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Chitosan: Development of Nanoparticles, Other Physical Forms and Solubility with Acids

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Abstract. Chitosan is a modified form of Chitin. It is a modified carbohydrate polymer derived by hydrolyzing the aminoacetyl groups of chitin. Chitosan is a biodegradable natural polymer which is, biocompatible, non-toxic. It also shows anti-bacterial properties. This polysaccharide is available in different forms such as nanoparticles, solution, powder, flake, fiber, film, etc. Due to its wide range of physical forms and good reactivity with other compounds, chitosan can produce various blends. Nanoparticles of various natural biopolymers have emerged as potential carrier for drugs in oral controlled drug delivery. Nanostructured drug carriers allow the delivery of not only small-molecule drugs but also of nucleic acids and proteins. Chemical modification of nanoparticles of chitosan is useful for the association of bioactive molecules to polymer and controlling the drug release profile. In recent years focus on chitosan is shifted to its derivatives. This versatile material has broad applications in many different fields. Various physical forms of chitosan and its blends together with other derivatives such as composites and graft copolymers have been developed to overcome limitations of different polymeric materials such as poor mechanical properties and to improve its functionality towards specific applications. Nanoparticles of chitosan and it derivatives are extensively exploited in the field of oral drug delivery. The progress made in converting chitosan and its blends into nanoparticles forms as well as the preparation methods are studied. For preparation of these blends and nanoparticles of chitosan need to be dissolved in for reactions. We also studied its dissolution behavior with different acids. It shows quite interesting results.

Introduction

Nanotechnology, most explored field in science and technology! Researchers widely exploring nanotechnology for widely different applications. Nanotechnology and nanopolymers have created a tremendous interest in many areas such as the pharmaceutical industry and drug delivery innovation among others. Various natural and synthetic polymers have been used as a promising tool for nanoscale drug carrier systems, especially in oral administration for poorly absorbed therapeutic drugs [1]. In this paper, we will be emphasizing the role of chitosan and its derivatives as a drug carrier, giving special attention to prepare various forms used in controlled oral drug delivery.

Chitosan [2-amino-2-deoxy-β-D-glucan] is a naturally occurring cationic polysaccharide, obtained from chitin. It is derived by the alkaline deacetylation of chitin. Chitin found in exoskeleton of marine crustacean such as shrimps and crabs. It is also found in insects. Chitosan has some very interesting combination of biopharmaceutical properties like pH sensitivity, bioactivity, biocompatibility and low toxicity. However, chemical versatility of chitosan to form derivatives or cross-links, permeation-enhancing capability and ability to control the release of therapeutic agents make it an ideal candidate for fabricating oral nanoparticulate drug delivery system [91]. Moreover, chitosan is metabolized by certain human enzymes, especially lysozyme, and is considered as biodegradable [4]. Good adhesion and sorption properties are the major reasons for its multiple applications [1,2]. Due to these favorable properties, the interest in chitosan and its derivatives as

excipients in drug delivery has been increased in recent years [5,6]. The interest in this natural polysaccharide arises mainly from the fact that it allows the production of biocompatible and biodegradable drug delivery systems. It is extremely important that chitosan is water soluble and positively charged. These properties enable it to interact with negatively charged polymers, macromolecules and polyanions on contact in an aqueous environment. Another main reason for this increasing interest of chitosan is its wide range of physical forms which can be obtained by using an appropriate technological process. Many studies have reported the use of chitosan in the formation of gels, particles, and microspheres. Chitosan has already been used in a variety of fields such as drug delivery, medicine, food, agriculture, wastewater treatment, paper industry and cosmetics etc [3-7].

However chitosan is not having all bushes. It also suffers from some limitations like low solubility at a physiological pH of 7.4, rapidly adsorbing water and higher swelling degree in aqueous environments etc. due to low solubility its use as absorption enhancer is limited in nasal or peroral delivery systems. Due to rapidly adsorbing water and higher swelling degree in aqueous environments, its leading to fast drug release. It limits use of chitosan for the preparation of sustained release systems. In order to overcome these problems, a number of chemically modified chitosan derivatives have been synthesized and tested. Chemical modification of chitosan improves its solubility and widens its applications [9,10]. There are free amino and hydroxyl groups on Chitosan, which enable substitution or modification with different chemical entities to form Chitosan derivatives with desired properties for oral drug delivery.

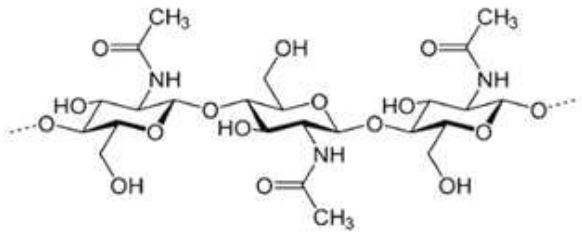


Fig. 1: Chemical structure of chitin.

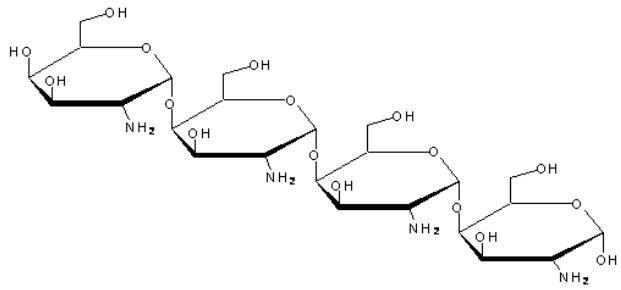


Fig. 2: Chemical structure of chitosan.

Due to the importance of the chitosan in various fields of science and technology, this paper attempts to present the recent developments with chitosan nanoparticles as well as its blends in different physical forms, which are utilized for the controlled oral delivery of different therapeutic agents and their in vitro and in vivo implications. These developments may be physical or chemical. Most of the chemical modifications need to prepare acidic solution of chitosan. So solubility of chitosan is also studied and discussed here.

A typical proton NMR spectrum of chitosan is shown in Figure 3. The signal at δ 3.20 ppm was attributed to H-2 of GlcN residue. The intense band at 4.8 – 5.30 ppm is related to OH groups and HDO (solvent). In this region, as observed more clearly from an extended spectrum, some different anomeric protons (H-1 of GlcN and GlcNAc units) are appeared at 4.88–5.00 ppm. The DA is calculated from the integral ratio between protons of acetyl group and the GlcN protons. The degree of deacetylation (DDA) is calculated from the integral ratio between the proton on C- 2 and the glucose unit protons. The 1H-NMR spectra also allowed us to propose a method to determine the degree of substituent (DS) value and the determination is based on the ratio between the protons of the substituent and the protons of the pyranose unit.

Physical Forms of Chitosan and its derivatives (General Preparation and Characterization):

Chitosan and its derivatives are prepared in various physical forms like nanoparticles, powder, beads (resins), microspheres, films, hydrogels, membranes and fibers. The selection of the form depends on applications criteria. To produce different physical shapes, chitosan is to be mixed with the blend component in the liquid phase itself. Then with different techniques physical shapes are produced. Scanning electron microscope photograph is shown in fig4.

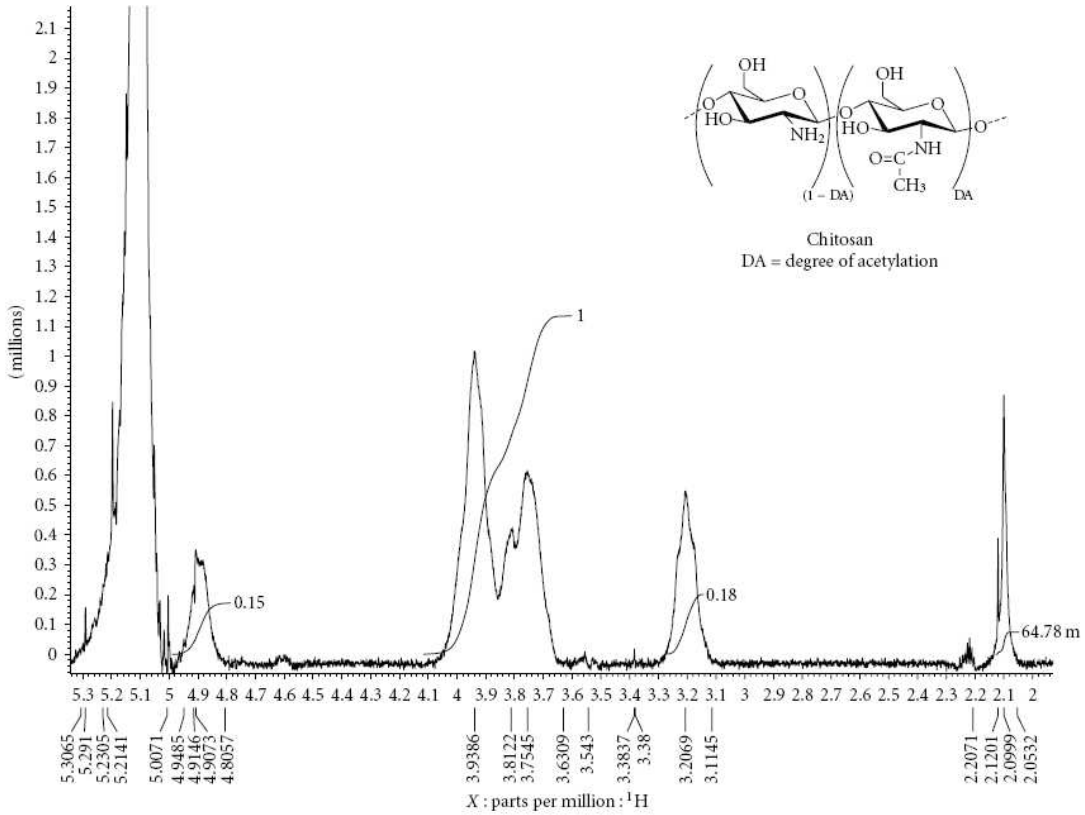


Fig. 1 ^{1}H-NMR spectrum (300 MHz) of chitosan in 0.5% CD_3COOD/D_2O at 25°C.

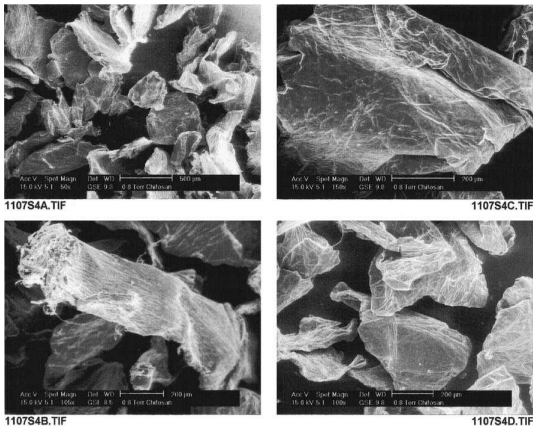
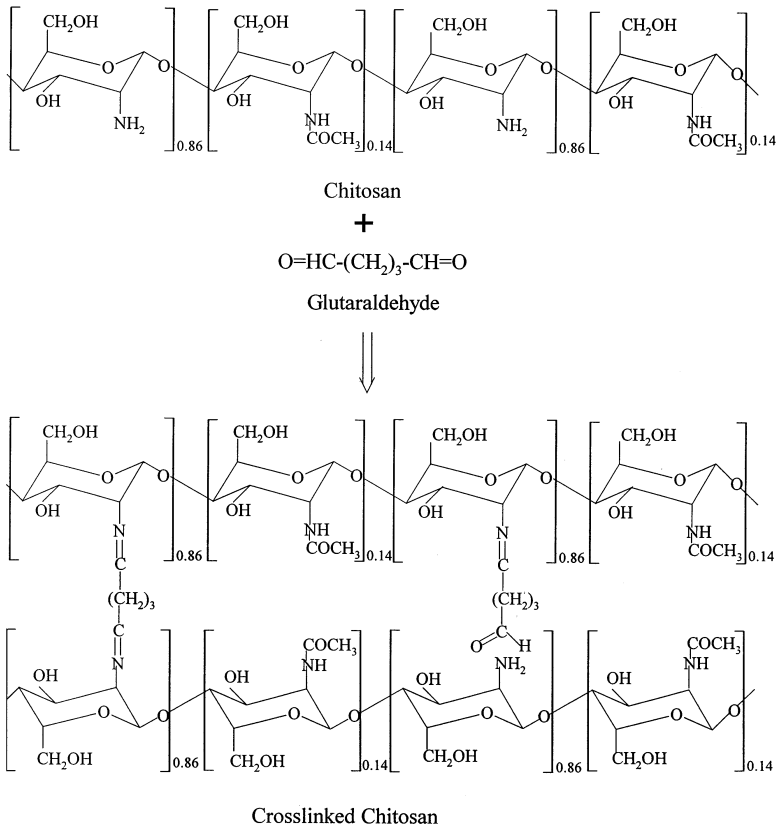


Fig. 4. Chitosan nanoparticles.

Nanoparticles of unmodified chitosan and its derivatives: Several researchers have earlier utilized chitosan for preparing micro and nanoparticles of different drugs, proteins and peptides. Pan and co-workers first prepared nanoparticles only with chitosan in an attempt to improve the oral bioavailability of insulin. They prepared insulin loaded chitosan nanoparticles by ionotropic gelation method using tripolyphosphate (TPP) anions for cross-linking.[91]

Recently, chitosan - nanoparticles of catechin have been developed with same method. These nanoparticles demonstrated high encapsulation efficiency of about 90% with good mucoadhesive property and controlled drug release of only 32% over a period of 24 h. This effect was attributed to better cross-linking of chitosan with TPP during nanoparticle formation. In addition, enhanced interaction due to hydrogen bonding was observed between the phenolic groups of catechin and unreacted amino groups of chitosan.



Though all these studies with unmodified chitosan used almost similar preparatory conditions and formulations parameters, the results indicate that the encapsulation efficiency and release properties of the nanoparticles were more depended on the nature of the drug molecule itself rather than the inherent property of the chitosan - nanoparticles system. Thus, to effectively control the release of its content, it was required to develop some advance chitosan nanoparticulate systems which could precisely anticipate the drug release on oral administration, yet enhance the bioavailability of the drug. Several therapeutic agents have been orally delivered using nanoparticles prepared from different chitosan derivatives and chitosan complexes.

Numerous researchers developed nanoparticulate systems with large variety of chitosan derivatives like:

- Trimethyl Chitosan,
- Quaternized Derivatives of Chitosan,
- Alginate-Modified Chitosan,
- Derivatives Combined with Different Peptides,
- N,O-Carboxymethyl Chitosan,
- Methyl Methacrylate Derivatives,
- Chitosan–Heparin,
- Chitosan–Cyclodextrin Complex,
- Thiolated Chitosan,
- Pegylated Chitosan,
- Lauryl Succinyl Chitosan, etc.

Chitosan is generally accepted as a non-toxic and biocompatible polymer. It is also approved material by Food and Drug Administration (FDA - USA) for medical applications. The LD50 of Chitosan is very low, it is 16 g/kg body weight in mice after oral administration, which is nearly equivalent to household sugar or salt. It is not showing any side effects in human up to 4.5 g/day oral administration of chitosan. However, upon longer usage upto 12 weeks, it showed symptoms of mild nausea and constipation in humans. Chitosan alone is considered safe for oral administration, but its properties may change completely upon chemical modification. Moreover, it is well-known

that the pharmacokinetic properties of a drug or excipients change considerably when included in a nanoparticulate system. This is because the size, charge and surface modifications of the nanoparticles often decide their fate in vivo[91].

The preparation of various chitosan physical forms is summarized in Figure:

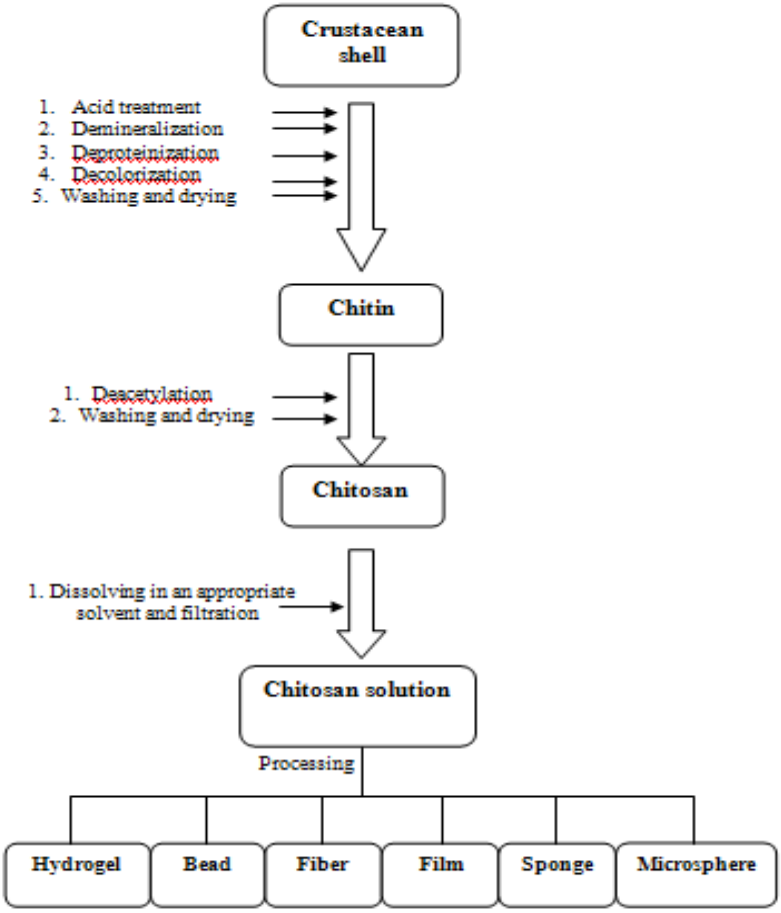


Fig. 5: Routes for preparation of various chitosan physical forms.

Beads (Resins)/microspheres of Chitosan derivatives: Peniche et al and Genta et al prepared beads of chitosan derivatives by various methods, such as solvent evaporation, coacervation, emulsion methods etc. [8, 76] Out of all these methods, emulsion method can produce the smaller and uniform beads of chitosan derivatives. Bodemier *et al* reported the preparation of chitosan beads. For the drug loaded beads, chitosan is dissolved in acetic acid stir it for 6-7 h for cross-linking. Then, known amount of drug is added to the chitosan - acid solution and stir it for 2 h. pH of the drug loaded chitosan solution is maintained around 4 – 4.5 with dilute alkali solution.

After that, this drug-chitosan solution is filled in a syringe and added dropwise into sodium tripolyphosphate solution for preparation of beads. After few minutes these beads separated from solution by filtration. Washed with distilled water and kept at 50°C for 4 h to dry and then at room temperature for 12 h under air flow.

Lim et al. prepared chitosan microspheres by emulsification ionotropic gelation, and reported that the Span 85 concentration had a marked effect on the surface of the microspheres.[92] At high Span 85 concentration, the microspheres had collapsed surfaces. The same effect can be seen in the following Fig. 6. Scanning electron micrograph of microspheres of chitosan microspheres prepared by emulsification ionotropic gelation is shown here.

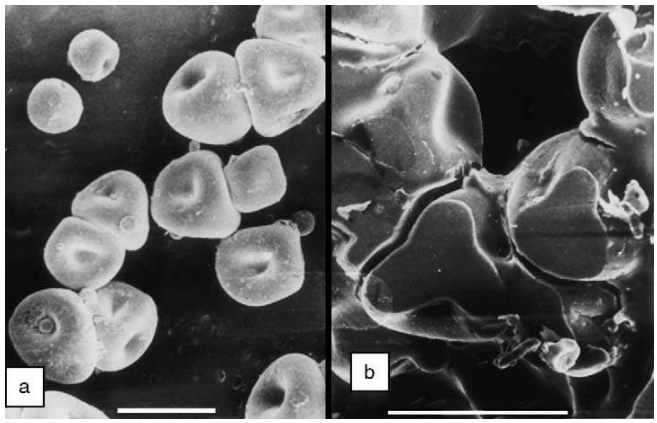


Fig. 6. Scanning electron micrograph of (a) external surface and (b) internal structure of unloaded chitosan microspheres at 1.5% (w/v) Span 85 concentration.

Many researchers reported preparation of beads of chitosan derivatives with different compounds. Details are as below:

- The chitosan/alginate microparticles [11]
- chitosan/xanthan microspheres [12] and
- chitosan/gelatin microspheres [13-14]
- chitosan/cellulose blend beads were prepared via homogeneous dissolution of chitosan and cellulose in methylmorpholine-N-oxide.

In one of the study scanning electron microscopy indicated that the roughness of chitosan microspheres surface increased with increase in drug loading. Fig. 7a shows the surface topography of unloaded chitosan microspheres while Fig. 7b shows the surface of drug (nifedipine) loaded chitosan microspheres.

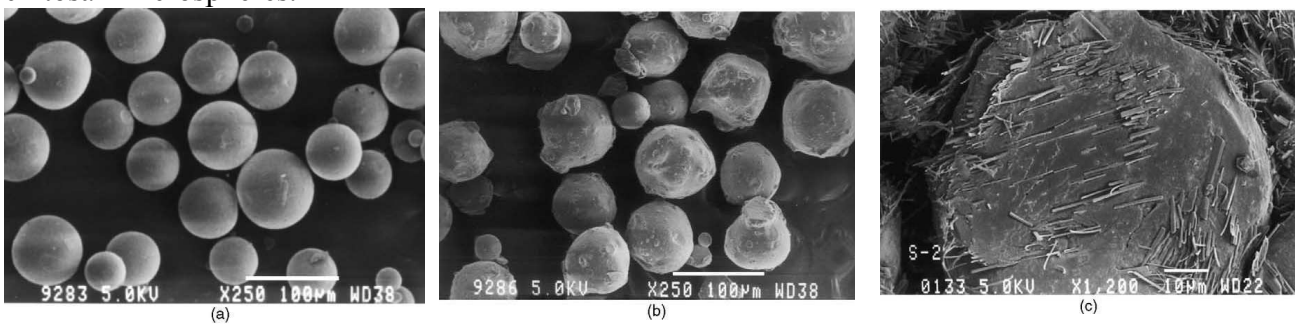


Fig. 7. Scanning electron micrograph of (a) unloaded chitosan microspheres; (b) drug (nifedipine)loaded (27.1%) chitosan microspheres; (c) internal structure of drug (nifedipine)loaded chitosan microspheres.

Twu et al reported that the microspheres prepared by a spray-drying process have a spherical geometry and a smooth surface morphology. [77]

Hydrogels: Hydrogels are important due to their interesting property of absorbing water. These biopolymers are having very specific and wide applications in biomedical, pharmaceutical, tissue engineering etc areas. Berger et al reported different methods for preparation of hydrogels from chitosan.[79] Koseva et al and Kumbar et al reported chitosan is dissolved in organic solvent to prepare emulsions or coacervates and it is followed by cross-linking of the polymer. [81,39]

Currently many researchers are working on blend or crosslinking of chitosan and its derivatives to improve the hydrogel stability. Some prepared non-covalent cross-linked chitosan derivative hydrgels as below: [15-33, 78]

- chitosan/alginate
- chitosan/carboxymethyl-cellulose
- chitosan/dextran sulfate
- chitosan/carboxymethyl-dextran
- chitosan/heparin
- chitosan/carrageenan
- chitosan/pectin
- chitosan/collagen
- chitosan/xanthan
- Crosslinking of chitosan hydrogel
- chitosan/PVA hydrogel membranes with formaldehyde

Films/membranes: Films of chitosan is one of the most commonly used physical form in many applications like skin care, cosmetics, membranes etc. Kanke et al and Bonvin et al reported film of chitosan prepared by casting technique.[35]. Many researchers studied and reported different properties of chitosan films like it clear, homogeneous and flexible, with good oxygen barrier and mechanical properties. Butler et al reported that it is having relatively low water vapor barrier characteristics.[36] Muzzarelli et al and Hirano et al reported that the films of chitosan are dense and do not possess pores.[37,38,83] Upon heating chitosan degrades before melting, so for casting into films it is dissolved in some solvents prior to.

Butler et al and Chen et al reported that properties of chitosan films depend on their morphology, which in turn depends on the molecular weight of chitosan.[40-41] It also depends on source and degree of deacetylation of chitosan. Samuels et al and Lim et al reported that method of film preparation, free amine regenerating mechanism and most importantly by the type of dissolving solvent also affect morphology of chitosan film and in turn its properties too. [9, 45]

Generally chitosan is dissolved in Acetic acid for formation of film or membranes. Austin et al. reported preparation of chitosan films from 5% w/v chitosan solution dissolved in 4% v/v aqueous acetic acid followed by coagulatation in aqueous base solution. Averbach et al. also reported casting chitosan films from 10% aqueous acetic acid solutions on stainless steel plates and drying them at 125^0C.[46] Khan et al reported casting chitosan from other acid solutions such as lactic acid yielded softer and more pliable bioadhesive films compared with those prepared from acetic acid.[47]

Trung *et al* reported that chitosan membranes prepared with various degrees of deacetylation of 75%, 87% and 96% (same molecular weight).[84] They found that membranes obtained from chitosan of higher degree of deacetylation exhibited higher tensile strength and higher elongation at break. In addition, membranes casted from chitosan with 75% degree of deacetylation displayed higher permeability and higher water absorption.

Chen et al and Blair et al prepared chitosan membranes from higher molecular weight chitosan and showed that membranes were having higher tensile strength and percent elongation compared to those obtained from low molecular weight ones.[42]

Elhefian *et al* reported the surface investigation of chitosan film with two fatty acids i.e., stearic (C18) and arachidic (C20) acids monolayers by means of Langmuir-Blodgett (LB) technique and atomic force microscopy (AFM). It was found that smoother and more homogeneous surfaces were achieved after transferring a layer of the fatty acids onto chitosan films.

The properties of films of blend or composite of chitosan with other bipolymers is found better compare to pure chitosan film. Some of the blends are as below:[49-51,85]

- Blended films such as chitosan/pectin laminated films
- chitosan/cellulose
- chitosan/methylcellulose films
- chitosan and poly (ethylene oxide)
- chitosan/agar, chitosan/PVA and chitosan/agar/PVA blended films.

Characterization with the FTIR results, thermal curves and morphology showed that the interaction (intermolecular hydrogen bonding) among the functional groups of the blend components can occur.

The films and membranes made of derivatives of chitosan with other synthetic polymers found improvement in the biocompatibility and expanded use as biomaterials. Miya et al prepared membranes from blends of chitosan and polyvinyl alcohol (PVA).[86] They found membranes to be clear and homogeneous and have mechanical resistance greater than that of the pure components. Uragami et al reported that such membranes were capable of transport of halogenated ions and diffusion of cattle serum albumin and vitamin B12 when crosslinked.[53]

Nasir *et al* reported that in chitosan/ poly (ethylene oxide) blended membrane the addition of PEO with higher molecular weight reduces the percentage of water adsorption of the obtained blended membranes whereas the addition of lower molecular weight PEO improves the porosity of chitosan as revealed by scanning electron microscopy.[85] The results of structural analysis by x-ray diffraction showed that chitosan-PEO blended membrane with higher water adsorption ability has lesser degree of amorphosity. The blend membrane showed intermolecular interactions between chitosan and higher molecular PEO chains which caused an important alteration in chitosan structure and decreased the permeability of the membrane.

Fibers: Kunike prepared Fiber from chitosan solution first time as early as 1926.[54] Fibers possesses many advantages such as superior mechanical properties compared with the same material in bulk form. However, due to the high production cost, other weaknesses of pure chitosan, researchers looked into blends or composites of this polymer with other yarns.

Many researchers reported variety of Fibers from chitosan derivatives.

- fibers of chistosan blends with collagen [41-44]
- starch [86]
- poly(ethylene oxide) [56]
- poly(vinyl alcohol) [11-58]
- silk fibroin [87]
- alginate [60]
- Chitosan-poly (L-glutamic acid) and Chitosan-polyacrylic acid fiber [61]
- collagen-chitosan complex nanofibers by electrospinning.
- Jaipura et al reported that there is potential for various applications in industrial applications due to their enhanced tensile strength and environmental biodegradability.[61]
- Lertviriyasawat et al reported structures of Electrospun nano fibers and nanofibrous of chitosan. [88]
- Desai et al reported nanofiber of chitosan/polyethylene oxide blends were made by high electrospinning for applications in air and water filtration. They prepared non-woven fibers by electrospinning blend solutions of chitosan and poly acrylamide with various blend ratios. Zhang et al reported that the introduction of an ultrahigh-molecular weight poly (ethylene oxide) into aqueous chitosan solutions remarkably enhanced the formation of nanofibrous structure and led to much lower loading of the water soluble fiber-forming aiding agent of PEO down to 5 wt % as compared to previous high PEO loadings in the electrospun chitosan nanofibers.[62-63]
- Pillai and Sharam reviewed the applications of chitin and chitosan nanofibers.

Surface characteristics of electrospun chitosan fibers are presented in Figure 8. These fibers were prepared from aqueous acetic acid solution of 7 wt% chitosan. In the SEM photograph relatively smooth as well as coarse regions are seen. Pore sizes are also different. Figures 8b and 8d show scaffold-like structures in the coarse regions. These easily visible differences could be produced by variations in the processing. An interesting explanation was given by Gholipour et al. [93]. They have observed that increasing the concentration of chitosan produces brittle fibers; even viscosity also. Due to high viscosity of pure chitosan fibers cannot even be electrospun. They have noted that

an increase in the number of amino groups in acidic media causes a corresponding increase in the density of electrical charges on the surface of the jet, and therefore electrical field effects are stronger [93].

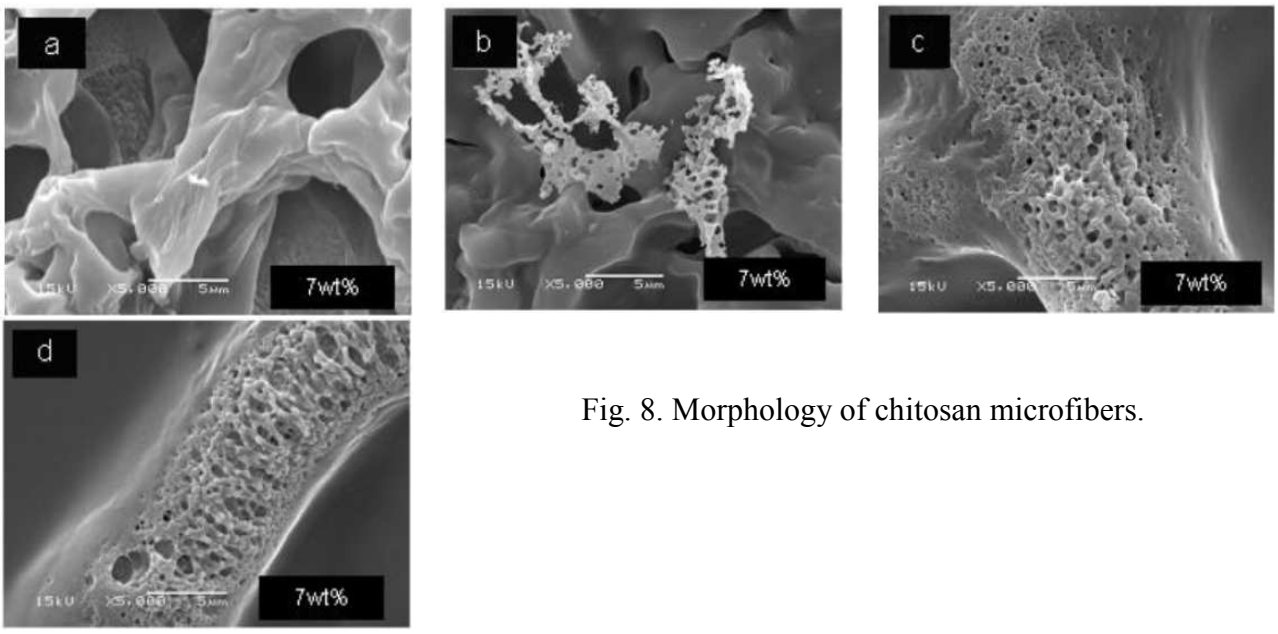


Fig. 8. Morphology of chitosan microfibers.

Sponge: Sponge of chitosan derivatives are widely used in drug carrier systems. As chitosan is having some interesting properties like biodegradability, biocompatibility, antibacterial activity and non-toxicity together with film forming capability. Poole et al reported that sponge is produce by foaming the solution. However, chitosan solutions cannot be foamed alone. Therefore, it is often combined with another good foaming polymer such as gelatin, which is also a cheap biodegradable polymer with good foaming properties. Thacharodi et al reported that when blended with chitosan, gelatin makes a good contact with chitosan and forms polyionic complexes of slower rate of dissolution than chitosan at the appropriate pH value.[89]

Oungbho reported that such property could be utilized in drug release to a wound while the spongy form absorbs the wound fluid. [64]

Tabata et al and Jameela et al reported that the release profile and biodegradability of both polymers can be effectively controlled by crosslinking.[64- 65] Leffler et al reported that the release profile was found to be affected by the type of acid used during the preparation. [66] Different researchers studied different combination to produce better quality sponge. Some are as below:[67-74]

- chitosan/alginate sponges were also studied in various occasions
- Chitosan and sodium alginate blended sponge incorporating silver sulfadiazine was reported by Kim *et al* for wound dressing application.
- Yang *et al.* also prepared hepatocyte-loaded alginate/galactosylated chitosan sponges for the growth of liver tissue.
- Sponge of blended chitosan-fibroin that could be suitable for a future construction of dressing materials has been investigated by Strobin *et al*.

Composition of chitosan/fibroin sponges affect their structures of surface and cross-section. The sponges exerted reasonable strength and elasticity which gives a well grounded chance to use them for the construction of dressings.

Solubility of chitosan

Chitosan is a semi-crystalline polymer, a weak base, which is insoluble in water, alkali or aqueous solution above pH 7, and common organic solvents due to its stable and rigid crystalline structure. Chitosan is normally polydispersed and has the ability to dissolve in certain inorganic and

organic acids such as hydrochloric acid, phosphoric acid, lactic acid, propionic acid, succinic acid, acetic acid, tartaric acid, citric acid and formic acid at certain pH values after prolonged stirring.[48] Nitric acid could dissolve chitosan, but after dissolution, white gelatinous precipitate would occur.[39] Sulphuric acid does not dissolve chitosan because it would react with chitosan to form chitosan sulphate, which is a white crystalline solid.[39] The solubility of chitosan also depends on the p*K*a of these acids and their concentrations. Investigation of chitosan dissolution characteristics revealed that its dissolution rate varied according to the type of acid used. Chitosan behaves as a sphere in aqueous acetic acid solution or as an expanded random coil in urea.[90]

For each solvent system, polymer concentration, pH, counter-ion concentration and temperature would affect the solution viscosity. For example, at pH value below 4, most of the amino groups of chitosan are supposed to be protonated, and since this effect promotes electrostatic repelling between charged groups of the same sign, it leads to enhanced swelling of the polymer network.[90] While Muzzarelli et al reported that at pH 5.2, an unstable structure is generated. Upon neutralization with an excess NaOH, the ionic strength of the solution increases and therefore the size of the aggregates decreases due to compaction of the macromolecular coils.[83]

The free amino groups form intermolecular hydrogen bonds with the oxygen of the adjacent chains. At pH value greater than 6.5, which is approximately the pKa of the amino group in chitosan, the size of the aggregates increases and phase separation occurs. The polymer coagulates and can be recovered as an amorphous solid.[90]

The uniqueness of chitosan depends on the distribution of the acetyl groups remained along the chain but mostly depends on the free amino (-NH2) groups which is important in forming conformational features through intra and / or intermolecular hydrogen bonding. This makes it soluble in acidic solutions below pH of approximately 6.5 and thereby overcoming associative forces between chains. Amino groups make chitosan a cationic polyelectrolyte (p*K*a ≈ 6.5), one of the few found in nature. In contrast, other polysaccharides are either neutral or negatively charged. The basicity gives chitosan singular properties: chitosan is protonated upon dissolution in aqueous acidic medium at pH < 6.5, but when dissolved possesses high positive charge on –NH3 + groups and the resultant soluble polysaccharide is positively charged. As a result, it adheres to negatively charged surfaces. Chitosan aggregates with polyanionic compounds, and chelates heavy metal ions. Both the solubility in acidic solution and aggregation with polyanions impart chitosan with excellent gel-forming properties.[90]

Even though chitosan is known to have important functional activities, the poor solubility of chitosan is the major limiting factor in its utilization. This interferes with the biomedical application of chitosan, especially at the physiological pH value (7.4) where chitosan is insoluble and ineffective as an absorption enhancer.[90] Hence, improving the solubility of chitosan is crucial if this plentiful resource is to be utilized across a wide pH range. Despite this limitation, various applications of chitosan and modified chitosan have been reported. [90] Chitosan possesses distinct chemical and biological properties. In its linear polyglucosamine chains of high molecular weight, chitosan has reactive primary amino and hydroxyl groups, amenable to chemical modification and provide a mechanism for side group attachment using a variety of mild reaction conditions. Modification of chitosan provides a powerful means to promote new biological activities and to modify its mechanical properties. The general effect of addition of a side chain is to disrupt the crystal structure of the material and hence increase the amorphous fraction. This modification generates a material with lower stiffness and often altered solubility. [90]

Solubility with acids: We had discussed that for formation of different physical forms of chitosan, it is required to make solution of it. Chitosan is biopolymer, it is not dissolve in water. So to make solution for different processes, we need to have data regarding its solubility. In many processes, it is dissolved in acidic media. So we studied the solubility of Chitosan with different acids. Most common laboratory acids are taken Acetic Acid, Sulphuric acid, Nitric Acid and Hydrochloric acid.

Materials and experimental methods:

Acetic acid (AA) and Hydrochloric acid (HA) purchased from CDH and used as that were supplied. Sulphuric acid (SA) and Nitric acid (NA) purchased from Merck and used as that were supplied. Chitosan was supplied by Cochin Fishries as gift sample.

Acid was taken in predefined quantity and diluted with water as per information given in table 2. Then 0.1 gm chitosan was added in it. Stirring was carried out as per time duration mentioned in table 2. All samples were studied for atmospheric temperature (i.e 32 C), 50 C and 80 C. Stirring was maintained at 500- 600 rpm. All the samples were neutralized after cooling and found the chitosan reprecipitated. The samples were studied under SEM. After getting soluble and reprecipitated, the surface found getting more smooth.

Results:

The following table 1 gives information regarding its solubility experiments:

Table 1 Results of Solubility Experiments.

Acid type	Acid ml	Water ml	Chitosan g	Stirring min	Temperature ^{0}C	Result
AA	5	95	0.1	60	Atmospheric	dissolved
AA	5	95	0.1	30	50	dissolved
AA	5	95	0.1	30	80	dissolved
HA	5	95	0.1	60	Atmospheric	Not dissolved
HA	5	95	0.1	30	50	dissolved
HA	5	95	0.1	30	80	dissolved
SA	5	95	0.1	60	Atmospheric	Not dissolved
SA	5	95	0.1	30	50	Not dissolved
SA	5	95	0.1	30	80	dissolved
NA	5	95	0.1	60	Atmospheric	Not dissolved
NA	5	95	0.1	30	50	dissolved
NA	5	95	0.1	30	80	dissolved

HA: Hydrochloric Acid, AA: Acetic Acid, SA: Sulphuric Acid, NA: Nitric Acid

Morphology observations

Analysis of the morphologies of chitosan before and after solubilize in acid is obtained by Scanning Electron Microscopy (SEM), is shown in fig 8. It shows that the surface of pure chitosan found rough compared to the chitosan after dissolution and precipitation. The surface become smoodh. It may be due to removal of some impurity molecultes attached on the surface of the chitosan molecules.

Applications:

According to the literature, most of the applications of chitosan and its blends are in the pharmaceutical, biomedical and industrial fields depending on the type of the physical form. For example, chitosan beads have been receiving increasing interest in drug release applications. It has also been reported that chitosan beads are useful in heavy metals removal. Sponge of chitosan blends are also of current interest as wound dressing. Following table summarizes the applications of chitosan and its blends in different physical forms.

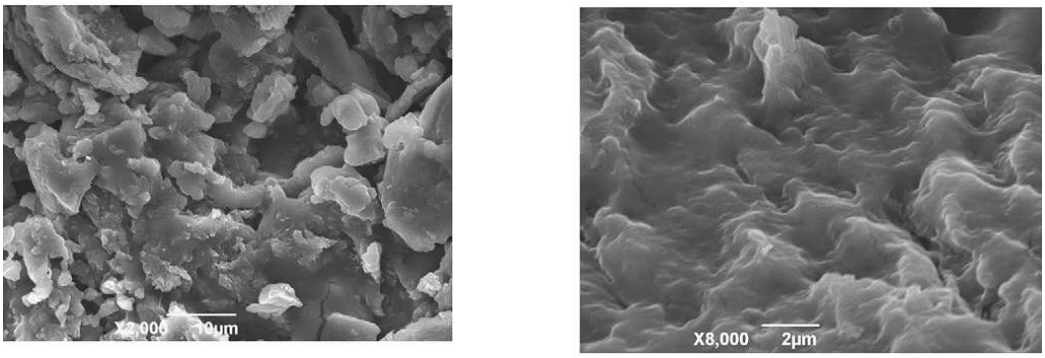


Fig 10. Chitosan nanoparticles surface before dissolution in acids.

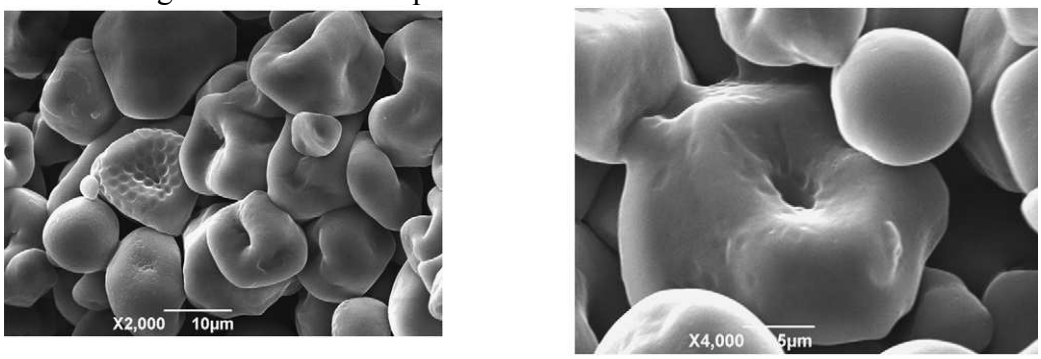


Fig. 11. Chitosan nanoparticles surface after dissolution in acids and precipitated with alkali.

Table 2: Some applications of chitosan and its blends in different physical form. [90]

Chitosan Derivatives	**Physical form**	**Application**	**Team of researchers**
Chitosan and chitosan-alginate	Beads	Removal of Cu(II) ions	(Wan Ngah, 2008)
Ionic cross-linked chitosan	Beads	Drug release	(Shu, 2002)
cross-linked chitosan-alginate	Beads	Drug release	(Anal, 2005)
chitosan and cross-linked chitosan beads	Beads	Removal of Cu(II) ions	
Ionically and chemically cross-linked chitosan	Beads	Remove the anionic dyes	(Chiou, 2004)
Glutaraldehyde-activated alginate-chitosan Gel	beads	Immobilization of antibodies	(Albarghouthi, 2000)
Chitosan and cross-linked chitosan beads with glutaraldehyde (GLA), epichlorohydrin(ECH) and ethylene glycol diglycidylethe	Beads	Adsorption of Fe(II) and Fe(III) ions	(Wan Ngah, 2005)
Chitosan cross-linked with sodium tripolyphosphate	Beads	Drug release	(Srinatha, 2008)
Chitosan	Beads	Color removal	(Wu, 2001)
Chitosan	Beads	Drug release	(Sezer, 1995)
Chitosan	Beads	Sorption of Cr(VI)	(Kousalyaa, 2010)
Chitosan/calcium phosphate composite materials and drug carrier	Fiber	Biomedical implant	(Matsuda, 2004)
Chitosan-poly (Lglutamic acid) and Chitosan-polyacrylic acid	Fiber	Industrial applications	(Jaipura, 2006)
Chitosan flakes and cross-linked chitosan beads with glutaraldehyde	Flakes and beads	Adsorption of p-nitrophenol	(Wan Ngah, 2006)

Chitosan/carrageenan cross-linked with glutaric acid and glutaraldehyde	hydrogel beads	Drug release	(Piyakulawat, 2007)
Chitosan	Hydrogel beads	Adsorption of nitrate	(Chatterjee, 2009)
Chitosan	Hydrogel beads	Fulvic acid adsorption	(Wang, 2008)
Chitosan	Hydrogel beads	Encapsulate proteins.	(Alsarra, 2004)
Chitosan	Hydrogel beads	Fulvic acid removal	(Sun, 2008)
κ-carrageenan/chitosan	Membrane spherical capsules	Drug release	(Tomida, 1994)
Chitosan	Microspheres	Drug release	(Li, 1992)
Chitosan	Microspheres	Encapsulation	(Kosaraju, 2006)
Chitosan cross-linked with glutaraldehyde	Microspheres	Drug release	(Genta, 1998)
Collagen-chitosan	Nanofibers	Tissue engineering	(Chen, 2007)
Chitosan	Porous beads	Cu (II) ion adsorption	(Zhao, 2007)
Chitosan-gelatin	Sponge	Biomedical applications (absorbs the wound fluid)	(Oungbho, 1997)
Chitosan /sodium alginate + silver sulfadiazine	Sponge	Wound dressing application	(Kim, 1999)
Chitosan-fibroin blend	Sponge	Dressing materials	(Strobin, 2006)

Conclusions:

Due to the importance of chitosan forms in several fields, we have attempted to shortly review the recent studies on the nanoparticulate forms of chitosan and its blends. These nanoparticles are potential carriers for various drug molecules, peptides, proteins and DNAs. It is found having quite attractive stability, enhanced paracellular transportation and mucoadhesion compared to conventional drug carriers. These nanoparticulate drug carriers could effectively protect drug molecules from degradation against pH variation or enzymatic actions in GI tract. In addition to this, these nanoparticles of chitosan and its derivatives could alter the rate of release of drug encapsulated in it and play better role of drug release rate controller. Overall, it can be concluded that the chitosan-based nanoparticles have a promising future as an oral controlled drug delivery system. We also focus on the solubility of chitosan with most common acids. Results show that except acetic acid, all other acids are not showing solubility at atmospheric condition. But when we increase temperature of the solution, it starts to soluble. With increase in temperature, we also found that it would need less time to get dissolved. These studies clearly revealed the strong relation between the physical form, solubility and application of chitosan.

References:

[1] Ravi Kumar, M.N.V., 2000. React & Funct. Polym., 1: 46-1

[2] Pillai, O. and R. Panchagnula, 2001. Curr.Opin. Chem. Biol., 5: 447

[3] Khor, E. and L.Y. Lim, 2003. Biomaterials., 24: 2339

[4] Yuan, S. and T.J. Wei, 2004. Bioact.Compat.Polym., 19-467

[5] Crini, G., 2006. Bioresour. Technol., 97-1061

[6] Chiou, M.S., P.Y. Ho and H.Y. Li, 2004. Dyes and Pigments., 60-69

[7] Griesbach, U., 1999. Panzer C and Wachter R. Cosmet. Toilet., 114-81

[8] Genta, I., M. Constantini, A. Asti, B. Conti and L. Montanari, 1998. Carbohydr. Polym., 36-81

[9] Lim, L.Y., L.S.C. Wan and P.Y. Thai, 1997. Drug. Dev. Ind. Pharm., 23-981

[10] Huguet, M.L., A. Groboillot, R.J. Neufeld, D. Poncelet and E.J. Dellacherie, 1994. Appl. Polym. Sci., 51-1427

[11] Jia, Y.T., J. Gong, X.H. Gu, H.Y. Kim, J. Dong and X.Y. Shen, 2007. Carbohydr. Polym., 67-403

[12] Chellat, F., M. Tabrizian, S. Dumitriu., E. Chornet, C.H. Rivard and L.J. Yahia, 2000a. Biomed. Mater.Res., 53-592

[13] Yao, K.D., M.X. Xu, Y.J. Yin, J.Y. Zhao and X.L. Chen Polym.l, 1996. Int. 39-333

[14] Yuji, Y.J., M.X. Xu, X. Chen and K.D. Yao, 1996. Chin. Sci. Bull., 41-1266

[15] Berger, J., M. Reist, J.M. Mayer, O. Felt, N. Peppas, 2004a. A and Gurny R. Eur. J.Pharm. Biopharm., 57-19

[16] Arguelles-Monal, W., O.L. Hechavarria, L. Rodriguez and C. Peniche, 1993. Polym. Bull., 31-471

[17] Fukuda, H., 1980. Bull. Chem. Soc. Jpn., 53-837

[18] [Fukuda, H. and Y. Kikuchi, 1978. Bull. Chem. Soc. Jpn., 51-1142

[19] Sakiyama, T., H. Takata, T. Toga and K.J. Nakanishi, 2001. Appl. Polym. Sci., 81-667

[20] Sakiyama, T., C. Chu, T. Fujii and T.J. Yano, 1993. Appl. Polym. Sci., 50-2021

[21] Kikuchi Y and Noda A. J. Appl. Polym. Sci. 1976, 20, 2561

[22] Kratz, G., M. Back, C. Arnander and O. Larm, 1998. Scand. J. Plast. Reconstr. Surg. Hand. Surg., 32-301

[23] Chang, K.L.B. and J.Lin, 2000. Carbohydr. Polym., 43-163

[24] Mireles, C., M. Martino, J. Bouzas and J.A. Torres, 1992. Complex Formation of Chitosan and Naturally Occurring Polyanions. In Chitin and chitosan; Elsevier Applied Science: London. 506

[25] Zhang, Q., L. Liu, L. Ren and F.J. Wang, 1997. Appl. Polym. Sci., 64-2127

[26] Chellat, F., M. Tabrizian, S. Dumitriu, E. Chornet, P. Magny, C.H. Rivard, *et al.* 2000b. J. Biomed. Mater.Res., 51-107

[27] Chu, C., T. Sakiyama and T. Yano, 1995. Biosci.Biotech.Biochem., 59-717

[28] Dumitriu, S., E. Chornet, 1998. Adv. Drug Del. Rev., 31-223

[29] Dumitriu, S. and E. Chornet, 1996. Functional Versatility of Poly Ionic Hydrogels; Ed.; R.A.A. Muzzarelli, Chitin Enzymology, Italy, Atec Edizioni., 2: 543-564

[30] Dumitriu, S., E. Chornet, 1998. Adv. Drug Del. Rev., 31-223

[31] Dumitriu, S. and E. Chornet, 2000. Chitin Enzymology., 2-527

[32] Dumitriu, S., P. Magny, D. Montane, P.F. Vidal and E.J. Chornet, 1994. Bioact. Comp. Polym., 9-184

[33] Singh, A., S. Narvi, P.K. Dutta and N.D. Pandey, 2006. Bull. Mater. Sci., 3-233

[34] Yang, M.J., W.Y. Su, T.L. Leu and M.C.J. Yang, 2004. Memb. Sci., 236-39

[35] Kanke, M., H. Katayama, S. Tsuzuki and H. Kuramoto, 1989. Chem. Pharm. Bull., 37-523

[36] Butler, B.L., P.J. Vergano, R.F. Testin, J.M. Bunn and J.L.J. Wiles, 1996. Food Sci., 61-953

[37] Chen RH. and H.D. Hwa, 1996. Carbohydr. Polym., 29-353

[38] Genta, I., P. Giunchedi, F. Pavanetto, B. Conti, P. Perugini and U. Conte, R.A.A. Muzzarelli, and M.G.Peter, 1997. Eds.; Chitin Handbook; Atec: Italy, 391

[39] Kumbar, S.G., A.R. Kulkarni and T.M.J. Aminabhavi, 2002. Microencapsul., 19-173

[40] Butler, B.L., P.J. Vergano, R.F. Testin, J.M. Bunn and J.L.J. Wiles, 1953. Food Sci., 61-953

[41] Chen, Z.G., X.M. Mo and F.L. Qing, 2007. Mater. Lett., 61-3490

[42] Chen, Z.G., X.M. Mo and F.L. Qing, 2006. Tissue Eng., 12-1074

[43] Chen, Z.G., X.M. Mo and F.L. Qing, 2007. Mater. Lett., 61-3490

[44] Chen, Z., X. Mo, C. He and H. Wang, 2008a. Carbohydr.Polym., 72-410

[45] Samuels, R.J.J., 1981. Polym.Sci., Part B. Polym. Physic., 19-1081

[46] Averbach, L.B., 1975. The Structure of Chitin and Chitosan; MITSea Grant Program, Report No. NITSG 674, Cambridge, Massachusetts, 75-17

[47] El-hefian, E.A., M.M. Nasef, A.H. Yahaya and R.A. Khan, 2010. J. Chil. Chem. Soc., 55-130

[48] Khan, T.A., K.K. Peh and H.S.J. Ch'ng, 2000. Pharm. Pharmaceut. Sci., 3-303

[49] Hoagland, P.D. and N.J. Parris, 1996. Agric. Food. Chem., 44-1915

[50] Hasegawa, M., A. Isogai, F. Onabe, M. Usuda and R.H. Atalla, 1992. J. Appl. Polym. Sci., 45-1873

[51] Kittur, F.S., K.R. Kumar and R.N.Z. Tharanathan, 1998. Lebensm Unters A. Forsch, 206-44

[52] Kittur, F.S., K.R. Kumar and R.N.Z. Tharanathan, 1998. Lebensm Unters A. Forsch 206-44

[53] Uragami, T., F. Yoshida and M.J. Sugihara, 1983. Appl. Polym. Sci., 28-1361

[54] Kunike, G., 1926. Kunsideide (Chemiefasern)., 8.182

[55] Sun, X.F., S.G. Wang, X.W. Liu, W.X. Gong, N. Bao and Y. Ma, 2008. Physicochem. Eng. Aspects., 324-28

[56] Wang, S.G., X.F. Sun, X.W. Liu, W.X. Gong, B.Y. Gao and N. Bao, 2008. Chem. Eng. J., 142-239

[57] Zhou, Y.S., D.Z. Yang and J. Nie, 2006. J. Appl. Polym. Sci., 102-5692

[58] Bhattarai, N., D. Edmondson, O. Veiseh, F.A. Matsen and M.Q. Zhang, 2005. Biomaterials., 26-6176

[59] Liao, I., C.A. Wan, E.K.F. Yim and K.W.J. Leong, 20005. Control. Release., 104-347

[60] Jaipura, L., A. Harad and S. Maitra, 2006. Asian Text. J., 15-55

[61] Desai, K. and K. Kit, 2008. Polymer., 49-4046

[62] Desai, K., K. Kit, J. Li and S. Zivanovic, 2008. Biomacromol., 9-1000

[63] Oungbho, K. and B.W. Muller, 1997. Int. J. Pharm., 156-229

[64] Jameela, S.R., T.V. Kumary, A.V. Lal and A.J. Jayakrishnan, 1998. Control. Release., 52-17

[65] Leffler, C.C. and B.W. Müller, 2000. Int. J. Pharm., 194-229

[66] Shapiro, L. and S. Cohen, 1997. Biomaterials., 18-583

[67] Kataoka, K., Y. Suzuki, K. Ohnishi, K. Suzuki, M. Tanihara, C. Ide, K. Endo and Y.J. Nishimura, 2001

[68] Singla, A.K. and M.J. Chawla, 2001. Pharm. Pharmacol., 53-1047

[69] Kumar, R.M.N.V., 2000. React. Fun. Polym., 46-1

[70] Coppi, G., V. Iannuccelli, B. Leo, M.T. Bernabei and R. Cameroni, 2002. J. Microencapsul., 19-37

[71] Lai, H.L., A. Abu'Khalil and D.Q.M. Craig, 2003. Int. J. Pharm., 251-175

[72] Dai, M., X.L. Zheng, X. Xu, X.Y. Kong, X.Y. Li, G. Guo, F. Luo, X. Zhao, Y.Q. Wei and Z.J. Qian, 2009. Biomed. Biotech., doi:10.1155/2009/595126

[73] Kim, J.J., H.C. Lee, J.S. Oh, B.A. Shin, C.S. Oh, R.D. Park, K.S. Yang and C.S. Cho, 1999. J. Biomater. Sci. Polym. Edn., 10-543

[74] Yang, J., T.W. Chung, M. Nagaoka, M. Goto, C.S. Cho and T. Akaike, 2001. Biotech. Lett., 23-1385

[75] Strobin, G., M. Kucharska, M. Ciechaiska, D. Wawro, W. Stiplewski, J. Jóiwicka and S. Sobczak, 2006. Pol. Chitin Soc., XI-61

[76] Peniche, C., 2003. W. Argu¨elles-Monal, H. Peniche and N. Acosta, 2003. Macromol. Biosci. 3-511

[77] Twu, Y.K., H.I. Huang, S.Y. Chang and S.L. Wang, 2003. Carbohydr.Polym., 54-425

[78] Takka, S. and F.J. Acarturk, 1999. Microencapsul., 16-275

[79] Berger, J., M. Reist, J.M. Mayer, O. Felt and R. Gurny, 2004b. Eur. J. Pharm. Biopharm., 57-35

[80] Ikeda, S., H. Kumagai, T. Sakiyama, C. Chu and K. Nakamura, 1995. Biosci. Biotech. Biochem., 59-1422

[81] Koseva, N., N. Manolova, N. Markova, T. Radoucheva and I. Rashkov, 1999. Polym. Bull., 43-101

[82] Muzzarelli, R.A.A, 1977. Chitin; Pergamon Press Ltd: Hungary., 83

[83] Wang, Q., N. Zhang, X. Hu, J. Yang and Y. Du, 2007. Eur. J. Pharm. Biopharm., 66-398

[84] Trung, T.S., W.W. Thein-Haw, N.T. Qui, C.H. Ng and W.F. Stevens, 2006. Bioresour. Technol., 97-659

[85] Mohd, Nasir N.F., Mohd N. Zain, M.G. Raha and N.A. Kadri, 2005. Am. J. Appl. Sci., 2-1578

[86] Miya, M., A.S. Yoshikawa, R.Iwamoto and S. Mima 1983. KobunshiRonbunshu. 40-645

[87] Park, S.I. and Y. Zhao, 2004. J. Agric. Food Chem., 52-1933

[88] Lertviriyasawat, S., A. Chuachamsai and P. Danwanichakul, 2008. Thammasat. Int. J. Sc. Tech., 13-48

[89] Thacharodi, D. and K.P. Rao, 1995. Int. J. Pharm., 120-115

[90] El-hefian,E.A., Nasef, M.M. and Yahaya, A.H., Australian Journal of Basic and Applied Sciences, 5(5): 670-677, 2011

[91] Chaudhury, A. and Das,S., AAPS PharmSciTech, Vol. 12, No. 1, (March 2011), p10-20

[92] V.R. Sinha et al. / International Journal of Pharmaceutics 274 (2004) 1–33

[93] Espíndola et al, Int. J. Mol. Sci. 2011, 12.1908-1920

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