolecular interactions between the polymers limited the molecular movement of matrix. Hence, crystallinity had decreased to a great extent making the blend as an efficient adsorbent.

On looking at the XRD pattern of NCS/PU/PPG- glu (1:1:1) ternary blend at long last presumed that the prepared blend is the one that has cross connecting operator's glutaraldehyde which was found to have amorpous nature.

ANTIBACTERIAL ACTIVITY

Novel scientific strategies for the valuating of the prepared ternary blends with biological activity needed the introduction of wide-scale screening programs. During the past decade, life-threatening infectious diseases caused by gram positive and gram-negative pathogenic bacteria have increased to an alarming level around the world. The antimicrobial activity limits or prevents microbial growth by extending the lag period and reducing the growth rate or decreasing live count of microorganisms.[41] Most microbes are harmful and can cause numerous disease infections such as diarrhea, respiratory illness, whooping cough and fever. [42]

The antimicrobial activity limits or prevents microbial growth by extending the lag period and reducing the growth rate or decreasing live count of microrganisms. Antimicrobial samples were required to prevent microbial growth in food for food packaging industry, wound dressing in medical devices and clothing in textile industry and footwear industry.[43] The primary goals of acne treatment are resolution of inflammatory lesions, prevention of future comedo formation, and prevention of persistent inflammation.[44] Therefore, for acne therapy, agents with both antimicrobial and anti-inflammatory properties are highly effective. As chitosan has demonstrated antimicrobial activity against various pathogens, including *Staphylococcus aureus* [45,46] and *E. coli*. [47,48]

In addition, chitosan may enter the nuclei of bacteria and fungi and inhibit mRNA and protein synthesis by binding to microbial DNA.[49,50] When nanoscaled, chitosan has a higher surface-to-volume ratio, translating into higher surface charge density, increased affinity to bacteria and fungi, and greater antimicrobial activity.

The bioefficacy of the NCS/PU/PPG-glu ternary blends prepared in various ratios were tested against bacteria such as *Staphylococcus aureus* as a Gram positive model bacterium and *E.coli* as a Gram negative model bacterium. For the Disc diffusion method, the bacterial suspension prepared was inoculated onto the entire surface of a Mueller-Hinton agar (MHA) plate (pH 5.9) with a sterile cotton-tipped swab to form an even lawn. And the sample was placed on the surface of each MHA plate using a sterile pair of forceps. The plates were incubated aerobically at 37 °C for 24 hours. The diameter of inhibition zone was measured after 24 hours incubation using a ruler or caliper.

The antibacterial activities of the prepared ternary polymeric samples NCS/PU/PPG with and without gluteraldehyde prepared in various ratios are predicted from the diameter of zone of inhibhition values measured in mm. The zone of inhibition values of the prepared ternary polymeric sample against the growth of selected bacteria is given in (Table –2 and Fig – 15 and 16) .

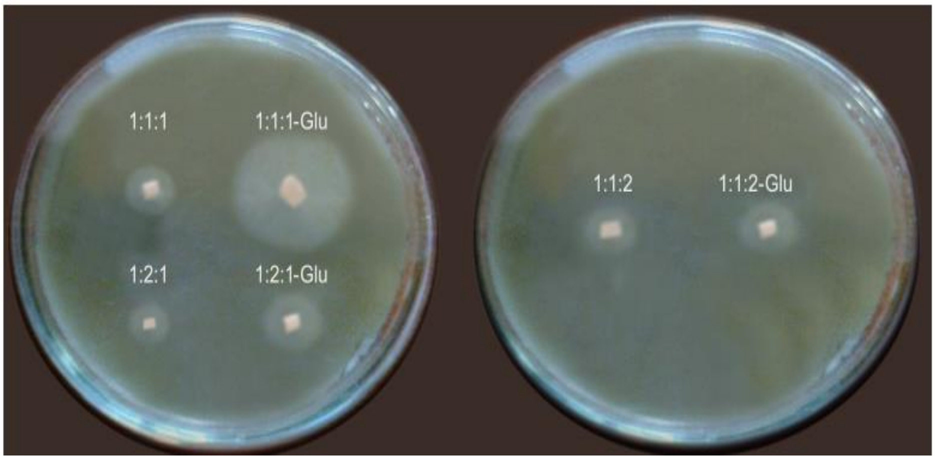

Figure -15: Antibacterial activity of NCS/PU/PPG with and without gluteraldehyde prepared in various compositions against *Bascillus Subtilis*

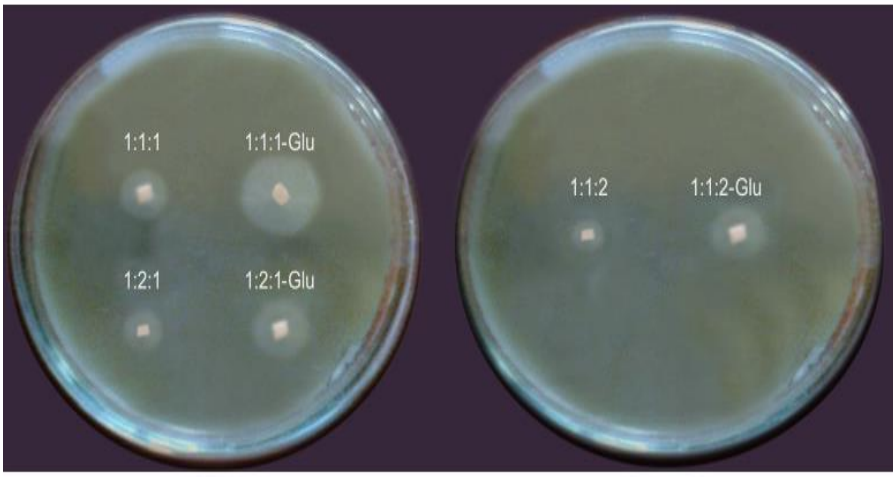

Figure -16: Antibacterial activity of NCS/PU/PPG with and without gluteraldehyde prepared in various compositions against *E.Coli*

Table – 2: Antibacterial activity data for the NCS/PU/PPG with and without gluteraldehyde

Organism (Bacteria)	**Diameter of zone of Inhibition (mm)**						
	NCS/ PU/ PPG (1:1:1)	**NCS/PU/ PPG-glu (1:1:1)**	**NCS/ PU/ PPG (1:2:1)**	**NCS/PU/ PPG-glu (1:2:1)**	**NCS/ PU/ PPG (1:1:2)**	**NCS/PU/ PPG-glu (1:1:2)**	**Control Ciproflaxicin**
Bascillus subtilis	12	17	9	13	9	11	17.0±0.6
E. coli	11	19	9	10	9	11	15.4±0.6

From the measured values of zone of inhibhition results (Table –2 and Fig – 15,16)**,** it was evident that all the NCS/PU/PPG-glu ternary blends prepared in various ratios shows a good antibacterial activity against both the *Bascillus subtilis* and *E. coli*, the bacterial species under study.

LiFeng Qi et al., (2004) reported that the nanochitosan exhibited higher antibacterial activity than chitosan itself.[51] The reason behind this may be due to the fact that the negatively charged surface of the bacterial cell wall interacts effectively to a greater degree with the polycationic nanochitosan (NH_3^+) and hinder the growth of the microorganism.[52] This interaction caused disruption on the microbial cells, which then changed their metabolism and led to cell death. [53]

Moreover, the antibiotic property of the nanoparticles ternary blend against the gram positive was much more pronounced than against the gram negative. It is clear from the experiment that Gram-positive bacteria exhibited the most susceptibility to the ternary blend in comparison to gram negative. This is due to a ternary blend, which may be a result of their cell wall plasmolysis or the separation of cytoplasm from their cell wall.[54]

Hence, based on the reports the NCS/PU/PPG blends prepared with and without gluteraldehyde in various ratios, the blend prepared in the ratio 1:1:1 posseses higher antibacterial activity towards both *Bascillus Subtilis* and *E.Coli*.

ANTIFUNGAL ACTIVITY

Basically, the antifungal activity is contributed by the polycationic nature of chitosan. Therefore, chitosan exhibits natural antifungal activity without the need of any chemical modification.[55]

There are three mechanisms proposed as the inhibition mode of chitosan. In the first mechanism, plasma membrane of fungi is the main target of chitosan. The positive charge of chitosan enables it to interact with negatively charged phospholipid components of fungi membrane. This will increase the permeability of membrane and causes the leakage of cellular contents, which subsequently leads to cell death. [56,57]

For the second mechanism, chitosan acts as a chelating agent by binding to trace elements, causing the essential nutrients unavailable for normal growth of fungi.[58] Lastly, the third mechanism proposed that chitosan could penetrate cell wall of fungi and bind to its DNA. This will inhibit the synthesis of mRNA and, thus, affect the production of essential proteins and enzymes.[59]

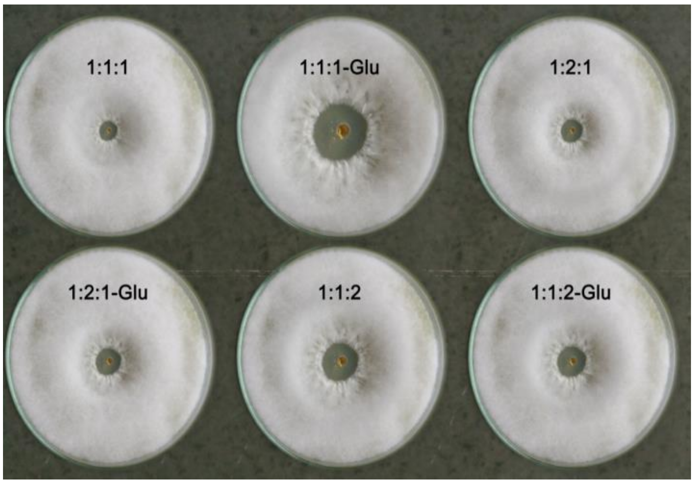

Figure- 17: Antifungal activity data for the NCS/PU/PPG with and without gluteraldehyde on *Aspergillus niger*

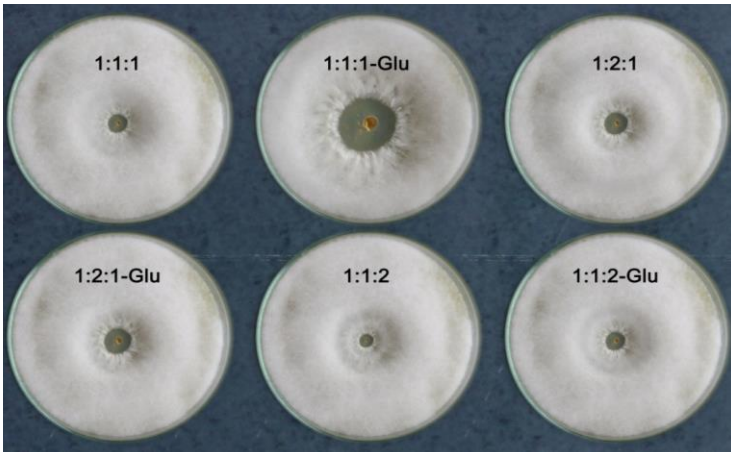

Figure -18: Antifungal activity data for the NCS/PU/PPG with and without gluteraldehyde on *Aspergillus flavus*

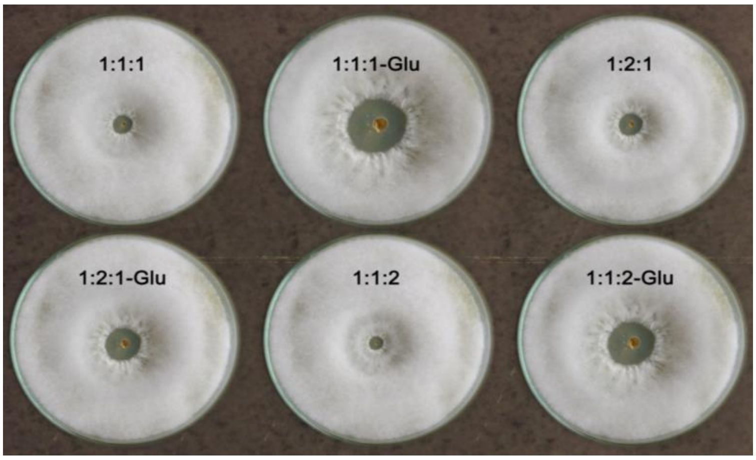

Figure -19: Antifungal activity data for the NCS/PU/PPG with and without gluteraldehyde on *Penicillium notatum*

Table –3: Antifungal activity data for the NCS/PU/PPG with and without gluteraldehyde

Microorganisms	Zone of inhibition (mm)		
	Aspergillus niger	*Aspergillus flavus*	*Penicillium notatum*
NCS/PU/PPG (1:1:1)	1.3	1.5	1.5
NCS/PU/PPG-glu (1:1:1)	2.9	3	3
NCS/PU/PPG (1:2:1)	1.3	1.5	1.5
NCS/PU/PPG-glu (1:2:1)	2	2	2
NCS/PU/PPG (1:1:2)	1	1.3	1.5
NCS/PU/PPG-glu (1:1:2)	2	1.9	2.5

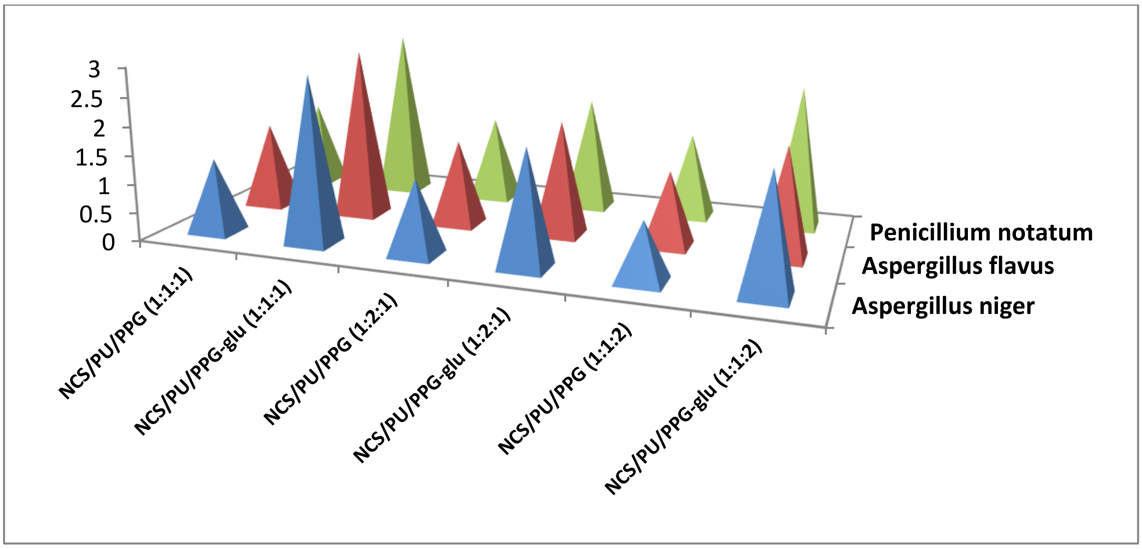

Figure -20: Antifungal activity data for the NCS/PU/PPG with and without gluteraldehyde

Chitosan has been proven to have antifungal activity, and therefore it has attracted a great attention from many researchers and proved that the effectiveness of chitosan did not depend solely on the chitosan formulation, but also on the type of fungus.[60]

A study carried out by Ma and Lim, reported that cellular uptake of chitosan nanoparticles into cells was higher than that of chitosan molecules as the bulk chitosan molecules were located extracellularly. This suggested that chitosan nanoparticles might be able to diffuse into fungal cell and hence disrupt the synthesis of DNA as well as RNA. This could explain a better antifungal activity of chitosan nanoparticles compared to its free polymer or solution form. [61]

A better antifungal activity by the parent compound was correlated with that of water insolubility of chitosan which precipitates and stacks on the microbial cell surface as the physiological pH in microbial cells is around neutral. The formation of impermeable layer will block the channels on the cell surface and hence prevent the transportation of essential nutrients which are crucial for survival of microbial cells. Contrary to that, the water-soluble chitosan derivatives are unable to form such layer, and therefore they exert no antimicrobial activity.

Particle surface charge plays a role in the inhibitory effect of chitosan nanoparticles by contributing a positive charge to improve the interaction between nanoparticles and negatively charged microbial cell surface.[62] This in turn alters fungi cell membrane permeability which eventually induces leakage of intracellular material and coincides with the previous reported study which showed that chitosan particles would only inhibit microbial growth when they were positively charged.[63]

In order to confirm antibacterial activity and antifungal activity against pathogenic bacteria and fungi such as *Aspergillus niger, Aspergillus flavus, Penicillium notatum* on agar plates the samples, NCS/PU/PPG (1:1:1), NCS/PU/PPG (2:1:1), NCS/PU/PPG (1:1:2), NCS/PU/PPG-glu (1:1:1), NCS/PU/PPG-glu (2:1:1) and NCS/PU/PPG-glu (1:1:2) were exposed to the selected bacteria and fungi (Table-3, and Fig-17-20).

For both antibacterial and antifungal activities the prepared solid samples were directly placed on the agar plates and the significant activities of the samples were compared. The findings of this study show that polymeric surfaces can be effectively modified and converted to be antimicrobial. When nanoscaled, chitosan and its blends will have a higher surface-to-volume ratio, translating into higher surface charge density, increased affinity to bacteria and fungi, and greater antimicrobial activity. [64]

CONCLUSION

The results of this study showed the potent anti-microbial effect of of the prepared ternary blends of various ratios NCS/PU/PPG (1:1:1) (1:2:1) (1:1:2) with and without crosslinking against *Bacillus subtilis, Escherichia coli*. Similarly, in all series, sample exhibited better antifungal activity against *Aspergillus flavus, Aspergillus niger and Penicillium notatum* also can be used for future application in antimicrobial therapy.

REFERENCES:

[1] Pandey S., Ramontja J., Sodium alginate stabilized silver nanoparticles – silicaanohybrid and their antibacterial Characteristics. Int J Biol Macromol., 93(Pt A):712-723, (2016).

[2] Angebault C., Andremont A., Antimicrobial agent exposure and the emergence and spread of resistant microorganisms: issues associated with study design. Eur J Clin Microbiol., 32: 581-595, (2013).

[3] Chen Y. and Chou C. (2005). Factors affecting the susceptibility of Staphylococcus aureus CCRC 12657 to water soluble lactose chitosan derivative. Food Microbiol, 22, 29, (2005).

[4] Roller S. and Covill N., The antimicrobial properties of chitosan in mayonnaise and mayonnaise-based shrimp salads. J. Food Prot, 63: 202 (2000).

[5] Sudarshan N. Hoover D. and Knorr D. (1992). Antibacterial action of chitosan. Food Biotechnol. 6, 257, (1992).

[6] Kompella U.B., Subconjunctival nano- and microparticles sustain retinal delivery of. budesonide, a corticosteroid capable of inhibiting VEGF expression. Invest Ophthalmol Visual Sci, 44: 1192- 201, (2003).

[7] Zheng Y., Lv H., Wang Y., Lu H., Qing L., Xi T., Performance of novel bioactive hybrid hydrogels in vitro and in vivo used for artificial cartilage. Biomed Mater, 4:15015, (2009).

[8] Thandapani Gomathi., Govindarajan C., Rose H., Maximas H.R., Sudha P.N., Mohamed Imran P.K., Jayachandran Venkatesan, and Se- Kwon Kim. Studies on drug-polymer interaction, in vitro release and cytotoxicity from chitosan particles excipient. Int. j. pharm, 468: 1- 2, (2014).

[9] Moawed E.A., Sorption behaviour, diffusion mechanism of the iron metal ions in the environmental samples onto polyurethane foam. Anal. Chem. Indian J, 10: 93–100, (2011).

[10] Pawlak A and Mucha M., Thermogravimetric and FTIR studies of Chitosan blends. ThermochimicaActa, 396.153-166, (2003).

[11] Almedia E.V.R., Frollini E., Castellan A., Coma V., Chitosan, sisal cellulose, and biocomposite chitosan/sisal cellulose flims prepared from thiourea/NaOH aqueous solution. Carbohydrate polymers, (80), 655-664, (2010).

[12] Anna Regiel-Futyra., Małgorzata Kus-Liśkiewicz., Victor Sebastian., Silvia Irusta., Manuel Arruebo., Grażyna Stochel and Agnieszka Kyzioł.,. Development of Noncytotoxic Chitosan–Gold Nanocomposites as Efficient Antibacterial Material ACS. Appl Mater Interfaces. 21, 7(2): 1087–1099, (2015).

[13] Helander I., Nurmiaho-Lassila E., Ahvenainen R. Rhoades J. and Roller S., Chitosan disrupts the barrier properties of the outer membrane of gram-negative bacteria. Int. J. Food Microbiol, 71, 235, (2001).

[14] Morimoto M., Saimoto H., Usui H., Okamoto Y., Minami S., and Shigemasa Y., (2001). Biological activities of carbohydrate-branched chitosan derivatives. Biomacromolecules, 2, 1133, (2001).

[15] Saranya M., Srinivasan Latha., Gopal Reddi M.R., Gomathi T., Sudha P.N., and Sukumaran Anil., Adsorption Studies of Lead (II) from aqueous solution onto Nanochitosan /Polyurethane /Polypropylene glycol ternary blends. International Journal of Biological Macromolecules, 1436-1448, (2017).

[16] Colleen Gorey., Isabel C., Escobar, N-isopropylacrylamide (NIPAAM) modified cellulose acetate ultrafiltration membranes. J. Membr. Sci, 383,272–279, (2011).

[17] Ping Qu., Huanwei Tang., Yuan Gao., Li-ping Zhang., Siqun Wang., Polyether sulfone composite membrane blended with cellulose fibrils, BioResource., 5, 2323–2336, (2010).

[18] Emmanuel Chinedum Ibezim., Cristina Tristao Andrade., Cristina Marcia., Bianca Barretto., Damian Chukwu Odimegwu., Felipe Forte De Li., Malonically Cross-linked Chitosan/TPP Microparticles for the Controlled Delivery of Pyrimethamine. Ibnosina J.Med. B.S., 3(3), 77-88, (2011).

[19] Lee J. Y. Nam S. H., Im S. Y., Park Y. J., Lee Y. M., Seol Y. J., Chung C. P., Lee S. J., Enhanced bone formation by controlled growth factor delivery from chitosan-based biomaterials. J. Control. Release, 78, 187–97, (2002).

[20] Hosseini S.F, Zandi M., Rezaei M., and Farahmandghavi F., Two-step method for encapsulation of oregano essential oil in chitosan nanoparticles: preparation, characterization and in vitro release study. Carbohydr. Polym., 95 (1), 50–56, (2013).

[21] Milosavljevi N.B., Lj M Kljajevi Popovi I.G., Filipovi J.M. and Kalagasidis Krusi M.T. (2010). Chitosan, itaconic acid and poly (vinyl alcohol) hybrid polymer networksof high degree of swelling and good mechanical strength. Polym. Int, 59, 686–694, (2010).

[22] Wan Ngah W.S., and Fatinathan S., Adsorption characterization of Pb(II) and Cu(II) ions onto chitosan-tripolyphosphate beads: Kinetic, equilibrium andthermodynamic studies. J. Environ. Manage, 91, 958–969, (2010).

[23] Sankalia M.G. Mashru R.C. Sankalia J.M. Sutariya V.B., (Reversed chitosan-alginate polyelectrolyte complex for stability improvement of alpha-amylase: optimization and physicochemical characterization. Eur J Pharm Biopharm, 65: 215–232, (2007).

[24] Adavan Kiliyankil Vipin., Baiyang Hu., Bunshi Fugetsu., Prussian blue caged in alginate/calcium beads as adsorbents for removal of cesium ions from contaminated water. Journal of Hazardous Materials, 258– 259, 93– 101, (2013).

[25] Lee J. Y., Nam S. H., Im S.Y., Park Y. J., Lee Y. M., Seol Y. J., Chung, C. P., Lee S. J., Enhanced bone formation by controlled growth factor delivery from chitosan-based biomaterials. J. Control. Release, 78, 187–97, (2002).

[26] Vedia Nuket Tirtom., Ays Dincer., Seda Becerik., Tulin Aydemir and Ali Celik., Comparative adsorption of Ni(II) and Cd(II) ions on epichlorohydrin crosslinked chitosan–clay composite beads in aqueous solution. Chem. Eng. J., 197, 379–386, (2012).

[27] Riham R., Mohamed Fekry A.M., Antimicrobial and Anticorrosive Activity of Adsorbents Based on Chitosan Schiff's Base. Int J Electrochem Sci, 6, 2488-2508, (2011).

[28] Panya Wongpanit., Yasuhiko Tabata., Ratana Rujiravanit., Miscibility and biodegradability of silk fibroin/carboxymethyl chitin blend films. Macromolecular Bioscience, 7(12), 1258–1271, (2007).

[29] Raghunadh Acharyulu S., Gomathi T. and Sudha P. N. (2013). Physicochemical characterization of cross-linked chitosan-polyacrylonitrile polymer blends, Der Pharmacia Lettre, 5 (2), 354-363.

[30] Pradhan D.K. Samantaray B.K. Choudhary R.N.P., Thakur A. K., Effect of Plasticizer on structure- property relationship in composite polymer electrolytes. Journal of Power Sources, 139, 384-393, (2005).

[31] Rajendran S., Sivakumar M. and Subadevi R. Investigations on the effect of the various plasticizers in PVA–PMMA solid polymer blend electrolytes. Materials Letter, 58(5) 641–649, (2004).

[32] EraricarSalleh., Ida IdayuMuhamad., and NozieannaKhairuddin., "Structural Characterization and Physical Properties of Antimicrobial (AM) Starch-Based Films", World Academy of Science, Engineering and Technology 55, (2009).

[33] Wu F. C., Tseng R.L., Juang R.S., Kinetics of colour removal by adsorption from water using activated clay. Environmental Technology, 22(6): 721-729, (2001).

[34] Martinez-Camacho A.P., Cortez-Rocha M.O., Ezquerra-Brauer J.M., Graciano-Verdugo A.Z., Rodriguez-Felix F., Castillo-Ortega M.M., Yepiz-Gomez M.S. and Plascencia-Jatomea M., Chitosan composite films: Thermal, structural, mechanical and antifungal properties. Carbohydr. Polym., 82(2), 305–315, (2010).

[35] Tang C., Zhang Q., Wang K., Fu Q. and Zhang C., Water transport behavior of chitosan porous membranes containing multi-walled carbon nanotubes (MWNTs). J. Membr. Sci., 337, 240–247, (2009).

[36] Vijayalakshmi K., Gomathi T. and Sudha P. N. Preparation and characterization of nanochitosan/sodium alginate/ microcrystalline cellulose beads. Der Pharmacia Lettre, 6 (4): 65-77, (2014).

[37] Zhang X., Yang D., Nie J., Chitosan/polyethylene glycol diacrylate films as potential wound dressing material. Int. J. Biol. Macromol., 43, 456, (2008).

[38] Jaworska M., Kula K., Chassary P., Guibal E., . Influence of chitosan characteristics on polymer properties: II. Platinum sorption properties. Polymer International, 52, 206–212, (2003).

[39] Elmotasem H. Chitosan–alginate blend films for the transdermal delivery of meloxicam Chitosan–alginate blend films of meloxicam. Asian J. Pharm. Sci., 3 (1), 12-29. Formation of ionic polysaccharide gels, Patent WO 99/07416, 2008.

[40] Zong Z., Kimura Y., Takahashi M., and Yamana H. (2000). Characterization of chemical and solid-state structures of acylatedchitosans. Polymer, 41: 899, (2000)

[41] Han J. H., Antimicrobial food packaging. Food Technol, 54: 56–65, (2000).

[42] Adegboye M.F., Babalola O.O and Akinpelu D.A., Issues of resistance of pathogens to antimicrobial agents. Scientific research and essays, 7(41): 3468-3478, (2012).

[43] Pielesz A., Machnicka A., Sarna E., Antibacterial activity and scanning electron microscopy (SEM) examination of alginate-based films and wound dressings. Ecol. Chem. Eng. S, 18: 2. (2011).

[44] Lee L., The clinical spectrum of neonatal lupus. Arch Dermatol Res., 301:107–110, (2009)

[45] Jia Z., shen D., Xu W., Synthesis and antibacterial activities of quaternary ammonium salt of chitosan. Carbohydr Res, 333:1–6, (2001).

[46] Qi L, Xu Z, Jiang X, Li Y, Wang M. (2005). Cytotoxic activities of chitosan nanoparticles and copper-loaded nanoparticles. Bioorg Med Chem Lett. ;15:1397–1399

[47] Chen Y-M., Chung Y-C., Wang L-W et al., Antibacterial properties of chitosan in waterborne pathogen. J Environ Sci Health A Tox Hazard Subst Environ Eng, 37:1379–90,(2002).

[48] Sanpui P., Murugadoss A., Prasad PV et al., The antibacterial properties of a novel chitosan-Ag-nanoparticle composite. Int J Food Microbiol, 124:142–6, (2008).

[49] Blecher K., Nasir A., Friedman A., The growing role of nanotechnology in combating infectious disease Virulence 2:395–401, (2011).

[50] Ma Y., Zhou T., Zhao C., Preparation of chitosan-nylon-6 blended membranes containing silver ions as antibacterial materials. Carbohydr Res, 343:230–7,(2008).

[51] Lifeng Qi., Zirong Xu., Xia Jiang., Caihong Hu and Xiangfei Zo., Preparation and antibacterial activity of chitosan nanoparticles. Carbohydrate Research, 339: 2693–2700, (2004).

[52] Zheng L.Y., and Zhu J.F., Study on antimicrobial activity of chitosan with different molecular weights. Carbohydr. Polym, 54, 527–530, (2003).

[53] Leceta I., Guerrero P., Ibarburu I., Dueñas M.T. and de la Caba K. Characterization and antimicrobial analysis of chitosan-based films. Journal of Food Engineering, 116, 889–899, (2013).

[54] Nathanael AJ., Hong SI Mangalaraj D., Ponpandian N., Chen PC., Template-free growth of novel hydroxyapatite nanorings: formation mechanism and their enhanced functional properties Crystal Growth & Design. 12 (7), 3565-3574, (2012).

[55] Ziani K., Fernández-Pan I., Royo M., and Mate J. I., (2009). Antifungal activity of films and solutions based on chitosan against typical seed fungi. Food Hydrocolloids, vol. 23, no. 8, pp. 2309–2314, (2009).

[56] García-Rincón J. Vega-Pérez J. Guerra-Sánchez M. G. Hernández-Lauzardo A. N. Peña-Díaz A. and Velázquez-Del Valle M. G., Effect of chitosan on growth and plasma membrane properties of Rhizopus stolonifer (Ehrenb.:Fr.) Vuill. Pesticide Biochemistry and Physiology, vol. 97, no. 3, pp. 275–278, (2010).

[57] Liu H., Du Y., Wang X., and Sun L. Chitosan kills bacteria through cell membrane damage. International Journal of Food Microbiology, 95(2): 147–155, (2004).

[58] Roller S. and Covill N. The antifungal properties of chitosan in laboratory media and apple juice. International Journal of Food Microbiology, 47(1-2): 67–77, (1999).

[59] Kong M., Chen X. G., Xing K., and Park H. J., Antimicrobial properties of chitosan and mode of action: a state of the art review. International Journal of Food Microbiology, 144(1). 51–63, (2010).

[60] Ling Yien Ing, Noraziah Mohamad Zin, Atif Sarwar, and Haliza Katas. Antifungal Activity of Chitosan Nanoparticles and Correlation with Their Physical Properties. International Journal of Biomaterials, Article ID 632698, 9 pages, (2012).

[61] Ma Z, Lim LY. Uptake of chitosan and associated insulin in Caco-2 cell monolayers: a comparison between chitosan molecules and chitosan nanoparticles. Pharm Res, 20(11) 1812-9, (2003).

[62] Wiarachai O. Thongchul N. Kiatkamjornwong S. and Hoven V. P. Surface-quaternized chitosan particles as an alternative and effective organic antibacterial material. Colloids and Surfaces B, 92, 121–129, (2012).

[63] Vallapa N. Wiarachai O. Thongchul N. et al., Enhancing antibacterial activity of chitosan surface by heterogeneous quaternization. Carbohydrate Polymers, vol. 83, no. 2, 868–875, (2011).

[64] Qi L, Xu Z, Jiang X, Li Y, Wang M. Cytotoxic activities of chitosan nanoparticles and copper-loaded nanoparticles. Bioorg Med Chem Lett, 15: 1397–1399, (2005).