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Review of some Applications of Chitin/Chitosan with Metal/Metal Oxide Composite

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Abstract: Chitin and chitosan are biological polymers with promising commercial and biomedical applications. Researchers have long explored the properties and derivative functions of these two polymers. Because of their expansive use, chitin and chitosan are poised to become one of the most important natural resources in the future. Apart from the non-toxic and biodegradable properties of chitosan, using this polymer in manufacturing will promote sustainable industrial practices. Accordingly, the marine environment remains the main source of chitin and chitosan. Thus, these natural resources do not compete against other land resources. This review discusses the various applications of chitosan and compared with pristine Chitosan, Chitosan with metal/metal oxide composites are very efficient in Dye degradation, Antibacterial activity and Biosensor applications.

Keywords: Chitosan, composite, nanoparticles, Biosensor, Antibacterial activity

I. INTRODUCTION

Chitosan is a linear polymer of $\alpha(1\rightarrow4)$ -linked 2-amino-2-deoxy- β -D-glucopyranose and is easily derived by N-deacetylation, to a varying extent that is characterized by the degree of deacetylation, and is consequently a copolymer of N-acetylglucosamine and glucosamine.

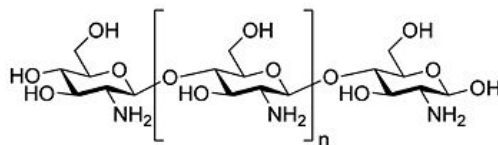


Figure.1 Structure of chitosan

Chitin is estimated to be produced annually almost as much as cellulose. Chitin and chitosan are the naturally derived abundant and renewable polymers and have excellent properties such as bio-degradability, bio-compatibility, non-toxicity and adsorption¹. Commercial chitosan is derived from the shells of shrimp and other sea crustaceans². Chitosan is produced commercially by deacetylation of chitin, which is the structural element in the exoskeleton of crustaceans (such as crabs and shrimp) and cell walls of fungi. The degree of deacetylation (%DD) can be determined by NMR spectroscopy, and the %DD in commercial chitosan ranges from 60 to 100%. On average, the molecular weight of commercially produced chitosan is between 3800 and 20,000 Daltons. A common method for the synthesis of chitosan is the deacetylation of chitin using sodium hydroxide in excess as a reagent and water as a solvent. This reaction pathway, when allowed to go to completion (complete deacetylation) yields up to 98% product³.

The amino group in chitosan has a pKa value of ~6.5, which leads to a protonation in acidic to neutral solution with a charge density dependent on pH and the %DA-value. This makes chitosan water soluble and a bioadhesive which readily binds to negatively charged surfaces such as mucosal membranes. Chitosan enhances the transport of polar drugs across epithelial surfaces, and is biocompatible and biodegradable. Purified quantities of chitosan are available for biomedical applications.

Chitosan and its derivatives, such as trimethylchitosan (where the amino group has been trimethylated), have been used in nonviral gene delivery. Trimethylchitosan or quaternized chitosan has been shown to transfect breast cancer cells, with increased degree of trimethylation increasing the cytotoxicity; at approximately 50% trimethylation, the derivative is the most efficient at gene delivery. Oligomeric derivatives (3-6 kDa) are relatively nontoxic and have good gene delivery properties⁴. Chitosan with iron oxide composites have recently attracted much attention since surface functionalization of the nanoparticles allows their covalent attachment, self assembly and organization on surface making them promising for the loading of biomolecules in a favorable microenvironment for the development of a biosensor⁵. In this review, discussed the synthesis of chitosan and chitin/ chitosan composite used for various applications.

II. SYNTHESIS OF CHITOSAN

The exploitation of chitinous materials seems to be an infinite treasure. The oligosaccharides of chitin/chitosan, prepared by hydrolyzing chitin/chitosan with chitinase/chitosanase, have various potential applications in the fields of food, agricultural, and pharmaceutical industries⁶. Chitin prepared from the shells of rice-field crabs (*Somanniathelphusa dugasti*) was converted into chitosan with a degree of acetylation of 0.21 and then sulphated with chlorosulphonic acid in N,N-dimethylformamide under semi-heterogeneous conditions to give 87% of water-soluble sulphated chitosan⁷. Chitin, a homopolymer of N-acetylglucosamine, is obtained from a variety of sources. They form the structural component of fungal cell wall and plants. They are commercially obtained from shrimp and crab shell waste from the fishing industry^{8,9}. The hydrothermal production of chitosan from the carapace of gray shrimp (*Palaemonidae*) for use as a coagulant in wastewater treatment is reported¹⁰.

Aminoderivatized chitosan derivatives were prepared in addition of amino functional groups at a hydroxyl site in the chitosan backbone. Six kinds of aminoderivatized chitosan such as aminoethyl-chitosan (AEC90), dimethylaminoethyl-chitosan (DMAEC90), and diethylaminoethyl-chitosan (DEAEC90), which were prepared from 90% deacetylated chitosan, and AEC50, DMAEC50 and DEAEC50, which were prepared from 50% deacetylated chitosan, were prepared and their reactive oxygen species (ROS) scavenging activities were investigated against hydroxyl radical, superoxide anion radical and hydrogen peroxide¹¹. Chitosan derived from crab shells, was used to prepare the graft polymer in aqueous solution with acrylamide (AM) and methacrylate ethyl trimethyl ammonium chloride (DMC) as raw materials and ceric ammonium nitrate (CAN) as initiator. The flocculation ability of the resulting polymer (PCAD) was studied in wastewater treatment experiments¹².

Chitosan (CS) was prepared from *Artemia urmiana* cyst shells using the same chemical process as described for the other crustacean species, with minor adjustments in the treatment conditions. The study results indicate that *Artemia urmiana* cyst shells are a rich source of chitin as 29.3-34.5% of the shell's dry weight consisted of this material¹³. Rifampicin-Chitosan complex is prepared by ultrasonic and characterized by modern analysis methods^{14,15}. A new process, the cost of the raw materials used for preparing chitosan was cut down 49%, the preparation time was shortened by one half, and the main properties of this chitosan such as viscosity, deacetylation and molecular weight all approached or exceeded those of the Sigma' commercial chitosan (Chitosan C-3646)¹⁶.

Hirano et al reported that novel chitin-silk fibroin fibres and chitin fibres were prepared by an environmental friendly wet-spinning method¹⁷. The degree of deacetylation, molar mass, and the yield of chitosans prepared from four fishery wastes (shrimp shell, lobster shell, crab shell, and cuttlebone) under the same conditions were compared¹⁸.

III. CHITOSAN, CHITOSAN-COMPOSITE AND DYE DEGRADATION

Chitosan has cellulose like structure with amino functions at C₂ carbons. Once adsorbed on fibers, it enhances the uptake of anionic dyes. A wide variety of fluorescent agents are used for whitening textile. A high affinity for fibers, high intensity of fluorescences and photostability are required of practical fluorescent agents. In contrast to dyestuffs little has been studied on the interaction between fluorescent agents and chitosan. The effect of chitosan on the ultraviolet and fluorescent spectra and photofading of fluorescent agents of a pyrazoline type in aqueous solution as well as on cotton and silk was investigated¹⁹. The composites of niobium (V) oxide and chitosan (Chit/Nb) were prepared with semiconductor loadings in the range 0-13.9% attached to the polymer surface. The Chit/Nb composites were used to photocatalyse the degradation of indigo carmine dye in aqueous solution by UV irradiation. The thermal stabilities of the Chit/Nb composites were slightly lower than that of the pure biopolymer, the materials presented high catalytic efficiencies that remained unaltered after 15 cycles of reuse²⁰. A natural polyelectrolyte microshell that was preformed by the alternate adsorption of the anionic alginate sodium (ALG) and the cationic chitosan (CHI) onto weakly cross-linked melamine formaldehyde (MF) colloidal particles, and the subsequent sacrifice of MF templates in 0.1 M HCl. The as-prepared microshells could accumulate rhodamine B (RhB) and fluorescein (Flu) efficiently in water under ordinary conditions by means of a simple mixing process. The photodegradation of the accumulated RhB and Flu was examined in the presence of Fe³⁺ and H₂O₂ under visible radiation. The accumulated RhB and Flu are rapidly degraded and the assembled shells maintain their intact spherical shape throughout the photoreaction process²¹. Cu(II)-chitosan complexes were used as heterogeneous catalysts for degradation of five model azo textile dyes in aqueous solution with hydrogen peroxide²².

Two different TiO₂-chitosan hybrid materials were synthesized (TiO₂-Chit A with 280 mg chitosan/g TiO₂ and TiO₂-Chit B with 46.76 mg chitosan/g TiO₂). Adsorption data obtained at different solution temperatures (25, 35, and 45°C) revealed an irreversible adsorption that decreases with the increment of T. The obtained parameters and correlation coefficient showed that the adsorption of both dyes on TiO₂-Chit A at the three work temperatures was best predicted by the Langmuir isotherm, while Sips equation adjusted better to adsorption data on TiO₂-Chit B. The adsorption enthalpy was relatively high and varied with T, indicating that interaction

between adsorbent and adsorbate molecules was not only physical but chemical. There is a change in the adsorption heat capacity related with intense hydrophobic interactions. The kinetic adsorption data were processed by the application of Lagergren and Avrami models. It was found that adsorption of both dyes on both adsorbents under the operating conditions was best predicted by Avrami model²³. Fe-immobilized polyelectrolyte microshells have been successfully constructed by alternative adsorption of Fe(III) and alginate sodium (ALG) onto the precursor shells composed of chitosan (CHI) and ALG templated on melamine formaldehyde (MF) particles. The photooxidative reaction occurring in the Fe-immobilized microshells can be performed at a wide range of pH from acid to neutral media, which is superior to the conventional Fenton reaction that allows taking effect only under acid condition of pH4²⁴. Chitosan capped CdS (CdS/CS) composite was prepared by biomimetic synthesis method under mild conditions. Visible light photocatalytic decolourization of Methyl Orange (MO) was carried out by employing this innovative composite catalyst. Results suggested that photocatalyst of CdS/CS is suitable for potential applications in organic waste removal from water by adsorption and photocatalysis²⁵. Higher-ordered 3D macroporous TiO₂-functionalized chitosan scaffolds were prepared by ice segregation induced self-assembly in which homogenous dispersions of 6, 27 or 200 nm sized TiO₂ nanoparticles and chitosan cross-linked with glycidoxypolytrimethoxysilane were unidirectionally frozen at -196°C. The hybrid scaffolds were shown to be reusable substrates for the photocatalytic degradation of methylene blue and Orange II dye molecules²⁶.

The crosslinked chitosan/nano-CdS (CS/n-CdS) composite catalyst prepared by simulating bio-mineralization process was extensively characterized. An azo dye, Congo Red (CR), was used as model pollutant to study its photocatalytic activity under visible light irradiation. The photocatalytic degradation was found to follow a pseudo-first-order kinetics according to Langmuir-Hinshelwood model. The dye could be decolourized more efficiently in acidic media than alkaline media²⁷. Chen et al reported that chitosan biopolymer and UV/TiO₂ to degrade textile wastewater and to measure the colour removal by UV-visible spectrophotometer. The operational parameters are chitosan, TiO₂, pH and reaction time. Single chitosan of 2500 ppm dose was used to remove Acid Blue 40 textile wastewater and to obtain a better efficiency. The result shows that the decolourization efficiency reached 98.8% elimination after 210 min of reaction time²⁸. The residual copper ions and iron ions concentration after the degradation of the dye reactive brilliant red were 0.396 mg/L and 0.105 mg/L respectively, which was lower than the regulation for drinking water²⁹. The use of photo-Fenton processes assisted by artificial or solar light, using immobilized iron on chitosan beads, crosslinked with glutaraldehyde, for the anthraquinone type compound Blue QR-19 standard dye degradation in aqueous solutions was studied. The obtained spheres showed a regular size and 4.0 mm diameter. The results showed 90% discolouration of the system within 180 minutes and a 60% total organic carbon (TOC) reduction for the photo-Fenton system using artificial light. For the system using sunlight, the total discolouration was achieved in 120 min and the TOC value decreased 70%. Also observed was that iron remained in the polymeric matrix after the treatment, thus allowing reuse³⁰. The laccase immobilized on magnetic chitosan beads was very effective for removal of textile dyes from aqueous solution which creates an important environmental problem in the discharged textile dyeing solutions³¹.

The technology of the selective biosorption and photocatalysis were coupled to synthesize a double functional Chitosan-TiO₂ adsorbent (CTA). CTA can not only degrade organic pollutants but also adsorb metal ions simultaneously. It was interesting that the photocatalysis of CTA for methyl orange (MO) was promoted when MO was coexisted with metal ions. In the presence of Ni²⁺, the degradation ratio of MO gradually increased from 78.9 to 94.2% with the increasing of Ni²⁺ concentration from 50 to 150 mg/L; furthermore with the presence of Ag⁺, the degradation ratio for MO increased from 65.9 to 79.3%³². By electrodisolution of copper, nanocomposites of copper oxide and chitosan can be formed. The particles have a maximum optical absorption at a wavelength of around 450 nm. Under light radiation, some 70% of the methyl orange present is decomposed after 90 min. This demonstrates that the photochemical activity of this composite is significantly higher than that of pure copper oxide³³.

A photocatalytic thin film of TiO₂ nanoparticles and polyaniline-grafted-chitosan (CPANI) was fabricated by layer-by layer approach. These results indicate that the presence of CPANI improves the adsorption of dye in the self-assembly. The effect of surface area and the amount of catalyst was also examined. The reusability of the thin films for dye degradation study ensures the stability of the self-assembly³⁴. The glow discharge plasma (GDP), which has thus far been studied for degradation of contaminants, was used for the first time to pre-treat chitosan for dye removal in aqueous solution. The results show that the GDP treatment changed the morphology and crystallinity of chitosan particles, and the number of -CH₂ and -CH₃ groups in the chitosan samples increased. These results show that GDP may be an attractive pretreatment technology for environmental adsorption materials³⁵. Reactive Red 195, which is an azoic anionic dye characterized by the presence of five sulphonic groups and one azoic group, is efficiently removed using chitosan. The increasing chitosan dose had a dramatic positive impact on the achieved colour removal, there was approximately a linear relationship between chitosan dose and colour removal of dye before colour removal reach maximum. Also, the increase of dye concentration led to the increase of chitosan dosage in order to get the same colour removal. 92

mg/L of chitosan dosage was sufficient to achieve complete remove of dye at initial concentration of dye at 200 mg/L. For the higher concentrations of dye, high dosages were necessary to reach complete colour removal. On the other hand, the use of adsorption interferents (Fe^{2+} , Na^+ , HCO_3^- and others) can be interesting, addition of ions had effect on the colour removal of Reactive Red 195³⁶. The immobilized laccase on CHX-g-p(IA)-Cu(II) membrane was more effective for removal of MO dye than removal of CB and RB5 dyes. Flexibility of the enzyme immobilized grafted polymer chains is expected to provide easy reaction conditions without diffusion limitation for substrate dye molecules and their products. The support described, prepared from green chemicals, can be used for the immobilization of industrially important enzymes³⁷. The synthesis of TiO_2 membranes on alumina supports by the spin-coating technique using the sol-gel method with water-soluble chitosan (WSC) as an additive was made. These properties included a high surface area (164-116 m^2/g) and porosity (47.3-52.2%), homogeneity without cracks and pinholes, thinness (0.8 μm), as well as high degradation of methyl orange (61.2-49.2%)³⁸.

A multilayer photoactive coating containing surface fluorinated TiO_2 nanoparticles and hybrid matrices by sol gel approach based on renewable chitosan was applied on poly(lactic acid) (PLA) film by a step wise spin-coating method. The photocatalytic activity of the multilayer coatings were investigated using methyl orange as a target pollutant; the results showed that PLA films coated with surface fluorinated particles exhibit higher activity than films with neat particles, because of a better dispersion of TiO_2 particles. The mechanical properties of PLA and films coated with fluorinated particles, irradiated by UV light were also investigated; the results showed that the degradation of PLA substrate was markedly suppressed because of the UV adsorptive action of the multilayer coating³⁹.

IV. ANTIBACTERIAL ACTIVITY OF CHITOSAN

The soluble and antibacterial chitosan derivative was prepared on the basis of the regioselective chemical modification. The results of platelet adhesion and the activated partial thromboplastin times (APTTs) indicate that grafting hydroxyethyl could improve anti-coagulation of chitosan. The antibacterial activity of HC against Enterococcus and E. coli had been much better owing to enhancing the degree of protonation⁴⁰. Damaging impact of the quaternized chitosan particles on the bacteria was also qualitatively determined by microscopic observation of the bacterial morphology⁴¹. Antimicrobial activities indicated that the antimicrobial activities of the derivatives increased with increasing the concentration. The antibacterial activity of schiff base of chitosan against E. coli was stronger, while acylated chitosan had better inhibiting effect on S. aureus than others. It was also found that the antifungal activities of the derivatives were stronger than that of chitosan, and schiff base of chitosan was obviously superior to acylated chitosan⁴². The functionalized PBSA-g-AA/chitosan composites showed markedly enhanced antibacterial properties due to the carboxyl groups of acrylic acid, which acted as coordination sites for the chitosan phase, allowing the formation of stronger chemical bonds⁴³. The antibacterial activity of arginine-functionalized chitosan was tested against pathogenic Escherichia coli O157 in chicken juice. The results imply that incorporating water soluble chitosan-arginine into packaging may help maintain both product shelf life and freshness as well as minimize the risk of food poisoning in both retail outlets and domestic homes⁴⁴. The effects of chitosan as the antimicrobial on Protein viscose fibers were studied. Antimicrobial flame-retardant protein viscose fibers are more efficient compared with original flame-retardant protein viscose fiber in improving the limiting oxygen index (LOI) of fiber. SEM images showed that the antimicrobial finishing and the softener were made in a same bath, which might be due to the formation of a protective layer or cross linking effect⁴⁵. The antibacterial properties to microfibrinous electrospun materials from styrene/maleic anhydride copolymers, quaternized chitosan derivatives (QCh) containing alkyl substituents of different chain lengths are covalently attached to the mats. A complete inhibition of the growth of bacteria, S. aureus (Gram-positive) and E. coli (Gram-negative), for a contact time of 30-120 min or a decrease of the bacterial titer by 2-3 log units is observed depending on the quaternization degree, the chain length of the alkyl substituent, and the molar mass of QCh. The modified mats are also effective in suppressing the adhesion of pathogenic S. aureus bacteria⁴⁶. Chitosan demonstrated its antibacterial activity as nanoparticle form and the activity was mainly influenced by its particle size⁴⁷. The chitosan content as well as its molecular weight has a direct influence on bacteria growth inhibition. The higher the chitosan content in the blend and the higher its initial molecular weight, the larger was the inhibition zone diameter. The bacteria growth inhibition was attributed to the diffusion of entrapped chitosan from the hydrogel blend to the culture medium⁴⁸. The antibacterial activity (a degree growth inhibition of more than 95%) of the promising Nan fibrous mats was revealed, through utilizing the colony counting method against Gram-positive bacteria S. aureus and Gram-negative bacteria Ecolab, which would open up wide applications on wound dressing, filtration and environmental purification⁴⁹. Chitosan had a stronger effect on the cell membrane of S. aureus than on that of P. aeruginosa due to the differences in their cell structures⁵⁰. The bacteriostatic activity of high molecular weight (M_w) chitosan derivative is higher on gram positive microbes while that of low M_w chitosan derivative is higher on gram negative bacteria⁵¹. Antimicrobial photodynamic inactivation (PDI) was shown

to be a promising treatment modality for microbial infections. The effect of chitosan in increasing the PDI efficacy against Gram-positive bacteria, including *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus pyogenes*, and methicillin-resistant *S. aureus* (MRSA), as well as the Gram-negative bacteria *Pseudomonas aeruginosa* and *Acinetobacter baumannii* was tested. The potentiated PDI effect of chitosan was related to the level of PDI damage and the deacetylation level of the chitosan. These results indicate that the combination of PDI and chitosan is quite promising for eradicating microbial infections⁵². A bio-composite scaffold containing chitosan/nano-hydroxyapatite/nano-silver particles (CS/nHAp/nAg) was developed by freeze drying technique, followed by introduction of silver ions in controlled amount through reduction phenomenon by functional groups of chitosan. The testing of the prepared scaffolds with Gram-positive and Gram-negative bacterial strains showed antibacterial activity. The scaffold materials were also found to be non-toxic to rat osteoprogenitor cells and human osteosarcoma cell line. Thus, these results suggested that CS/nHAp/nAg bio-composite scaffolds have the potential in controlling implant associated bacterial infection during reconstructive surgery of bone⁵³.

Zhao et al reported that the new chitosan ester derivative, chitosan p-hydroxybenzoate is synthesized, which is expected to have antimicrobial activity similar to parabens. Water solubility of the ester is slightly better than that of heptyl p-hydroxybenzoate, and its solubility in alcohol is greatly improved. Chitosan p-hydroxybenzoate shows broader spectrum antimicrobial activities. It has strong antimicrobial effects against Gram-negative, Gram-positive bacteria and yeasts. The MICs of chitosan p-hydroxybenzoate for *Staphylococcus aureus* and *Escherichia coli* are 0.01% and 0.025%, respectively⁵⁴. The antibacterial activity of the surface-quaternized chitosan film against *Staphylococcus aureus* and *Escherichia coli*, as model Gram-positive and Gram-negative bacteria, respectively, were superior to that of the virgin chitosan film. Thus, the introduction of additional positive charges to the chitosan surface via the versatile and yet simple process of heterogeneous quaternization can significantly improve the antibacterial activity of the chitosan surface, especially in a neutral environment⁵⁵. Owing to its high biodegradability, and nontoxicity and antimicrobial properties, chitosan is widely-used as an antimicrobial agent either alone or blended with other natural polymers⁵⁶.

Chemical modification of chitosan by introducing quaternary ammonium moieties into the polymer backbone renders excellent antimicrobial activity to the adducts. Derivatives with higher ES exhibited reduced antibacterial activity due to low quaternary ammonium moiety content. At the same degree of quaternization, all quaternized N-aryl chitosan derivatives bearing either electron-donating or electron-withdrawing substituents did not contribute antibacterial activity relative to chitosan Quat-188⁵⁷.

Antibacterial activities of six acid-soluble [two degrees of deacetylation (DD) × three viscosities] and two water-soluble chitosans (two DD with similar viscosities) were examined against eight gram-negative (*Pseudomonas fluorescens*, *Proteus vulgaris*, *Erwinia carotovora*, *Serratia marcescens*, *Escherichia coli*, *Vibrio parahaemolyticus*, *V. vulnificus*, and *Salmonella Typhimurium*) and six gram-positive bacteria (*Listeria monocytogenes*, *Staphylococcus aureus*, *Bacillus subtilis*, *B. cereus*, *Lactobacillus curvatus*, and *L. plantarum*). Based on MIC values, the acid-soluble chitosan with 99% DD and lower viscosity (17.9 mPa s) was most effective in inhibiting bacteria growth among eight chitosans tested⁵⁸. Essential oils are known to possess antimicrobial and antioxidant activity while chitosan is a biocompatible polymer with antibacterial activity against a broad spectrum of bacteria. The grafted eugenol and carvacrol conferred antioxidant activity to the chitosan nanoparticles, and the essential oil component grafted chitosan nanoparticles achieved an antibacterial activity equivalent to or better than that of the unmodified chitosan nanoparticles⁵⁹. Methylpiperazine and mono-quaternary dimethylpiperazine substituents do not contribute to activity against bacteria, whereas di-quaternary trimethylpiperazine moiety, will contribute to antibacterial activity⁶⁰. The nano indentation results suggested that despite little modification observed in the Gram-positive bacteria in morphological studies, cell wall damage had indeed occurred, since cell wall stiffness was reduced after chitooligosaccharide treatment⁶¹. The chemical and structural characteristics of chitosan-based matrices can be manipulated to influence the interaction of different bacterial species⁶². Chitosan, the deacetylated derivative of chitin, is a natural D-glucosamine polymer that can be extracted from the shells of seafood such as prawns crabs and lobsters. The antibacterial mechanism is proposed involving the cell wall disruption due to free amino groups present in chitosan⁶³.

Chitosan was obtained from cuticles of the housefly (*Musca domestica*) larvae. Antibacterial activities of different MW chitosans were examined against six bacteria. The minimum inhibitory concentrations of chitosans ranged from 0.03% to 0.25% and varied with the type of bacteria and MW of chitosan. Chitosan could cause leakage of cell contents of the bacteria and disrupt the cell wall⁶⁴.

The relationship between antibacterial activity of chitosans and their chemical structure (DA, degree of acylation and DP, degree of polymerization) was investigated. MICs (minimum inhibiting concentration) of four different series of 29 different 'pure' chitosans whose DA and DP were well characterized against four different bacteria (gram-positive bacterium *E. coli* K1, gram-negative bacteria *Bacillus cereus*, *Bacillus megaterium* and *Staphylococcus aureus*) were determined with micro plate reader. These results suggest that static electricity might be the main force of antibacterial activity of a chitosan⁶⁵. Chitosan is a well sought-after

polysaccharide in biomedical applications and has been blended with various macromolecules to mitigate undesirable properties. However, the effects of blending on the unique antibacterial activity of chitosan as well as changes in fatigue and degradation properties are not well understood⁶⁶. Chitosan/poly(acrylic acid)/poly(ethylene glycol) diacrylate (PEGDA) composite membranes with a bi-layer configuration were prepared and their potential application as an antibacterial material was examined. The antibacterial activities of the formed membranes were tested both with respect to a Gram-negative (*Escherichia coli*) and a Gram-positive (*Staphylococcus aureus*) bacteria^{67,68}. The hydrophobicity and cationic charge of the introduced substituent and the chain movement of chitosan derivatives strongly affect the antibacterial activity of quaternized chitosan derivatives against *S. aureus* and *E. coli*^{69,70}.

Chitosan generally showed stronger bactericidal effects with gram-positive bacteria than gram-negative bacteria in the presence of 0.1% chitosan. Antibacterial activity of chitosan was inversely affected by pH (pH 4.5-5.9 range tested), with higher activity at lower pH value⁷¹. Chen et al reported that the antibacterial effects of sulphonated and sulphobenzoyl chitosans were evaluated and compared with that of 69% deacetylated chitosan (DD69 chitosan). Minimal inhibitory concentrations of sulphonated chitosan (SC1, 0.63% sulphur content) against *Shigella dysenteriae*, *Aeromonas hydrophila*, *Salmonella typhimurium*, and *Bacillus cereus* were found to be lower than those of DD69 chitosan. High sulphur content in sulphonated chitosan adversely influenced its antibacterial effect. Sulphobenzoyl chitosan (SBC) has excellent water solubility and an antibacterial effect comparable to that of SC1. SBC at 1,000 and 2,000 ppm extended the shelf life of oysters at 5°C by 4 days at the former or by 7 days at least at the latter concentration. The growth of coliforms and *Pseudomonas*, *Aeromonas*, and *Vibrio* species on oysters was retarded by the addition of DD69 chitosan or SBC⁷².

The antibacterial action of chitosan hydroglutamate (CH), chitosan lactate (CL) and chitosan derived from fungal mycelia was examined against both gram-negative and gram-positive bacteria. Injury to chitosan-exposed *Staphylococcus aureus* MF-31 could not be demonstrated using the criterion sublethally stressed cells have increased sensitivity to high levels of sodium chloride⁷³.

V. ANTIBACTERIAL ACTIVITY OF METAL OXIDE WITH CHITOSAN

The polarized optical microscopy (POM) images show a batonet-like structure for Ag-chitosan nanocomposites film, while for the films cast from other metal (Au, Pt, and Pd)-chitosan nanocomposites, some branched-like structures with a few differences among them were observed under POM observation⁷⁴. Chitosan/layered silicate nanocomposites with different ratios were successfully prepared via solution-mixing processing technique. Unmodified Ca²⁺-rectorite and organic rectorite modified by cetyltrimethyl ammonium bromide were used. The results showed that chitosan chains were inserted into silicate layers to form the intercalated nanocomposites. The lowest minimum inhibition concentration (MIC) value of the nanocomposites against *Staphylococcus aureus* and *Bacillus subtilis* was 0.00313% (w/v), and the relative inhibition time (RIT) against *B. subtilis* with concentration of 0.00313% (w/v) was >120 h⁷⁵. Compared with pure chitosan films, chitosan films with silver showed both fast and long-lasting antibacterial effectiveness against *Escherichia coli*⁷⁶.

The antibacterial activity of CECh/PEO/AgNPs fibrous materials against *Staphylococcus aureus* showed that at AgNPs content of 5 wt % the mats had bacteriostatic, and at AgNPs content of 10 wt% - bactericidal activity⁷⁷. The films containing ZnO nanoparticles showed antibacterial activity toward the bacterial species *Staphylococcus aureus*. The observed antibacterial activity in the composite films prepared suggests that they may be used as hydrophilic wound and burn dressings⁷⁸.

Films of chitosan with both of zirconium and/or titanium at different ratios as model film applied to cotton fabric have been evaluated. Chitosan-titanium film showed the highest interaction with cotton fabric that lead to lower crystallinity index of fabric. The antibacterial activities of the prepared cotton fabrics containing the metal oxides using reduction of bacterial count method against G +ve bacteria (96-87%) and G -ve bacteria (93-70.5%) are depending on the nature of the deposited metal oxide, i.e. Zr > mixed > Ti > coated fabric > none. UV-cutting as well as incorporation of metal oxides enhanced both UV-protection and prominent antibacterial activities⁷⁹.

The synthesis of porous chitosan-silver nanocomposite consists of three-steps including silver ion-poly(ethylene glycol) matrix preparation, addition of chitosan matrix, and removal of poly(ethylene glycol) from the film matrix. The examined antibacterial activity results of these films revealed that porous chitosan-silver nanocomposite films exhibited superior inhibition⁸⁰. Antimicrobial activity of the prepared fabric samples treated with chitosan nanoparticles and CuO/chitosan nanocomposites against Gram-positive bacteria (*Staphylococcus aureus*) and Gram-negative bacteria (*Escherichia coli*) were investigated. Durability of treated cotton fabrics with nanocomposites has been evaluated⁸¹. New chitosan complex with 4-(ethoxycarbonyl) phenyl-1-amino-oxobutanoic acid (ETHA), as a matrix for silver nanoparticles to obtain a nanocomposite film by solution casting method was prepared. The nanocomposite film was screened for antibacterial activity with *Staphylococcus aureus* (gram positive), *Pseudomonas aurigionasa*

(gram negative) and *Escherichia coli* (gram negative) bacteria by adopting the disk diffusion method. The result of antibacterial study revealed that the prepared nanocomposite film may be a promising candidate for wide range of bio-medical applications⁸². New chitosan nanocomposites doped with silver nanoparticles were synthesized by a simple method. The chitosan particles were prepared by desolvation followed by crosslinking with poly(ethylene glycol-dialdehyde), this was prepared with poly(ethylene glycol) in the presence of a silver nitrate solution. These nanocomposites were shown to have antibacterial activity towards *Escherichia coli*⁸³.

VI. IRON OXIDE-CHITOSAN COMPOSITE FOR BIOSENSOR APPLICATIONS

Electrochemically fabricated nano-composite film of chitosan (CH)-iron oxide (Fe_3O_4) has been used to detect gonorrhoea, a sexually transmitted disease (STD) via immobilization of biotinylated probe DNA (BDNA) using avidin-biotin coupling for rapid and specific (mismatch-discriminating) DNA hybridization. The presence of Fe_3O_4 nanoparticles (~18nm) increases the electro-active surface area of the nano-biocomposite that provides desirable environment for loading of DNA with better conformation leading to increased electron transfer kinetics between the medium and electrode⁸⁴. A novel amperometric immunosensor was developed by immobilizing ferritin antibody (FeAb) on the surface of Fe_3O_4 magnetic nanoparticles/chitosan composite film modified glassy carbon electrode (GCE). The analytical results showed that the developed immunoassay was comparable with the radioimmunoassay (RIA), and the studied immunosensor exhibited good accuracy, high sensitivity, and long-term stability for 3 weeks, which implies a promising alternative approach for detecting ferritin in clinical diagnosis⁸⁵.

A novel tyrosinase biosensor based on Fe_3O_4 nanoparticles-chitosan nanocomposite has been developed for the detection of phenolic compounds. The large surface area of Fe_3O_4 nanoparticles and the porous morphology of chitosan led to a high loading of enzyme and the entrapped enzyme could retain its bioactivity. The tyrosinase biosensor exhibits good repeatability and stability. Such new tyrosinase biosensor shows great promise for rapid, simple, and cost-effective analysis of phenolic contaminants in environmental samples⁸⁶. The rabbit immunoglobulin antibodies (IgGs) have been immobilized onto nanobiocomposite film of chitosan (CH)-iron oxide (Fe_3O_4) nanoparticles prepared onto indium-tin oxide (ITO) electrode for detection of ochratoxin-A (OTA). Excellent film forming ability and availability of $-\text{NH}_2$ group in CH and affinity of surface charged Fe_3O_4 nanoparticles for oxygen support the immobilization of IgGs. Differential pulse voltammetry (DPV) studies indicate that Fe_3O_4 nanoparticles provide increased electroactive surface area for loading of IgGs and improved electron transport between IgGs and electrode. IgGs/CH- Fe_3O_4 nanobiocomposite/ITO immunoelectrode exhibits improved characteristics such as low detection limit (0.5 ng/dL), fast response time (18 s) and high sensitivity (36 $\mu\text{A}/\text{ng}/\text{dL}/\text{cm}^2$) with respect to IgGs/CH/ITO immunoelectrode⁸⁷. Glucose oxidase (GOx) has been immobilized onto this CH- Fe_3O_4 nanocomposite film via physical adsorption. This GOx/CH- Fe_3O_4 /ITO nanocomposite bioelectrode has response time of 5 s, linearity as 10-400 mg/dL of glucose, sensitivity as 9.3 $\mu\text{A}/(\text{mg}/\text{dL}/\text{cm}^2)$ and shelf life of about 8 weeks under refrigerated conditions. The value of Michaelis-Menten (Km) constant obtained as 0.141 mM indicates high affinity of immobilized GOx towards the glucose substrate⁸⁸.

Nucleic acid sensor has been fabricated via immobilization of single standard calf thymus deoxyribose nucleic acid (ssCT-DNA) onto chitosan (CH)-iron oxide (Fe_3O_4) nanoparticles based hybrid nanobiocomposite film deposited onto indium-tin-oxide (ITO) coated glass for pyrethroids [cypermethrin (CM) and permethrin (PM)] detection⁸⁹. Glucose oxidase (GOD) was simply mixed with Fe_3O_4 NPs and cross-linked on the Pt electrode with chitosan (Cs) medium by glutaraldehyde, and then covered with a thin nafion film. The biosensor showed high sensitivity (11.54 $\mu\text{A cm}^{-2} \text{mM}^{-1}$), low detection limit (6×10^{-6} M), and good storage stability⁹⁰. Urease (Ur) and glutamate dehydrogenase (GLDH) have been co-immobilized onto superparamagnetic iron oxide (Fe_3O_4) nanoparticles-chitosan (CH) based nanobiocomposite film deposited onto indium-tin-oxide (ITO) coated glass plate via physical adsorption for urea detection. It is shown that presence of Fe_3O_4 nanoparticles results in increased active surface area of CH- Fe_3O_4 nanobiocomposite for immobilization of enzymes (Ur and GLDH), enhanced electron transfer and increased shelf-life of nanobiocomposite electrode⁹¹. Yuan et al reported that the Fe_3O_4 nanoparticles and chitosan (CS) were mixed to form a matrix in which haemoglobin (Hb) can be immobilized for the fabrication of H_2O_2 biosensor. The Fe_3O_4 -CS-Hb film exhibited a pair of well-defined and quasi-reversible cyclic voltammetric peaks due to the redox of Hb-heme Fe (III)/Fe (II) in a pH 7.0 phosphate buffer⁹².

A sensitive and selective amperometric glucose biosensor was obtained by using the electrodeposition of Pt nanoparticles on iron oxide-multiwall carbon nanotubes/chitosan (FeyOx-MWCNTs/CS) magnetic composite modified glassy carbon electrode (GCE) followed by the adsorption of glucose oxidase (GOx) at the surface of the electrode. The proposed biosensor exhibit excellent electrocatalytic activity and good response performance to glucose. The proposed biosensor has good anti-interferent ability and long-term storage stability after coating with Nafion, and it can be used for the determination of glucose in synthetic serum⁹³.

The one-step synthesis is reported of a nanofilm composed of iron oxide and gold nanoparticles in a chitosan matrix that can act as a novel matrix for the immobilization of glucose oxidase (GOx) to fabricate a glucose biosensor. The use for the composite film strongly increased the effective electrode surface for loading of GOx. Under optimized conditions, the biosensor has a response time of 6 s and a linear response in the range between 3 μ M and 0.57 mM of glucose, with a detection limit of 1.2 μ M at a signal-to-noise ratio of 3. This novel and disposable mediatorless glucose biosensor may form the basis for a future mass-produced glucose biosensor⁹⁴.

VII. CONCLUSION

It can be deduced from this review, the synthesis of chitin and chitosan with composite can be very efficient in various applications. Available literatures also indicate that both chitin and chitosan are effective materials for application in dye degradation, Antibacterial activity and Biosensor. Modification of both chitin and chitosan had been reported to improve their nanomedicine and in future research the chitosan in organic electronics.

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