Chapter 13

Chitosan-Based Thermosensitive Materials

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Additional information is available at the end of the chapter

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Abstract

Thermosensitive polymers are materials capable of undergoing a reversible phase transition in aqueous media in response to a variation of the temperature. They have attracted high scientific interest for advanced applications in diverse areas, such as biotechnology, biomedical, environmental, food industry and other fields. At the same time, chitosan is a promising marine polysaccharide that has long been used in applications such as drug, peptide or gene delivery systems. Being the most abundant marine polysaccharide, chitin and chitosan do not exhibit thermoresponsive properties, but some of their derivatives do. In the present chapter, the efforts to produce chitosan-based thermosensitive materials are reviewed. Particularly, the properties and applications of chitosan-glycerophosphate thermogelling system are examined; the methods of synthesis of chitosan copolymers grafted with poly(*N*-isopropylacrylamide) or poly(*N*-vinylcaprolactam), their physicochemical properties and most of their prominent applications are discussed as well.

Keywords: chitosan, thermosensitive material, chitosan-glycerophosphate, chitosan-*g*-*N*-isopropylacrylamide, chitosan-*g*-poly(*N*-vinylcaprolactam)

1. Introduction

Smart polymers are usually defined as 'macromolecules capable of undergoing rapid, reversible phase transitions from a hydrophilic to a hydrophobic microstructure. These transitions are triggered by small shifts in the local environment, such as slight variations in temperature, pH, ionic strength, or the concentration of specific substances like sugars' [1]. Among them, thermosensitive water-soluble materials have attracted high scientific interest

for advanced applications in diverse areas, such as biotechnology, biomedical, environmental, food industry and other fields.

Chitosan (Cs) is a linear polymer obtained by extensive deacetylation of chitin, the most abundant marine polysaccharide. It is mainly composed by two kinds of β(1→4)-linked structural units: 2-amino-2-deoxy-D-glucose and *N*-acetyl-2-amino-2-deoxy-D-glucose. Chitosan is a very interesting polymer for biomedical applications because of its biocompatibility, biodegradability and low toxicity. It can also be applied for the treatment of residual waters, in agriculture, food, cosmetics and textile industries, among others [2].

Because of the polyelectrolyte nature of chitosan, these thermoresponsive materials are also sensitive to changes of pH in the medium. Aqueous polymer solutions that could be transformed in situ into hydrogels by changes in environmental conditions, such as temperature and pH, have an advanced scientific interest due to their specific technological applications as sensors, actuators, controllable membrane for separations and modulators for delivery of drugs for use in medicine, biotechnology and other fields.

A wide variety of chitosan thermosensitive materials has been generated, like nanostructures, scaffolds, membranes, cryogels and paramagnetic beads, to cite some of them. In the present chapter, the efforts to produce chitosan-based thermosensitive materials, as well as their most relevant physicochemical properties and applications, will be considered.

2. Chitosan-glycerophosphate system

2.1. Physicochemical characteristics and mechanism of gelation

Chitosan is an ionic polysaccharide that does not yield physical gels by itself. It was up to 2000, when Chenite et al. published a couple of articles describing one of the rare true physical chitosan hydrogels [3, 4]. They described a chitosan formulation with glycerophosphate (GP), which is capable to render physical gels upon heating. In fact, when adding glycerophosphate to chitosan aqueous solutions, the polymer remains in solution at neutral pH and room temperature, while a heat-induced gelation can be triggered upon heating around the physiological temperature. Due to these interesting characteristics, this system has found a wide interest in the biomedical field including drug delivery and tissue engineering.

The rheological behaviour of a Cs-GP nearly neutral aqueous solution (pH 7.15) exhibits a strong rise of the storage modulus, G', upon heating, indicating that the liquid solution turned into a solid-like gel in the vicinity of 37 °C. In turn, during the cooling run, there is a decrease of G', revealing a tendency of the gel to return to the liquid state [3]. Cho et al. have classified the viscoelastic behaviour of this system in three well-distinctive regions: (1) a liquid-like behaviour manifested at low temperatures, (2) the thermal gelation process characterized by an increment in G' and G'' moduli as the temperature rises and (3) a slow terminal gelation after the heat-induced cross-linking process [5].

The temperature of incipient gelation increases as the degree of deacetylation decreases, while the molecular weight showed no significant effect on the temperature of gelation [3]. If

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transparent hydrogels are needed to be prepared, it is necessary to previously reacetylate chitosan up to a degree of deacetylations 35–55%, under homogeneous conditions [6].

The morphology of Cs-GP hydrogel as observed by laser scanning confocal microscopy displays a heterogeneous microstructure, suggesting that the kinetic gelation mechanism of this system may be nucleation and growth. Power spectra reveal a fractal-like morphology in the gel [7].

The mechanism of gelation of chitosan in the presence of glycerophosphate involves several interactions such as screening of electrostatic repulsion, ionic cross-linking and hydrophobic and hydrogen-bonding interactions [5, 8].

The effects of experimental parameters on the characteristics of the gelation process have been documented. *Chitosan and glycerophosphate concentration*: the gelation temperature decreased with increasing both β-GP and polymer concentration, while the mechanical properties of the gel become enhanced due to an increase of intermolecular interactions and entanglements [8]. *Temperature*: it is interesting to note the behaviour of the pH and conductivity during heating. While any change in pH could not be appreciated, the conductivity displayed a monotonous increment as the temperature rises. This behaviour was associated to a reduction of chitosan solubility and an enhancement on the screening of electrostatic repulsion, therefore increasing hydrophobic interactions and improving conditions for gel formation [5]. According to the same authors, temperature most probably modifies hydrogen bond distribution and favours polymer-polymer interactions over those of polymer-solvent interactions. *Ionic strength*: an increment of the ionic strength gives favourable conditions for gel formation due to the higher screening of electrostatic repulsions, thus promoting the hydrophobic interactions in the polymer physical network [5].

In a continuation of these works, Cho et al. used oscillatory shear data to verify the scaling behaviour at the gel point. Their conclusions showed that the power-law index was dependent to some extent on chitosan concentration and temperature [9].

Moreover, an interesting research published by Supper et al. [10] demonstrated the specific role of the polyol-phosphate molecules on the thermo-physical gelation process. On the one hand, the phosphate moiety neutralizes the charges along the Cs chains up to the physiological pH, keeping them in the solution state at room temperature. On the other hand, the polyol unit is responsible for the thermal sensitivity of the Cs solution. Both the chemical structure and size of the polyol moiety play a significant role in controlling the gelation process [10]. In **Figure 1** this gelation mechanism is clearly depicted.

Similar conclusions have been achieved by Qiu et al. when they investigated the influence of urea and isobutanol on the thermogelling process of Cs-GP system. Urea appears to be unfavourable to the gelation process, because it disrupts hydrogen bonding and retards the formation of hydrophobic domains, but the addition of isobutanol speeded the sol-gel transition by strengthening the hydrophobic interactions. This opposite effect opens the possibilities of tuning the gelling process of Cs-GP system [11].

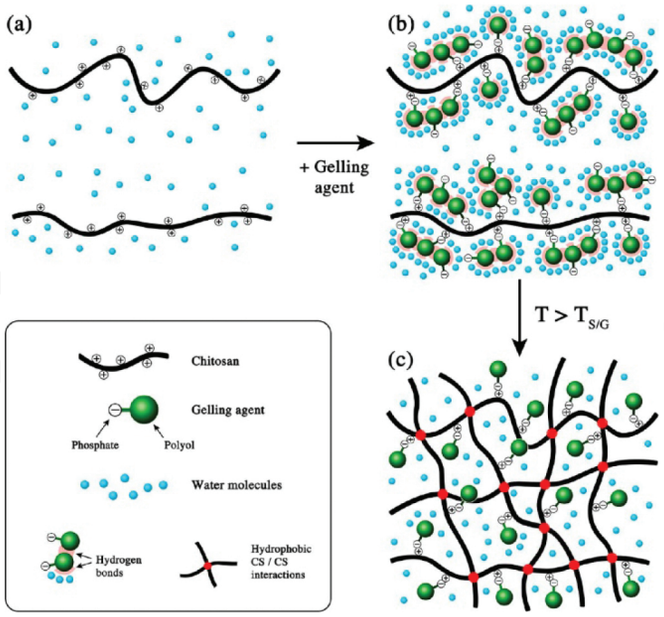

Figure 1. Representation of the gelation mechanism of Cs/polyol-phosphate solutions according to Supper et al. [10]. Reprinted with permission from Ref. [10]. Copyright 2013, American Chemical Society.

2.2. Applications

For biomedical purposes, the sterilization of the Cs-GP system and other biomedical characteristics are important to be considered. There have been documented some investigations about the influence of different sterilization procedures on the thermogelling ability of this system. Autoclaving and steam sterilization may significantly affect the molecular weight and viscosity of chitosan, but do not impair its ability to form gels upon heating [12, 13]. However, γ-radiation markedly changed its thermogelling properties [13]. Regarding the angiogenic potential of Cs-GP system, results indicate that this system does not contribute to enhance angiogenesis, while the presence of human bone marrow-derived mesenchymal stem cells resulted in an increased angiogenic response after 3 days of placement on the chick chorioallantoic membrane [14]. A lower acetylation of chitosan seems to be desirable for better biocompatibility. At the same time, even though biodegradation is slower, fewer fragments are generated, and this seems to lead to a minor pronounced immune response [15]. Newer studies in this sense confirm that Cs-GP is a biocompatible hydrogel, extracts of which can stimulate mesenchymal stem cell proliferation at certain concentrations. According to authors' conclusions, 'this material is therefore a promising vehicle for cell encapsulation and injectable tissue-engineering applications' [16].

Chitosan thermosensitive hydrogel has the advantage to form in situ a hydrogel at physiological temperature, avoiding the necessity of surgical implants. This fact underlies its applications in biomedical field including local drug delivery and tissue engineering [17]. Cs-GP system has a great potential as scaffold material in tissue engineering and regenerative medicine due to its good biocompatibility, minimal immune reaction, high antibacterial nature, good adhesion to cells and the possibility to be moulded in various geometries [18–26]. For

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similar reasons, the other key pharmaceutical application of this material is a smart-controlled release system [4, 18, 20, 27–38], because the hydrogel is able to keep the drug level within the therapeutic window during extended periods of time, thus avoiding frequent low doses and undesirable secondary effects in patients. Important and interesting patents of biomedical applications of this system have been revised recently [39].

Composite nanomaterials of Cs-GP hydrogel and silver nanoparticles (NPs) with potential applications in medicine due to their antibacterial activity have also been documented [40, 41].

3. Chitosan thermosensitive derivatives

Chitosan macromolecule is prone to chemical modifications, due to the high reactivity of their functional groups: primary and secondary hydroxyl groups at C-3 and C-6 positions, respectively, and the highly reactive amino group at C-2 position. The chemical structure of chitosan is represented in **Figure 2**. The most common purposes for modifying chitosan include improvement of its solubility at neutral or alkaline pH and to impart specific functional properties [42]. Among the different chemical approaches to modify chitosan, the grafting procedures open a huge range of possibilities to achieve versatile molecular designs for new advanced materials.

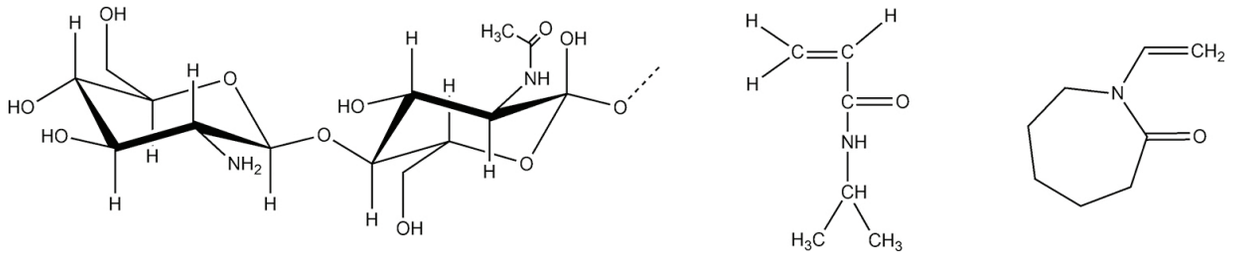

Figure 2. Structural units of chitosan, *N*-isopropylacrylamide and *N*-vinylcaprolactam.

A graft copolymer contains a long sequence of structural units (often referred to as the backbone polymer, in this case chitosan), with one or more branches (grafts) of long sequences of another monomer. Grafting copolymerization can be conducted in both heterogeneous and homogeneous conditions [43]. The graft copolymers can be principally synthesized by three strategies: *grafting through*, *grafting from* and *grafting onto*, in dependence of the method of preparation. The *grafting from* reaction is based on the in situ polymerization of the grafting monomers from a preformed macromolecular backbone that is chemically modified by the introduction of active sites. The *grafting onto* is an interesting technique that consists in a coupling reaction between end-functional groups of the graft chains onto pendant functional groups of the backbone chain [44]. This procedure has become an efficient tool for the preparation of graft copolymers with well-defined structure.

Poly(*N*-isopropylacrylamide), PNIPAm, and poly(*N*-vinylcaprolactam), PVCL, are two well-recognized water-soluble thermosensitive polymers (**Figure 2**). They both undergo hydro-

philic to hydrophobic phase transition when temperature increases, exhibiting lower critical solution temperature (LCST) behaviour. Below the LCST, the polymer is soluble, but when temperature increases, they experience a reversible volume phase transition.

Chitosan has been grafted with PNIPAm and PVCL. Other chitosan derivatives such as chitosan-poly(ethylene glycol) copolymer and *N*-isobutyryl chitosan also display thermoreversible behaviour.

In this section, an overview about the methods to synthesize chitosan-based copolymers will be described, as well as their physicochemical properties and potential applications.

3.1. Chitosan-*graft*-poly(*N*-isopropylacrylamide)

3.1.1. Synthesis

The *grafting from* approaches to modify chitosan with NIPAm could be performed under simple homogeneous conditions by one-pot free radical polymerization. With this purpose cerium ammonium nitrate has been frequently employed as initiator [45], as well as a variety of thermal initiators such as azobisisobutyronitrile (AIBN) [46] and ammonium or potassium persulfate [47–50]. Cerium ion is an efficient redox agent capable of undergoing radical polymerization under soft conditions, in acidic aqueous media at low temperatures [51–54].

This copolymer has also been prepared by the *grafting onto* strategy. With this purpose, a two-step synthesis should be conducted. Firstly, carboxyl-terminated PNIPAm is obtained by free radical polymerization in the presence of 3-mercaptopropionic acid as chain-transfer agent. Then, the homopolymer is grafted onto chitosan chain using a condensing agent such as EDC/NHS [55, 56].

Nowadays, more organized polymer structures are required to attend specific biotechnological applications. The conventional radical polymerization employed to prepare PNIPAm-COOH homopolymers by the *grafting onto* strategies does not allow a control over the degree of polymerization of PNIPAm chains, and hence no predefined molecular architecture of chitosan-*g*-PNIPAm copolymer could be synthesized.

Atom transfer radical polymerization (ATRP) is an alternative in which the polymer length is controlled by the synthesis of well-defined graft chains. Bao et al. have obtained a Cs-*g*-PNIPAm copolymer by the following approach: azide-ended PNIPAm homopolymer was firstly prepared through ATRP. Simultaneously, alkynyl pendant Cs derivative is prepared by the amidation of Cs with 4-pentynoic acid in the presence of EDC/NHS. Finally, the Cs copolymer was completed by the click reaction of alkynyl Cs with PNIPAm-N_3 under mild click chemistry conditions. The click reaction proved to be an efficient coupling method for grafting Cs [57]. The same research group has also reported the synthesis of a comb-type Cs(-*g*-PDMAEMA)-*g*-PNIPAm terpolymer using a similar strategy of copolymerization [58].

Chen et al. have proposed a four-step route where the grafting reaction was directed towards the C-6 position; the method involves [59]:

- Protection of amino groups of chitosan by *N*-phthaloylation.
- Preparation of the bromoisobutyryl-terminated *N*-phthaloyl chitosan macroinitiator.
- Synthesis of the copolymer via ATRP from the chitosan macroinitiator with NIPAm.
- Removal of the *N*-phthaloyl groups regenerates amino groups.

Don et al. synthesized Cs-*g*-PNIPAm by means of a two-step route: in order to include vinyl carboxylic acid groups in the backbone, chitosan was first modified with maleic anhydride to produce MA-Cs. In the second step, NIPAm monomer was grafted onto MA-Cs via UV-initiated free radical polymerization [60]. Later, this group proposed a new grafting route in which chitosan amino groups were protected by *N*-phthaloylation, and then the vinyl functional group was introduced at the C-6 position by reaction with *m*-tetramethylxylene isocyanate, followed by deprotection of amino groups. Finally, PNIPAm was grafted to the vinyl Cs by UV-initiated free radical polymerization [61].

3.1.2. Properties

Due to the interesting dual-responsive behaviour of Cs-*g*-PNIPAm copolymers, the properties of these materials have been thoroughly studied using different techniques such as micro-differential scanning calorimetry (μDSC), dynamic light scattering (DLS), NMR, UV-Vis spectroscopy and rheological measurements, among others [46, 51, 52, 54, 58–60, 62–65].

During heating of Cs-*g*-PNIPAm solutions, μDSC traces show a sharp endothermic peak associated with the phase transition at LCST. Associated enthalpy values are proportional to NIPAm content in the copolymer. Comparable exothermic peaks were obtained during cooling, giving rise to fully reversible transition [52].

Hydrophilic and hydrophobic interactions are important factors governing thermosensitive properties of NIPAm polymers. At temperatures below LCST, water molecules form regular ice-like structures around hydrophobic methyl groups. An increase in temperature results in a breakdown of the hydrophobic hydration. As a result, hydrophobic interactions between methyl groups from different NIPAm-grafted blocks are promoted, giving rise to a polymer network. From a thermodynamic point of view, such a phase transition should generate a conformational entropy loss upon polymer association, which should be compensated by the translational-entropy gain of expelled water molecules. Therefore, there is a total entropy increment upon phase transition that overcomes the observed endothermic enthalpy, thus giving rise to a decrement in Gibbs free energy [52, 66].

Variation of viscoelastic G′ and G″ moduli during heating also confirms the existence of a sol-gel transition: as the phase transition takes place, there is a marked increase in storage modulus and a moderate decrement in loss modulus (**Figure 3a**). This phenomenon has been interpreted as the result of the formation of hydrophobic junctions at the expense of the net amount of sol fraction, giving rise to the formation of more elastic networks [52].

It is well known that the LCST value of PNIPAm may be changed conveniently if the hydrophobicity of the system is altered, either by changes in macromolecular composition or by

changes in overall hydrophilicity of the surrounding environment [50, 66–68]. This response is also observed for Cs-*g*-PNIPAm copolymers. Wang et al. successfully regulated the LCST adding acrylamide as a comonomer during the synthesis. By this way, the LCST of Cs-*g*-poly(NIPAm-co-AAm) was increased from 33 to 38 °C when 5.5% of AAm was included [69]. An increment in LCST from 33 to 44 °C as the chitosan feed concentration with respect to NIPAm was raised up is also reported [67].

The influence of the environmental conditions on the LCST of Cs-*g*-PNIPAm is noticeable. For example, it was observed that the transition temperature of the copolymer shifted to lower temperatures with increasing concentration of alcohols [70]. Meanwhile, addition of salt to PNIPAm solutions is known to disrupt the regular ice-like structure of water molecules around NIPAm moieties resulting in a decrement of the transition temperature [71, 72]. Such an effect was appreciated for the copolymer: the addition of NaCl to hydrochloric stoichiometric solutions of the copolymer decreased the LCST and caused an increase in enthalpy change [52].

The fully reversible behaviour of the phase transition of this copolymer is an upmost property. On the one hand, it is noticeable from the fast and reversible variation of the viscoelastic moduli during heating-cooling cycles [52]. On the other hand, the same behaviour is evident from the continuous swelling-shrinking cycles, induced by stepwise periodic changes in temperature for a polyelectrolyte complex membrane of Cs-*g*-PNIPAm [63] (**Figure 3b**).

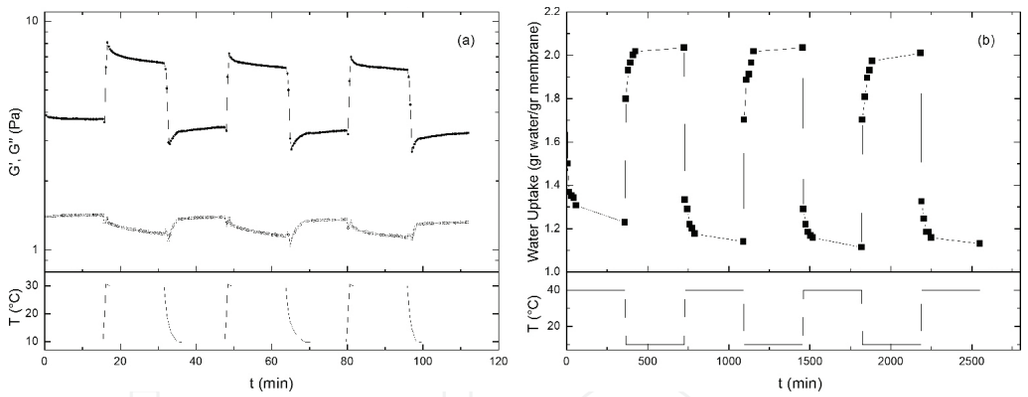

Figure 3. (a) Variations in mechanical moduli, G′ (,) and G″ (–), for 1% (w/w) solution of Cs-*g*-NIPAm in 10% aqueous acetic acid to stepwise periodic changes in temperature between 10 and 30 °C (ω = 1 rad s^{-1}; γ = 5%); reprinted with permission from Ref. [52]. Copyright 2009, American Chemical Society. (b) Variation of swelling in pure water (pH = 5.9) for a Cs-*g*-PNIPAm polyelectrolyte complex membrane to stepwise periodic changes in temperature between 10 and 40 °C; reprinted from Ref. [63]. Copyright 2011, with permission from Elsevier.

There are evidences that Cs-*g*-PNIPAm undergoes micellization processes above the phase transition temperature. On the one hand, Chen et al. noticed that aqueous solutions of Cs-*g*-PNIPAm (which amino groups were protected during the synthesis) showed pH-dependent behaviour, and at temperatures above the LCST, the copolymer self-assembled into micelles with chitosan core [59]. On the other hand, recent studies have demonstrated that during heating, Cs-*g*-oligo(NIPAm) copolymers self-assembled into aggregates due to the hydrophobization of NIPAm blocks. At 25 °C, oligo(NIPAm) chains are hydrophilic exhibiting expanded structures. In change, at 37 °C, a clear transition is observed, the side-chain segments

become hydrophobic and the molecules began to fold [54]. In the same way, Cs(-*g*-PDMAEMA)-*g*-PNIPAm terpolymer experiments thermo- and pH-responsive micellization behaviour in aqueous solutions. Moreover, this terpolymer could form three-layer *onion-like* micelles at 25 °C when pH is above 7 [58].

3.1.3. Applications

Cs-*g*-(PNIPAm) is a potential thermosensitive in situ gel-forming material for ocular drug delivery that may enhance ocular absorption, efficacy, bioavailability and pharmacokinetic properties. Experimental results suggested that, at physiological pH, the copolymer hydrogel can interact with the mucus and cornea cell membrane, increasing the drug residential time [46].

Chitosan/PNIPAm hydrogels have been considered as a useful tool to enhance oral bioavailability of low-solubility drugs such as naproxen [73], paclitaxel [69], caffeine [74], etc.

Raskin et al. have proposed a mucoadhesive amphiphilic nanogel based on the micellization of CS-*g*-oligo(NIPAm) and stabilized through the ionotropic gelation of chitosan. These polymeric micelles are self-assembled and positively charged nanocarriers with potential for improved mucosal administration of hydrophobic drugs [54].

A formulation of curcumin-loaded biodegradable thermoresponsive Cs-*g*-PNIPAm nanoparticles was prepared by ionic cross-linking method. The in vitro drug release was prominent at temperatures above LCST. The curcumin-loaded nanoparticles (NPs) showed specific toxicity on cancer cells and increased apoptosis on PC3 cells [55].

Gui et al. have developed some inorganic/organic hybrid composite hydrogels using Cs-*g*-NIPAm as a shell that combine thermo-/pH sensitivity, fluorescence and biocompatibility giving an attractive option for biological and biomedical applications [64, 75]. In the first report, experimental data evidence that Adriamycin-loaded microspheres could effectively improve drug release and accumulation in targeted tumour cells or tissues [64]. On the second article, authors prepared doxorubicin-loaded nanospheres that exhibited remarkable fluorescence/thermo-/pH sensitivity with high anticancer activity [75].

Recently, interpenetrated cryogel scaffolds of PNIPAm and chitosan have been prepared via free radical polymerization in the presence of cross-linkers. These materials were evaluated as potential bioartificial liver devices. The cell-seeded cryogel proved their capacity to successfully purify plasma, supporting liver function in terms of both detoxification and synthesis of important metabolites [65].

3.2. Chitosan-*graft*-poly(*N*-vinylcaprolactam)

3.2.1. Synthesis

To the best of our knowledge, the first report of the preparation of chitosan-*graft*-PVCL (Cs-*g*-PVCL) copolymer was documented by Kudyshkin et al. [76]. They reported the synthesis of this thermosensitive material by radical graft polymerization. This reaction was performed

under homogeneous conditions using a mixture of solvents at 60 °C. Unfortunately, this reaction is reported to be accompanied by a decrease in the molecular weight of chitosan, probably caused by the cleavage of the glycoside bonds by potassium persulfate [76, 77]. In the *grafting from* technique, the radical chain polymerization of a monomer is triggered by the thermal decomposition of the initiator. Simultaneously, the polymer radicals are formed by chain transfer between the propagating radical and polymer. It is well known that this method results in a mixture of homopolymer and graft copolymer, as well as ungrafted backbone polymer [43]. The *grafting from* approach allows the synthesis of graft copolymers by one-step reaction, but its principal drawback is the lack of control on the chain length of the grafted polymer.

A novel approach to obtain Cs-*g*-PVCL based on *grafting onto* copolymerization was documented by Prabaharan et al. This strategy consists of the reaction between functional groups from two different polymers. In this research, the first carboxyl-terminated PVCL homopolymer (PVCL-COOH) was synthesized by radical chain polymerization, using AIBN as initiator and 3-mercaptopropionic acid as chain-transfer agent. Then, PVCL-COOH chains were grafted onto chitosan backbone via amidation reaction using EDC/NHS as coupling agents [78].

After this pioneering work, the synthesis of the chitosan-*graft*-PVCL by the *grafting onto* approach has been documented by other authors [79–85]. The formation of amide bonds between PVCL-COOH and chitosan amino groups has been confirmed by FTIR [78, 80, 85], ^{1}H-NMR [78, 85] and Raman [85] spectroscopy techniques. EDC/NHS system is the activator agent usually used by many authors. The main drawback of this system is the formation of the secondary products, which are difficult to remove leading to low yields of functionalization [86, 87]. In this regard, DMTMM entails some benefits over the former system because it selectively promotes the formation of the amide bond in aqueous solution in a wide range of pH. The coupling reaction is thought to be initiated by addition of a carboxylate anion to DMTMM to give an activated ester, which undergoes attack by an amino to give the corresponding amide [88, 89].

The main advantage of the *grafting onto* procedure is the possibility to control the molecular architecture of the copolymer. In this sense, our group has reported the synthesis of PVCL-COOH samples with different molecular weights by means of controlled radical polymerization and their subsequent grafting onto the chitosan backbone using DMTMM as activator agent. This approach allows a control of the chain length of the grafted PVCL chains, as well as the spacing between grafted side chains onto the chitosan backbone [85].

Cs-*g*-PVCL copolymer has also been synthesized by gamma radiation [90]. This method allows obtaining functionalized materials without remaining residues. The reaction solution is irradiated with a ^{60}Co γ-source using doses between 10 and 50 kGy. The ionizing radiation is a powerful tool to achieve functionalization of polymers requiring no additional reactants [91]. However, it has been documented that γ-radiation leads to the scission of 1–4 glycosidic bond of chitosan, thus reducing the molecular weight [92, 93].

3.2.2. Properties

The Cs-*g*-PVCL system shows properties of both, chitosan and PVCL. As it is well known, chitosan is a pH-sensitive, non-toxic, biodegradable, biocompatible linear polyelectrolyte [2], while PVCL is a non-ionic, biocompatible, thermosensitive water-soluble polymer with a phase transition temperature in the physiological range [94]. This graft copolymer showed pH and temperature sensitiveness that could be very interesting for the development of smart-controlled release systems, as well as active scaffold for tissue engineering and regenerative medicine.

The grafting parameters, such as grafting percentage and grafting efficiency, are greatly influenced by the reaction conditions [95]. These parameters have been evaluated by ^{1}H-NMR [78], thermogravimetric analysis (TGA) [85] and gravimetrically [79, 85, 90].

Kholmuminov et al. observed a relatively rapid decrease on the effective viscosity of Cs-*g*-PVCL solutions at shear rates up to 100 s^{-1}. These authors suggested that this behaviour is related to the presence of flexible PVCL chain blocks that easily unwind and orient in a flow when increasing the shear rate [77].

The phase transition behaviour of Cs-*g*-PVCL in aqueous solutions has been investigated by turbidimetry [78–80, 85], DSC [79, 85] and DLS [96]. The LCST of this copolymer has been estimated between 32 and 42 °C, and it was close to the transition of the corresponding homopolymers.

It has been demonstrated that the properties of the Cs-*g*-PVCL are controlled by the molecular architecture of the copolymer: length of the grafted side chains and their spacing along the chitosan backbone [85].

On the one hand, the temperature of the phase transition of the copolymer depends on the spacing between grafted PVCL chains along chitosan backbone. Studies by μDSC evidenced that the longer the spacing between PVCL-grafted chains, the lower the LCST of the polymer, as well as the enthalpy associated with the phase transition (**Figure 4a**) [85]. It is obvious that as the spacing between grafted chains is smaller, the phase transition is more cooperative, thus facilitating the hydrophobic intercatenary interactions between PVCL chain segments during the dehydration process at the critical temperature. From a thermodynamic point of view, the phase transition produces a loss of conformational entropy due to the aggregation of PVCL chain segments. This phenomenon must be counterbalanced by the translational-entropy gain when water molecules are expelled out from the excluded volume of PVCL macromolecules during phase separation. Thus, the larger the hydrophobic portions are, the greater the entropy gain is, which explains why the cooperative hydrophobic interactions are favoured when the PVCL chains are closer in space [85].

On the other hand, the longer the grafted PVCL chain, the lower the phase transition temperature (**Figure 4b**) [85, 96]. This phenomenon has been attributed to the fact that increasing the PVCL chain length, the polymer-polymer interactions become more and more cooperative. As a result, longer hydrophobic segments appear, thus favouring polymer-polymer long-range interactions giving rise to phase separation phenomena at lower temperatures [85].

Since this system exhibits a dual temperature and pH sensitiveness, the ionic strength and pH of the medium have also an influence on the properties of the Cs-*g*-PVCL aqueous solutions, as evidenced from DLS and ζ-potential studies [96]. According to the DLS measurements, at temperatures above the phase separation, the size of the macromolecular aggregates is greater as the medium is more acidic, and a slight increment on the transition temperature is also observed (**Figure 4d**) [96]. Concerning the influence of the ionic strength on the copolymer solutions, a noticeable effect of the electrostatic screening on the hydrodynamic sizes of the copolymer coils, which causes that the phase transition takes place at lower temperatures, giving bigger macromolecular aggregates (**Figure 4e**) was found [96].

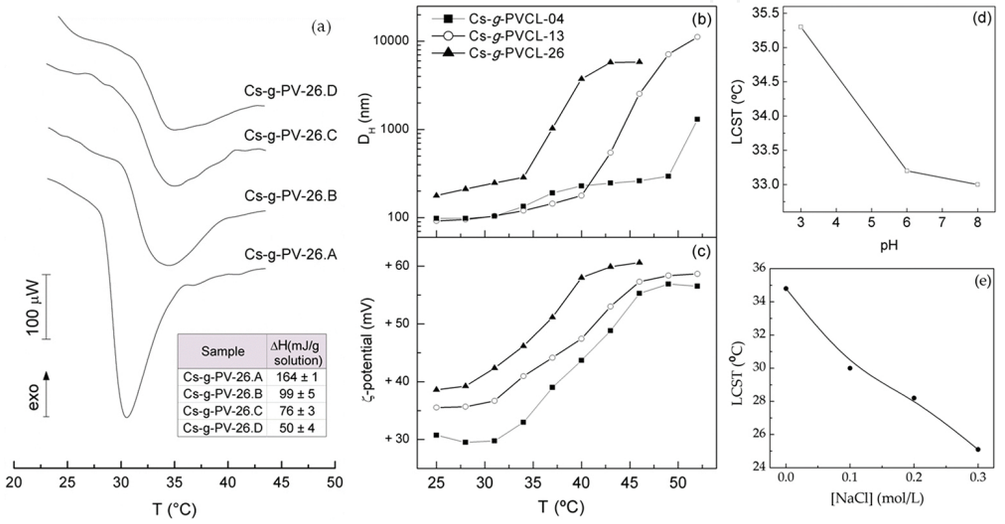

Figure 4. (a) Heating μDSC scans of 10 wt% aqueous solutions of Cs-*g*-PV-26.A, Cs-*g*-PV-26.B, Cs-*g*-PV-26.C and Cs-*g*-PV-26.D samples (heating rate, 0.6 °C min^{-1}; reference, water). Reprinted from Ref. [85]. Copyright 2015, with permission from Elsevier. Dependence of the (b) hydrodynamic diameter, D_H, and (c) ζ-potential of Cs-*g*-PVCL on temperature of aqueous solutions of Cs-*g*-PVCL for different number-average molecular weights of PVCL-grafted chains. Cs-*g*-PVCL samples with Mn 04, 13, and 26 kDa at 2 mg mL^{-1} (pH 6). Heating rate, 0.25 °C min^{-1}. Variation of LCST of the Cs-*g*-PVCL-26 solution (2 mg mL^{-1}) with (d) pH of the medium and (e) ionic strength. Heating rate, 0.25 °C min^{-1}. Reprinted from Ref. [96]. Copyright 2016, with permission from Springer.

Pérez-Calixto et al. have recently addressed the preparation of *N*-vinylcaprolactam and *N*,*N*-dimethylacrylamide (DMAAm) binary-grafted system onto cross-linked chitosan by γ-radiation. The incorporation of DMAAm hydrophilic comonomer increased the phase transition temperature from 34 to 37 °C, as well as the swelling degree due to intermolecular interactions with amino groups of chitosan molecule [97].

It is important to remark that Fernández-Quiroz et al. have reported that all the Cs-*g*-PVCL copolymers they synthesized were soluble in water at neutral pH, at room temperature. From a thermodynamic point of view, this fact suggests a significant improvement of the copolymer solvation via hydrogen bonding between PVCL-nitrogen unshared electron pair and water molecules. A better polymer-solvent interaction induces higher entropy of mixing giving rise to the dissolution of the macromolecule. This property could be of considerable interest for biomedical applications, where it is important to keep a pH near to the physiological one [85].

3.2.3. Applications

Thermoresponsive chitosan derivatives based on Cs-*g*-PVCL have been chiefly studied for biomedical applications as drug delivery and tissue engineering. Their biocompatibility, low toxicity, pH and temperature sensitiveness have drawn a great scientific interest.

In this sense, beads and nanomaterials based on Cs-*g*-PVCL copolymer have been prepared by ionotropic gelation using sodium tripolyphosphate (TPP) as ionic cross-linking agent [78, 80–84]. Studies carried out by Prabaharan et al. indicated that the copolymer has a swelling degree higher at pH 2.2 than at pH 7.4, decreasing with increased environmental temperature. These copolymer beads exhibited a slower release of ketoprofen, as compared with chitosan, revealing a different release profile in dependence on the pH and temperature. According to MTT assay, the copolymer showed no obvious cytotoxicity [78].

Rejinold et al. studied the behaviour of Cs-*g*-PVCL nanoparticles as 5-fluorouracil carrier for its delivery to cancer cells. The copolymer showed a temperature-induced phase transition in the range of 38–45 °C in aqueous solutions, and its NPs display nearly spherical shape with an average diameter of 150 nm, which increased up to 180–200 nm when loaded with the drug. According to the drug delivery, cytotoxicity, in vitro cell uptake and apoptosis studies, these NPs could be a promising candidate for cancer drug delivery [80].

Recently, several studies relative to Cs-*g*-PVCL nanoparticles loaded with curcumin (Cs-*g*-PVCL-CRC-NPs) have been investigated as a potential anticancer drug delivery carrier system [81–84]. These NPs exhibit similar morphology to those in Ref. [80]. The rate of drug release was dependent on pH and temperature of the medium and showed an increase in delivery at temperatures above LCST in acidic pH conditions. These NPs exhibited specific toxicity to cancer cells at above their LCST [84].

In other articles, this group also describes Cs-*g*-PVCL-CRC-NPs in combination with metallic nanoparticles to assess their feasibility as a potential system for radio frequency (RF)-assisted cancer therapy [81–83]. With this purpose, gold nanoparticles (Au-NPs) were incorporated in Cs-*g*-PVCL-CRC-NPs in order to induce RF-assisted heating. These NPs showed uniform spherical shape, with particle size around 170 nm and ζ-potential of +18 mV. These NPs proved to be beneficial for combined RF therapy for treating breast and colon cancers [81, 83].

In a recent study, iron oxide nanoparticles (Fe_3O_4 NPs) were also incorporated to Cs-*g*-PVCL-CRC-NPs. Fe_3O_4 NPs require lower-background RF heating than the Au-NPs. In this report, 80 W for 2 min was required to heat the system up to 42 °C, and curcumin was controlled and released in cancer cells. These nanoparticles showed cellular internalization on array of the cancer cells, which decrease in cell viability and increase in cellular apoptosis [82].

Indulekha et al. documented the behaviour of a thermoresponsive polymeric gel based on Cs-*g*-PVCL as an on-demand transdermal drug delivery carrier for pain management. In this article, the delivery behaviour was analyzed by loading acetamidophenol (a model hydrophilic drug) and etoricoxib (a model hydrophobic drug). This material showed a pulsatile drug release (ON-OFF mechanism), giving an enhanced release for both drugs at temperature above LCST and pH 5.5. Histopathological results proved that the gel is biocompatible [79].

3.3. Other chitosan derivatives

Selective modification of chitosan via *N*-acylation with carboxylic anhydrides in dilute acetic acid/methanol under mild conditions has been documented by Hirano et al. [98–100]. These gels are colourless, transparent, rigid and stable on heating. Among them, the gel formation during the *N*-isobutyrylation of chitosan has been investigated in our laboratory [101]. *N*-Isobutyryl chitosan has structural and steric similarities with NIPAm.

Even when it is known that *N*-acylation leads to irreversible gel formation, *N*-isobutyryl chitosan behaves as a thermally sensitive hydrogel material similar to other thermally synthetic systems such as PNIPA. Indeed, after the derivatization reaction, the gel was thoroughly washed with water at 60 °C in order to get rid of methanol, acetic acid and any excess of reactant. When this hydrogel was submitted to cyclic stepwise changes in temperature, there is a pulsatile reversible response of the viscoelastic moduli, which persisted after four cycles [101].

Lastly, it should be mentioned that other chitosan-*g*-poly(ethylene glycol) thermoreversible copolymers have been also recently proposed [102–104], which are potentially suitable in biomedicine, especially for drug release and tissue engineering applications.

4. Concluding remarks

In this chapter, a review about the methods of synthesis of chitosan thermosensitive derivatives is presented, as well as their featured properties and key potential applications.

When adding glycerophosphate to chitosan solutions at room temperature, the polymer remains in solution at neutral pH, while a gelation can be triggered upon heating around the physiological temperature. This thermo-gelation process involves several interactions such as screening of electrostatic repulsion, "ionic" cross-linking, hydrophobic effect and hydrogen-bonding interactions.

Some chitosan derivatives have also been extensively investigated. Among them, chitosan-*grafted*-poly(*N*-isopropylacrylamide) and poly(*N*-vinylcaprolactam) are two well-recognized chitosan-based thermosensitive materials (**Figure 5a** and **b**, respectively). Other derivatives such as chitosan-*g*-poly(ethylene glycol) and *N*-isobutyryl chitosan also display thermoreversible behaviour. These polymer materials exhibit a lower critical solution temperature behaviour. Below the LCST, polymer chains are soluble, but when temperature increases, they experience a hydrophilic-to-hydrophobic phase transition. In all cases, hydrophobic interactions play a key role, which are associated to a dehydration process at the critical temperature. Thus, the phase transition produces a loss of conformational entropy due to the aggregation of grafted chain segments in an extended polymer network. This phenomenon is counterbalanced by the translational-entropy gain when water molecules are expelled out from the excluded volume of the copolymers during phase separation.

Due to the polyelectrolyte nature of chitosan, all these materials are sensitive to changes in both temperature and pH. A great variety of thermosensitive materials have been produced,

such as nanostructures, micelles, membranes, hydrogels, cryogels and paramagnetic beads, to cite some of them. These characteristics make them susceptible to be used in advanced and exciting technological applications such as sensors, actuators, controllable membrane for separations and in biomedical and biotechnological fields including drug delivery and tissue engineering (**Figure 5**).

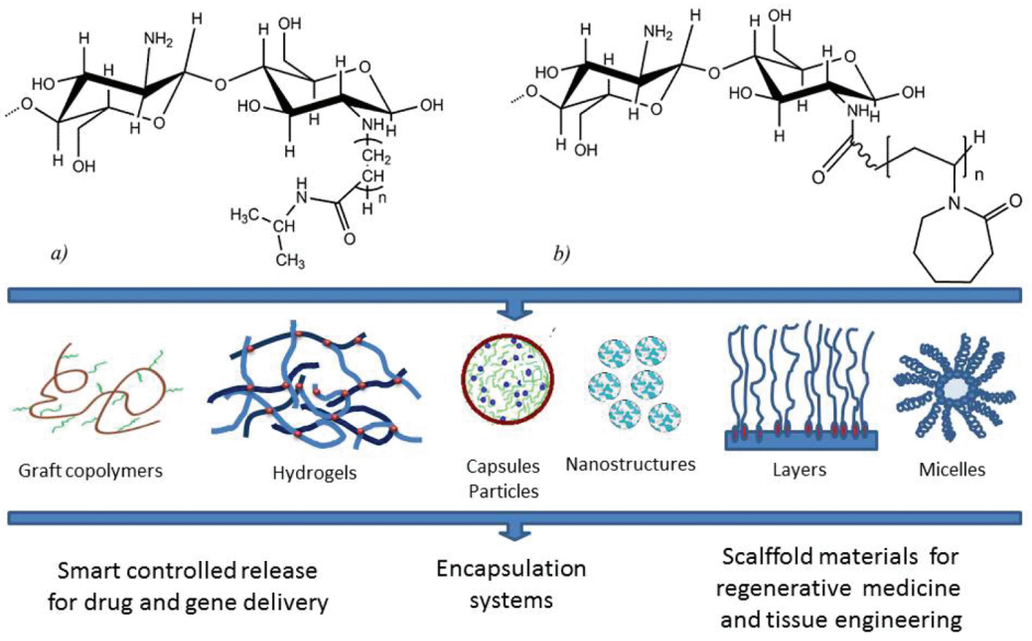

Figure 5. Chemical structure of (*a*) chitosan-*g*-PNIPAm, (*b*) chitosan-*g*-PVCL copolymers and some of their key applications.

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References

[1] Igor Galaev, Bo Mattiasson, editors. Smart Polymers: Applications in Biotechnology and Biomedicine. Second edition. Boca Raton, FL: CRC Press, Taylor & Francis Group; 2008.

[2] Peniche C, Argüelles-Monal W, Goycoolea FM. Chitin and chitosan: major sources, properties and applications. In: Gandini A, Belgacem M, editors. Monomers Polym. Compos. Renew. Resour., Amsterdam: Elsevier; 2008, p. 517–42.

[3] Chenite A, Chaput C, Wang D, Combes C, Buschmann MD, Hoemann CD, Leroux JC, Atkinson BL, Binette F, Selmani A. Novel injectable neutral solutions of chitosan form biodegradable gels in situ. Biomaterials 2000;21:2155–61.

[4] Ruel-Gariépy E, Chenite A, Chaput C, Guirguis S, Leroux J-C. Characterization of thermosensitive chitosan gels for the sustained delivery of drugs. Int J Pharm 2000;203:89–98.

[5] Cho J, Heuzey M-C, Bégin A, Carreau PJ. Physical gelation of chitosan in the presence of β-glycerophosphate: the effect of temperature. Biomacromolecules 2005;6:3267–75.

[6] Berger J, Reist M, Chenite A, Felt-Baeyens O, Mayer JM, Gurny R. Pseudo-thermosetting chitosan hydrogels for biomedical application. Int J Pharm 2005;288:17–25.

[7] Crompton KE, Prankerd RJ, Paganin DM, Scott TF, Horne MK, Finkelstein DI, Gross KA, Forsythe JS. Morphology and gelation of thermosensitive chitosan hydrogels. Biophys Chem 2005;117:47–53.

[8] Cho J, Heuzey M-C, Bégin A, Carreau PJ. Chitosan and glycerophosphate concentration dependence of solution behaviour and gel point using small amplitude oscillatory rheometry. Food Hydrocoll 2006;20:936–45.

[9] Cho J, Heuzey M-C. Dynamic scaling for gelation of a thermosensitive chitosan-β-glycerophosphate hydrogel. Colloid Polym Sci 2008;286:427–34.

[10] Supper S, Anton N, Seidel N, Riemenschnitter M, Schoch C, Vandamme T. Rheological study of chitosan/polyol-phosphate systems: influence of the polyol part on the thermo-induced gelation mechanism. Langmuir 2013;29:10229–37.

[11] Qiu X, Yang Y, Wang L, Lu S, Shao Z, Chen X. Synergistic interactions during thermosensitive chitosan-β-glycerophosphate hydrogel formation. RSC Adv 2011;1:282–9.

[12] Jarry C, Chaput C, Chenite A, Renaud M, Buschmann M, Leroux J. Effects of steam sterilization on thermogelling chitosan-based gels. J Biomed Mater Res 2001;58:127–35.

[13] Jarry C, Leroux J-C, Haeck J, Chaput C. Irradiating or autoclaving chitosan/polyol solutions: effect on thermogelling chitosan-β-glycerophosphate systems. Chem Pharm Bull (Tokyo) 2002;50:1335–40.

[14] Ahmadi R, Burns AJ, de Bruijn JD. Chitosan-based hydrogels do not induce angiogenesis. J Tissue Eng Regen Med 2010;4:309–15.

[15] Molinaro G, Leroux J-C, Damas J, Adam A. Biocompatibility of thermosensitive chitosan-based hydrogels: an in vivo experimental approach to injectable biomaterials. Biomaterials 2002;23:2717–22.

[16] Ahmadi R, de Bruijn JD. Biocompatibility and gelation of chitosan–glycerol phosphate hydrogels. J Biomed Mater Res A 2008;86A:824–32.

[17] Zhou HY, Jiang LJ, Cao PP, Li JB, Chen XG. Glycerophosphate-based chitosan thermosensitive hydrogels and their biomedical applications. Carbohydr Polym 2015;117:524–36.

[18] Hoemann CD, Sun J, Légaré A, McKee MD, Buschmann MD. Tissue engineering of cartilage using an injectable and adhesive chitosan-based cell-delivery vehicle. Osteoarthritis Cartilage 2005;13:318–29.

[19] Crompton KE, Goud JD, Bellamkonda RV, Gengenbach TR, Finkelstein DI, Horne MK, Forsythe JS. Polylysine-functionalised thermoresponsive chitosan hydrogel for neural tissue engineering. Biomaterials 2007;28:441–9.

[20] Cheng Y-H, Yang S-H, Su W-Y, Chen Y-C, Yang K-C, Cheng WT-K, Wu S-C, Lin F-H. Thermosensitive chitosan–gelatin–glycerol phosphate hydrogels as a cell carrier for nucleus pulposus regeneration: an in vitro study. Tissue Eng Part A 2009;16:695–703.

[21] Nair S, Remya NS, Remya S, Nair PD. A biodegradable in situ injectable hydrogel based on chitosan and oxidized hyaluronic acid for tissue engineering applications. Carbohydr Polym 2011;85:838–44.

[22] Dessì M, Borzacchiello A, Mohamed THA, Abdel-Fattah WI, Ambrosio L. Novel biomimetic thermosensitive β-tricalcium phosphate/chitosan-based hydrogels for bone tissue engineering. J Biomed Mater Res A 2013;101:2984–93.

[23] Niranjan R, Koushik C, Saravanan S, Moorthi A, Vairamani M, Selvamurugan N. A novel injectable temperature-sensitive zinc doped chitosan/β-glycerophosphate hydrogel for bone tissue engineering. Int J Biol Macromol 2013;54:24–9.

[24] Rahmati M, Samadikuchaksaraei A, Mozafari M. Insight into the interactive effects of β-glycerophosphate molecules on thermosensitive chitosan-based hydrogels. Bioinspir Biomim Nanobiomater 2016;5:67–73.

[25] Iliescu M, Hoemann CD, Shive MS, Chenite A, Buschmann MD. Ultrastructure of hybrid chitosan–glycerol phosphate blood clots by environmental scanning electron microscopy. Microsc Res Tech 2008;71:236–47.

[26] Cui J, Jiang B, Liang J, Sun C, Lan J, Sun X, Huang H, Sun K, Xu X. Preparation and characterization of chitosan/β-GP membranes for guided bone regeneration. J Wuhan Univ Technol Mater Sci Ed 2011;26:241–5.

[27] Ruel-Gariépy E, Leclair G, Hildgen P, Gupta A, Leroux J-C. Thermosensitive chitosan-based hydrogel containing liposomes for the delivery of hydrophilic molecules. J Control Release 2002;82:373–83.

[28] Ruel-Gariépy E, Shive M, Bichara A, Berrada M, Le Garrec D, Chenite A, Leroux J-C. A thermosensitive chitosan-based hydrogel for the local delivery of paclitaxel. Eur J Pharm Biopharm 2004;57:53–63.

[29] Zan J, Chen H, Jiang G, Lin Y, Ding F. Preparation and properties of crosslinked chitosan thermosensitive hydrogel for injectable drug delivery systems. J Appl Polym Sci 2006;101:1892–8.

[30] Cheng Y-H, Yang S-H, Lin F-H. Thermosensitive chitosan-gelatin-glycerol phosphate hydrogel as a controlled release system of ferulic acid for nucleus pulposus regeneration. Biomaterials 2011;32:6953–61.

[31] Lee S, Saito K, Lee H-R, Lee MJ, Shibasaki Y, Oishi Y, Kim B-S. Hyperbranched double hydrophilic block copolymer micelles of poly(ethylene oxide) and polyglycerol for ph-responsive drug delivery. Biomacromolecules 2012;13:1190–6.

[32] Khodaverdi E, Tafaghodi M, Ganji F, Abnoos K, Naghizadeh H. In vitro insulin release from thermosensitive chitosan hydrogel. AAPS PharmSciTech 2012;13:460–6.

[33] Liu W-F, Kang C-Z, Kong M, Li Y, Su J, Yi A, Cheng X-J, Chen X-G. Controlled release behaviors of chitosan/α, β-glycerophosphate thermo-sensitive hydrogels. Front Mater Sci 2012;6:250–8.

[34] Depani BP, Naik AA, Nair HA. Preparation and evaluation of chitosan based thermoreversible gels for intraperitoneal delivery of 5-fluorouracil (5-FU). Acta Pharm 2013;63:479–491.

[35] Kushwaha SKS, Rai AK, Singh S. Formulation of thermosensitive hydrogel containing cyclodextrin for controlled drug delivery of camptothecin. Trop J Pharm Res 2014;13:1007–12.

[36] Supper S, Anton N, Seidel N, Riemenschnitter M, Curdy C, Vandamme T. Thermosensitive chitosan/glycerophosphate-based hydrogel and its derivatives in pharmaceutical and biomedical applications. Expert Opin Drug Deliv 2014;11:249–67.

[37] Faikrua A, Wittaya-areekul S, Oonkhanond B, Viyoch J. A thermosensitive chitosan/corn starch/β-glycerol phosphate hydrogel containing TGF-β1 promotes differentiation of MSCs into chondrocyte-like cells. Tissue Eng Regen Med 2014;11:355–61.

[38] Wu J, Su Z-G, Ma G-H. A thermo- and pH-sensitive hydrogel composed of quaternized chitosan/glycerophosphate. Int J Pharm 2006;315:1–11.

[39] Tahrir FG, Ganji F, Ahooyi T. Injectable thermosensitive chitosan/glycerophosphate-based hydrogels for tissue engineering and drug delivery applications: a review. Recent Pat Drug Deliv Formul 2015;9:107–20.

[40] Tsai M-L, Chang H-W, Yu H-C, Lin Y-S, Tsai Y-D. Effect of chitosan characteristics and solution conditions on gelation temperatures of chitosan/2-glycerophosphate/nanosilver hydrogels. Carbohydr Polym 2011;84:1337–43.

[41] Chang H-W, Lin Y-S, Tsai Y-D, Tsai M-L. Effects of chitosan characteristics on the physicochemical properties, antibacterial activity, and cytotoxicity of chitosan/2-glycerophosphate/nanosilver hydrogels. J Appl Polym Sci 2013;127:169–76.

[42] Lizardi-Mendoza J, Argüelles Monal WM, Goycoolea Valencia FM. Chemical characteristics and functional properties of chitosan. In: Silvia Bautista-Baños, Gianfranco Romanazzi, Antonio Jiménez-Aparicio, editors. Chitosan Preserv. Agric. Commod., San Diego: Academic Press; 2016, p. 3–31.

[43] Odian G. Principles of Polymerization. Hoboken, New Jersey: John Wiley & Sons; 2004.

[44] Zhang M, Müller AHE. Cylindrical polymer brushes. J Polym Sci Part Polym Chem 2005;43:3461–81.

[45] Jenkins DW, Hudson SM. Review of vinyl graft copolymerization featuring recent advances toward controlled radical-based reactions and illustrated with chitin/chitosan trunk polymers. Chem Rev 2001;101:3245–74.

[46] Cao Y, Zhang C, Shen W, Cheng Z, Yu L, Ping Q. Poly(N-isopropylacrylamide)–chitosan as thermosensitive in situ gel-forming system for ocular drug delivery. J Control Release 2007;120:186–94.

[47] Wang L-Q, Tu K, Li Y, Fu J, Yu F. Micellization behavior of temperature-responsive poly (N-isopropylacrylamide) grafted dextran copolymers. J Mater Sci Lett 2002;21:1453–5.

[48] Lee C-F, Wen C-J, Chiu W-Y. Synthesis of poly(chitosan-N-isopropylacrylamide) complex particles with the method of soapless dispersion polymerization. J Polym Sci Part Polym Chem 2003;41:2053–63.

[49] Nie P, He X, Chen L. Temperature-sensitive chitosan membranes as a substrate for cell adhesion and cell sheet detachment. Polym Adv Technol 2012;23:447–53.

[50] Liu S, Zhang J, Cui X, Guo Y, Zhang X, Hongyan W. Synthesis of chitosan-based nanohydrogels for loading and release of 5-fluorouracil. Colloids Surf Physicochem Eng Asp 2016;490:91–7.

[51] Kim SY, Cho SM, Lee YM, Kim SJ. Thermo- and pH-responsive behaviors of graft copolymer and blend based on chitosan and N-isopropylacrylamide. J Appl Polym Sci 2000;78:1381–91.

[52] Recillas M, Silva LL, Peniche C, Goycoolea FM, Rinaudo M, Argüelles-Monal WM. Thermoresponsive behavior of chitosan-g-N-isopropylacrylamide copolymer solutions. Biomacromolecules 2009;10:1633–41.

[53] Mu B, Liu P, Li X, Du P, Dong Y, Wang Y. Fabrication of flocculation-resistant ph/ionic strength/temperature multiresponsive hollow microspheres and their controlled release. Mol Pharm 2012;9:91–101.

[54] Raskin MM, Schlachet I, Sosnik A. Mucoadhesive nanogels by ionotropic crosslinking of chitosan-g-oligo(NiPAam) polymeric micelles as novel drug nanocarriers. Nanomed 2016;11:217–33.

[55] Sanoj Rejinold N, Sreerekha PR, Chennazhi KP, Nair SV, Jayakumar R. Biocompatible, biodegradable and thermo-sensitive chitosan-g-poly (N-isopropylacrylamide) nanocarrier for curcumin drug delivery. Int J Biol Macromol 2011;49:161–72.

[56] Antoniraj MG, Kumar CS, Kandasamy R. Synthesis and characterization of poly (N-isopropylacrylamide)-g-carboxymethyl chitosan copolymer-based doxorubicin-loaded polymeric nanoparticles for thermoresponsive drug release. Colloid Polym Sci 2015;294:527–35.

[57] Bao H, Li L, Leong WC, Gan LH. Thermo-responsive association of chitosan-graft-poly(N-isopropylacrylamide) in aqueous solutions. J Phys Chem B 2010;114:10666–73.

[58] Bao H, Li L, Gan LH, Ping Y, Li J, Ravi P. Thermo- and pH-responsive association behavior of dual hydrophilic graft chitosan terpolymer synthesized via ATRP and click chemistry. Macromolecules 2010;43:5679–87.

[59] Chen C, Liu M, Gao C, Lü S, Chen J, Yu X, Ding E, Yu C, Guo J, Cui G. A convenient way to synthesize comb-shaped chitosan-graft-poly (N-isopropylacrylamide) copolymer. Carbohydr Polym 2013;92:621–8.

[60] Don T-M, Chen H-R. Synthesis and characterization of AB-crosslinked graft copolymers based on maleilated chitosan and N-isopropylacrylamide. Carbohydr Polym 2005;61:334–47.

[61] Don T-M, Chou S-C, Cheng L-P, Tai H-Y. Cellular compatibility of copolymer hydrogels based on site-selectively-modified chitosan with poly(N-isopropyl acrylamide). J Appl Polym Sci 2011;120:1–12.

[62] Bae JW, Go DH, Park KD, Lee SJ. Thermosensitive chitosan as an injectable carrier for local drug delivery. Macromol Res 2006;14:461–5.

[63] Recillas M, Silva LL, Peniche C, Goycoolea FM, Rinaudo M, Román JS, Argüelles-Monal WM. Thermo- and pH-responsive polyelectrolyte complex membranes from chitosan-g-N-isopropylacrylamide and pectin. Carbohydr Polym 2011;86:1336–43.

[64] Gui R, Wang Y, Sun J. Encapsulating magnetic and fluorescent mesoporous silica into thermosensitive chitosan microspheres for cell imaging and controlled drug release in vitro. Colloids Surf B Biointerfaces 2014;113:1–9.

[65] Jain E, Damania A, Shakya AK, Kumar A, Sarin SK, Kumar A. Fabrication of macroporous cryogels as potential hepatocyte carriers for bioartificial liver support. Colloids Surf B Biointerfaces 2015;136:761–71.

[66] Feil H, Bae YH, Feijen J, Kim SW. Effect of comonomer hydrophilicity and ionization on the lower critical solution temperature of N-isopropylacrylamide copolymers. Macromolecules 1993;26:2496–500.

[67] Jaiswal MK, Banerjee R, Pradhan P, Bahadur D. Thermal behavior of magnetically modalized poly(N-isopropylacrylamide)-chitosan based nanohydrogel. Colloids Surf B Biointerfaces 2010;81:185–94.

[68] Li G, Song S, Zhang T, Qi M, Liu J. pH-sensitive polyelectrolyte complex micelles assembled from CS-g-PNIPAM and ALG-g-P(NIPAM-co-NVP) for drug delivery. Int J Biol Macromol 2013;62:203–10.

[69] Wang Y, Xu H, Wang J, Ge L, Zhu J. Development of a thermally responsive nanogel based on chitosan-poly(N-isopropylacrylamide-co-acrylamide) for paclitaxel delivery. J Pharm Sci 2014;103:2012–21.

[70] Liu W, Huang Y, Liu H, Hu Y. Composite structure of temperature sensitive chitosan microgel and anomalous behavior in alcohol solutions. J Colloid Interface Sci 2007;313:117–21.

[71] Yang Y, Zeng F, Tong Z, Liu X, Wu S. Phase separation in poly(N-isopropyl acrylamide)/water solutions. II. Salt effects on cloud-point curves and gelation. J Polym Sci Part B Polym Phys 2001;39:901–7.

[72] Eeckman F, Amighi K, Moës AJ. Effect of some physiological and non-physiological compounds on the phase transition temperature of thermoresponsive polymers intended for oral controlled-drug delivery. Int J Pharm 2001;222:259–70.

[73] Li G, Guo L, Chang X, Yang M. Thermo-sensitive chitosan based semi-IPN hydrogels for high loading and sustained release of anionic drugs. Int J Biol Macromol 2012;50:899–904.

[74] Lee C-F, Wen C-J, Lin C-L, Chiu W-Y. Morphology and temperature responsiveness-swelling relationship of poly(N-isopropylamide-chitosan) copolymers and their application to drug release. J Polym Sci Part Polym Chem 2004;42:3029–37.

[75] Gui R, Wang Y, Sun J. Embedding fluorescent mesoporous silica nanoparticles into biocompatible nanogels for tumor cell imaging and thermo/pH-sensitive in vitro drug release. Colloids Surf B Biointerfaces 2014;116:518–25.

[76] Kudyshkin VO, Milusheva RY, Futoryanskaya AM, Yunusov MY, Rashidova SS. Synthesis of graft copolymers of N-vinylcaprolactam on chitosan. Russ J Appl Chem 2007;80:1750–2.

[77] Kholmuminov AA, Kudyshkin VO, Futoryanskaya AM, Avazova OB, Milusheva RY, Rashidova SS. Rheological properties of solutions of chitosan and its graft copolymer with N-vinylcaprolactam. Polym Sci Ser A 2010;52:939–41.

[78] Prabaharan M, Grailer JJ, Steeber DA, Gong S. Stimuli-responsive chitosan-graft-poly(N-vinylcaprolactam) as a promising material for controlled hydrophobic drug delivery. Macromol Biosci 2008;8:843–51.

[79] Indulekha S, Arunkumar P, Bahadur D, Srivastava R. Thermoresponsive polymeric gel as an on-demand transdermal drug delivery system for pain management. Mater Sci Eng C 2016;62:113–22.

[80] Sanoj Rejinold N, Chennazhi KP, Nair SV, Tamura H, Jayakumar R. Biodegradable and thermo-sensitive chitosan-g-poly(N-vinylcaprolactam) nanoparticles as a 5-fluorouracil carrier. Carbohydr Polym 2011;83:776–86.

[81] Sanoj Rejinold N, Thomas RG, Muthiah M, Chennazhi KP, Manzoor K, Park I-K, Jeong YY, Jayakumar R. Anti-cancer, pharmacokinetics and tumor localization studies of pH-, RF- and thermo-responsive nanoparticles. Int J Biol Macromol 2015;74:249–62.

[82] Sanoj Rejinold N, Thomas RG, Muthiah M, Lee HJ, Jeong YY, Park I-K, Jayakumar R. Breast tumor targetable Fe_3O_4 embedded thermo-responsive nanoparticles for radio-frequency assisted drug delivery. J Biomed Nanotechnol 2016;12:43–55.

[83] Sanoj Rejinold N, Thomas RG, Muthiah M, Chennazhi KP, Park I-K, Jeong YY, Manzoor K, Jayakumar R. Radio frequency triggered curcumin delivery from thermo and pH responsive nanoparticles containing gold nanoparticles and its in vivo localization studies in an orthotopic breast tumor model. RSC Adv 2014;4:39408–27.

[84] Sanoj Rejinold N, Muthunarayanan M, Divyarani VV, Sreerekha PR, Chennazhi KP, Nair SV, Tamura H, Jayakumar R. Curcumin-loaded biocompatible thermoresponsive polymeric nanoparticles for cancer drug delivery. J Colloid Interface Sci 2011;360:39–51.

[85] Fernández-Quiroz D, González-Gómez Á, Lizardi-Mendoza J, Vázquez-Lasa B, Goycoolea FM, San Román J, Argüelles-Monal WM. Effect of the molecular architecture on the thermosensitive properties of chitosan-g-poly(N-vinylcaprolactam). Carbohydr Polym 2015;134:92–101.

[86] Reyes-Ortega F, Parra-Ruiz FJ, Averick SE, Rodríguez G, Aguilar MR, Matyjaszewski K, San Román J. Smart heparin-based bioconjugates synthesized by a combination of ATRP and click chemistry. Polym Chem 2013;4:2800–14.

[87] Farkaš P, Bystrický S. Efficient activation of carboxyl polysaccharides for the preparation of conjugates. Carbohydr Polym 2007;68:187–90.

[88] Kunishima M, Kawachi C, Monta J, Terao K, Iwasaki F, Tani S. 4-(4,6-Dimethoxy-1,3,5-triazin-2-yl)-4-methyl-morpholinium chloride: an efficient condensing agent leading to the formation of amides and esters. Tetrahedron 1999;55:13159–70.

[89] Kunishima M, Kawachi C, Hioki K, Terao K, Tani S. Formation of carboxamides by direct condensation of carboxylic acids and amines in alcohols using a new alcohol- and water-soluble condensing agent: DMT-MM. Tetrahedron 2001;57:1551–8.

[90] Montes JÁ, Ortega A, Burillo G. Dual-stimuli responsive copolymers based on N-vinylcaprolactam/chitosan. J Radioanal Nucl Chem 2014;303:2143–50.

[91] Wang J-P, Chen Y-Z, Ge X-W, Yu H-Q. Gamma radiation-induced grafting of a cationic monomer onto chitosan as a flocculant. Chemosphere 2007;66:1752–7.

[92] Choi W-S, Ahn K-J, Lee D-W, Byun M-W, Park H-J. Preparation of chitosan oligomers by irradiation. Polym Degrad Stab 2002;78:533–8.

[93] Hien NQ, Phu DV, Duy NN, Lan NTK. Degradation of chitosan in solution by gamma irradiation in the presence of hydrogen peroxide. Carbohydr Polym 2012;87:935–8.

[94] Imaz A, Forcada J. N-vinylcaprolactam-based microgels for biomedical applications. J Polym Sci Part Polym Chem 2010;48:1173–81.

[95] Jayakumar R, Prabaharan M, Reis RL, Mano JF. Graft copolymerized chitosan—present status and applications. Carbohydr Polym 2005;62:142–58.

[96] Fernández-Quiroz D, González-Gómez Á, Lizardi-Mendoza J, Vázquez-Lasa B, Goycoolea FM, Román JS, Argüelles-Monal WM. Conformational study on the thermal transition of chitosan-g-poly(N-vinylcaprolactam) in aqueous solution. Colloid Polym Sci 2016;294:555–63.

[97] Pérez-Calixto MP, Ortega A, Garcia-Uriostegui L, Burillo G. Synthesis and characterization of N-vinylcaprolactam/N,N-dimethylacrylamide grafted onto chitosan networks by gamma radiation. Radiat Phys Chem 2016;119:228–35.

[98] Hirano S, Ohe Y. Chitosan gels: a novel molecular aggregation of chitosan in acidic solutions on a facile acylation. Agric Biol Chem 1975;39:1337–8.

[99] Hirano S, Yamaguchi R. N-acetylchitosan gel: a polyhydrate of chitin. Biopolymers 1976;15:1685–91.

[100] Hirano S, Ohe Y, Ono H. Selective N-acylation of chitosan. Carbohydr Res 1976;47:315–20.

[101] Félix L, Hernández J, Argüelles-Monal WM, Goycoolea FM. Kinetics of gelation and thermal sensitivity of N-isobutyryl chitosan hydrogels. Biomacromolecules 2005;6:2408–15.

[102] Liang Y, Deng L, Chen C, Zhang J, Zhou R, Li X, Hu R, Dong A. Preparation and properties of thermoreversible hydrogels based on methoxy poly(ethylene glycol)-grafted chitosan nanoparticles for drug delivery systems. Carbohydr Polym 2011;83:1828–33.

[103] Bhattarai N, Matsen FA, Zhang M. PEG-grafted chitosan as an injectable thermoreversible hydrogel. Macromol Biosci 2005;5:107–11.

[104] Bhattarai N, Ramay HR, Gunn J, Matsen FA, Zhang M. PEG-grafted chitosan as an injectable thermosensitive hydrogel for sustained protein release. J Control Release 2005;103:609–24.

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