THE FORMATION OF HYDROGELS BASED ON CHITOSAN AND ITS WATER-SOLUBLE DERIVATIVES

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Abstract

This review considers articles on the formation of hydrogels based on chitosan as well as succinylated and quaternized chitosan derivatives. They are synthesized using low toxicity reagents, under ordinary conditions (low production costs). Chitosan derivatives are soluble in an extended range of pH values and characterized by mucoadhesiveness, bioavailability and biodegradability, which extends the potential of their medical applications.

One of the most important properties of chitosan and its derivatives is the ability to form hydrogels. Depending on the nature of the bonds that occur during formation, hydrogels are divided into chemically or physically crosslinked, or a mixture of the two. Chemically crosslinked gels have covalent bonds, while physically crosslinked gels are formed by noncovalent interactions, for example, ionic. Mixed hydrogels have both types of crosslinking.

Keywords: chitosan, succinyl chitosan, quaternized chitosan, gelation chitosan, hydrogels, crosslinking agent

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1. Introduction

Chitosan is one of the most widely used natural polysaccharides; it is characterized by high biocompatibility, and it is a biodegradable, nontoxic and adhesive biomaterial. Chitosan is derived from chitin – one of the most common organic materials, being an important component of the exoskeleton of animals, especially crustaceans, insects and molluscs [1, 2]. It is a linear polymer constructed from glucosamine and N-acetyl glucosamine units linked by β-(1→4)-glycosidic bonds. Physicochemical and biological properties of chitosan depend on structural parameters: molecular weight, degree of deacetylation and distribution of the two types of residues that make up the chain. Chitosan contains three types of reactive functional groups: amino/acetamido groups, primary and secondary hydroxyl groups at the C3 and C6 atoms in the units, respectively. Chemical modification of chitosan leads to the synthesis of new derivatives and aims to enhance the biological properties of the biopolymer as well as create new physicochemical characteristics. Due to its high biocompatibility, chitosan can be used in medical therapy, including wound healing. This is a complex and dynamic process of replacing damaged tissue layers. The skin acts as a barrier to the external environment, while damage to this barrier disrupts the innate immune system and increases the susceptibility to bacterial infection, leading to the development of a distinct immune response. Unlike most polysaccharides, chitosan has a positive charge and a pH similar to that of skin; these features allow it to interact with the surface of skin cells (negatively charged).

Chitosan can form moisture-retaining coatings, such as hydrogels, which help to maintain optimal moisture in the affected areas, contributing to their subsequent healing [3]. A hydrogel is a three-dimensional network obtained from hydrophilic polymers that can absorb and retain significant amounts of water or biological fluid while maintaining integrity [4]. Insoluble polymer hydrogels are used on a large scale in wound dressings. They have a significant potential in the absorption of wound exudate, regulating moisture content and oxygen transfer, helping to remove dead tissue and restore the epidermis. Hydrogels provide a cool sensation, relieve pain and minimise the risk of infection. In addition, their transparency allows one to monitor the state of the wound [5]. Hydrogels can be divided into natural, synthetic or mixed depending on the origin of the polymers that comprise the hydrogel. By the nature of the bonds arising during the formation, hydrogels can be classified as follows: physically crosslinked (noncovalent interaction, for example, ionic), chemically crosslinked (covalent bonds) or mixed with a combination of those and other methods of crosslinking [6]. Given that natural raw materials are the main source of chitosan, chitosan-based hydrogels can be defined as natural. Chitosan-based hydrogels are formed in several ways: by physical association, coordination with metal ions, or chemical crosslinking between chitosan and a crosslinking agent.

This review considers methods for the formation of hydrogels based on chitosan, succinyl and quaternized derivatives set forth in public domain articles.

2. Physically Crosslinked Hydrogels

Ho et al. [7] suggested using freeze gelation to obtain a bulk hydrogel, which was formed in a gelling solution consisting of ethanol (C₂H₅OH) and sodium hydroxide (NaOH) at a temperature below the freezing point of the polymer solution. The method was used to form a hydrogel from a frozen 2% solution of chitosan in acetic acid (1 M). The proposed method of freeze gelation does not require special equipment and allows one to obtain hydrogels with the exact form shape. However, strong bases, such as NaOH, which are often used in the preparation of hydrogels, affect their biocompatibility with the cells of a living organism.
In one study [8], a chitosan solution with a molecular weight (MW) of 187 kDa (viscosity) and a degree of deacetylation (DD) of 89.8% was frozen in the desired form in acetic acid and then placed in a gelling solution, replacing NaOH with sodium chloride (NaCl) and phosphate-buffered saline (PBS, pH 7.4), given the dependence of the solubility of chitosan on pH and ionic strength. The results showed that the microstructure and physical properties of the hydrogel can be adjusted by varying parameters such as concentration, temperature and pH during gelation. It was confirmed that the replacement of NaOH with a 2.5 M NaCl solution and PBS (pH 7.4) as the gelling solution facilitated the production of gels that lacked cytotoxicity on L929 cells (a mouse fibroblast line) in the MTT assay. The method of forming hydrogels based on the ability of chitosan to coordinate with metal ions was also used in another study [9]. The gelation mechanism was investigated based on the complexes of chitosan with calcium (Ca$^{2+}$) and copper (Cu$^{2+}$) ions. Chitosan with a MW of 563 kDa (viscosity) and a DD of 91% was used. A fibre-oriented chitosan hydrogel sample was prepared from a 5% solution of chitosan in 2% acetic acid. The corresponding salt was used as the source of Cu$^{2+}$ or Ca$^{2+}$ in the system, which was added to the chitosan solution to form the soluble Me$^{2+}$–CS complex. Samples with different concentrations of Me$^{2+}$ were obtained using a different molar ratio between Me$^{2+}$ and the chitosan amino group. A mould open on one side was used to form the gel, in which a solution of the Me$^{2+}$–CS complex was placed, immersed in a coagulation bath with a 10% aqueous solution of NaOH, the volume of which was many times greater than the volume of the sample (100 times). After a certain period of time, the gelation process was interrupted to transfer the form into a deionized water bath for the same period of time. The sequence of movements was repeated five times. The gel samples were washed repeatedly with deionized water after gelation, controlling the pH. The results revealed that an oriented hydrogel structure can be formed due to diffusion processes.

The formation of hydrogel biopolymer from methyl alcohol supersaturated with calcium chloride (CaCl$_2$) has been reported. Chitin/chitosan polymer chains capable of hydrophobic and electrostatic interactions formed hydrogels as a result of self-assembly with the addition of excess methyl alcohol [10].

Liu et al. used a simple and effective method to construct a three-dimensional polymer structure of a hydrogel by neutralization of chitosan [11]. As a result of a sharp increase in pH in the volume, the neutralization reaction between the proton and the hydroxyl group proceeded quickly and irreversibly. Sodium bicarbonate (NaHCO$_3$), a weak base, was chosen to neutralize the protonated chitosan amino groups (MW 500 kDa, DD 95%). The morphology of chitosan hydrogels and their properties depended on the concentration of NaHCO$_3$ (0.07–0.13 mol/L), which played an important role in the formation of interactions between chitosan chains and the duration of gelation.

Various approaches have been employed to produce hydrogels from chitosan using organic and inorganic phosphates, for example, glycerophosphates (GP), potassium dihydrogen phosphate (KH$_2$PO$_4$), tripotassium phosphate (K$_3$PO$_4$), sodium tripolyphosphate and dibasic sodium phosphate (Na$_2$HPO$_4$), which were used as gelling agents. A 2% chitosan solution – with a MW of 50–190 kDa, a viscosity of 20–300 cps and DD of 75–85% – in 0.1 M HCl and two salts of inorganic sodium phosphate, 0.7 M NaH$_2$PO$_4$ solution (weak base with a pK$_a$ of 7.2) and 0.7 M Na$_3$PO$_4$ (a stronger base with a pK$_b$ of 2.23) as gelling agents were used to obtain the hydrogel [12]. The effect of various pH values (from 5.0 to 7.0) and chitosan/salt ratios – 0.5, 1.25 and 2.0 – was studied, and the stability of the obtained hydrogels was determined. There was a clear interdependence of pH and phosphate concentration on gelation. According to the research findings, a pH close to 7.0 has a predominant effect on gelation. It was noted that the ratio between chitosan and inorganic salt is the determining factor because it has a greater effect on the
equilibrium state between the total positive charge of the polysaccharide and the negative charge of the salt than the type of inorganic phosphate.

Another study proposed a new process for mechanically initiating the formation of physical chitosan hydrogels (MW 550 kDa, DD 58%) in an aqueous solution (acetic acid, dissolution time 4 h, room temperature) without a crosslinking agent [13]. A physical hydrogel was formed by mechanical activation of a chitosan solution with a low DD in a rotor-stator device. The formation of a three-dimensional gel structure occurs within a short period of time. According to the results, gelation is ensured by the action of a high shear stress, which leads to the formation of hydrophobic associations from residual chitin blocks, the content of which in the structure of chitosan is approximately 42%. Therefore, the use of mechanical activation to change the structure of the polymer solution has an advantage over traditional technologies, due to the short-term processing (10 s), the absence of chemicals or toxic solvents.

Thangavel et al. obtained 0.25–1.0% hydrogels based on chitosan (MW 100–150 kDa, DD 85%) and L-glutamic acid using physical crosslinking (ionic interaction), in order to improve the wound healing ability [14]. Gels were formed from a 2% (w/w) solution of chitosan in 2% (w/w) lactic acid and L-glutamic acid (25, 50 and 100 mg in 1 mL, 1 M HCl) with glycerol (50% w/w). The results of the swelling of chitosan with and without L-glutamic acid hydrogels in phosphate buffer were presented. The absorption of the buffer was more than 100% after 1 h. In vitro hydrogel biodegradation was performed using 1 mg/mL lysozyme in PBS. Lysozyme hydrolyses the \( \beta-(1 \rightarrow 4) \)-glycosidic bond between D-glucosamine and N-acetyl-D-glucosamine units of the chitosan molecule at 37°C. The results showed that chitosan with L-glutamic acid hydrogels are biodegradable and can be used as dressings for wounds. Biocompatibility was studied by culturing a fibroblast cell line (NIH 3T3) for 24, 48 and 72 h. Hydrogels did not show significant differences in cell viability compared with the control (tissue culture plate). Cells cultured on chitosan or chitosan and L-glutamic acid hydrogels are viable and multiply at a speed comparable to that in the control. In animal experiments (experimental diabetic rats), the potential of hydrogels in the treatment of diabetic wounds was studied. The results of histopathology and immunohistochemistry demonstrated that the hydrogel accelerated vascularization and increased the number of macrophages necessary for the rapid healing of wounds in diabetic rats.

ChitoHeal®, a hydrogel based on chitosan, is commercially used to heal burn wounds. Studies in animals (rabbits) showed that the wounds treated with the drug were epithelized [15]. The obtained data confirmed that the use of ChitoHeal accelerates wound healing in New Zealand rabbits compared with the control group (utilizing traditional gauze dressings). Morphological, histological and molecular parameters were examined to evaluate the effect of chitosan-based gel on burn healing. Faster healing of wounds and epithelization of burn wounds can lead to a decrease in collagen deposition, preventing the formation of severe scars. The authors suggest that the results of this study can potentially be important for developing new wound healing strategies using chitosan gel.

3. Chemically Crosslinked Hydrogels

A number of researchers believe that it is necessary to use crosslinking agents to form a stable and biocompatible crosslinked product and obtain a complete chitosan hydrogel. The main requirement for crosslinking agents is low toxicity to living organisms. The most common crosslinking agent for chitosan is glutaraldehyde, used to produce hydrogels with stable chemical and physical characteristics. Nevertheless, the resulting hydrogels can exhibit a certain degree of toxicity, both due to the presence of unreacted glutaraldehyde.
molecules and by-products that are formed during the reaction [16]. These problems can be mitigated when using natural crosslinking agents. Sucrose was used as a crosslinking agent to obtain a polysaccharide hydrogel, based on chitosan with a low viscosity (MW ≈ 150 kDa, DD 81%). The synthesis scheme implied reductive alkylation of chitosan using polyaldehyde, obtained from the periodic oxidation of sucrose [17]. The swelling of the synthesized hydrogel, depending on the amount of crosslinking agent, in the concentration range (200–1000 ppm) and the formation conditions (pH from 2.0 to 10.0, at room temperature), was studied. The authors hypothesized that the hydrogel will have higher biodegradability and lower toxicity due to the natural origin of the crosslinking agent. A fully polysaccharide hydrogel can potentially be used as a wound dressing.

Nada [18] utilized chitosan (MW 310–375 kDa, viscosity 800–2000 cP, DD ~ 85%), which was previously functionalized with active double bonds by opening the glycidyl methacrylate ring, to obtain a hydrogel precursor. The structure of the obtained methacrylate-chitosan was fully confirmed. The synthesized chitosan derivative was crosslinked using maleic anhydride, and 0.17, 0.35, and 0.5 M crosslinking agent was used for free radical polymerization in the presence of potassium persulfate (24–48 mM) as a chemical initiator. The structure and properties of a chemically crosslinked hydrogel enriched with dicarboxylic groups along the methacrylate-chitosan chain were studied. The highest swelling coefficient (1450%) was observed for the hydrogel that was crosslinked with the least amount of maleic anhydride (0.17 M), in an acidic medium at pH 4, in which chitosan swells well (amino groups are protonated). By contrast, the lowest swelling occurred at pH 7 and 9. The swelling coefficient at pH 7 and 9 increased with higher maleic anhydride concentration, explained by an increase in the number of carboxyl groups (along the methacrylate-chitosan chain) that are capable of adsorbing more water molecules. Colourimetric (MTT assay) and biological analysis using the skin fibroblast cell line (hTERT-BJ1) showed the biocompatibility of chitosan-based hydrogels.

Sharma et al. reported that nontoxic chitosan (MW 96,950 Da, DD 85.8%) and acryloyl – phenylalanine-based hydrogels biocompatible with moderate mechanical strength – contribute to the optimal balance of hydrophilic and hydrophobic components. The synthesis was carried out under ordinary conditions for 15 min; ammonium persulfate (0.5% w/w) was added to an aqueous acetic acid solution of chitosan (1.5% w/v) and acryloyl-phenylalanine (1 mmol/mL) as an initiator of free radical polymerisation and N,N'-methylene bisacrylamide as a crosslinking agent, varying the concentration from 0.8 to 1.4% (w/w). The amino and carboxyl groups of the hydrogel side chains contributed to the formation of noncovalent interactions, namely hydrogen bonds. The researchers believed that they developed hydrogels with good adhesion along with good antimicrobial activity against Escherichia coli, Klebsiella pneumonia, Staphylococcus aureus, Candida albicans and Microsporum gypseum [19]. The obtained hydrogels are suitable for biomedicine: they have properties similar to the internal properties of living tissue, moderate mechanical strength and self-healing ability [20].

Chemical crosslinking, leading to the creation of hydrogels with improved mechanical properties and chemical stability, is carried out using both synthetic and natural crosslinking agents [21]. Vilchez et al. synthesized hydrogels from chitosan (MW ≈ 190–310 kDa, DD ~ 85%) by crosslinking with 0.05, 0.01, and 0.005% (w/w) genipin, a natural reagent. The choice of the crosslinking reagent was due to its nontoxicity compared with glutaraldehyde. Genipin reacts with the primary amino groups of chitosan under mild conditions. The time required to obtain hydrogels and their physicochemical properties, such as rheological analysis, stability at various pH values and water absorption, were studied depending on the genipin concentration. The morphology of lyophilized hydrogels was determined by scanning electron microscopy. The results showed that the
use of a low genipin concentration produced well-crosslinked hydrogels. The swelling of crosslinked hydrogels depended strongly on the degree of crosslinking and the pH of the medium. For hydrogels crosslinked by genipin (0.005%), the percentage of swelling in distilled water was 525% (pH 7), 1000% in a solution of acetic acid (pH 2.9) and 97% in a solution of sodium hydroxide. The free amino groups of chitosan are protonated in the acidic pH range, and thus adsorb more water, resulting in the hydrogel swelling, while at pH > 7.0, the amino group are not protonated, which leads to hydrogel shrinkage [22].

Dimida et al. obtained chitosan (chitosan medium MW and DD 75%) with different degrees of crosslinking using 0.25 and 0.5 M genipin (final concentration in solution) [23]. The chitosan solutions (1.5%, 0.1 M acetic acid) and genipin (3.7% or 7.5%) were stirred for 1 h after mixing. The crosslinking reaction can occur at chitosan amino groups or as a result of the polymerization reaction of the chitosan/genipin system, in which oxygen radicals were induced, and their formation was influenced by temperature and the presence of H+. The crosslinking reaction of chitosan with genipin depends on the pH of the medium; at pH > 7, crosslinking occurs according to the Schiff reaction, which leads to the formation of a crosslinked polymer network. Under acidic conditions, two reactions take place, both involving chitosan amino groups [24]. Moulds (diameter 22.5 mm) and heat treatment at 25, 37 and 50°C were used for the formation of hydrogels. The thermal stability of crosslinked chitosan samples was characterised by calorimetric analysis (differential scanning calorimetry), and the effect of pH and ionic strength on swelling was studied. The possibility of using hydrogels for biomedical applications was assessed.

Pomari et al. focused on pre-treatment in an ultrasonic bath (5 min, room temperature) before mixing the solutions and for a short crosslinking time (1 min) when preparing crosslinked hydrogels from chitosan (MW 190-310 kDa, weight, DD 75–85%) and genipin (8:1 molar ratio) in an aqueous solution of acetic acid (1%) [25]. Crosslinked chitosan hydrogels were then formed at room temperature for 7 days in plastic tubes, where contact was limited to the surface hydrogel with the atmosphere (O₂), as suggested by another study [26]. According to Butler et al., the blue colouration of the formed hydrogels is explained by oxygen-radical-induced polymerization of genipin and dehydrogenation of intermediate compounds [27]. The obtained crosslinked hydrogels were characterized by weak mechanical properties, which may hinder their use in those cases when it is exposed to stress such as tension, compression, shear and torsion. Hence, the authors proposed forming hydrogels using a reinforcing filler – cellulose nanocrystals with a content of 0, 2, 4 and 6% (w/w).

Delmar et al. investigated the effect of minor changes in pH (in the acidic region) on the formation of chitosan hydrogels (MW 207 kDa, DD 77.6%) crosslinked by genipin [28]. A change in the pH of a chitosan solution even by half a unit led to a sharp change in the gelation time and gel properties. The pH value was crucial for reproducible and reliable results. The swelling ability of hydrogels was studied; it depended on pH even after a long gelation period (90 h). A comparison of any two hydrogels crosslinked at the same pH but swollen in different media revealed a higher swelling at pH 1.2 compared with pH 7.4 due to the greater number of protonated amino groups at a lower pH. It was noted that time is an important factor in creating hydrogels. The authors speculated that the ability to adjust the properties of hydrogels by changing the conditions for their preparation for 1 week, is a powerful and useful tool in the formation of hydrogels.

It should be noted that some properties, such as colloidal stability (swelling), degradability (under acidic conditions), and very weak aqueous solubility at pH > 6.2 limit the use of chitosan as a biomaterial. Chitosan dissolves well in aqueous solutions at pH < 4, when all amino groups are protonated and the intermolecular hydrogen bonds in
the polymer are weakened due to the electrostatic repulsion of the similar charges, which is accompanied by its dissolution. Therefore, it is preferable to use chitosan derivatives of various functionality that are water-soluble in a neutral medium to create materials and medical preparations e.g. polysols with inorganic and organic acids or N- and O-substituted chitosan derivatives [29, 30].

4. Hydrogels Based on Succinylated and Quaternized Water-Soluble Chitosan Derivatives in an Extended Range of pH Values

4.1. Succinyl Derivatives

Succinyl derivatives are acyl derivatives of chitosan synthesized without the use of toxic reagents under ordinary conditions (low-cost production). The introduction of succinyl groups into chitosan extends the range of solubility and provides low cytotoxicity of the biopolymer. Bashir et al. synthesized N-succinyl chitosan from chitosan with a MW of 190–310 kDa (weight), viscosity of 200-800 cP and a DD of 75%–85% [31]. The succinylation reaction proceeded by replacing a hydrogen atom from the amino group with the formation of an amide bond and the opening of the anhydride ring. Hydrogels from a 3% (w/w) solution of chitosan succinyl were obtained by the Schiff reaction between the remaining unsubstituted amino groups and aldehyde groups of a 1% (w/w) solution of glutaraldehyde, as a crosslinking agent, with thorough stirring, at 50°C. The physicochemical properties of the hydrogel were determined using spectral methods; the surface morphology was observed using a field emission scanning electron microscope, the mechanical properties were studied and swelling in buffer solutions of pH 1.2 and pH 7.4 was evaluated. Morphological studies showed the presence of highly porous structures that absorbed and retained a large amount of water due to the porosity. Hydrogels showed a low degree of swelling at pH 1.2 and high swelling at pH 7.4, which indicated the porous structure of the hydrogels. The number of protonated amino groups decreased while the carboxyl groups increased after chitosan modification, contributing to swelling at pH 7.4. The results obtained in the study of mechanical properties confirmed that hydrogels behave as elastic materials. The authors suggest that hydrogels from chitosan succinyl can be used in biomedical application.

The degree of substitution (DS) and DD in the structure of chitosan succinyl play an important role in the formation of wound healing hydrogels. In another work [32], acetyl and succinylated chitosan derivatives with different DD (43%–82%) and DS (0.14–0.79) were obtained from chitosan with a MW of 1200 kDa and DD of 94%. The effect of succinyl substituents and free amino groups on the ability to absorb (Ra) and retain (Rh) moisture was studied. The maximum values of Ra and Rh were obtained under conditions of high relative humidity with a chitosan DD of about 50%; however, the values of Ra and Rh decreased as DD changed. Moisture retention for N-succinyl chitosan with DS from 0.65 to 0.79 was the greatest. The authors noted that the results are comparable to moisture retention by hyaluronic acid, which is the main component of the extracellular matrix. They believe that crosslinked hydrogels of N-succinyl chitosan can be obtained using crosslinking agents, e.g. glutaraldehyde, genipin, etc.

4.2. Quaternized Derivatives

It is known that the introduction of quaternary ammonium groups into the structure of chitosan solves the solubility issue in a wide pH range and increases the antibacterial activity, expanding the potential for medical use [33, 34]. Shi et al. [35] reported the preparation of hydrogels based on quaternized chitosan (HTCC) and chitosan with a MW of 300 kDa and a DD of 95% (based on which the derivative was synthesized), with
α-β-glycerophosphate (GP). Gels were obtained by ionic interaction of constituent components under ordinary conditions. The authors noted that gelation mainly depended on the concentration and ratio of the components. Gel formation with an HTCC/GP component ratio of 1/2.5 occurred instantly (< 1 min), and it took 28 min to obtain the gel from chitosan, with the same ratio. Thermogravimetric analysis showed high stability of HTCC/GP hydrogels. The cytotoxicity of HTCC/GP and CS/GP hydrogels was measured in NIH3T3 cell cultures (RPMI-1640 medium) to record their vital activity. HTCC/GP hydrogel was characterised by lower cytotoxicity compared to CS/GP hydrogel. Morphological surface studies using scanning electron microscopy confirmed the three-dimensional porous structure of the gel with pore sizes ranging from 5 to 40 µm. Due to this structure, cells – adhered to the surface – could migrate into the gel while maintaining viability. The cytotoxicity did not exceed 80% with an increase in the concentration of HTCC/GP hydrogel from 1 to 10 mg/mL. The authors concluded that the water-holding ability, antimicrobial activity, absorption properties, biocompatibility and proliferative potential of cells for gels, based on quaternized chitosan, are higher than for gels prepared from chitosan.

The possibility of using a thermosensitive hydrogel formed from chitosan (MW 1080 kDa, DD 90%), HTCC and α-β-glycerophosphate was investigated in [36]. 0.1% solution of chlorhexidine was added to gel to enhance the antibacterial activity against pathogens present in the oral cavity. Thermal gelation was used to obtain hydrogels. The time required for gel formation was decreased from 20 to 3 min with an increase in temperature from 25 to 37°C [37]. Studies showed that the optimal composition required for gel formation corresponded to the CS/HTCC component ratio (5:1 w/w) with the addition of α, β-GP (final concentration of 8.33% in the gel) and 0.1% chlorohexidine. The obtained gel inhibited the growth of gram-negative periodontal pathogens – *Porphyromonas gingivalis*, *Prevotella intermedia* and *Aggregatibacter actinomycetemcomitans* – twice as much compared with the gel from CS/GP – 0.1% chlorohexidine and four times as much compared with 0.1% chlorohexidine. The authors suggest that the thermosensitive hydrogel is an excellent vehicle for local drug delivery during periodontal therapy.

Palacio et al. synthesized a quaternized chitosan derivative with a DS of 94% using CS (Mv 2,639 kDa, DD 97%) [38]. Hydrogels were formed (by mixing different molar ratios components – 1.0, 0.5, 0.25, 0.125 and 0.067 – in a solution of 1.7 M NaCl) based on the derivative and polyelectrolyte copolymers (CP), which differed in the ratio of the charges, CP1 (1:2 -/+), CP2 (2:1 -/+ and CP3 (1:1 ±). Hydrogels had different structures and variations in the total charge. Hydrogels obtained as a result of polyelectrolyte complexation had the following stability order CP2 > CP3 > CP1, which is associated with large electrostatic interactions. In addition, the hydrogel of quaternized chitosan with polyelectrolyte copolymer CP2, obtained by mixing the components at a molar ratio of 0.065, was the most stable. The obtained hydrogels were characterized by thermogravimetric analysis and spectral methods, while the structure was studied using scanning electron microscopy; solubility in water at various temperatures and pH values were also evaluated.

Wang et al. [39] investigated the effect of HTCC, temperature, valency of cations, anion type, inorganic salt concentration and DS on viscoelastic properties. Derivatives were synthesized from chitosan (viscous MW 520 kDa, DD 91%). The DS markedly affected the apparent viscosity. The viscoelastic properties of HTCC contributed to the formation of the gel structures, which was confirmed by atomic force microscopy results. The content of 20 and 40 mmol/L NaCl, as well as 10, 20 and 30 mmol/L CaCl₂, in HTCC solutions promoted electrostatic repulsion between HTCC molecules, inducing ‘entanglement’ of HTCC molecules and an increase in apparent viscosity as well as viscoelastic properties. An increase in the

12  Progress on Chemistry and Application of Chitin and its Derivatives, Volume XXV, 2020  
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concentration of NaCl from 40 to 60 mmol/L caused the screening of electrostatic repulsion of HTCC molecules, inducing the formation of dispersed aggregates, a decrease in apparent viscosity and viscoelastic properties. As a result, the HTCC molecules took an elongated shape (stretched), with an increase of C3 from 0.5 to 0.96, and the hydrophobic interaction between the methyl groups promoted the formation of physical crosslinks, causing an increase in viscosity; in addition, the larger the substitution, the higher the viscosity.

5. Conclusion
This review examined articles related to the formation of chitosan-based hydrogels. Hydrogels were obtained by physical or chemical crosslinking using various crosslinking mechanisms: covalent, ionic, by formation of hydrogen bonds or hydrophobic associations – using crosslinking reagents of both synthetic and natural origin. Emphasis has been placed on the formation of hydrogels based on succinylated and quaternized chitosan derivatives, water-soluble in an extended range of pH values. The described hydrogels obtained from chitosan and derivatives are characterized by a three-dimensional network structure and the ability to absorb and retain a significant amount of moisture or biological fluid while maintaining their integrity. They are characterized by mucoadhesiveness and biodegradability, characteristics that improve the potential of their medical applications.

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