



RESEARCH ARTICLE

PHOTOCROSSLINKING AND ANTIMICROBIAL ACTIVITY OF NATURALLY OCCURRING POLYMER  
POLY N- ACETYL D- GLUCOSAMINE

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ABSTRACT

Photocrosslinkable polymers have attracted considerable attention for their application in surface coatings, printings, printing plates; photo resists optical data storage high thermal stability and superior chemical resistance. In the present work poly N- acetyl D-glucosamine is taken and dissolved in DMAc. For the spectral characterization UV-visible spectroscopy and IR – Infrared spectroscopy were used. photocrosslinking studies was carried out using UV lamp. Antimicrobial studies were carried out and assayed against four species of clinically relevant bacteria Escherichia coli, Bacillus cereus, Klebsiella pneumoniae and Streptococcus faecalis and antifungal activity against three species, Mucor sps, Aspergillus Flavus, and Aspergillus niger.

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INTRODUCTION

Photo cross linkable polymers have attracted considerable attention for their application in surface coatings, printings, printing plates, photo resists optical data storage high thermal stability and superior chemical resistance [1, 2]. The photo cross linkable substances which are frequently employed include derivatives of acetylene, azides, acrylate esters, arylidenes, chalcones, cinnamate ester, siloxanes, and stillbenes and vinyl ethers. Hay *et.al* [3]

Chitin and chitosan are considerably versatile and promising biomaterials. The de acetylated chitin derivative, chitosan is more useful and interesting bioactive polymer. Chitin is the second most ubiquitous natural polysaccharide after cellulose on earth and is composed of Poly N-acetyl D-glucosamine. It is often considered as cellulose derivative, even though it does not occur in organisms producing cellulose. It is structurally identical to cellulose, but it has acetamide groups (-NHCOCH<sub>3</sub>) at the C<sub>2</sub> positions. Chitin is estimated to be produced annually almost as much as cellulose. It has become of great interest not only as an under-utilized resource but also as new functional biomaterial of high potential in various and the recent progress in chitin chemistry is quite significant.

Chitin is a white, hard, inelastic, nitrogenous polysaccharide found in the exoskeleton as well as in the internal structure of invertebrates. The waste of this natural polymer is a major source of surface pollution in coastal areas. The production of chitosan from crustacean shells obtained as a food industry waste is economically feasible, especially if it includes therecovery of carotenoids. The shells contain considerable quantities of astaxanthin, a carotenoid that has so far not been synthesized, and which is marketed as a fish food additive in aquaculture.

Chitin and chitosan the naturally abundant and renewable polymers have excellent properties such as biodegradability, biocompatibility, non-toxicity, and absorption [4]. The reaction of chitosan is considerably more versatile than cellulose due to the presence of -NH<sub>2</sub> groups. Various efforts have been made to prepare functional derivatives of chitosan by chemical modification [5], graft reactions, ionic interactions, and only few of them are found to dissolve in conventional organic solvents [6]. Institute for marine resource and environment, Japan [7,8] studied mechanic-chemical preparation of a novel composite under a dry and solid state. They synthesized a new type of polysaccharide composite by ball-milling a polysaccharide with synthetic polymer.

Structure of Poly N-Acetyl D-Glucosamine

The thermal behavior and molecular motion of the synthetic polymer in the composite are entirely different from those of original one. These results suggest strong interactions between a polysaccharides and synthetic polymer and thus compatibilization of the polysaccharides and synthetic polymer. Chitin (C<sub>8</sub>H<sub>13</sub>O<sub>5</sub>N)<sub>n</sub> was first isolated and characterized from mushrooms, the earliest known polysaccharide, by a French chemist Henri Braconnot in 1811 [9]. It has been discovered to be the second most abundant natural biopolymers in the world [10], amounting in marine biomass alone to approximately 106-107 tons. Chitin is a lone-chain homo polymer of N-acetyl D-glucosamine.

Application of Chitin and Chitosan

- ❖ Industrial application
- ❖ Water engineering
- ❖ Paper industry
- ❖ Textile industry

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- ❖ Food processing
- ❖ Agriculture
- ❖ Photography
- ❖ Chromatographic separation
- ❖ Solid state batteries
- ❖ Chitosan gel for led and nlo application
- ❖ Biomedical application
- ❖ Wound dressing and wound healing
- ❖ Drug delivery system

Chitin----> Deacetylation (NaOH)----> Washing and Dewatering----> Chitosan.

## MATERIALS

(N, N-Dimethyl acetamide (DMAc) (BDH, Analar), is used as the solvent for UVspectral characterization and in photo crosslinking studies. The naturally occurring polymer poly N-acetyl D- glucosamine was purchased from Loba Chemie and used for characterization studies as received.

**Table 3** Inhibition effect of Poly N-acetyl D-glucosamine on the growth of following microorganisms (antibacterial activity)

S. No	Microorganisms	50 µg/mL	100 µg/mL
1	Escherichia coli	5 mm	6 mm
2	Bacillus cereus	4 mm	4 mm
3	Klebsiella pneumonia	4 mm	5 mm
4	Streptococcus faecalis	4 mm	5 mm

## RESULT AND DISCUSSON

### Preliminary Test

#### Solubility

For the spectral characterization regarding UV visible spectroscopy and photo crosslinking studies the polymer poly N-acetyl D-glucosamine commercially called a chitin was dissolved in DMAc. Most of the naturally occurring polysaccharides e.g., cellulose, dextrin, pectin, alginic acid, Agar, agarose and carragenas are natural and acidic in a nature, where as chitin and chitosan are examples of highly basic polysaccharides. Their properties include solubility in various media, solution viscosity, polyelectrolyte behavior, and poly oxysalt formation, ability to from films, metal chelating, optical and structural characteristics [11]. Although the properties of chitin are also present in cellulose the characteristics properties of chitin and chitosan are not shared by cellulose [12].

**Table 4** Inhibition effect of Poly N-acetyl D-glucosamine on the growth of following microorganisms (antifungal activity)

S. No	Microorganisms	50 µg/mL	100 µg/mL
1	Mucor sps	4 mm	5 mm
2	Aspergillus Flavus	4 mm	6 mm
3	Aspergillus niger	5 mm	6 mm

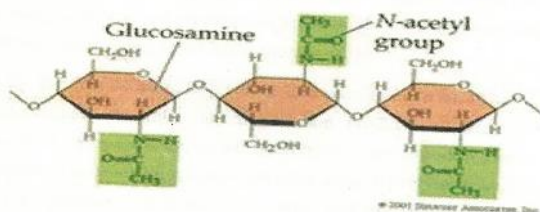
Chitin is highly hydrophobic and it is insoluble in water and most organic solvents. It is soluble in hexafluoro propanol, hexa fluoroacetone and dimethyl acetamide (DMAc) containing 5 percent lithium chloride (LiCl) [13]. Recently the dissolution of chitosan in N-methyl morpholine-N-oxide (NMMO)/H<sub>2</sub>O has been reported by Dutta et.al [14, 15]. The hydrolysis of chitin with concentrated acids under drastic condition produces relatively the pure amino sugar, D-glucosamine.

Depending on the extent of deacetylation chitin contains 5 to 8 percent (w/v) nitrogen, which is mostly in the form of primary aliphatic amino group as found in chitosan. Highly benzoylated chitin is soluble in benzyl alcohol, dimethylsulphoxide, formic acid and dichloro acetic acid. Special attention has been given to the chemical modification of chitin since it has greatest potential to be fully exploited. Reactions with pure chitin have been carried out mostly in the solid state owing to the lack of solubility in ordinary solvents 5 percent deacetylated chitin has been to be soluble in water.

## MATERIAL AND METHODS

This chapter comprises of the list of essential chemicals used in the present work for the characterization of the polymer and various analytical techniques employed for the characterization. The method employed in the study of antibacterial activity is also described briefly. Chitin and chitosan are natural resources waiting for a market. They were waste product of the crabbing and shrimp canning industry. The US department of commerce reported in 1973 that there were over 1, 50,000Mt chitin produced by processing waste from shellfish, krill, clams, oysters, squid and fungi.

(c) Chitin



Commercially chitin and chitosan are of great importance owing to their relatively high percentage of nitrogen (6.89 percent) compared to synthetically substituted cellulose. The crustacean shells mainly involve the removal of proteins and the dissolution of calcium carbonate that is present in crab shells in high concentrations. The resulting chitin is deacetylated in 40 percent sodium hydroxide at 120 °C for 1-3 this treatment produces 70 percent deacetylated chitin. The following four steps in chronological order of the process needed to produce chitosan from crustacean shells are deproteinization, demineralization, decolouration and deacetylation.

**Table 1** Assignment of characteristic frequency in IR spectra Poly N-acetyl D-glucosamine polymer (chitin)

Absorption frequency (ν) cm <sup>-1</sup>	Assignment
1150 – 1060	C-O stretch of acyclic group
1350 – 1250	O-H bend
1680 – 1440	CH <sub>2</sub> and CH <sub>3</sub> bend
1550 – 1510	N-H bend of N-acetyl group
1678 – 1630	C=O stretch of N-acetyl group

**Table 2** Absorbance values at different intervals of UV irradiation

Time (min)	Absorbance (A <sub>t</sub> )	
	At 250 nm	At 312 nm
0	1.700	0.800
5	1.600	0.700
10	1.500	0.600
15	1.400	0.550
20	1.300	0.500

Crustacean shells----> Size reduction----> Protein separation--> (NaOH)----> Washing and Dewatering Decolouration---->

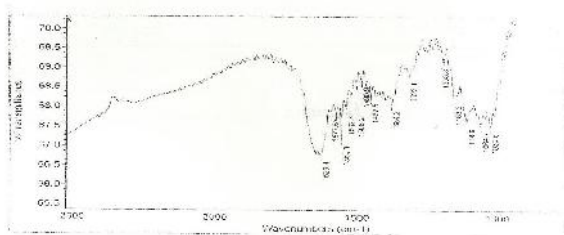


Figure - 1

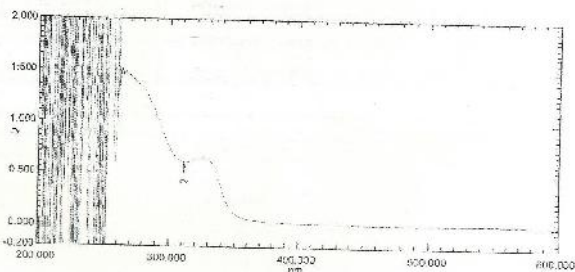


Figure - 2

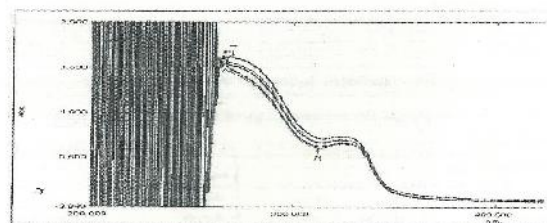


Figure - 3

Relative change in the UV Spectral intensity with irradiation Time at 250 nm and 312 nm

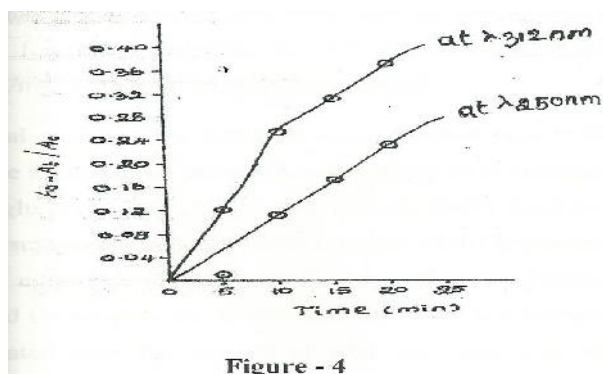


Figure - 4

This water [14] soluble form of chitin is a useful attracting material for its smooth modifications, through various reactions in solution phase.

### Spectral Characterization

#### Infrared spectroscopy

It is obvious from the spectra that the C-O Stretching -CH-O-CH- of poly N-acetyl D-glucosamine was observed around 1150 – 1060  $\text{cm}^{-1}$ . It may be noted that the strong absorption at 1550 – 1510  $\text{cm}^{-1}$  shows N – H bend of N-acetyl group and absorption band at 1629  $\text{cm}^{-1}$  shows C=O stretch of N-acetyl group. The O-H bend was observed around 1350 – 1250  $\text{cm}^{-1}$ . There is relatively intense absorption band at 1480-1440  $\text{cm}^{-1}$  attributed to the  $\text{CH}_2$  and  $\text{CH}_3$  bend of poly N-acetyl D-glucosamine. Thus a satisfactory IR spectral assignment has been carried out and the results are given in the Table 1.

#### UV-Visible spectroscopy

The ultraviolet-visible spectroscopy is a valuable tool for the identification of multiple bonded conjugated aromatic and hetero atomic systems [16, 17]. The UV spectra of the polymer chitin were recorded in DMAc solution. The spectral data of the UV irradiated poly N-acetyl D-glucosamine are presented in figure 2. It is obvious from the spectral data poly N-acetyl D-glucosamine show n- $\pi^*$  transition. Both the n- $\pi^*$  and  $\pi-\pi^*$  transitions shift to longer wavelength and the absorption maxima of the poly N-acetyl D-glucosamine at  $\lambda_{\text{max}} 312 \text{ nm}$  indicated  $\pi-\pi^*$  transition and the peak at  $\lambda_{\text{max}} 250 \text{ nm}$  show n- $\pi^*$  transition.



Escherichia coli Bacillus cereus



Klebsiella pneumoniae Streptococcus faecalis

Figure - 5

#### Photo cross linking studies by UV spectroscopy

The last few years have seen many advances in the field of organic onto electronics specifically, light emitting devices using polymers and other smaller molecules [18]. The performance of such devices has improved considerable due to optimization of material interfaces and enhancement of quantum yield. Among many photochemical reactions in polymeric materials the photo crosslinking by UV light exposure has been highlighted in many practical applications [19, 20].

The UV-Visible spectra of the chitin in DMAc solution [ $0.1 \text{ g dL}^{-1}$ ] were recorded after UV irradiation a 160 W medium-pressure mercury lamp at regular time intervals. This mercury lamp emits radiation in the range of 200 nm to 600 nm which is convenient for photo crosslinking study.

The UV absorbance change at the characteristic wavelength was monitored during UV irradiation and the decrease in absorbance at the characteristic wavelength at  $\lambda = 250 \text{ nm}$  and  $\lambda = 312 \text{ nm}$  with time indicates that there is a steady rate of photo crosslinking on irradiation. The photosensitivity of the poly N-acetyl D-glucosamine (chitin) is mainly attributed to the  $\pi$ -electron density of the photoactive chromosphere of N-acetyl group.

#### Studies on antimicrobial activity

In the study, the antibacterial activity of naturally occurring Poly N-acetyl D-glucosamine were predicted and assayed

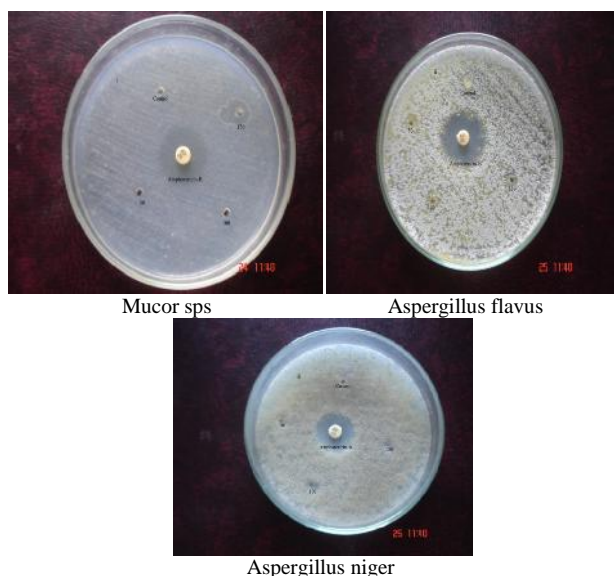


Figure 6

against four species of clinically relevant bacteria *Escherichia coli*, *Bacillus cereus*, *Klebsiella pneumonia*, *Mucor sps*, *Aspergillus Flavus*, and *Aspergillus niger*. Inhibition zones were recorded as diameter of growth free zones and the diameter of the well in millimeter (mm) for each polymer at two different concentrations of 50  $\mu$ g/mL, 100  $\mu$ g/mL, at the end of incubation period.

Antimicrobial analysis was followed using standard agar well diffusion method to study the antibacterial and antifungal activity of compounds (Perez et, al 1990 Erdemoglu et, al 2003 Bagamboula et, al 2004). Each bacterial and fungal isolate was suspended in Brain Heart Infusion (BHI) broth and diluted to approximately  $10^5$  colony forming unit (CFU) per mL. Mac Farland standard number 5 was used to compare the growth pattern of the microorganism. They were flood – inoculated on to the surface of BHI agar and then dried. Five-millimeter diameter wells were cut from the agar using a sterile cork-borer and 30  $\mu$ L (50  $\mu$ g compounds in 1 ml of solvent- DMSO) of the sample solution were poured into the wells.

The plates were incubated for 18 h at 37<sup>0</sup>C for bacteria and at room temperature for fungi. Antimicrobial activity was evaluated by measuring the diameter of the zone of inhibition in mm against the test microorganisms. DMSO was used as solvent control. Ciprofloxacin was used as reference antibacterial agent. Amphotericin B was used as reference antifungal agent. The tests were carried out in triplicate [21,22,23]. The standard antibiotic disc (Ciprofloxacin) inhibited the growth of *Escherichia coli* by 14 mm, *Bacillus cereus* by 16 mm, *Klebsiella pneumonia* by 15 mm and *Streptococcus faecalis* by 13 mm.

The zone of inhibition at different concentration against the

Test bacteria are given in Table 3.

Poly N-acetyl D-glucosamine shows inhibition against *Escherichia coli* at concentration 50  $\mu$ g/mL by 5 mm and at 100  $\mu$ g/mL by 6 mm, *Bacillus cereus* at 50  $\mu$ g/mL by 4 mm and at 100  $\mu$ g/mL by 4 mm, *Klebsiella pneumonia* at 50  $\mu$ g/mL by 4 mm and at 100  $\mu$ g/mL by 5 mm, *Streptococcus faecalis* at 50  $\mu$ g/mL by 4 mm and at 100  $\mu$ g/mL by 5 mm.

A noteworthy observation from data revealed that Poly N-acetyl D-glucosamine showed good inhibitory activity against all the tested pathogens suggesting that the presence of ether group enhances the antibacterial activity more over poly N-acetyl D-glucosamine exhibited substantial antifungal effect against three species *Mucor sps*, *Aspergillus Flavus* and *Aspergillus niger*.

Poly N-acetyl D-glucosamine shows inhibition against *Mucor sps* at 50  $\mu$ g/mL by 4 mm and at 100  $\mu$ g/mL by 5 mm, *Aspergillus Flavus* at 50  $\mu$ g/mL by 4 mm and at 100  $\mu$ g/mL by 6 mm, *Aspergillus niger* at 50  $\mu$ g/mL by 5 mm and at 100  $\mu$ g/mL by 6 mm. The results of present assay revealed that the polymer showed considerable antifungal activity.

In general, the presence of ether linkage and longer alkyl chain enhanced the antimicrobial activity for Poly N-acetyl D-glucosamine.

## CONCLUSION

The chitinous solid waste fraction of average Indian landing of shellfish was ranged from 60,000 to 80,000 t. The three parts of our motherland, India, are surrounded by ocean and its inner land is also very much rich with ponds, lakes, and lagoons. The proper utilization of those water resources (aquaculture) in terms of research in chitin and chitosan can bring the economic and academic prosperity of the nation. Chitin and chitosan are now produced commercially in India.

In recent years, chitin and derivatives- as a high potential resource as well as multiple functional substrates-have generated attractive interest in various fields such as biomedical, pharmaceutical, food and environmental industries. While chitin is an insoluble polymer in water, which is the major limiting factor for its utilization in living systems.

In the present work an attempt has been made to increase the understanding of the importance and characteristics of the chitin by describing photo crosslinking and biological properties. Investigation on photosensitivity of the poly N-acetyl D-glucosamine shows a significant photo crosslinking on exposure to UV radiation. Thus the influence of microstructure of the poly N-acetyl D-glucosamine on the rate of photo crosslinking was established. The results of the study on the rate of photo crosslinking lead towards establishing a suitable photoactive chromophore.

The present investigation on the antimicrobial activity of poly N-acetyl D-glucosamine was found to be considerably efficient against tested microorganisms. The result revealed that the presence of long alkyl chain and ether linkage enhanced the antimicrobial activity.

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