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Review

Chitosan as a Natural Copolymer with Unique Properties for the Development of Hydrogels

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Received: 30 April 2019; Accepted: 23 May 2019; Published: 29 May 2019

Abstract: Hydrogel-based polymers are represented by those hydrophilic polymers having functional groups in their chain such as amine (NH_2), hydroxyl [-OH], amide (-CONH-, -$CONH_2$), and carboxyl [COOH]. These hydrophilic groups raise their potential to absorb fluids or aqueous solution more than their weights. This physicochemical mechanism leads to increased hydrogel expansion and occupation of larger volume, the process which shows in swelling behavior. With these unique properties, their use for biomedical application has been potentially raised owing also to their biodegradability and biocompatibility. Chitosan as a natural copolymer, presents a subject for hydrogel structures and function. This review aimed to study the structure as well as the function of chitosan and its hydrogel properties.

Keywords: hydrogel; chitosan; swelling behavior

1. Introduction

In recent decades, advances in the field of biomaterials has grown so much, leading to concentration on the synthesis of alternative biocompatible materials or to improving the characters of these present materials. Chitosan, as a mucopolysaccharide having structural characteristics similar to glycosamines, seems to be an ideal biopolymer with a wide variety of biomedical and industrial applications [1]. It is one of the most abundant polysaccharides on Earth and a natural cationic copolymer. Chitosan is the alkaline deacetylated product of chitin, derived from the exoskeleton of crustaceans, insects and fungal cell walls. Chitin consists of a sugar backbone with β-1,4-linked glucosamine units and a high degree of acetylation (see Figure 1; all figures have been drawn by the authors) [2]. It is also a derivative of cellulose with the hydroxyl groups replaced by amine groups, thereby making it a polycation. Chitosan is the major derivative of chitin, which is composed of randomly distributed *N*-acetyl glucosamine and D-glucosamine, varying in composition, sequence and molecular chain length [3,4].

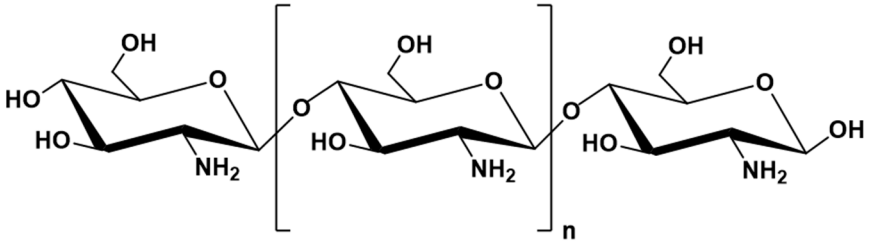

Figure 1. Chitosan chemical structure.

Many beneficial pharmacological properties have been suggested for chitosan due to its biocompatibility, non-toxicity, biodegradability, antibacterial activity, antioxidant activity and muco-adhesive properties [5]. Furthermore, it has been introduced extensively in the pharmaceutical industry including the formation of tablets as a controlled release dosage form [6], a gel absorption enhancer [7], for drug dissolution in wound-healing products and in developing micro/nanoparticles. Chitosan is a weak polybase with a pKa around 6.5, implying that its charge density varies in the pH range of 6–6.5. This imparts pH-responsiveness, which is beneficial for various therapeutic applications. For this reason, chitosan-soluble and -insoluble transition occurs at pH between 6 and 6.5, which is a convenient range for biological applications (see Figure 2).

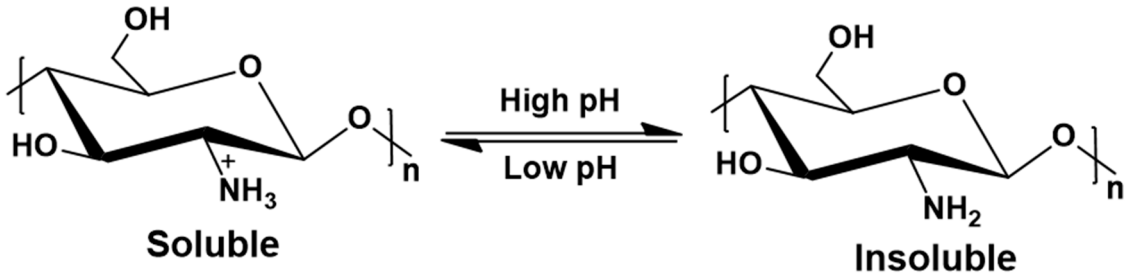

Figure 2. Nature of chitosan below its pKa.

The high charge density of chitosan at pH levels below the pK a results in polyelectrolyte formation, whereas a low charge density at neutral pH contributes to its low cytotoxicity and facilitates the intracellular release of biomolecules. It is reported that the low charge density leads to low solubility, and aggregation. Thus, the poor stability of chitosan-based formulations depends on the type of chitosan applied [8]. The degree of de-acetylation and molecular weight might alter the cationic properties of chitosan by varying the positive charge density and affect its cell-dependent transfection efficiency [4]. The cationic nature of chitosan enables the formation of polyelectrolyte complexes with the negatively charged biomolecules, allowing for the interaction with cell membranes and more efficient transfection.

2. Determination of De-Acetylation of Chitosan

The degree of de-acetylation of chitosan was determined by the titrimetric method. In this method, 1% chitosan solution was prepared using acetic acid that was added to phosphoric acid in the ratio of 1:1 (*v*/*v*) and the mixture was further titrated against 0.1 M NaOH using phenolphthalein as an indicator. The degree of de-acetylation of the chitosan can be obtained using the following formula,

$$\text{Degree of deacetylation} = 100 - 2.303\frac{V_\infty - V_o}{m}$$

where (m) represents amount of chitosan (mg) used and (V), represents difference of the 0.1 M NaOH used between the chitosan solution and standard.

3. The Limitations of Chitosan

In therapeutic applications, chitosan limitations are caused by decreasing its solubility and by increasing its swelling degree in aqueous environments. Consequently, this leads to rapid drug release (i.e., chitosan is used as the continuous matrix) [9]. For instance, chitosan has often been reported with low limitation to pass the colonic region due to its high solubility in gastric fluids, sometimes resulting in burst release of the drug at the stomach [10]. It is well known that chitosan can be insoluble at acidic fluids through chemical cross-linking of the microsphere with aldehydes. However, it is not effective in preventing the release of the encapsulated drugs [11]. Additionally, several factors might affect

chitosan-intrinsic properties including the low mechanical resistance, and there is no control for its hydrogel pore size and toxicity of cross-linking [12].

Although many investigators have attempted to solve these limitations by performing several fabrication methods, chitosan properties are not completely being optimized. For instance, chitosan nanoparticles produced by the ionic gelatin method suffered from poor stability in acidic conditions and difficulty in entrapping high molecular weight drugs [8]. Nanoparticles produced by the micro-emulsion method have disadvantage due to usage of organic solvent, a lengthy process and a complex washing step [13]. In recent work, Hanafy and his coworkers have reported the possible strengthening of chitosan properties by doping with polygacalcturonic acid [14].

4. Chitosan in Nanoscience

Nanobiomaterials have been recently used to transport and release the drugs in the target site owing to their possible degradation in the biological system. They are accustomed to switch and handle drugs that are critical for the furtherance of human health and developing the quality of life. In recent years, chitosan-based nanomaterials have been paid weighty attention and used in different biomedical arenas. For example, chitosan-based nanoparticles, scaffolds, microfluidcs, lab on chip, and organ on chip, have been established and widely used for various biomedical applications [15].

Several drugs and polyphenolsencapsulated inside chitosan moieties were synthesized such as catechin and epigallocatechin [16], tamoxifen [17], alendronate sodium [18], insulin [19] and peptide [20]. These nanoparticles are delivered in severalapproachesincluding injection drug delivery [21], topical drug delivery [22], colon-targeted drug delivery [23], carcinoma therapy [24] and gene delivery [25]. They are further administrated as oral delivery, injectable delivery, or in cream like form (See Table 1).

Table 1. Chitosan assembling structure and its role in drug delivery system (adapted from [10]).

Morphology	The Role Chitosan Played	Preparation Method	Application
Nanogels	Chitosan-carbon dot hybrid nanogels	Covalent cross-linking	Photothermal–chemo therapy
	pH responsive eucalyptus oil coated double walled biodegradable nanogels	Ion cross-linking	Controlled drug delivery
	PEGylated and fluorinated chitosan nanogel	Covalent modification	Targeted drug delivery
	Reversible swelling-shrinking nanogel	Covalent modification/cross-linking	Character of deep tumor penetration
Micelles	Chitosan-based pH-sensitive polymeric micelles	Covalent modification/self-assembly	Colon-targeted drug delivery
	pH-responsive aerobic micelles	Ion cross-linking	Photodynamic therapy
	Chitosan-pluronic micelles	Covalent modification/self-assembly	Drug delivery for glioblastoma cancer
	Multifunctional nanoparticles	Covalent modification/self-assembly	Targeted photothermal therapy
	Chitosan grafted methoxy poly(ethylene glycol)-poly(ε-caprolactone)	Covalent modification/self-assembly	Ocular delivery of hydrophobic drug
Nanofibers	Biomimetic mineralization of carboxymethyl chitosan nanofibers	Electrospinning process	Improve osteogenic activity
Liposomes	Arginine-modified nanostructured lipid carriers	Covalent modification/self-assembly	Anticancer drug delivery
	Glycosaminoglycan modified chitosan liposome	Covalent modification	Antimalarial drug delivery
	Aptamer-modified liposomal complexes	Covalent modification/other processing	Reverse drug resistance in lung cancer
	Gold nanoshell-coated liposomes	Covalent modification/electrostatic adsorption	Photothermal and chemotherapy
	Glycol chitosan-coated liposomes	Covalent modification/self-assembly	pH-responsive drug-delivery

Table 1. *Cont.*

Morphology	The Role Chitosan Played	Preparation Method	Application
Nanosphere	Magnetic nanoparticle-loaded chitosan-deoxycholic acid nanodroplets	Covalent modification, self-assembly	siRNA Delivery
	Smart pH-responsive nanocarrier	Covalent modification/electrostatic adsorption	Targeted delivery of ursolic acid
	Thermoresponsivenanospheres	Covalent modification/emulsification/solvent evaporation method	Release drug for the treatment of osteoarthritis
Nano-particles	Uniform core-shell nanoparticles	Ion crosslinking	Enhance oral delivery of insulin
	N-trimethyl chitosan nanoparticles	Covalent modification/self-assembly	Oral delivery to treat breast cancer
	Chitosan-modified PLGA nanoparticles	Ion crosslinking	Tumor-targeted drug delivery
	EGFR-targeted chitosan nanoparticles	Covalent modification/self-assembly	SiRNA delivery
	Indomethacin-conjugated chitosan oligosaccharide nanoparticle	Covalent modification/self-assembly	Prodrug and tumor-targeted drug delivery
Inorganic nano-materials	Viable smart targeted nanoenvelope delivery system	Covalent modification/self-assembly	Dox encapsulated and targeted therapy
	Multifunctional magnetic nanoparticles	Covalent modification/ sonication treatment	Thermo-Chemotherapy Intracellular Imaging
	Combinatorial nanocarrier	Covalent modification/ion crosslinking	Drug delivery for breast cancer
	Magnetic thymine-imprinted chitosan nanoparticles	Physical adsorption	Gene therapy
	Functional hollow microspheres constructed from MOF shells	Covalent modification/Physical adsorption	Drug delivery and targeted transport

5. Nanochitosan Synthesis Approaches

Many methods have been applied to fabricate chitosan-based nanogels having several service abilities. These nanogels are auspicious as active vehicles for the different biological applications such as drug delivery, cell culture, bioimaging and therapy.

5.1. Ionotropic Gelation

Gelation is one of the common and modest methods for fabrication of chitosan nanoparticles. These nanoparticles can be formedby electrostatic interaction between amino group of chitosan and cross-linking agent (tripolyphosphate; (TPP)). In this method, chitosan and cross-linking agent is dissolved separately in acetic acid and water respectively. Then, cross-linking agent is added further to chitosan solution (in the presence or absence of surfactants, i.e., Tween 80, Polyethylene glycol) to form nanoparticles under mechanical stirring (see Figure 3). In this case, the amount and type of surfactants (stabilizers) can control the size and surface charges of the nanoparticles formed [26].

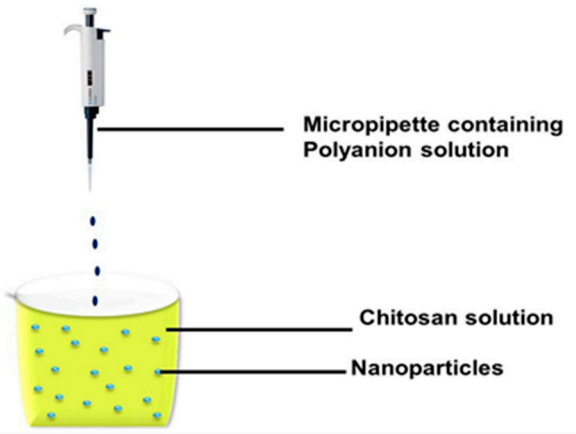

Figure 3. Ionotropic gelation technique.

5.2. Ionic Gelation Method

Chitosan nanoparticles formed by the ionic gelatin method depend mainly on the electrostatic interaction using reversible physical cross-linkers instead of chemical cross-linkers. TPP is also used in this method due to its non-toxicity and fast ability to form chitosan-based nanogels via its ionic interaction with chitosan. The nanogel formed can be controlled via changing different parameters such as weight ratio, molar ratio, temperature, time charge density of TPP/chitosan and pH. For example, it has been reported that TPP/chitosan nanogel produced via ionic gelation could enhance the stability and bio-viability of drugs [27].

5.3. Emulsification Solvent Diffusion Method

This technique depends on using different types of organic solvents that are partially miscible with water. Briefly, the organic solvent is injected into an aqueous solution containing chitosan and stabilizing agent, which leads to the formation of an oil/water (O/W) emulsion mixture. This process was performed by using stirring followed by ultra-sonication and/or ultra-homogenization. Consequently, the nanoparticles formed due to precipitation of stabilizing agent (i.e., polymer) arise as a result of diffusion of organic solvent into water (see Figure 4). In general, this method is appropriate for hydrophobic drugs and showed a high percentage of drug encapsulation. However, this approach has some limitations including harsh reaction conditions such as presence of different organic solvents and high shear forces, which leads to synthesis of some aggregated and agglomerated nanoparticles [28].

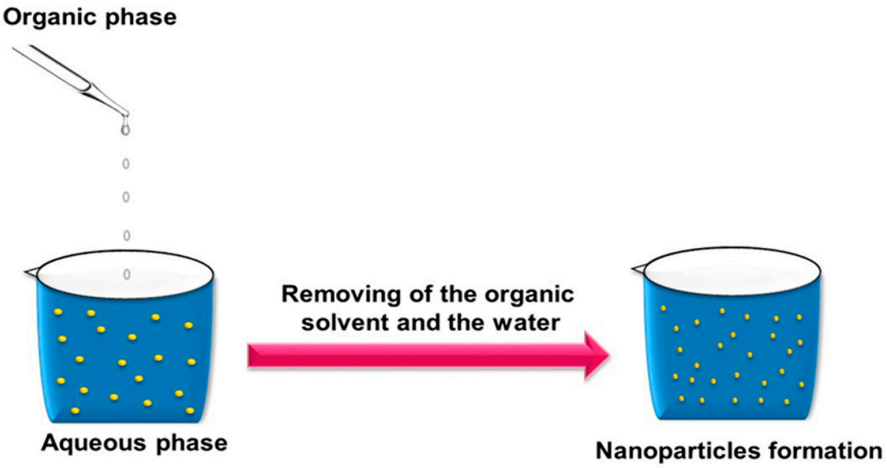

Figure 4. Emulsification solvent diffusion method.

5.4. Spray-Drying

Spray-drying is a well-known method, and it has been used widely for fabrication of micro-particles (i.e., powders, pellets) than nanoparticles. This method is based on atomized droplets drying in a hot air stream. Many parameters should be controlled to get the particle size of interest, the tap size, atomization pressure, inlet air temperature, spraying flow rate and degree of cross-linking. Briefly, chitosan is dissolved in aqueous acetic acid solution and then the drug is dissolved in the solution followed by adding the cross-linker. The solution is atomized in a hot air stream leading to small droplet formation, then the solvent evaporates rapidly and free-flowing particles are formed [29].

5.5. Sieving Method

This is a simple, new and not a regular procedure to produce chitosan micro-particles. It can then be scaled up easily [30]. Micro-particles are prepared by using a fit cross-linker to form glassy hydrogels from chitosan solution (4% acetic acid). They are passed through a sieve method (see Figure 5). Through using this method, clozapine (as a typical antipsychotic medication) has been

encapsulated into moieties of chitosan gel resulting in irregular shaped microparticles, having diameter ~540–700 nm. The results pointed to a slow release of clozapine in in vivo studies.

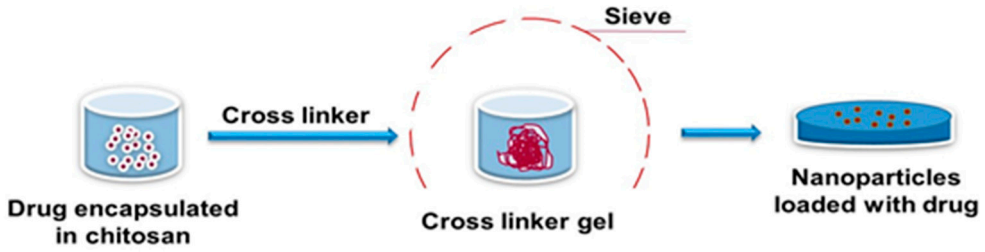

Figure 5. Emulsification solvent diffusion method.

5.6. Reverse Micellar Method

The reverse micellar method is a mixture of water, oil and surfactant giving thermodynamically stable reverse micelles with very important properties. Such a reverse micellar medium can be used to give extremely fine polymeric nanoparticles with narrow size distribution, avoiding the regular emulsion in the polymerization methods resulting in a large and broad size range of nanoparticles (200–450 nm). As a result of the Brownian motion, the micellar droplets undergo incessant combination subsequently with re-separation on a time between millisecond and microsecond. This condition can exhibit a fast dynamic equilibrium preserving the size, polydispersity and thermodynamic stability of those nanoparticles. In general, reversed micelles are prepared by dissolving the surfactant used in an organic solvent followed by the addition of chitosan and a drug under a uniformed vortex leading to the formation of a transparent solution. Then, a cross-linking agent is added with constant stirring overnight. The extreme drug amount that can be dissolved in this technique differs from one drug to another and should be determined by increasing its amount until converting the clear micro-emulsion into a glowing solution. For example, doxorubicin–dextran encapsulated into chitosan nanoparticles was prepared by this approach [31].

5.7. Self-Assembly

Recently, this method has been used to obtain novel materials through the different types of bonds such as electrostatic interactions and van der Waals interactions, exhibiting versatility and simplicity [14]. Many organic and inorganic materials have been presumed to fabricate chitosan-based nanogels by using the self-assembly method [32]. Chitosan nanoparticles produced by self-assembly can be further functionalized, allowing them to be more suitable for biomedical applications. This condition results in improvement of their circulation in the blood stream and increasing their targeted therapy. Peptides and proteins can also be assembled with chitosan or its derivatives via electrostatic interactions forming nanogels [33–35]. New, highly luminescent nanogels that were made-up by self-assembly exhibited a support in the rapid formation of luminescent Au nanoclusters. So, the nanogelscould be saturated with Au nanoclusters of different emission colors and different thiolate ligands, which improves the photoluminescence properties.

6. Hydrogels

A hydrogel consists of networks matrix formedby cross-linked polymers with variable dimensions starting from the nano to micro-scale, containing a great number of hydrophilic sets. Furthermore, they possess a great appetite for water without being dissolved through as a result of the different types of bonds formed among the polymer chains. While water is allowed to pass simply through the hydrogel networks resulting in high water adsorption and swelling [36]. Upon that, polymers of a hydrophilic property can take various water quantities more than their original weight by a million times [37] depending on the density of the hydrophilic sets that form the polymer [38]. When it is fully swollen, a hydrogel confers physical properties similar to those of the living tissues such as the softness

and elasticity. This results in reduction of the interfacial tension with biological fluids and water, which helps in minimizing the irritation of the surrounding tissues in implantation processes. Furthermore, hydrogels can mold the shape of any space in which they are performed [39]. Their rubbery nature and low interfacial tension between their surfaces and the biological fluids enabled them to decrease the adsorption of proteins and cell union, minimizing the immune system's negative response. This makes them an excellent choice for drug delivery. Different types of polymers can be used to fabricate the hydrogel, for example, polyethylene glycol and polyvinyl alcohol for their muco- and bio-adhesive merits. This allows drugs to penetrate the tissues and perform its function in a good way by increasing its residence duration [40]. A hydrogel is mechanically and compositionally similar to the extracellular matrix that help them to be used as supporting material in addition to its usage in a drug delivery system [41].

6.1. Synthesis of the Hydrogel

Various parameters are considered as chief constituents for the hydrogel preparation such as, the amount of water expected to be absorbed by the hydrogel and the process of initiating the chains of the polymeric network. Furthermore, a hydrogel is prevented from being dissolved due to the functional groups of the polymer used during formation of the hydrogel. This assembly can be performed in two ways: the first is by physical reversible cross-linking (e.g., hydrogen bonds) and the second by chemical irreversible cross-linking (e.g., covalent bonds) [42]. In the hydrogel synthesis process, the chains of the polymer used react reciprocally with solvent molecules and become fully solvated. During this, a pulling back force is formed by the cross-linking components tocontrol the polymer chains (Flory's rubber elasticity theory) [43]. Moreover, the balance of the expanding and withdrawing forces reach equilibrium at a specific temperature. The swelling feature of a hydrogel is the main reason for being used in variable applications and the swelling ratio affects different features of a hydrogel such as surface wet nature and the mechanical and optical properties [44]. The polymer's molecular weight is another parameter that should be taken into consideration because it regulates the charges present on the polymer, and the cross-linking density. Each one of these features can outline the bonds among polymer chains. In this case, polymers with a small molecular weight are essential at higher concentrations to give stability for the gel. Polymers with a large molecular weight may create healthier polymers with various cross linkages [39]. The material's pore size and the drug hydrodynamic size are the most vital items because they may control and regulate encapsulated drug release out of the hydrogel [45]. One of the most brilliant components to form a hydrogel is chitosan due to its great properties including non-toxicity, bioavailability, and the capability of being sterilized. Chitosan has a great reputation in the biological activities (e.g., medical and biotechnologicalapplications) [46]. Chitosan hydrogel can be synthesized in countless varieties including the shape, the size, texture, fibers, powders, and liquids [42].

6.2. Cross-Linking in Nanoparticles' Preparation

The most interesting properties of the hydrogel are related to the choice of suitable cross-linkers, whereas, in the presence of an agent, intermolecular links producedamong the reacting molecules can further interact with itself and/or with large lined chains in basic media. This action generates a new linkage among the polymer chains resulting in reduction of the interconnecting action. It also can affect the polymers matrix if the degree of cross-linking is high, giving it an insoluble property in different types of solvents [47].

7. Classification of Cross-Linking Agents

7.1. Physical Cross-Linking

They can be produced by their combination with the anionic molecules across hydrogen bonding or by hydrophobic associations. The key advantage of this technique is that toxic cross linkers

are prevented, allowing the avoidance of any negative effects on the biocompatibility. In addition, the hydrogels cross-linked by this method are self-healing. In this type, a cross-linking network made by the association with the polysaccharides is formed with ions on the surface. The greater the ions concentration, the more time is consumed to perform the whole cross-linking action of this polysaccharide. Physical cross-linking produces reversible and pH sensitive nanoparticles allowing them an advantage in controlling sensitive release stimulation. For this reason, TPP and calcium ions can be used as ionic and inorganic cross linkers, respectively [48].

7.2. Chemical Cross-Linking

Numerous methods of chemical cross-linking can be performed including condensation and addition reactions. In this type, produced hydrogels are mostly with uniform properties, generating the main advantage for this type compared to the physical cross-linking type. However, great caution should be exercised especially for biomedical applications by using chemicals that do not generate any toxicity as much as possible. For example, polysaccharides can react with the cross linker by chemical covalent bonds forming intermolecular or intramolecular combinations, allowing nanoparticles to be more stable in their structure. With severe pH changes, the stiff network provides water and bioactive components absorption without nanoparticle dissolution. This type is also affected by the concentration of the cross linker and the cross-linking process time [49–52]. For example, on the chemical cross-linking agents, glutaraldehyde, formaldehyde and cinnamaldehyde were used [53].

8. Conclusions

Hydrogel systems produced by using natural polymers have been recommended for bio medical applications. In this case, chitosan is one of the natural polysaccharides containing *N*-acetyl-D-glucosamine and D-glucosamine units produced by the de-acetylation of chitin. It has many properties for biological applications owing to its non-toxicity, biocompatibility and biodegradability. Moreover, it is a pH-sensitive polymer that readily dissolves at low pH whereas it is insoluble at high pH. Considering these unique properties, chitosan and its derivatives have been investigated widely as a potential polymer for hydrogel-based biomedical applications.

Author Contributions: F.S.E-b. wrote the review; M.E.M. revised the review; S.L. guided the review. N.A.N.H. designed the review; M.E.-K. supervised the review. All authors read and approved the final review version.

Funding: This work was completely supported by Nanomedicine Department, Nanoscience and Nanotechnology Institute, Kafrelsheikh University, Egypt.

Acknowledgments: Hanafy would like to thank president of Kafrelsheikh University and director of Nanoscience and Nanotechnology Institute, Maged El Kemary, for his generous help, advice, and support. The highest appreciation and gratitude go to the wonderful advisor and mentor, Stefano Leporatti, for his care, support and guidance. Hanafy would like to give great thanks to Magdy Mahfouz for his great efforts in his revision and correction. Finally, Hanafy would to thank his student, Fatma El Banna, for her activity and this promising work.

Conflicts of Interest: Authors declare no conflict of interest.

References

1. Chen, M.C.; Mi, F.L.; Liao, Z.X.; Hsiao, C.W.; Sonaje, K.; Chung, M.F.; Hsu, L.W.; Sung, H.W. Recent advances in chitosan-based nanoparticles for oral delivery of macromolecules. *Adv. Drug Deliv. Rev.* **2013**, *65*, 865–879. [CrossRef]
2. Engkagul, V.; Klaharn, I.Y.; Sereemaspun, A.; Chirachanchai, S. Chitosan whisker grafted with oligo (lactic acid) nanoparticles via a green synthesis pathway: Potential as a transdermal drug delivery system. *Nanomedicine* **2017**, *13*, 2523–2531. [CrossRef]
3. Motiei, M.; Kashanian, S.; Lucia, L.A.; Khazaei, M. Intrinsic parameters for the synthesis and tuned properties of amphiphilic chitosan drug delivery nanocarriers. *J. Control. Release* **2017**, *260*, 213–225. [CrossRef] [PubMed]

4. Shoueir, K.; El-Sheshtawy, H.; Misbah, M.; El-Hosainy, H.; El-Mehasseb, I.; El-Kemary, M. Fenton-like nanocatalyst for photodegradation of methylene blue under visible light activated by hybrid green DNSA@ Chitosan@ MnFe2O4. *Carbohydr. Polym.* **2018**, *197*, 17–28. [CrossRef] [PubMed]
5. Nimesh, S.; Thibault, M.; Lavertu, M.; Buschmann, M. Enhanced Gene Delivery Mediated by Low Molecular Weight Chitosan/DNA Complexes: Effect of pH and Serum. *Mol. Biotechnol.* **2010**, *46*, 182–196. [CrossRef]
6. Rajitha, P.; Gopinath, D.; Biswas, R.; Sabitha, M.; Jayakumar, R. Chitosan nanoparticles in drug therapy of infectious and inflammatory diseases. *Expert Opin. Drug Deliv.* **2016**, *13*, 1177–1194. [CrossRef]
7. Xiao, B.; Chen, Q.; Zhang, Z.; Wang, L.; Denning, T.; Kang, Y.; Merlin, D. TNFalpha gene silencing mediated by orally targeted nanoparticles combined with interleukin-22 for synergistic combination therapy of ulcerative colitis. *J. Control. Release* **2018**, *287*, 235–246. [CrossRef] [PubMed]
8. Mohammed, M.A.; Syeda, J.T.M.; Wasan, K.M.; Wasan, E.K. An Overview of Chitosan Nanoparticles and Its Application in Non-Parenteral Drug Delivery. *Pharmaceutics* **2017**, *9*, 53. [CrossRef] [PubMed]
9. Park, J.H.; Saravanakumar, G.; Kim, K.; Kwon, I.C. Targeted delivery of low molecular drugs using chitosan and its derivatives. *Adv. Drug Deliv. Rev.* **2010**, *62*, 28–41. [CrossRef]
10. Li, J.; Cai, C.; Li, J.; Li, J.; Li, J.; Sun, T.; Wang, L.; Wu, H.; Yu, G. Chitosan-Based Nanomaterials for Drug Delivery. *Molecules* **2018**, *23*, 2661. [CrossRef]
11. Croisier, F.; Jérôme, C. Chitosan-based biomaterials for tissue engineering. *Eur. Polym. J.* **2013**, *49*, 780–792. [CrossRef]
12. Gonçalves, I.C.; Henriques, P.C.; Seabra, C.L.; Martins, M.C.L. The potential utility of chitosan micro/nanoparticles in the treatment of gastric infection. *Expert Rev. Anti. Infect.* **2014**, *12*, 981–992. [CrossRef]
13. Martins, A.F.; de Oliveira, D.M.; Pereira, A.G.; Rubira, A.F.; Muniz, E.C. Chitosan/TPP microparticles obtained by microemulsion method applied in controlled release of heparin. *Int. J. Biol. Macromol.* **2012**, *51*, 1127–1133. [CrossRef]
14. Hanafy, N.A.; De Giorgi, M.L.; Nobile, C.; Cascione, M.; Rinaldi, R.; Leporatti, S. CaCO3 rods as chitosan polygalacturonic acid carriers for brompyruvic acid delivery. *Sci. Adv. Mater.* **2016**, *8*, 514–523. [CrossRef]
15. Ahsan, S.M.; Thomas, M.; Reddy, K.K.; Sooraparaju, S.G.; Asthana, A.; Bhatnagar, I. Chitosan as biomaterial in drug delivery and tissue engineering. *Int. J. Biol. Macromol.* **2018**, *110*, 97–109. [CrossRef] [PubMed]
16. Dube, A.; Nicolazzo, J.A.; Larson, I. Chitosan nanoparticles enhance the intestinal absorption of the green tea catechins (+)-catechin and (−)-epigallocatechingallate. *Eur. J. Pharm. Sci.* **2010**, *41*, 219–225. [CrossRef] [PubMed]
17. Barbieri, S.; Buttini, F.; Rossi, A.; Bettini, R.; Colombo, P.; Ponchel, G.; Sonvico, F. Ex vivo permeation of tamoxifen and its 4-OH metabolite through rat intestine from lecithin/chitosan nanoparticles. *Int. J. Pharm.* **2015**, *491*, 99–104. [CrossRef] [PubMed]
18. Miladi, K.; Sfar, S.; Fessi, H.; Elaissari, A. Enhancement of alendronate encapsulation in chitosan nanoparticles. *J. Drug Deliv. Sci. Technol.* **2015**, *30*, 391–396. [CrossRef]
19. Caramella, C.M.; Rossi, S.; Ferrari, F.; Bonferoni, M.C.; Sandri, G. Mucoadhesive and thermogelling systems for vaginal drug delivery. *Adv. Drug Deliv. Rev.* **2015**, *92*, 39–52. [CrossRef]
20. Yang, Y.; Zhu, H.; Wang, J.; Fang, Q.; Peng, Z. Enzymatically Disulfide Crosslinked Chitosan/Hyaluronic Acid Layer-by-Layer Self-Assembled Microcapsules for Redox-Responsive Controlled Release of Protein. *ACS Appl. Mater. Interfaces* **2018**, *10*, 33493–33506. [CrossRef]
21. Chen, W.L.; Li, F.; Tang, Y.; Yang, S.D.; Li, J.Z.; Yuan, Z.Q.; Liu, Y.; Zhou, X.F.; Liu, C.; Zhang, X.N. Stepwise pH-responsive nanoparticles for enhanced cellular uptake and on-demand intracellular release of doxorubicin. *Int. J. Nanomed.* **2017**, *12*, 4241–4256. [CrossRef]
22. De la Fuente, M.; Ravina, M.; Paolicelli, P.; Sanchez, A.; Seijo, B.; Alonso, M.J. Chitosan-based nanostructures: A delivery platform for ocular therapeutics. *Adv. Drug Deliv. Rev.* **2010**, *62*, 100–117. [CrossRef]
23. Woraphatphadung, T.; Sajomsang, W.; Rojanarata, T.; Ngawhirunpat, T.; Tonglairoum, P.; Opanasopit, P. Development of Chitosan-Based pH-Sensitive Polymeric Micelles Containing Curcumin for Colon-Targeted Drug Delivery. *AAPS PharmSciTech* **2018**, *19*, 991–1000. [CrossRef]
24. Wu, J.; Tang, C.; Yin, C. Co-delivery of doxorubicin and interleukin-2 via chitosanbased nanoparticles for enhanced antitumor efficacy. *Acta Biomater.* **2017**, *47*, 81–90. [CrossRef]
25. Shen, J.W.; Li, J.; Zhao, Z.; Zhang, L.; Peng, G.; Liang, L. Molecular dynamics study on the mechanism of polynucleotide encapsulation by chitosan. *Sci. Rep.* **2017**, *7*, 5050–5058. [CrossRef]

26. Calvo, P.; Remunan-Lopez, C. Chitosan and chitosan ethylene oxide propylene oxide block copolymer nanoparticles as novel carriers for proteins and vaccines. *Pharm. Res.* **2003**, *14*, 1431–1436. [CrossRef]
27. Ravi, H.; Baskaran, V. Chitosan Nanoparticles—An Emerging Trend in Nanotechnology. *Food Hydrocoll.* **2015**, *43*, 717–725. [CrossRef]
28. Shen, S.; Li, Y.; Xiao, Y.; Zhao, Z.; Zhang, C.; Wang, J.; Li, H.; Liu, F.; He, N.; Yuan, Y.; et al. Folate-conjugated nanobubbles selectively target and kill cancer cells via ultrasound-triggered intracellular explosion. *Biomaterials* **2018**, *181*, 293–306. [CrossRef]
29. Huang, Y.; Yeh, M.; Chiang, C. Formulation factors in preparing BTM-chitosan microspheres by spray drying method. *Int. J. Pharm.* **2002**, *242*, 239–242. [CrossRef]
30. Agnihotri, S.A.; Aminabhavi, T.M. Controlled release of clozapine through chitosan microparticles prepared by a novel method. *J. Control. Release* **2004**, *96*, 245–259. [CrossRef]
31. Mitra, S.; Gaur, U.; Ghosh, P.C.; Maitra, A.N. Tumour targeted delivery of encapsulated dextran-doxorubicin conjugate using chitosan nanoparticles as carrier. *J. Control. Release* **2001**, *74*, 317–323. [CrossRef]
32. Oh, N.M.; Oh, K.T.; Baik, H.J.; Lee, B.R.; Lee, A.H.; Youn, Y.S.; Lee, E.S. A self-organized 3-diethylaminopropyl-bearing glycol chitosan nanogel for tumor acidic pH targeting: In vitro evaluation. *Colloids Surf. B* **2010**, *78*, 120–126. [CrossRef]
33. Hanafy, N.A.; Dini, L.; Citti, C.; Cannazza, G.; Leporatti, S. Inhibition of Glycolysis by Using a Micro/Nano-Lipid Bromopyruvic Chitosan Carrier as a Promising Tool to Improve Treatment of Hepatocellular Carcinoma. *Nanomaterials* **2018**, *8*, 34. [CrossRef]
34. Pereira, P.; Correia, A.; Gama, F.M. In Vivo Imaging of Glycol Chitosan-Based Nanogel Biodistribution. *Macromol. Biosci.* **2016**, *16*, 432–440. [CrossRef]
35. Wang, Y.; Xu, S.; Xiong, W.; Pei, Y.; Li, B.; Chen, Y. Nanogels fabricated from bovine serum albumin and chitosan via self-assembly for delivery of anticancer drug. *Colloids Surf. B* **2016**, *146*, 107–113. [CrossRef]
36. Hoffman, A.S. Hydrogels for biomedical applications. *Adv. Drug Deliv. Rev.* **2002**, *54*, 3–12. [CrossRef]
37. Hamidi, M.; Azadi, A.; Rafiei, P. Hydrogel nanoparticles in drug delivery, Hydrogel nanoparticles in drug delivery. *Adv. Drug Deliv. Rev.* **2008**, *60*, 1638–1649. [CrossRef] [PubMed]
38. Peppas, N.A.; Hilt, J.Z.; Khademhosseini, A.; Langer, R. Hydrogels in biology and medicine: From molecular principles to bionanotechnology. *Adv. Mater.* **2006**, *18*, 1345–1360. [CrossRef]
39. Peppas, N.A.; Bures, P.; Leobandung, W.; Ichikawa, H. Hydrogels in pharmaceutical formulations. *Eur. J. Pharm. Biopharm.* **2000**, *50*, 27–46. [CrossRef]
40. Khan, F.; Tare, R.S.; Oreffo, R.O.; Bradley, M. Versatile biocompatible polymer hydrogels: Scaffolds for cell growth. *Angew. Chem. Int. Ed. Engl.* **2009**, *48*, 978–982. [CrossRef]
41. Tessmar, J.K.; Gopferich, A.M. Matrices and scaffolds for protein delivery in tissue engineering. *Adv. Drug Deliv. Rev.* **2007**, *59*, 274–291. [CrossRef]
42. Risbud, M.V.; Hardikar, A.A.; Bhat, S.V.; Bhonde, R.R. pH-sensitive freeze-dried chitosan–polyvinyl pyrrolidone hydrogels as controlled release system for antibiotic delivery. *J. Control. Release* **2000**, *68*, 23–30. [CrossRef]
43. Flory, P.J. *Principles of Polymer Chemistry*; Cornell University Press: New York, NY, USA, 1953.
44. Lou, X.; Dalton, P.D.; Chirila, T.V. Hydrophilic sponges based on 2-hydroxyethyl methacrylate: Part VII: Modulation of sponge characteristics by changes in reactivity and hydrophilicity of crosslinking agents. *J. Mater. Sci. Mater. Med.* **2000**, *11*, 319–325. [CrossRef]
45. Lin, C.C.; Metters, A.T. Hydrogels in controlled release formulations: Network design and mathematical modeling. *Adv. Drug Deliv. Rev.* **2006**, *58*, 1379–1408. [CrossRef]
46. Denkbas, E.B.; Ottenbrite, R.M. Perspectives on: Chitosan drug delivery systems based on their geometries. *J. Bioact. Compat. Polym.* **2006**, *21*, 351–368. [CrossRef]
47. Muzzarelli, R.A.A.; Muzzarelli, C. Chitosan chemistry: Relevance to the biomedical sciences. In *Polysaccharides 1: Structure, Characterization and Use*; Springer: Berlin/Heidelberg, Germany, 2005; pp. 151–209.
48. Crini, G. Recent developments in polysaccharide-based materials used as absorbents in waste water treatment. *Prog. Polym. Sci.* **2005**, *30*, 38–70. [CrossRef]
49. Peniche, H.; Peniche, C. Chitosannanoparticles: A contribution to nanomedicine. *Polym. Int.* **2011**, *60*, 883–889. [CrossRef]
50. Bumgardner, J.D.; Ong, J.L.; Yang, Y. The effect of cross-linking of chitosan microspheres with genipin on protein release. *Carbohydr. Poly.* **2007**, *68*, 561–567.

51. Hanafy, N.A.; Leporatti, S.; El-Kemary, M. Mucoadhesive Hydrogel Nanoparticles as Smart Biomedical Drug Delivery System. *Appl. Sci.* **2019**, *9*, 825. [CrossRef]
52. Shoueir, K.; Kandil, S.; El-hosainy, H.; El-Kemary, M. Tailoring the surface reactivity of plasmonic Au@ TiO2 photocatalyst bio-based chitosan fiber towards cleaner of harmful water pollutants under visible-light irradiation. *J. Clean.* **2019**, *230*, 383–393. [CrossRef]
53. Laroui, H.; Dalmasso, G.; Nguyen, H.T.; Yan, Y.; Sitaraman, S.V.; Merlin, D. Drug-loaded nanoparticles targeted to the colon with polysaccharide hydrogel reduce colitis in a mouse model. *Gastroenterology* **2010**, *138*, 843–853. [CrossRef]