INTERNATIONAL JOURNAL OF RESEARCH IN PHARMACY AND CHEMISTRY

Available online at www.ijrpc.com

Research Article

CHARACTERIZING FORMULATIONS CONTAINING DERIVATIZED CHITOSAN WITH POLYMER BLENDING

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ABSTRACT

Structural modifications of chitosan through Derivatization, substitution, grafting, cross linking, complexation and proving to cope with blend of biodegradable polymer like poly lactic acid, polyvinyl chloride *etc,* for required pharmaceutical properties by preparing it to a specified dosage form *via*, microspheres, nanoparticles, Mucoadhesive formulations, films and characterizing for its usage in exploring the wide applications of chitosan. Schemes of synthesizing the derivatives were discussed along with the suitable blending to make it either hydrophilic or lipophillic to suit the site of absorption. The effects of complexing with metallic ions were exhibited and extent of complexes with Cu (II), Co (II), Al (II) *etc*, and explored its effects as antibacterial activity on *Streptococcus aureus*, *Escherichia coli* and *Salmonella enteric* serovar Typhimurium. The anti-oxidant activity was also studied on metal complexes.

Keywords: Derivatization, Blending, Nanoparticles, Films and Complexations.

INTRODUCTION

Chitosan is a naturally occurring, bio-degradable, non-toxic, non-allergenic biopolysaccharide derived from chitin, found in abundance in nature. Chemical structure of chitin consists of linear repeating units of 2-acetamido-2-deoxy- D-glucopyranose attached through β-(1-4) linkages. Studies aimed at deriving newer applications of chitosan are hence of interest. Chitin is one of the most abundant polysaccharides and can be found in various invertebrates and lower plants. Chitosan is obtained by N-deacetylation of chitin, even though the reaction is never complete. There is not yet a formal nomenclature for describing chitosan of various degrees of deacetylation. Chitosan is known to be non-toxic, odorless, biocompatible with living tissues, biodegradable .In the present study, substituted chitosan derivatives were synthesized to produce water soluble derivatives at neutral pH. [Fig 1].

Chitosan, a white flaky solid, is difficult to manipulate with because of the solubility problems in neutral water, bases, and commonly used organic solvents. The pKa value of the primary amino groups in chitosan is determined to be around 6.5. As a result, even though chitosan and its derivatives are soluble in pH values of lower than 6.0, many of its applications in neutral or basic medium, including those of physiological relevance, may not be realized, for the pH under such situations will trigger an immediate precipitation. On the other hand, acidic solutions, in which chitosan is fairly soluble, may not be desirable in many of its applications, especially those in medicine, cosmetics, and food. There have been two

major approaches documented in literature towards improving the solubility of chitosan at neutral pH. First is to chemically Derivatized chitosan[1] (for example with substituents containing quaternary amino groups, carboxymethylation, and sulfatation) such that the substituent added is strongly hydrophilic. The second approach uses chitosan with an average 50% deacetylation prepared by homogenous processing of chitin [2]. Thawatchai Phaechamud *et al.,* Prepared chitosan sponge containing antibacterial by dissolving the chitosan in acetic acid solution in different concentration of 4, 6, 10% and straining it using muslin cloth and placing these different percentage solution in well and freeze dried, and crossedlinked with gluteraldehyde, plain and cross-linked sponge are loaded with different concentration of anti-bacterial solution by using micro pipettes. The release of the drug from non-cross-linked sponge was slower than that from the cross-linked owing to the hydration and gel formation retarding the drug diffusion of the first system[3]. L. Ammayappan *et al.,* Decided to see the effect of chitosan alone and in combination with aloevera and curcumin on cotton, wool, and rabbit hair fibrous substrates by exhaustion method for the assessment of their antimicrobial activity. The sorption of chitosan on cellulose/protein fibrous substrates is due to ionic interaction between negative charges [hydroxyl anion in cellulose polymer ($-O^-$) and carboxylate anion ($-COO^-$) in the protein polymer] and protonated amino groups of chitosan (NH_3^+), hydrogen bonding and van der Waal's forces. As its affinity can generally regarded as weak, so is its antimicrobial activity is also weak when applied alone[4]. Eun-Jin Yang [5] *et al*., Examined the effect of chitosan oligosaccharides (COSs) with different molecular weights (COS-A, 10 kDa < MW < 20 kDa; COS-C, 1 kDa < MW < 3 kDa) on the lipopolysaccharide (LPS)- induced production of prostaglandin E_2 and nitric oxide and on the expression of cyclooxygenase-2 and inducible nitric oxide synthase in RAW264.7 macrophages and showed significant inhibition of PEG_2. No skin irritation and adverse reaction of COSs usage observed and suggested COS-A and COS-C be considered as possible anti-inflammatory candidates for topical application [Fig 2].

Edelio Taboada[6] *et al.,* Synthesized a new chitosan derivatives N-(3, 5-Diethylaminobenzyol) chitosan and N-(4-ethylaminobenzyol) chitosan with a degree of substitution of 29% & 60% respectively. Such derivatives could be used for metal-chelating polymers, as flocculants, and in biomedicinal applications because of the aryl amine moieties in their structures. Fig 3

Kevin R. Holme [7] *et al.*, Reduced chitosan by n-alkylation with 6-O-substituted galactose derivative bearing an iminodiacetate residue, giving hydrophilic branch and a metal chelating group. Cu(II) binding capacity showed better ion-exchange ability with less viscous (1% aqueous solution) compared to native chitosan. This encourages further research into the rational tailoring of chitosan for structure / property studies. [Fig 4**]**

The copper (II) uptake by derivative **7** compared to lyophilized chitosan reflects higher degree of substitution and has more than one ion binds per Derivatized residue, which indicates that additional binding to the backbone, over and above one equivalent binding per chelating branch, occurs I the derivative.

Xueqiong Yin [8] *et al.,* developed a new method of metal-coordinating controlled oxidative degradation of chitosan leading to low molecular weight chitosan with uniform molecular weight. Hydrogen peroxide was used to break chitosan chain at those weak points to get low molecular weight chitosan. Degradative speed of complexes is faster than chitosan, and mol wt distribution is much narrower than chitosan. Low molecular weight chitosan and its complexes with Cu (II) or Co (II) posses' good O_2 scavenging activity.

Jukka Holappa[9] *et al.,* Efficiently synthetic route was developed for mild chloroacylation of chitosan with different chloroacyl chlorides. The idea is to substitute the primary hydroxyl of the starting material with a triphenylmethyl moiety and chloroacylation step can be carried out fast in a homogenous solvent environment at low temperature. Full N-chloroacylation was obtained with this procedure without any O-acylation. Organo-soluble 6-O-triphenylmethylchitosan was used as a starting material for the acylation reactions resulting N-chloroacyl-6-triphenylmethylchitosan intermediates with same solubility and characterized by FT-IR. N-Methylpiperazine moieties were attached to make product efficiently soluble for further modifications. [Fig 5]

Yoshhie Torii *et al.,*[10] Synthesized chitosan by dichlorophthaloylation method. Preparation

of an intermediate for chemical modification by chemo selective protection of chitosan by dichlorophthaloyation. Preparation by either partial hydrolysis of N,O-dichlorophthaloyl-chitosan or by one-step N-dichlorophthaloylation in N.N-dimethylformamide/water the reaction similar to phthaloylation. [Fig 6]

However, several of the applications of chitosan cited in the literature utilize some form of chitosan derivative and hence to provide for the solubility, it may be necessary to incorporate ionic groups into the polymer backbone. Z. Lu, *et al.*,[11] Developed excipients that are capable of fulfilling multifunctional roles such as controlling the release of drug according to therapeutic needs, their scope in dosage form design can be enlarged through combining different polymers. When a polymer is cross-linked or complexed with an oppositely charged polyelectrolyte, a three dimensional network is formed in which the drug can be incorporated to control its release. Chitosan-Polycarbophil interpolyelectrolyte complexes showed zero order release kinetics and demonstrated high potential excipients for the production of swellable matrix systems with controlled drug release properties.

Qian *et al.*,[12] Synthesized L- Asparaginase with N, O- Caroxymethyl Chitosan and fond the antitumor activity of L-asparaginase with water soluble N, O- Carboxymethyl chitosan retained more than 80% of it activity. The in vivo half life and other properties were influenced by the extent of the molecular weight of modified chitosan. [Fig 7]

DUAN Lihong[13] *et al.,* synthesized an improved adsorbent for heavy metal ions by condensation reaction of chitosan with salicylaldehyde in ethanol to form Sciff's base. The effect of irradiating the reaction using ultrasonic liquid processor was contrasted with conventional methods. The reaction conditions, including solvents, ultrasonic power density and irradiation time, pH, and reactant ratio, were optimized by orthogonal design. A shorter reaction time and higher product yield were obtained using ultrasonic-assisted synthesis compared with traditional method. [Fig 8]

Among these, Polymers blending is an attractive alternative for producing new polymeric materials with tailored properties without having to synthesize totally new materials. Other advantages for polymer blending are simple and inexpensive. Nowadays, natural polymers are increasingly used due to their natural abundance and costs effective.

Polylactic acid is proving to be a viable alternative to petrochemical-based plastics for many applications. It is produced from renewable resources and is biodegradable, decomposing to give H_2O, CO_2, and humus, the black material in soil. In addition, it has unique physical properties that make it useful in diverse applications including paper coating, fibers, films, and packaging[14].

MICRO / NANOSPHERES:

Pinwipha Piyakulawat *et al.*,[15] Formulated Chitosan / Carrageen beads. Complex polyelectrolyte chitosan and carrageen beads were prepared to get optimal formulation composition for release of the drug. Comparing the release study of the beads with or without cross linking agent and percent of the drug content were studied; which showed the release of ≈8 and ≈24 hrs release respectively. The difference in the drug release behavior attributed to the difference in ionic interactions between oppositely charged ions and to the concentration of drug in beads, which depended on composition of formulation and pH of the dissolution.

Shantha R bhattaria *et al.*,[16] Synthesized n-hexanoyl chitosan **s**tabilized magnetic iron oxide nanoparticles were formulated and characterized for its uptake by the cells using mouse macrophage cell line and found rapidly associate with Raw cells, and saturations was typically reached within 24 hrs of incubation at 37^{0}C. Nearly 8.53 ± 0.31pg iron/cell were bound. [Fig 9]

They were prepared by precipitation method showed highly crystalline, super paramagnetic, behavior. It also displayed high stability, nontoxicity, enhancement of MR images and potential endocytose the macrophage cell line. Iron oxide nanoparticles exhibited strong bands in low frequency region below 800 cm-1 due to oxide Skeleton. Amide I, II, III was shifted due to interaction with iron oxide nanoparticles.

Ali Demir Sezer *et al.*,[17] studied different delivery systems, encapsulated plasmid such as fucoidan-chitosan (Fucosphere) and chitosan microspheres were prepared and particle physicochemical properties evaluated. Chitosan being a polycationic polymer, have the capacity to act as cationic vector which electrostatically interact with DNA to form complex or involve in particulate system with

DNA. Though polycationic chitosan has lower transfection efficiency of the gene delivery, by modifying with chemical agents or ligands by using polyion complexation of negatively charged fucoidan with chitosan. The described formulation and process allowed achieving high encapsulation efficiency and low burst effect. It was observed that the plasmid release kinetic fit to zero-order release profiles.

Alessandro Nasti *et al.*,[18] Studied the influence of a number of orthogonal factors (pH, concentration, ratio of components, different methods of mixing) in the preparations of chitosan/TPP (Triphosphate) nanoparticles and in their coating with hyaluronic acid (HA), aiming at the minimization of size polydispersity, the maximization of zeta potential and long term stability. [Fig 10]

Sanat Kumar Basu *et al.*,[19] formulated chitosan/gelatin microspheres: Complex forming tendency of oppositely charged macro molecules like chitosan in acidic solutions at pH 5-6 and gelatin (Type B) which is negatively charged at pH above isoelectric point to prepare microspheres for controlled release by complex and simple coacervation method which differ by with or without combination of gelatin. Reversible physical crosslinking by electrostatic interaction, instead of chemical (Gluteraldehyde) is applied to avoid possible toxicity of reagent and other undesirable effects. All the microspheres showed release of drug by a fickian diffusion mechanism.

M Sivabalan *et al.*,[20] prepared chitosan/eudragit nanoparticles were prepared by emulsion-droplet coalescence method by dissolving chitosan in 1% acetic acid and drug in phosphate buffer saline. The solution was added to liquid paraffin containing surfactant tween 20. Mixture is homogenized to w/o emulsion. Similarly, eudragit emulsion in 3M sodium hydroxide solution was prepared. The two emulsions were mixed in homogenizer. Resulted in coalescence of droplets and solidified to get nanoparticles.

Amal El-Kamel *et al.*,[21] Prepared chitosan with sodium alginate beads. Cationic chitosan was crosslinked with gluteraldehyde alone with sodium alginate with or without microcrystalline cellulose. Some formulation contained even sodium Carboxymethyl cellulose. Formulation containing 6% chitosan, 24% sodium alginate, 30% Sodium CMC, and 20% MCC showed adequate release properties, lower swelling index and good bio-adhesion. Release mechanism were least affected by aging.

Wanlop Weecharangsan[22] *et al.,* To evaluate chitosan lactate of different molecular weight chitosan lactate as a DNA complexing agent in transfecting COS-1 cells affecting the cell viability by spray-drying method yielded nanosized particles and characterized for particle size, size distribution, zeta potential. Chitosan lactate has an advantage for ease of processing as it is water soluble and safe efficient gene carrier.

Lu HUANG *et al.*,[23] An oleic acid grafted chitosan nanoparticles were synthesized with different degree of amino substitution can be grafted with oleic acid by 1-ethyl-3(3-dimethylaminopropyl) carbodiimide mediated coupling method. Characterized for amide linkage between amino group of chitosan and carboxyl group of oleic acid using FT-IR, zetasizer and antibacterial activity against *E coli* and *S aureus.* Results showed particle size range of 60-200 nm. [Fig 11]

Angela M. de [24] *et al.,* Campos chitosan nanoparticles: Fluorescent (CS-fl) nanoparticles were prepared by ionotropic gelation. To assess the potential of chitosan (CS) nanoparticles for ocular drug delivery by investigating their interaction with the ocular mucosa *in vivo* and also their toxicity in conjunctival cell cultures.

Shanta Raj Bhattarai *et al.*,[25] Synthesised *n*-acylated chitosan stabilized iron oxide nanoparticles. Hydrophobically modified polycations (*N*-acylated chitosan, *Nac*) stabilized iron oxide nanoparticles (IOPs) as a three dimensional (3D) nano-matrix for the controlled fabrication of hydroxyapatite (HAP). Among three different fatty acid chlorides (hexanoyl, octanoyl, and myristoyl chloride) modified chitosan (*Nac*-6, *Nac*-8, and *Nac*-14, respectively), demonstrated the *Nac*-6-IOPs as a novel 3D nano-matrix for the controlled fabrication of HAP due to its well dispersibility and stability in aqueous medium (pH 7.4)

Yan Chen *et al.*,[26] Formulated nanoparticles to examine the effect of charge ratio of chitosan – dextran sulfate on its formation and properties for delivery of small and large molecules like Rhodamine 6G and bovine serum albumin. Nanoparticles were prepared by complex coacervation process. Drug entrapment, zeta potential and particle size were keenly studied for influence of charge ratio of two ionic

polymers as a system for controlled delivery of both small and large molecules, including proteins.

William J. Trickler *et al.,*[27] chitosan – glyceryl monooleate**.** To enhance the cellular accumulation of drug with chitosan- glyceryl monooleate nanostructure for localized delivery of chemotherapeutics potentially targeting cancerous tissues through synergistic bio-adhesive properties of the combination. The delivery system was prepared by emulsion solvent evaporation method. The nanostructure topography, size, and surface charge were determined by atomic force microscopy (AFM), and zeta meter. The drug delivery demonstrated capability to entrap both hydrophilic and lipophillic compounds to potentially provide effective treatment.

Jochen Haas, *et al.,*[28] Prepared poly-(ε-caprolactone) particles by an emulsion-diffusion-evaporation method using a blend of poly-(vinyl alcohol) and chitosan derivatives as stabilizers. Derivatives are chitosan hydrochloride and trimethyl chitosans with varying degree of quaternization. Particle characteristics- size, zeta potential, surface morphology, cytotoxicity, and transfection efficiency- were investigated. In immobilization using gel electrophoresis, it showed that cationic nanoparticles(NP) form stable complexes with DNA at ratio of 3:1(NP:DNA). These nanoplexes showed a significant higher transfection efficiency on COS-1 cells than naked DNA.

CHEN ChangJing *et al.,*[29] Prepared chitosan-poly(acrylic acid)-calcium phosphate hybrid nanoparticles based on the mineralization of calcium phosphate on the surface of chitosan-poly (acrylic acid) nanoparticles achieved by directly adding ammonia or by thermal decomposition of urea. The size morphology and ingredient of chitosan-poly (acrylic acid)-calcium phosphate hybrid nanoparticles were characterized by dynamic light scattering, transmission electron microscope, scanning electron microscope, thermogravimetry analysis and X-ray diffractometer. TGA results revealed that the hybrid nanoparticles contained approximately 23% inorganic component, which was consistent with the ratio of starting material. XRD results indicated dicalcium phosphate crystals were a dominant component of mineralization. The porous structure of hybrid nanoparticles might be greatly useful in pharmaceutical and other medical applications.

Brigitta Loretz *et al.,*[30] studied the potential of different molecular-weight chitosan-EDTA conjugates as a carrier matrix for nanoparticulate gene delivery systems. Covalent binding of EDTA to more than one chitosan chain provides a cross-linked polymer that is anticipated to produce stable particles generated via coacervation and characterized for size and zeta potential. Lactate dehydrogenase assay performed to determine toxicity. Low viscous, 68% amino group produced highest complexing efficiency resulting in nanoparticles of 43 nm mean size and exhibited zeta potential of +6.3 mV. Nanoplexes showed 35% improved in vitro transfection efficiency compared to unmodified chitosan nanoparticles. The chitosan-EDTA conjugates may be promising polymer for gene transfer. [Fig 12]

Gianni Ciofani *et al.,*[31] reported that the cytocompatibility of ceramic material barium titanate nanoparticles, using a dispersion method based on non-covalent binding to glycol-chitosan because of cytocompatibility and biological inertial character allowed even at high concentration, widely used chemotherapy drug Doxorubicin showed highly enhanced complexation with barium titanate nanoparticles and can be realistically exploited as alternative cellular nanovector. The hydrophobic interaction between the nanoparticles surface and aromatic ring of drug showed the possibilities of obtaining non-covalent supra-molecular complexes. [Fig 13]

Yongliang Wang *et al.,*[32] studied based on chelation effect between iron ions and amino groups of chitosan, in situ mineralization of magnetite nanoparticles in chitosan hydrogel. The mineralized nanoparticles were nonstoichiometric magnetite with unit formula of $Fe_{2.85}O_4$ and coated by a thin layer of chitosan. First iron ions were chelated by the amino groups of chitosan, and the complex was fabricated; on encountered OH^- , the iron ions chelated by the amino groups, which providing nucleation site for magnetite crystals. [Fig 14]

Wie liang XU *et al.,*[33] Prepared cyclomaltoheptaose-β-cyclodextrin crosslinked chitosan derivative via glyoxal or glutaraldehyde abd characterized by IR spectra. The microspheres of crosslinked chitosan were spherical and smooth outer structure; however in case of the incorporation of β-cyclodextrin the outer structure altered to

rough and multiporous surface. The water insoluble chitosan-linked cyclodextrin beads have highly porous structure. Weak acidity and optimum temperature about 80-90^{0}C was favorable for crosslinking reaction. This shows feasibility of converting naturally occurring entities, like chitosan, into useful sorbents. Which have varied and far-reaching applications.

Natapron Sowasod *et al.*,[34] formulated nanoencapsulate curcumin in chitosan cross-linked with tripolyphosphate and to characterize various batches of nanocapsules had average size range of 253.8 – 415.2 nm. And yield of encapsulation was at 18.56-96.28%.

COATINGS / FILMS

Gurpreet Kaur *et al.*,[35] aimed at formulating tablets comprising of coating susceptible to microbial enzyme degradation for releasing budesonide in the colon. Tablets coated with synthesized Chitosan – Chondroitin Sulfate. Interpolymer complex between chitosan and chondroitin sulfate was characterized using FTIR. Formation of bonds between $–COO^-$ and $–OSO_3^-$ groups of CS and $–NH_3$ groups of CH were evident of intermolecular complexed films. The pH sensitive swelling exhibited by these films was observed to be function of CH concentration. [Fig 15] [Fig 15 a]

ClaudiaCOlonna, *et al.,* [36] Prepared 5-methyl-pyrrolidinone chitosan films by casting method using tripolyphosphate as crosslinking agent loaded with protein for mucosal administration. By setting the crosslinking proportion one can release proteins at particular pH. The results showed that the myoglobin release was achieved only through chitosan cross-linking; the best result in release rate control, good bioadhesion were obtained by cross-linking performed at pH 6.5.

JIAO YenPeng *et al.*,[37] Synthesized carboxymethyl chitosan and n-methylene phosphonic chitosan with poly (l-lacticacid). Carboxymethyl chitosan was used as a surface modifier for poly (L-Lactic acid) films by entrapment method were the radio labeled (^{125}I) protein fibronectin was used to trace the adsorption tendency; at isotherm adsorption which showed greater adsorption with modified chitosan at less concentration whereas Carboxymethyl chitosan showed higher adsorption at higher concentration. [Fig 16]

Manisara Peesan *et al.*,[38] studied the contribution of the blend films of hexanoyl-chitosan and polylactide were prepared by solution-casting technique from the corresponding blend solutions in chloroform. 20% Hexanoyl-chitosan content exhibited the degradation temperature greater then those of pure components. All blend films showed one glass transition temperature indicating partial miscibility between hexanoyl-chitosan and polylactide molecules in bulk amorphous phase. Both the tensile strength and Young's modulus was found to decrease from that of pure polylactide to that of pure hexanoyl-chitosan with increasing hexanoyl-chitosan content. [Fig 17]

Tamaki Wada *et al.*,[39] made use of chitosan to introduce formaldehyde adsorption abilities into emulsion binder for interior finishing coatings. To produce stable chitosan-hybridized acrylic emulsion, of the two methods; the pre-emulsion dropping method is superior to the monomer dropping method. The adsorption performance for formaldehyde in chitosan-hybridized acrylic resin films increased with increasing chitosan content; the films also had adsorption abilities for hydrogen sulfide and ammonia. Tensile strength and elongation at breaking point decreased with increase in chitosan content. [Fig 18] [Fig 18a]

Gan Wang *et al.*,[40] Formulated nerve repair tubes using 1-ethyl-3(3-dimethylaminopropyl) Carbodiimide Hydrochloride cross-linked Carboxymethyl chitosan with improved bio-degradability compared to chitosan alone. Carboxymethyl chitosan films showed no cytotoxicity effect on Schwann cell. Carboxymethyl chitosan tubes prepared by using tube moulds showed better nerve regeneration performance than chitosan tubes and demonstrated equivalence to nerve autografts.

R. K Mishra *et al.,* [41] developed and characterized the chitosan / phosphomolybdic acid (PMA) based composite membranes by FTIR spectroscopy indicating the proper molecular interactions between chitosan and PMA, XRD spectroscopy indicating semi crystalline nature, DSC study showed the presence of single glass transition temperature at 156^o C in the 20% PMA doped membrane, which indicates miscible nature of chitosan and PMA blend.

MUCOADHESIVE FORMULATIONS

MR Rekha *et al.,*[42] aimed to study the development of a Mucoadhesive particulate drug delivery system with water soluble anionic chitosan derivative succinyl chitosan particles**.** This Derivatization formed water soluble product which was prepared same as above using Na- TPP as cross linking. The limited release of insulin in gastric acidic media is worth mentioning as it minimizes the loss and enhances the bioavailability of insulin. Hence succinyl chitosan particles seems to be a promising candidate for oral peptide delivery system.

Maria Cristina Bonferoni *et al.,*[43] Formulated chitosan gels for lactic acid delivery with varied molecular weight chitosan and lactic acid of two different concentration based gels were studied for its formulation and mucoadhesion properties. Based on the ratio it confirms that release mechanism is not only by diffusion but also by ionic displacement. [Fig 19]

S. K. Jain *et al.,*[44] utilized the mucoadhesion properties and formulated Mucoadhesive chitosan microspheres by emulfication method to explore the possibilities of non invasive delivery of insulin and characterized for various physiochemical attributes and mucoadhesion. Glutaraldehyde cross-linked microspheres showed better reduction of blood glucose level than citric acid cross-linked microspheres.

Martin Werle *et al.,* [45] Analysized the polymers exhibiting markable Mucoadhesive properties are chitosan, poly (acrylic acid) and in first instance thiomers. The Mucoadhesiveness of chitosan, which displays protonated primary amino groups at a pH below 6.5, is mediated by ionic interactions between the cationic polymer and anionic pars of the mucus, such as sialic acid. Other mechanisms which can cause mucoadhesion are dehydration processes or an interpenetration of polymeric chain into the mucus. Chitosan-thiobutylamidine displays up to 100-fold improved mucoadhesion properties compared to an unmodified chitosan. [Fig 20]

MISCELLANEOUS

Kai Zhang *et al.,* [46] in an effort to develop biomaterials to meet the guided tissue regeneration for periodontal tissue recovery, a homogenous Chitosan/ Hydroxyapatite membrane with potential applications has been prepared via in situ compositing. The membrane has been designed to have smooth – rough asymmetric structure as a biomaterial, compactable with required tensile strength for guided tissue generation for periodontal tissue recovery resulting inductive effect for cell growth.

Yhua Chang *et al.,*[47] Designed a novel thermosensitive hydrogel system composed of N-trimethyl chitosan chloride and β-Glycerophosphate and characterized for morphology and rheological characters; results reveled that at N-trimethyl chitosan chloride and β-Glycerophosphate system was liquid with low viscosity at low temperature, which allowed it to be an ideal injectable material with liquid status for long period at 4^0C and transformed rapidly to gel status within 1 min upon heating it to 37^0 C. This hydrogel promises to be an ideal vehicle for drug release, tissue repairing and regeneration. [Fig 21]

MAO Jinshu *et al.,*[48] In view of development in the field of tissue engineering, the interaction between biomaterials and cells hyaluronic acid was incorporated into chitosan-gelatin system and cell apoptosis was Analysized; hyaluronic acid shortened the adaptation period of cells on the material surface, and then cells enter the normal cell cycle quickly and also inhibits cell apoptosis triggered by the membranes. Thus hyaluronic acid improves the cell compatibility of chitosan-gelatin system and benefits the design of biomimetic materials.

Jariya kowapradit *et al.,*[49] Investigated the effect of methylated N-(4-N,N-dimethylamino-benzyl) chitosan, on paracellular permeability of Caco-2 cell monolayer's and its toxicity towards cell lines. Degree of quaternization and extent of dimethylaminobenzyl substitution were evaluated. The results revealed that, at pH 7.4, methylated N-(4-N, N-dimethylaminobenzyl) chitosan appeared to increase cell permeability in a concentration-dependent manner, and this effect was relatively reversible at lower doses of 0.05-0.5 mM. The cytotoxicity of methylated N-(4-N,N-dimethylaminobenzyl) chitosan depended on concentration, %DQ, and %ES. These studies demonstrate that novel modified chitosan has potential as an absorption enhancer. [Fig 22]

Noppakun Sanpo *et al.,*[50] Analysized the antibacterial behavior of chitosan copper complex powder synthesized by in-house powder processing techniques, and their composite coatings were investigated against

Escherichia coli. Subsequently used these composite powders as feedstock to generate antibacterial coating via cold spray technology. Analysized by FESEM/EDX and FTIR. G. Cardenas *et al.*,[51] Prepared and characterized colloidal Cu nanoparticles/chitosan composite film by solution casting technique with microwave heating. The antibacterial activity of films against *Staphylococcus aureus* and *Salmonella enteric* serovar Typhimurium, were also tested. Incorporation of colloidal Cu nanoparticles on chitosan matrix improved the barrier properties of films, decreasing oxygen and water vapor permeability and increasing protection against UV light. The composite films were effective in alteration of cell wall and reduction of microbial concentration in liquid culture for both bacteria.

CONCLUSION

Chitosan has a wide variety of applications in exploiting its biodegradable nature and structural compatibility in derivatizing to water soluble, N-Sulfonated, Quaternized, Carboxylated chitosan.

- For its antimicrobial and antibacterial activity.
- Its application in cosmetic as artificial skin and wound dressings.
- One of its applications as a waste water treatment by its complexing capability with many of the bivalent metallic Cat ions like Cu II, Co II, Au II, etc.,

Polymer blending, crosslinking and grafting to exploit its applications as microspheres, nanoparticles, films, Mucoadhesive formulations and scaffolds to extend the release pattern and tissue regeneration.

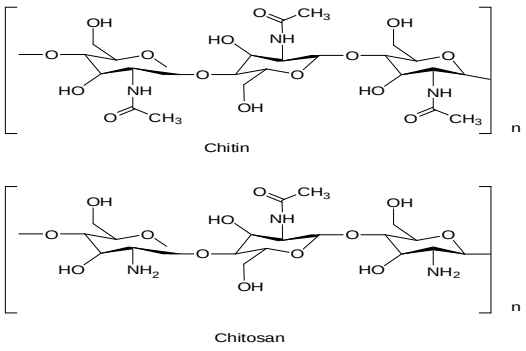
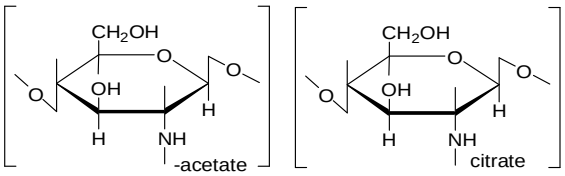


Fig.1



Chitosan Oligosaccharides (COS-A and CoS-C)

Fig. 2

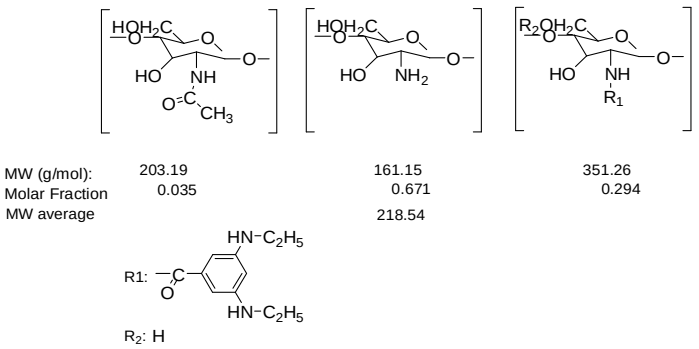

MW (g/mol): 203.19 161.15 351.26
Molar Fraction 0.035 0.671 0.294
MW average 218.54
R1:
R2: H

Fig. 3

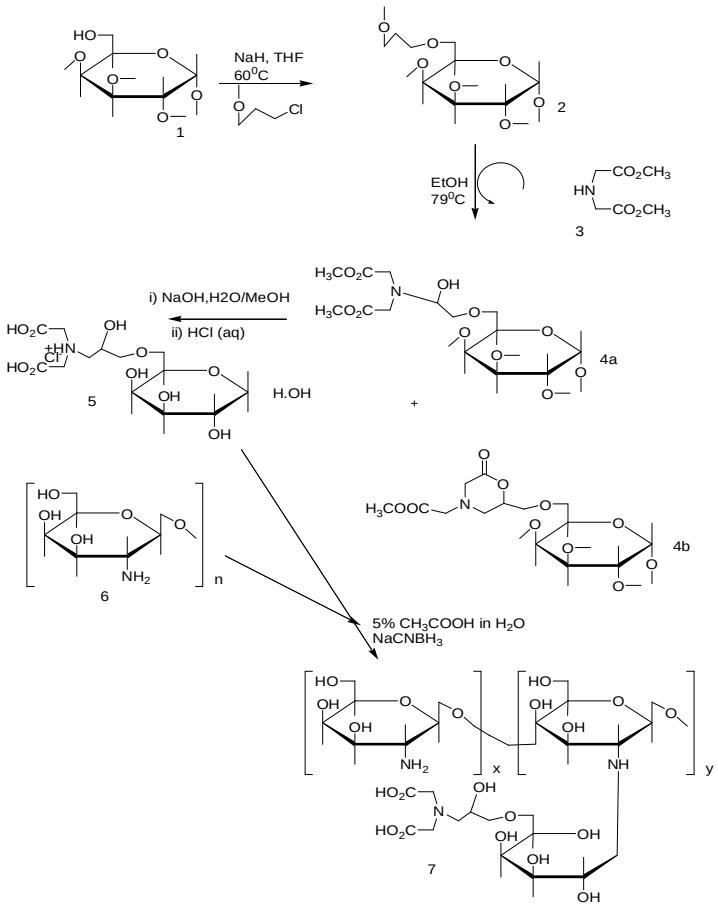
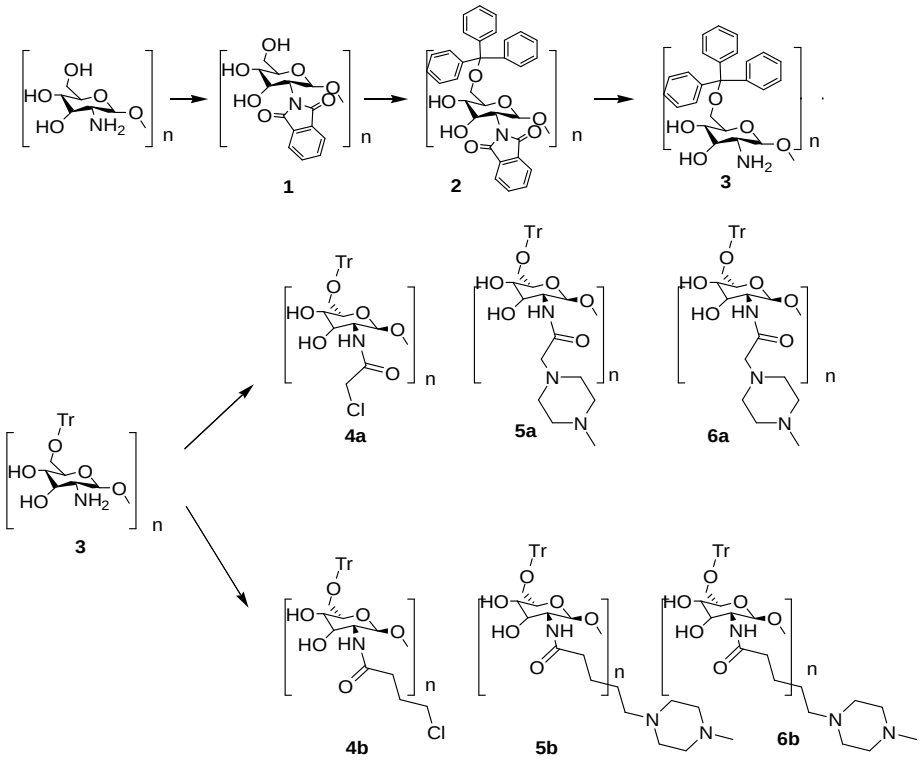

NaH, THF
60°C
EtOH
79°C
i) NaOH,H2O/MeOH
ii) HCl (aq)
5% CH3COOH in H2O
NaCNBH3

Fig. 4



Preparation of Methylpiperazine Derivative of Chitosan **6a, 6b** via N-Chloroacyl-6-O-triphenylmethylchitosan **4a,4b**

Fig. 5

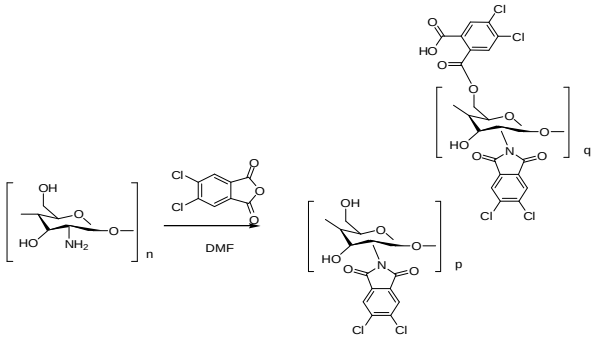


Fig. 6

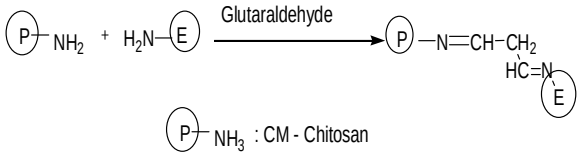


Fig. 7

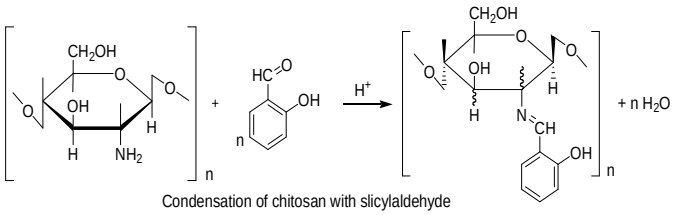
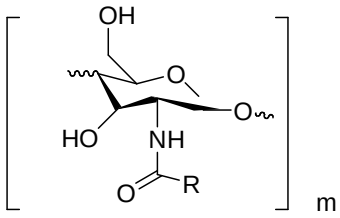


Fig. 8



Modified Chitosan R = - $(CH_2)_4CH_3$ caproyl chitosan

Fig. 9

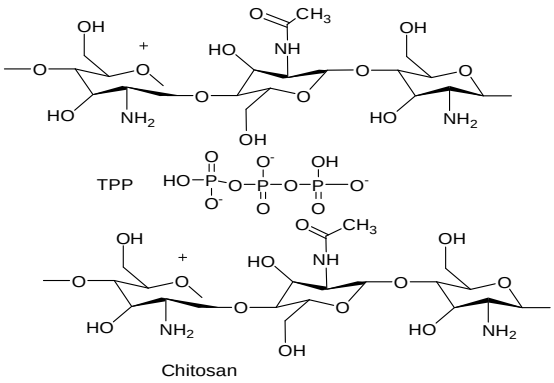


Fig.10

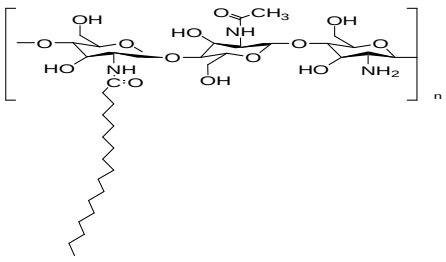
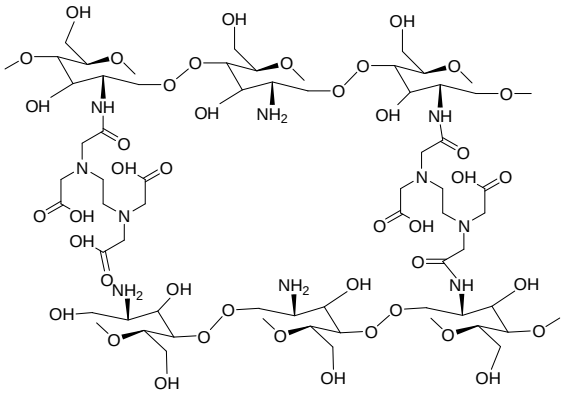


Fig. 11



Chitosan-EDTA conjugates and intermolecular cross-linking via covalent binding of EDTA to more than 1 amino group

Fig. 12

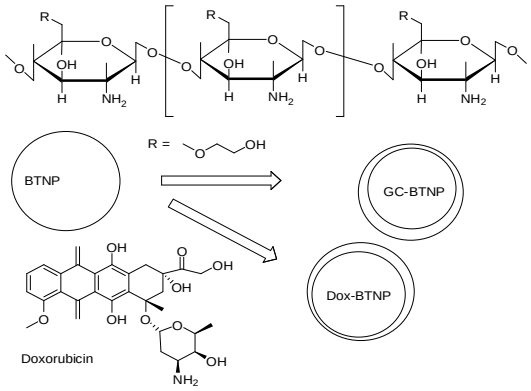


Fig. 13

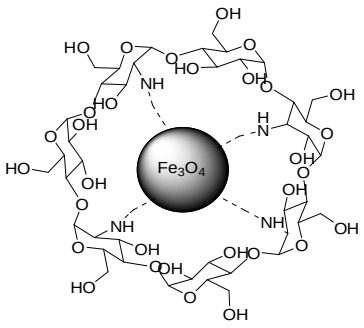


Fig. 14

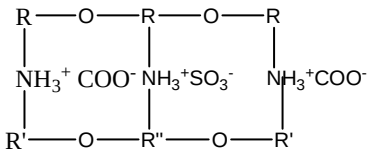


Fig.15

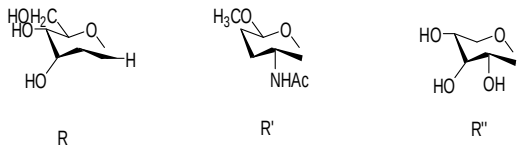


Fig.15a

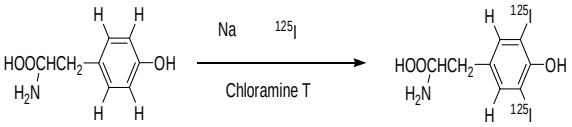


Fig.16

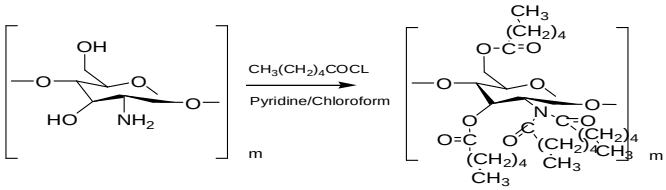


Fig. 17

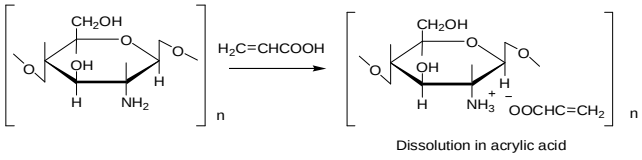
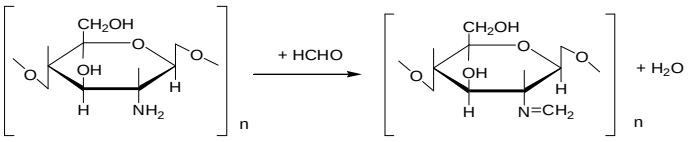


Fig.18



Reaction between chitosan and formaldehyde

Fig.18a

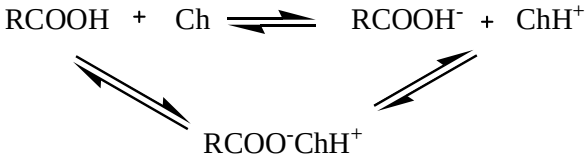
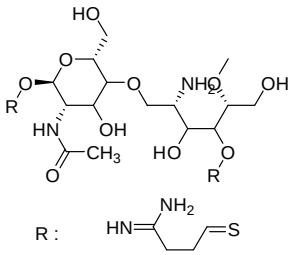
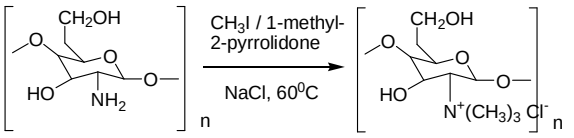


Fig. 19



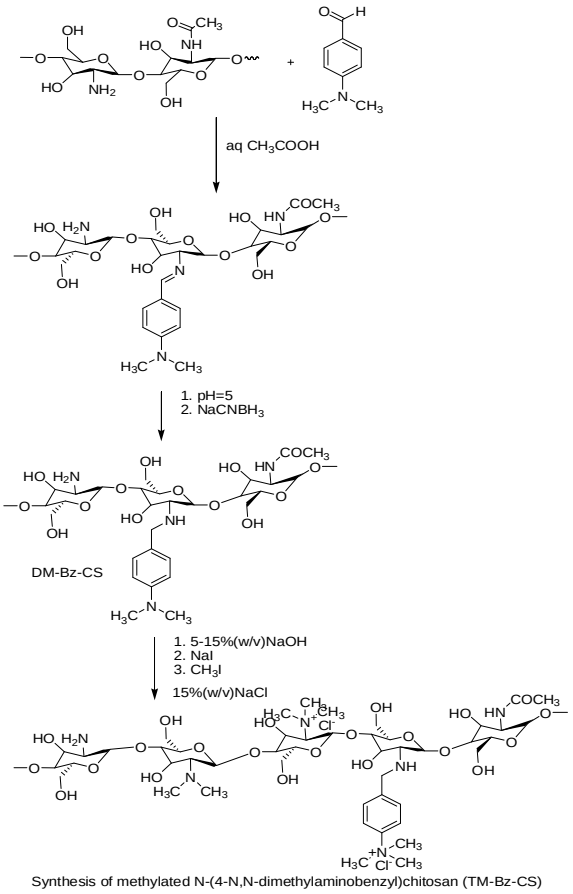
thiobutylamidine

Fig. 20



N-Trimethyl chitosan chloride

Fig. 21



Synthesis of methylated N-(4-N,N-dimethylaminobenzyl)chitosan (TM-Bz-CS)

Fig. 22

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