Akakuru et al., Biochem Pharmacol (Los Angel) 2018, 7:1
DOI: 10.4172/2167-0501.1000241

Biochemistry & Pharmacology: Open Access

Research Article Open Access

The Chemistry of Chitin and Chitosan Justifying their Nanomedical Utilities

Akakuru OU[1,2*], Louis H[2,3], Amos PI[4], Akakuru OC[5], Nosike EI[6] and Ogulewe EF[7]

[1]Ningbo Institute of Materials Technology and Engineering, Chinese Academy of Sciences, Zhejiang, China
[2]Department of Pure and Applied Chemistry, University of Calabar, Cross River State, Nigeria
[3]CAS Centre For Excellence in Nanoscience, National Centre for Nanoscience and Technology, University of Chinese Academy of Sciences, Beijing, China
[4]Department of Chemistry, Modibbo Adama University of Technology, Yola, Adamawa State, Nigeria
[5]Department of Geography and Environmental Studies, Alvan Ikoku Federal College of Education, Owerri, Nigeria
[6]Department of Chemistry, Federal University of Technology, Owerri, Imo State, Nigeria
[7]Department of Chemistry, Madonna University, Okija, Anambra State, Nigeria

Abstract

Chitin and chitosan are among the most commonly used natural polymers in nanomedicine because they display very attractive characteristics for drug delivery and have proven very effective when formulated in nanoparticle forms. Properties such as the cationic character and the solubility of chitosan in aqueous medium have been reported as determinants of the success of this polysaccharide. However, its most attractive property relies on its ability to adhere to mucosal surfaces, leading to prolonged residence time at drug absorption sites and enabling higher drug permeation. This is because chitin and chitosan are able to interact with anionic agents and form water-soluble barriers which participate in drug release. The wide nanomedical applications of chitin and chitosan are due not only to their excellent biocompatibility, biodegradability, non-toxicity, ordourless nature and economic efficiency but also due to their distinct chemical structure with high percentage of primary amino groups and acetamido groups in chitosan and chitin respectively, for easy binding to bio-molecules such as DNAs and proteins. This review highlights the properties and modifications of chitin and chitosan which are responsible for the wide range of applications of these materials, particularly in nanomedicine for drug delivery and gene therapy, thereby encouraging more research into the exploration of their properties and modifications for improved applications.

Keywords: Chitin; Chitosan; Nano-medicine; Nano-particles; Chemistry; Gene therapy

Introduction

Polymers are macromolecules composed of repeating structural units of monomers connected by covalent chemical bonds. The reaction which results in the process of connecting the monomers to form polymers is known as polymerization reaction. There are many types of polymers formed by this process and they exist in two broad areas which are natural and synthetic polymers [1]. Natural polymers such as proteins, chitin, chitosan, collagen, silk, keratin, carbohydrates, starch, glycogen, are widely used materials for conventional coating materials for controlled active-ingredient release and even as drug delivery systems because they are chemically inert, nontoxic, less expensive, biodegradable, eco-friendly and readily available [2].

Among the naturally occurring polymers, chitin, chitosan, starch and their derivatives have been studied by many researchers due to their wide range of applications in agriculture for the controlled release of pesticides and/or fertilizers to water and soil environments, in engineering and chemistry for dye and metal-ion removal due to their adsorptive properties, and in pharmacology for the controlled release of drugs and wound healing due to their hydrogel properties [3-6]. The difference between the chemical structures of chitin and chitosan lies in the degree of deacetylation, as the percentage of acetyl groups in the compound determines if it is a chitin or chitosan - chitosan is usually a 50 - 100% deacetylated form of chitin [7].

The application of chitin and chitosan in nanomedicine had been widely reported for the targeted delivery of drugs in nanoparticle formulations and also for gene therapy [8-12].

Chitin

The origin of chitin dates back to the year 1811 when it was first isolated and characterized from mushrooms, by the French Chemist Henri Braconnot [13]. Chitin is the second most abundant naturally occurring polysaccharide which is the structural element in the exoskeleton of animals, especially in crustaceans (snails, crabs, shrimps, etc.), molluscs, arthropods, cuticles of insects, scales of fish, and in the internal structure of invertebrates [14]. Chitin can also be found in fungi as it is the principal fibrillar polymer in the cell wall of certain fungi where it adds some form of structural rigidity to such fungi and also makes fresh mushroom to be crisp. It is synthesized from the units of N-acetyl-D-glucosamine [(1-4)-linked 2-acetamido-2-deoxy-β-D-glucan], derived from cellulose, where the hydroxyl groups in cellulose have been replaced by acetamido groups [2].

Structure and sources of chitin

The structure of chitin is similar to that of cellulose, the only difference being the replacement of the hydroxyl group at the C-2 position of cellulose by an acetamido group in chitin [15]. Chitin occurs in two main polymorphic forms - α and β - based on the source of the chitin. The α form is the most abundant and can be found in fungal and yeast cell walls, krill, lobster, tendons and shell of crabs, shells of shrimps, cuticle of insects, and in some marine living organisms. β-chitin is found together with the proteins in squid pens, monocrystalline spines excreted by the diatom *Thalassiosira fluviatilis*,

***Corresponding author:** Akakuru OU, Ningbo Institute of Materials Technology and Engineering, Chinese Academy of Sciences, Zhejiang, China, Tel: +8613291965229; E-mail: ozioma.akakuru@yahoo.com

Received: January 05, 2018; **Accepted:** March 15, 2018; **Published** March 23, 2018

Citation: Akakuru OU, Louis H, Amos PI, Akakuru OC, Nosike EI, et al. (2018) The Chemistry of Chitin and Chitosan Justifying their Nanomedical Utilities. Biochem Pharmacol (Los Angel) 7: 241. doi: 10.4172/2167-0501.1000241

tubes synthesized by pogonophoran and vestimetiferan worms, aphrodite chaetae, and in the lorica built by some seaweeds or protozoa. The minor form (ϒ chitin) seems to be a variant of the α form. These forms of chitin can be differentiated by infrared and solid-state nuclear magnetic resonance (NMR) spectroscopy with X-ray diffraction [16]. The chemical structure of chitin is shown in Figure 1.

Solubility of chitin

Despite the variations in crystallinity, the chitin forms are insoluble in all the usual solvents. Insolubility of chitin remains the major setback in the commercial utilization of chitin [15]. The mechanism for the solid-state transformation of β-chitin into α-chitin which occurs by the treatment of β-chitin with aqueous HCl (concentration more than 7 M) and washing with water, as well as the solubility study of chitin-LiCl complex (soluble in dimethylacetamide and N-methyl-2-pyrrolidone), have been reported [16]. This solubility problem has resulted in only few information being available on the physical properties of chitin.

Characterization of chitin

Proton nuclear magnetic resonance (^{1}H-NMR) spectroscopy: Chitin can be dissolved rapidly in concentrated acid if it is first wetted with dilute acid. One advantage of using concentrated DCl as solvent is that the resonance of the solvent (HDO) does not interfere with any of the carbohydrate protons, as the solvent protons resonate at 9.2 ppm (probably a weighted average of the signals from water and acid protons due to the fast exchange between the two). The spectra shows the characteristic resonances in the anomeric region of the acetylated α- and the β-anomer at 5.43 and 5.05 ppm, respectively. H-1 of internal de-*N*-acetylated units resonate at 5.07 ppm, overlapping with the β-anomeric proton, while H-1 of internal acetylated units resonate at at 4.91 ppm. H-2 of internal de-*N*-acetylated units resonate at 3.44 ppm. Acetyl-protons are found at 2.62 ppm while the remaining ring protons appear between 3.6 and 4.4 ppm [17].

^{1}H-NMR spectra of chitin in concentrated DCl can also be used to give an indication of the purity of the chitin sample, as methyl-proton resonances from protein present in the sample would appear between 1.0 and 1.5 ppm [17].

Carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectroscopy: When recorded at 7.05 T, there are only 8 signals for the 8 carbon atoms of α- and β-chitins. C1 occurs at approximately 109 ppm, C2 at 58 ppm, C3 at 75 ppm, C4 at 85 ppm, C5 at 77 ppm, C6 at 60 ppm, carbon of CH_3 at 22 ppm and the carbonyl carbon at 177 ppm. Thus, in both allomorphs, the N-acetyl D-glucosamine moiety can be considered as the magnetic independent residue, in full agreement with the crystal structure of α- and β-chitin where this residue is also the crystallographic independent unit [16].

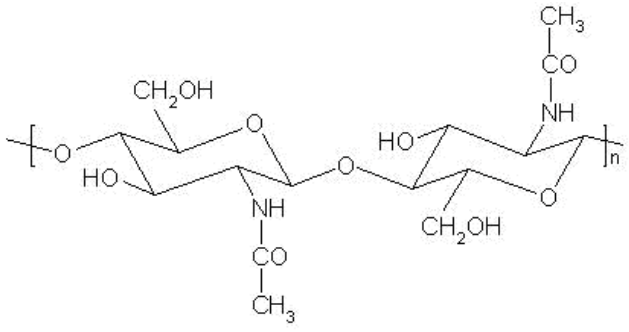

Figure 1: Structure of chitin.

Fourier transform infrared (FTIR) spectroscopy: Chitin exhibits an amide II band of 1558.48 cm^{-1} (a characteristic band of N-acetylation), an amide I band of 1651.07 cm^{-1}, a characteristic band at 3448cm^{-1} attributed to –NH and –OH groups stretching vibration, an aliphatic C-H stretching band at 2885 cm^{-1}, a characteristic carbonyl (C=O) stretch at 1627 cm^{-1} attributed to the vibrations of the amide I band, a band at 1427 cm^{-1} corresponding to the CH_3 group, a band at 1558 cm^{-1} corresponding to the N-H deformation of amide II, and vibration bands at 1072 cm^{-1} showing C-O-C vibration inside the chitin ring [18].

Applications of chitin

Chitin has a wide range of application in numerous areas. It has been widely used to immobilize enzymes and whole cells enzyme immobilization has applications in the food industry, such as clarification of fruit juices and processing of milk when α- and β-amylases or invertase are grafted on chitin. Chitin films and fiber have been used in medicine and pharmacy as wound-dressing and slow drug release materials. Chitin has been used to prepare affinity chromatography column to isolate lectins and determine their structure. Chitin-based materials have been applied in the adsorptive removal of heavy metals for water treatment [16].

In recent years, chitin is being investigated for immuno-stimulating and anticancer application [19].

Chitin derivatives

The main derivative of chitin is chitosan [16] while other derivatives include glucosamine, oligosaccharides, tosyl chitin, trityl chitin, fluorinated chitin, N- and O-sulphated chitin, phosphoryl chitin, etc. [20,16].

Glucosamine: The origin of glucosamine was initiated by Ledderhose, a premedical student at Gottinghen University in 1876 by mineral acid hydrolysis of the lobster shell and was named 'glucosamin'. This amino sugar was believed to have the D-glucose configuration and was synthesized by Fisher and Leuchs in 1903. It was not until 1939 that the configuration was unequivocally proved by synthesis.

Glucosamine is the principal component of O-linked and N-linked glycosaminoglycans (keratin sulphate, heparin sulphate, chondroitin sulphate, etc). Glucosamine glycans form the matrix of all connective tissue. Glucosamine is also the precursor for the biosynthesis of all the hexosamines that will form sialic acids and proteoglycans [21,22].

Glucosamine is prepared by the degradation of chitin by heating with concentrated hydrochloric acid. Crude glucosamine hydrochloride can be purified by filtration through celite and activated carbon. Final purification of the crude glucosamine can be done by dissolving in hot water and adding ethanol. Sibi and co researchers have also reported that glucosamine can also be prepared from chitosan [23].

An estimated 75% of chitin produced is used to manufacture products for the nutraceutical market. Currently the major driving force in the market is the increasing sales of glucosamine as a dietary supplement. Approximately 65% of the chitin produced is converted into glucosamine [24]. The chemical structure of glucosamine is shown in Figure 2.

Uses of glucosamine: Glucosamine derivatives are important intermediates in the synthesis of oligosaccharides and glucoconjugates. Both natural and synthetic glucosamine have demonstrated potential anti-coagulation and immune-modulatory activity and are used clinically to treat heart diseases, arthritis, and kidney disorders [25].

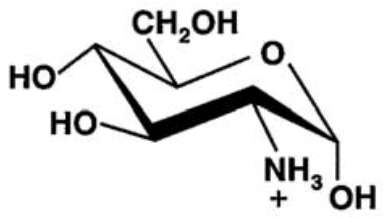

Figure 2: Structure of glucosamine (protonated).

Glucosamine hydrochloride has been reported to be a potential tumor growth inhibitor under certain circumstances. It has also been reported to promote absorption of antibiotics in the blood stream. It is also known to have anti-cancer, anti-inflammatory, and antibacterial effects [24].

Glucosamine can be characterized by FTIR spectroscopy and high performance liquid chromatography (HPLC).

Tosyl chitin: Tosylation of α-chitin with tosyl chloride is performed by interfacial condensation with an aqueous alkali chitin solution and a chloroform solution of tosyl chloride. During the reaction, partial deacetylation takes place because of the alkaline medium and subsequent acetylation followed by O-deacetylation is necessary. Chitin derivatives having p-toluenesulphonyl (tosyl) groups are versatile precursors for controlled further modifications, since they are soluble in organic solvents and highly reactive [20].

Trityl chitin: α-chitin cannot be tritylated directly with trityl chloride, hence trityl chitin is prepared through five-step reactions - deacetylation of chitin, N-phthaloylation, tritylation, dephthaloylation, and final N-acetylation. As in other modifications, however, β-chitin allows direct tritylation in pyridine. Although no appreciable substitution is observed at 90°C without a catalyst, the reaction proceeds smoothly in the presence of DMAP [20].

Chitosan

Chitosan, a naturally occurring polysaccharide is a cationic polysaccharide composed of glucosamine and N-acetylglucosamine units linked by β(1-4) glycosidic bonds obtained by the alkaline deacetylation of chitin [26]. The unique properties of chitosan including bioavailability, biodegradability, biocompactility, bioactivity, non-toxicity as well as good adhesion for sorption are the major reasons for its multiple applications [27]. Other reasons for the increasing interest of chitosan is its wide range of physical forms and various possible chemical modifications obtained using technical processes, in order to increase its solubility and widen its applications [14,28]. The chemical structure of chitosan is shown in Figure 3.

The processing of chitosan from crustacean shells mainly involves the removal of protein and the dissolution of calcium carbonate that is present as binders in crab shells in high concentration. The resulting chitin is deacetylated in 40% NaOH at 120°C for 1-3 hours. This treatment produces 70% deacetylated chitosan [29].

The process can be summarized as follows (Figure 4):

Characterization of chitosan

Chitosan is mainly characterized by the FTIR spectroscopy and determination of its degree of deacetylation. The degree of deacetylation of chitosan is a calculated measure of the extent of conversion of acetyl groups in chitin to amine groups of chitosan. High quality chitosan are those with degree of deacetylation greater than 85% [30].

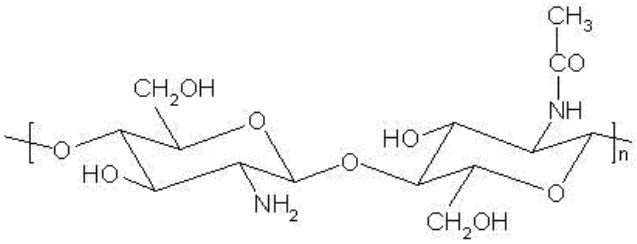

Figure 3: Structure of chitosan (partially deacetylated chitin).

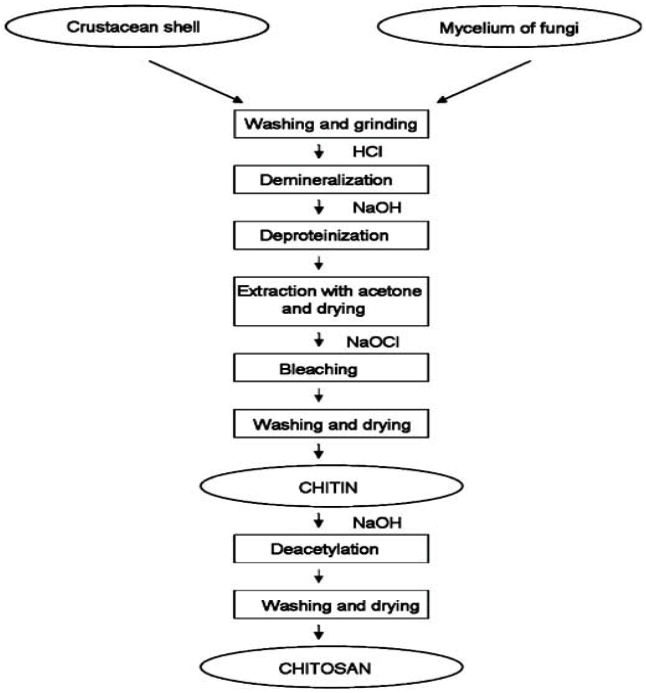

Figure 4: The processing of chitosan from crustacean shells.

FTIR analysis of chitosan shows broad absorption band in the range of 3000 to 3500 cm^{-1} which is attributed to O-H stretching vibrations and the 3263 cm^{-1} to vibration of N-H [28,30]. The stretching vibrations of methylene C-H occur at 2854 cm^{-1} and the absorption peak at 1558 cm^{-1} corresponds to the N-H bending vibrations. The amide II band is used as the characteristic band of N-acetylation. In comparison with that of chitin, the spectra for chitosan shows the different vibration that occurs after deacetylation pocess, which was not the emergence of the C=O vibration at 1627 cm^{-1} region, which indicates that the C=O has been reduced in chitosan, as well as the emergence of absorption band at 894 cm^{-1} on chitosan which is the vibration for NH_2 [18].

^{1}H-NMR analysis of chitosan showed peaks (singlets) corresponding to protons of glucosamine units, N-actyl-glucosamine, and acetyl group of N-acetyl-glucosamine at approximate chemical shift values of 4.7 ppm, 4.4 pm, and 1.8 ppm respectively [16].

Kafshgari and coworkers [30] had characterized the morphology of chitosan nanoparticles by transmission electron microscopy (TEM) and the mean particle size as well as the size distribution of the nanoparticles by dynamic light scattering (DLS).

Solubility of chitosan

Solubility according to an IUPAC definition is the analytical composition of a saturated solution expressed as a proportion of a designated solvent, which depends on the physical size of the polymer. The solubility of chitosan is a very difficult parameter to control. It is related to the degree of deacetylation, the ionic concentration, the pH, the nature of the acid used for protonation, the intra-chain hydrogen bonds involving the hydroxyl groups, and the distribution of acetyl groups along the chain, as well as the conditions of isolation and drying of the polysaccharide. Examination of the role of the protonation of chitosan in the presence of acetic acid and hydrochloric acid on solubility showed that the degree of ionization depends on the pH and the pK of the acid. The solubility of chitosan is usually tested in acetic acid by dissolving it in 1% or 0.1 M acetic acid [16].

Modifications of chitosan

Modifications of chitosan are increasingly studied as it has potential of providing new applications. The poor solubility, low surface area and porosity of chitosan are the major limiting factors in its utilization. Also, its fundamental skeleton does not change but brings new or improved properties. Derivatization of chitosan is the introduction of small functional groups on the reactivity of the primary amino groups and the secondary hydroxyl groups using various methods [14,31].

Chitosan, an unbranched cationic polymer, has three types of reactive functional groups that allows for further chemical modifications under mild reaction conditions to alter its properties i.e. amino or amido group at C-2 positions as well as both 1° and 2° hydroxyl groups at C-6 and C-3 positions respectively. An example is the introduction of carboxymethyl group on the chitosan structure which drastically increases the solubility of the chitosan at neutral and alkaline pH values without affecting it cationic character [14,31].

Graft copolymerization: This is the most attractive technique or method used to improve chitosan solubility and widen its application. Grafting of chitosan allows the formation of a functional derivative by covalent bonding of a molecule- the graft onto the chitosan backbone. Chitosan has two types of reactive groups that can be grafted; the free amine group on acetylated or deacetylated units and the hydroxyl groups [14,31]

Blending: Modification of chitosan by means of blending is an attractive method that has been extensively used for providing new desirable properties of chitosan. This is mainly due to its simplicity, availability of a wide range of synthetic and natural polymers for blending and its effectiveness for practical utilization [14,31].

Blending of chitosan with other polymers such as starch and crosslinking them are both convenient and effective methods of improving the physical and mechanical properties of chitosan for practical applications. Chitosan has been successfully blended with starch for the effective release of metformin hydrochloride [28].

Chitosan complex formation with metals: Chitosan-metal complexes have been reported for the metal salts of $CuSO_4.5H_2O$, $CoCl_2.6H_2O$, $NiCl_2.6H_2O$, $CdSO_4.8H_2O$, $Pb(CH_3CO_2)_2.3H_2O$, $AgNO_3$, $HgCl_2$, $Fe(NH_4)_2(SO_4)_2.6H_2O$, and $K_2Cr_2(SO_4)_4.24H_2O$, and in all cases, quantitative determination of the metal ions contained in the chitosan was conducted by using atomic absorption spectroscopy. Chitosan is known to have good complexing ability; the $-NH_2$ groups on the chain are involved in specific interactions with metals. Many papers are concerned with complexation for the recovery of heavy metals from various waste waters [16].

Chitosan-based materials

Chitosan hydrogels: One of the forms in which chitosan is used in medical application is as a hydrogel. A hydrogel (also called aquagel) is a network of polymer chains that are hydrophilic, sometimes found as colloidal gel in which water is the dispersion medium. Chitosan hydrogels, like other hydrogels, contain much water. Part of this water is tightly bound to the polymer and the rest is present as free water [7]. Water in crosslinked and uncrosslinked chitosan hydrogels gives rise to a three-dimensional network. Chitosan-based hydrogels have been reported to exhibit good biocompatibility, low degradation and processing ease [7,14]. The ability of these hydrogels to swell in water and dehydrate depends on its composition, biodegradability, and environment. These dependences have been exploited to facilitate a range of applications such as drug release [7,14]. The swelling ability, swelling kinetics and mechanism of uncrosslinked and glutaraldehyde-crosslinked chitosan had also been reported [8].

Chitosan-crosslinked blends: Stimuli-responsive hydrogels can also be obtained by blending biopolymers with synthetic thermo-responsive materials by various mechanisms of chemical crosslinking. When we have an assembly of two crosslinked polymers and at least one of which is synthesized and crosslinked in the presence of the other, this system is called an interpenetrated network (IPN). If only one component of the assembly is crosslinked leaving the other in the linear form, the system is termed as semi-IPN [14].

Blending of chitosan with other polymers such as starch and crosslinking them are both convenient and effective methods of improving the physical and mechanical properties of chitosan for practical applications. Corsslinking of chitosan can be achieved by reacting chitosan with epichlorohydrin [32,33], ethyleneglycol diglycidyl ether [33], sodium tripolyphosphate [34], or glutaraldehyde [18,35].

Applications of chitosan

Drug delivery: The release of drug from chitosan based dosage form depends upon the morphology, size, density, and extent of crosslinking of the particulate system, physico-chemical properties of drug as well as the polymer characteristics such as whether it is hydrophilic or hydrophobic, has gel formation ability, swelling capacity, muco-adhesive or bioadhesive properties and also on the presence of other excepients present in the dosage form [2,8,19]. Chitosan and its derivatives such as trimethylchitosan (where the amino group has been substituted with three alkyl groups), have been used in non-viral gene delivery system and has shown to transfect breast cancer cells; with increased degree of trimethylation increasing the cytotoxicity and at approximately 50% trimethylation, the derivative is the most efficient at gene delivery. Oligomeric derivatives are relatively non-toxic and have also been reported to have good gene delivery properties [36]. Chitosan has been used for the release of metformin hydrochloride [35], insulin, gentamicin sulphate, diclofenac, clarithromicin, cimetidine, famotidine, bovine serum albumin, clozapin, ovalbumin, doxorubicin, ofloxacin, 5-fluorouracil [2].

Metal-ion removal: The high sorption capacities of modified chitosan for metal ions are of great use for the recovery of valuable metals or the treatment of contaminated effluents. A great number of chitosan derivatives have been obtained with the aim of adsorbing metal ions by including new functional groups on the chitosan backbone. The new functional groups are incorporated into chitosan to increase the density of sorption sites, to change pH range for metal sorption and

to change the sorption sites in order to increase sorption selectivity for the target metal [14]. Chitosan has been reported to be effective for the removal of copper (ii) ions (Ngah et al.,), zinc (ii), and lead (ii) ions [32] in solution.

Dye removal: Chitosan, due to its high contents of amine and hydroxyl functional groups, has an extremely high affinity for many classes of dyes including disperse, reactive, anionic, vat, sulphur and naphthol. The only class for which chitosan has low affinity are cationic dyes. Carboxyl groups grafted onto chitosan may also serve as electron donors in an alkaline environment to confer chitosan the ability to adsorb cationic dyes from aqueous solutions. Modified chitosan gel beads with phenol derivatives were found to be effective in adsorption of cationic dyes, such as crystal violet (CV) and Bismarck brown Y (BB) [37].

Agricultural uses: Chitosan has a rich history of being researched for applications in agriculture, biology, and horticulture dating back to the 1980s. Chitosan increases photosynthesis, promotes and enhances growth of plants, stimulates nutrient uptake, increases germination and sprouting, and boosts plant vigor [38]. When used as seed treatment or seed coating on cotton, corn, seed potatoes, soybeans, sugar beets, tomatoes, wheat, and many other seeds, it elicits an innate immunity response in developing roots which destroys parasitic cyst nematodes without harming beneficial nematodes and organisms [38].

Other applications of chitosan: Chitosan has also found applications in industries such as in cosmetics, water engineering, paper industry, textile industry, food processing, photography, chromatographic separations, solid state batteries, and chitosan gel for light-emitting devices (LEDs) [27] and environmental-friendly and biodegradable flexible organic thin-film transistors [39].

Conclusion

It can be deduced from this review that the difference between the chemical structures of chitin and chitosan lies in the degree of deacetylation. Available literatures also indicate that both chitin and chitosan are effective materials for application in nanomedicine for drug delivery, tissue engineering and related applications. Modification of both chitin and chitosan had been reported to improve their range of applications even beyond nanomedicine. The recent application of chitosan in organic electronics is also an area that requires more research.

References

1. Tirrell M (1995) Chemical engineering of polymers: production of flexible, functional materials. Chem Engg Sci 50: 4123-4141.
2. Bansal V, Sharma KP, Sharma N, Pal PO, Malviya R (2011) Applications of chitosan and chitosan derivatives in drug delivery. Adv Biochem Res 5: 28-37.
3. Aranaz I, Mengibar M, Harris R, Panos I, Miralles B, et al. (2009) Functional characterization of chitin and chitosan. Curr Chem Biol 3: 203-230.
4. Bajer D, Kaczmarek H (2010) Study of the influence of UV radiation on the biodegradable blends based on chitosan and starch. Progress on Chemistry and Applications of Chitin and its Derivative 15: 17-24.
5. Oshita K, Oshima M, Gao YH, Lee, KH, Motomizu S (2002) Adsorption behavior of mercury and precious metals on crosslinked chitosan and the removal of ultratrace amounts of mercury in concentrated hydrochloric acid by a column traetmant with crosslinked chitosan. Japanese J Analyt Sci 18: 1121-1125.
6. Ostrawka-Czubenko J, Pieróg M, Gierszewska-Drużyńs M (2011) State of water in non-cross linked and crosslinked hydrogel chitosan membranes-DSC studies. Progress on Chemistry and Application of Chitin and Its Derivatives 16: 147-156.
7. Rohindra DR, Nand AV, Khurma IR (2004) Swelling properties of chitosan hydrogels. South Pacific J Nat Sci 22: 32-35.
8. Akakuru OU, Isiuku BO (2017) Chitosan hydrogels and their glutaraldehyde-crosslinked counterparts as potential drug release and tissue engineering systems - synthesis, characterization, swelling kinetics and mechanism. J Phys Chem Biophys 7: 1-7.
9. Csaba N, Koping-Hoggard M, Alonso MJ (2009) Ionically crosslinked chitosan/tripolyphosphate nanoparticles for oligonucleotide and plasmid DNA delivery. Int J Pharm 382: 205-214.
10. Grenha A (2012) Chitosan nanoparticles: a survey of preparation methods. J Drug Target 20: 291-300.
11. Gupta NV, Natasha S, Getyala A, Bhat RS (2013) Bioadhesive vaginal tablets containing spray dried microspheres loaded with clotrimazole for treatment of vaginal Candidiasis. Acta Pharmaceutica 63: 359-372.
12. Kang BS, Lee SE, Ng CL, Kim JK, Park JS (2015) Exploring the preparation of albendazole-loaded chitosan-tripolyphosphate nanoparticles. Materials 8: 486-498.
13. Park BK, Kim M (2010) Applications of chitin and its derivatives in biological medicine. International J Mol Sci 11: 5152-5164.
14. Alves NM, Mano JF (2008) Chitosan derivatives obtained by chemical modifications for biomedical and environmental applications. Int J Biol Macromol 43: 401-414.
15. Bhatt LR, Kim BM, Hyun K, Kwak GB, Lee CH, et al. (2011) Preparation and characterization of chitin benzoic acid esters. Molecul 16: 3029-3036.
16. Rinaudo M (2006) Chitin and chitosan: properties and applications. Prog Polym Sci 31: 603-632.
17. Cardenas G, Cabrera G, Taboada E, Miranda SP (2004) Chitin characterization by SEM, FTIR, XRD, and 13C crosspolarization/mass angle spinning NMR. Appl Polym Sci 93: 1876-1885.
18. Rumengan IFM, Suryanto E, Modaso R, Wullur S, Tallei TE, et al. (2014) Structural characteristics of chitin and chitosan isolated from the biomass of cultivated rotifier, Brachionus rotundiformis. Int J Fish Aquat Sci 3: 12-18.
19. Park I, Cheng J, Pisano AP (2007) Low temperature, low pressure Nano imprinting of chitosan as a biomaterial for bio nanotechnology applications. Appl Phys Lett 90: 1-3.
20. Goosen M (1996) Applications of chitin and chitosan. 1st Edition, CRC Press, Taylor and Francis Group, United Kingdom.
21. Deal CL, Moskowitz RW (1999) Nutraceuticals are therapeutic agents in arthritis. The role of glucosamine, chondroitin sulphate, and collagen hydrolysate. Rheum Dis Clin North Am 25: 379-395.
22. Kelly GS (1998) The role of glucosamine sulphate and chondroitin sulphates in the treatment of degenerate joint diseases. Alt Med Rev 3: 27-39.
23. Sibi G, Dhananjaya K, Ravikumar KR, Mallesha H, Venkatsesha RT (2013) Preparation of glucosamine hydrochloride from crustacean shell waste and its quantitation by RP-HPLC. American-Eurasian J Sci Res 8: 63-67.
24. Sandford PA (2002) Commercial sources of chitin & chitosan and their utilization. Adv Chitin Sci 6.
25. Petitou M, Boeckel CAA (1997) Heparin: from the original 'soup' to well-designed heparin mimetics. Pure Appl Chem 69: 1839.
26. El-Hefian EA, Nasef MM, Yahaya AH (2011) Chitosan physical forms: a short review. J Basic Appl Sci 5: 670-677.
27. Pillai O, Rari P (2001) Insulin therapies: past, present and future. Drug Discov Today 6: 1056.
28. Okolo PO, Akakuru OU, Osuoji OU, Jideonwo A (2013) Studies on the properties of chitosan-starch beads and their application as drug release material. Bayero J Pure Appl Sci 6: 118-126.
29. Dutta PK, Dutta J, Tripathi VS (2004) Chitin and chitosan: chemistry, properties and applications. J Sci Industrial Res 63: 20-31.
30. Kafshgari MH, Khorram M, Khodadoost M, Khavari S (2011) Reinforcement of chitosan nanoparticles obtained by an ionic crosslinking process. Iranian Polymer J 20: 445-456.
31. Lim LY, Khor E (2003) Implantation and application of chitin and chitosan. Biomaterials 24: 2339-2349.

32. Arh-Hwang C, Sheng-Chang L, Chia-Yuan C, Chia-Yun C (2007) Comparative adsorption of copper (II), zinc (II) and Lead (II) ions in aqueous solution on the crosslinked chitosan with epichlorohydrin. J Hazard Mater 154: 184-191.

33. Ngah WSW, Endud CS, Mayanar R (2002) Removal of copper (II) ions from aqueous solution onto chitosan and crosslinked chitosan beads. Reactive and Functional Polymers 50: 181-190.

34. Tiwary AK, Rana V (2010) Crosslinked chitosan films: effect of cross-linking density on swelling parameters. Pak J Pharm Sci 23: 443-448.

35. Kumari K, Rani U (2011) Controlled release of metformin hydrochloride through crosslinked blends of chitosan-starch. Adv Appl Sci Res 2: 48-54.

36. Kean T, Roth S, Thanou M (2005) Trimethylated chitosans as non-viral gene delivery vectors: cytotoxicity and transfection efficiency. J Control Release 103: 643-653.

37. Crini G, Badot PM (2008) Application of chitosan, a natural aminopolysaccharide, for dye removal from aqueous solutions by adsorption processes using batch studies: a review of recent literature. Progress in Polymer science 33: 399-447.

38. Linden J, Stoner R, Knuston K, Gardner-Hughes C (2000) Organic disease control elicitors. Agro Food Industry Hi-Te 12-15.

39. Du BW, Hu SY, Singh R, Tsai TT, Lin CC, et al. (2017) Eco-friendly and biodegradable biopolymer chitosan/Y_2O_3 composite materials in flexible organic thin-film transistors. Materials 10: 1026.