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Development of dual-sensitive smart polymers by grafting chitosan with poly (*N*-isopropylacrylamide): an overview

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Abstract

A great deal of research on polymers over the past two decades has been focused on the development of stimuli-responsive polymers to obtain materials able to respond to specific surroundings. In this paper, an overview is presented of the concepts, behavior and applicability of these "smart polymers". Polymers that are temperature- or pH-sensitive are discussed in detail, including the response mechanisms and types of macromolecules, because they are easy to handle and have a wide range of applications. Finally, the combination of pH and temperature responsive properties by means of graft copolymerization of chitosan with poly (*N*-isopropylacrylamide) (PNIPAM) was chosen to represent some synthetic routes and properties of dual-sensitive polymeric systems developed currently.

Keywords: *smart polymer, thermosensitive, pH-responsive, N-isopropylacrylamide, chitosan.*

1. Introduction

Recently, scientists all over the world have been attempting to synthesize polymers capable of mimic the stimuli-responsive property present in common biopolymers of living organisms, in order to reach scientific and industrial applications. Frequently named as smart polymers, those materials are able to undergo fast, abrupt and reversibly alteration in their structure/properties as a response to small changes in the environment[1-4]. This behavior can be employed in a wide range of applications, such as controlled drug delivery[5-8], chromatographic separation[9,10], water remediation[11-13], enhanced oil recovery[14,15], catalysis[16,17], sensors[18,19] and tissue engineering[20,21].

The stimuli can be classified as physical, such as temperature[22-24], electric/magnetic fields[25,26] and light[27]; chemical, such as pH[28,29] and ionic strength[30]; and biochemical, such as enzymes[31] and antigens[32]. These unique macromolecules are also known as intelligent polymers[33], responsive polymers[34], sensitive polymers[35], stimuli-sensitive polymers[36], stimuli-responsive polymers[37], environmentally sensitive polymers[38] and environmentally responsive polymers[39,40].

These materials have been designed in many forms, depending on the desired application. They can be polymers chains dissolved in solutions, chemically crosslinked hydrogels, physical gels, micelles and even chains immobilized or grafted onto solid surfaces[2,4,10]. Furthermore, regardless the physical form, the smart polymers can be conjugated with biomolecules or synthetic substances, and the activity of the conjugates is going to depend on the polymer-conjugate interactions and the response of the polymer to the stimulus applied[2,4].

In order to improve and increase their applicability, several smart polymers have been developed to combine two or more mechanisms of responsiveness in only one material[41,42]. A quite common approach involves preparing polymers sensible to both temperature and pH stimuli[43-55], since they are typical variables parameters in biological and chemical systems, and they can also be easily controlled *in vitro* and *in vivo* conditions[56-59].

The aim of this paper is to present the methods of obtainment and properties of smart polymers in a compact form. It is not intended to be a complete review and therefore the selection of cited literature is of some extent personal. Our presentation is focused mainly on copolymers of chitosan with poly (*N*-isopropylacrylamide) (PNIPAM), due to the large number of studies found in the literature on these copolymers, but many properties are universal and can also be applied to other pH and temperature sensitive polymers.

2. Thermosensitive Polymers

Temperature is one of the most interesting properties investigated in responsive polymer systems[2,24,59-65]. The thermosensitive polymers are well-known by possessing a large alteration in their structure as a response to slight changes in temperature. When a polymer is dissolved in an appropriate solvent, it may become insoluble upon increase or decrease in temperature and, thus, precipitate from the solution[66]. Solubility of most polymers increases with increasing temperature[59,63]. When phase separation happens with decreasing temperature, polymer system presents an *upper critical solution temperature* (UCST)[67]. However, some polymers exhibit a peculiar behavior, in which a phase separation occurs with rising temperature. The temperature in which this occurs is called *lower critical solution temperature* (LCST)[4,10,59]. The LCST behavior in water has attracted much attention due to the great applicability[66], such as in wastewater treatment[68], chromatographic separation[69], enzyme immobilization[70] and tissue engineering[71].

From a thermodynamic point of view, the solubilization at low temperatures occurs due to the polymer-solvent hydrogen bonding that leads to a negative enthalpy of mixing. On the other hand, even with a moderate gain in compositional entropy as a consequence of the mixing process, the entropy of organization required to achieve this polymer-solvent hydrogen bonding is unfavorable (negative entropy). Thus, the free energy of dissolution, ΔG, given by $\Delta H - T\Delta S$, can change from negative (solution) to positive (phase transition) as the temperature is increased[59,66,72].

The *N*-alkyl-substituted polyacrylamides are an especially relevant family of thermosensitive polymers, as seen by a huge numbers of publications. Interestingly, the LCST in this group of polymers varies according to the type of *N*-substituted groups, ranging from materials insoluble at room temperature to other with high LCST[65]. They include poly (*N*-isopropylacrylamide)[2,56,62,65,73-75], poly (*N,N*-diethylacrylamide) [2,56,59,62], poly (2-carboxyisopropylacrylamide), poly (*N*-(L)-(1-hydroxymethyl) propylmethacrylamide, poly (*N*-acryloyl-*N'*-propilpiperazine)[2], poly (*N*-ethylacrylamide), poly (*N*-methyl-*N*-ethylacrylamide), poly (*N*-n-propylacrylamide)[56,65], poly (*N*-ethylmethacrylamide), poly (*N*-methyl-*N*-isopropylacrylamide), poly (*N*-isopropylmethacrylamide), poly (*N*-n-propylmethacrylamide), poly (*N*-methyl-*N*-isopropylacrylamide), poly (*N*-cyclopropylacrylamide), poly (*N*-cyclopropymethacrylamide)[56], poly (*N,N*-bis(2-methoxyethyl) acrylamide), poly (*N*-(3-methoxypropyl) acrylamide), poly (ethoxypropylacrylamide)[65] and poly (aminomethoxypropylacrylamide)[76].

Thermosensitive polymers that do not contain acrylamide-based repeat units have also attracted significant attention, such as poly (*N*-vinylcaprolactam)[59,62,65], poly (2-ethyl-2-oxazoline)[65], poly (vinyl methyl ether)[59,65], poly (2-isopropyl-2-oxazoline), poly ((2-dimethylamino) ethyl methacrylate), poly (propylene oxide)[65] and poly (*N*-acryloylpiperidine)[56]. There are also polymers derived from natural sources with LCST behavior, such as methylcellulose, ethyl(hydroxyethyl)cellulose and hydroxypropylcellulose, that are of great interest for biomedical applications[65]. Some of thermoresponsive polymer structures are presented in Figure 1.

Despite the vast variety of thermoresponsive polymers, poly (*N*-isopropylacrylamide) (PNIPAM) is the most extensively studied one, as seen by the huge amount of publications dealing with this polymer. PNIPAM presents a LCST that lies between 30 and 35°C, depending on the precise microstructure of the macromolecule[77]. PNIPAM brings together an abrupt and reversible thermosensitive nature[78], with biocompatibility and LCST close to the human body[24], which turns it especially attractive in biomedical applications[79].

At temperatures below the LCST of PNIPAM, water-polymer interactions are dominant, leading to dissolution or swelling in water[78]. With rising temperature, the polymer-solvent hydrogen bonds are being disrupted, whereas polymer-polymer interactions are greatly increased, resulting in collapsed structures at the LCST[80,81]. Above the transition temperature, the globules are aggregated into a few PNIPAM chains at very dilute solutions, while higher polymer concentrations result in colloidal dispersions or even macroscopic precipitates[23,82,83].

Furthermore, as a consequence of LCST dependence on the balance between attractive polymer-polymer and polymer-solvent interactions[24,65], the transition temperature of PNIPAM can be adjusted by changing its molecular weight and concentration, by means of copolymerization of NIPAM with other comonomers, as well as through and addition of salts, surfactants and co-solvents[24,62,65,82-92].

3. pH-sensitive Polymers

pH-sensitive polymers experience abrupt alterations in their polymer-polymer and polymer-solvent interactions in response to small variations in the environmental pH[61]. This behavior is attributed to the presence of pendant weak basic or acid groups in the polymeric chains, that either accept or release protons, respectively, as a result of slight changes in the pH of the medium[62]. This occurs because the degree of ionization of weak acids or bases is highly modified by changing the pH around their pK_a value. Then, a large alteration in the hydrodynamic volume of the polymeric chains takes place, as a result of the quick variation in the charges of the pendant groups[2,63].

There are many polymers responsive to the environmental pH (Figure 2), including synthetic ones, such as poly (acrylic acid), poly (2-ethyl acrylic acid), poly (*N,N*-diethyl aminoethyl methacrylate) and poly (vinyl imidazole)[2], and those from natural sources, such as alginate[88,89], carboxymethylcellulose[90,91] and chitosan[92-97]. The pH-responsive polymers can also be classified as: (i) polyacids, which contain pendant weak acid groups, such as -COOH and $-SO_3H$; (ii) polybases, that possess pendant weak basic groups ($-NH_2$) in their chains[2,10]; or (iii) polyamphoterics, that bear both weak acid and basic groups[62].

When applying pH-responsive polymers, the polysaccharides are preferred in many applications, because they join pH-sensitiveness with inherent biological properties. One of the most outstanding pH-sensitive biopolymer is chitosan[98,99], a polysaccharide which naturally occurs in certain fungi[100], but is extensively obtained by the deacetylation of chitin[58,101,102], which is extracted from the shells of crustaceans, from the exoskeleton of many arthropods and from some fungi[103]. Despite the massive annual production and easy availability, due to its poor solubility in almost all common solvents, chitin does not find practical applications, except for being a source for obtaining chitosan[102-105].

Chitosan is a linear copolymer composed of two repeating units *i.e. N*-acetyl-2-amino-2-D-glucopyranose and 2-amino-2-deoxy-D-glucopyranose, linked by β-(1→4)-glycosidic bonds. Generally, when the content of 2-amino-2-deoxy-D-glucopyranose (degree of deacetylation – DD) in the polysaccharide chain is higher than 50 %, it becomes soluble in an aqueous acidic medium, as a result of the protonation of its amino groups and, in these conditions, it is named chitosan[106,107]. As a polysaccharide, chitosan exhibits attractive properties such as biocompatibility and biodegradability. Also, its degradation products are non-toxic, non-immunogenic and non-carcinogenic[62,106].

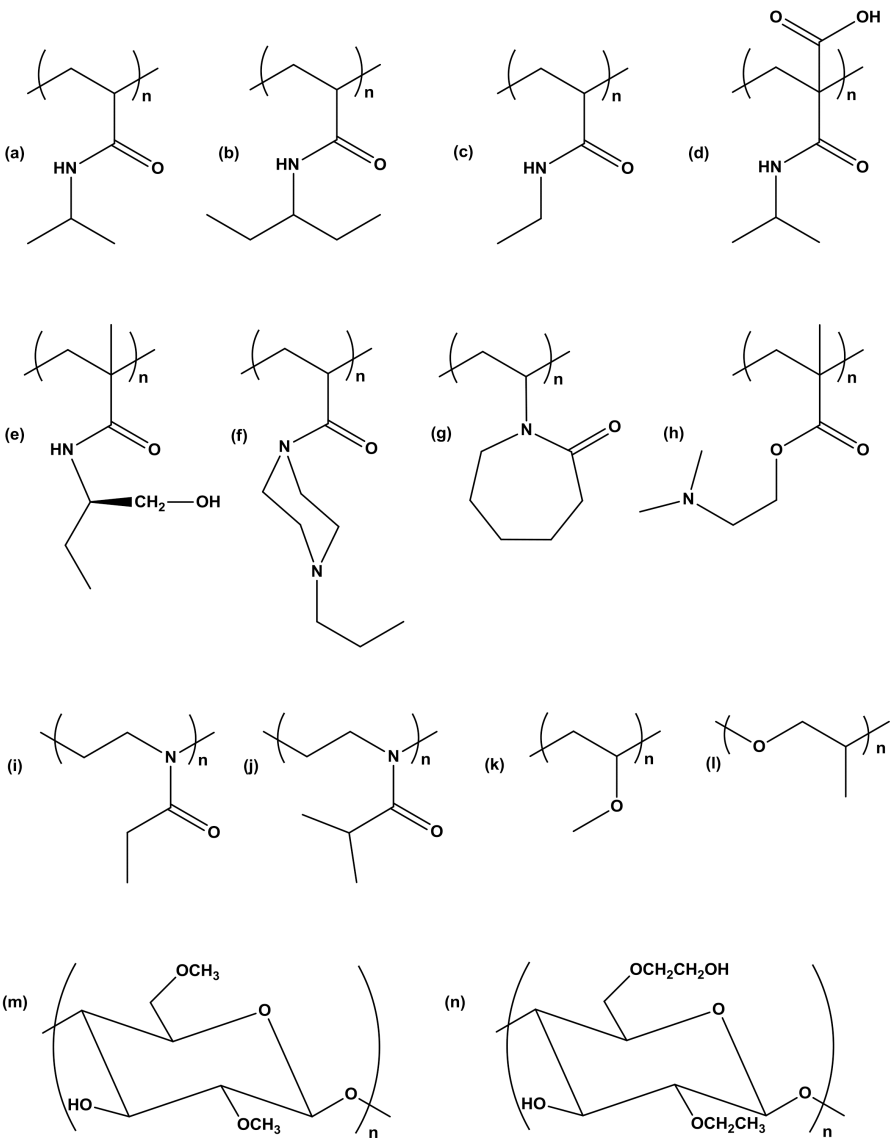

Figure 1. Chemical structure of some thermosensitive polymers: (a) poly (*N*-isopropylacrylamide); (b) poly (*N,N*-diethylacrylamide); (c) poly (*N*-ethylacrylamide); (d) poly (2-carboxyisopropylacrylamide); (e) poly (*N*-(L)-(1-hydroxymethyl) propylmethacrylamide; (f) poly (*N*-acryloyl-*N'*-propilpiperazine); (g) poly (*N*-vinylcaprolactam); (h) poly ((2-dimethylamino)ethyl methacrylate); (i) poly (2-ethyl-2-oxazoline); (j) poly (2-isopropyl-2-oxazoline); (k) poly (vinyl methyl ether); (l) poly (propylene oxide); (m) methylcellulose; (n) ethyl(hydroxylethyl)cellulose.

The pH-responsive property of chitosan is a consequence of protonation–deprotonation equilibrium of its amino groups (pK_a around 6) in aqueous media[58,102,108]. Besides, the presence of -OH and -NH_2 reactive groups makes of this polysaccharide very attractive for chemical modifications[109,110].

4. Combination of pH and Thermosensitive Properties Through Graft Copolymerization of Chitosan with PNIPAM

Nowadays, grafting PNIPAM side chains on the chitosan backbone constitutes a crescent area of research, since it bonds the most studied thermosensitive polymer with the most outstanding cationic polysaccharide, to reach dual temperature and pH responsive materials with remarkable properties[33,111-114].

To achieve those dual-responsive copolymers, researchers have applied some strategies. One of them involves a coupling reaction between PNIPAM bearing a reactive end group and chitosan, by using a condensing agent[115]. These condensing agents can catalyze the formation of amide bonds between carboxylic acid group of a carboxyl-terminated PNIPAM and amine groups of chitosan. In these cases, an end-functionalized PNIPAM has to be prepared before the graft copolymerization[116,117]. For instance, Rejinold *et al.* (2011) prepared PNIPAAM-COOH by using azobisisobutyronitrile (AIBN) and 3-mercaptopropionic acid, in isopropyl alcohol, at 75 °C; then, they grafted

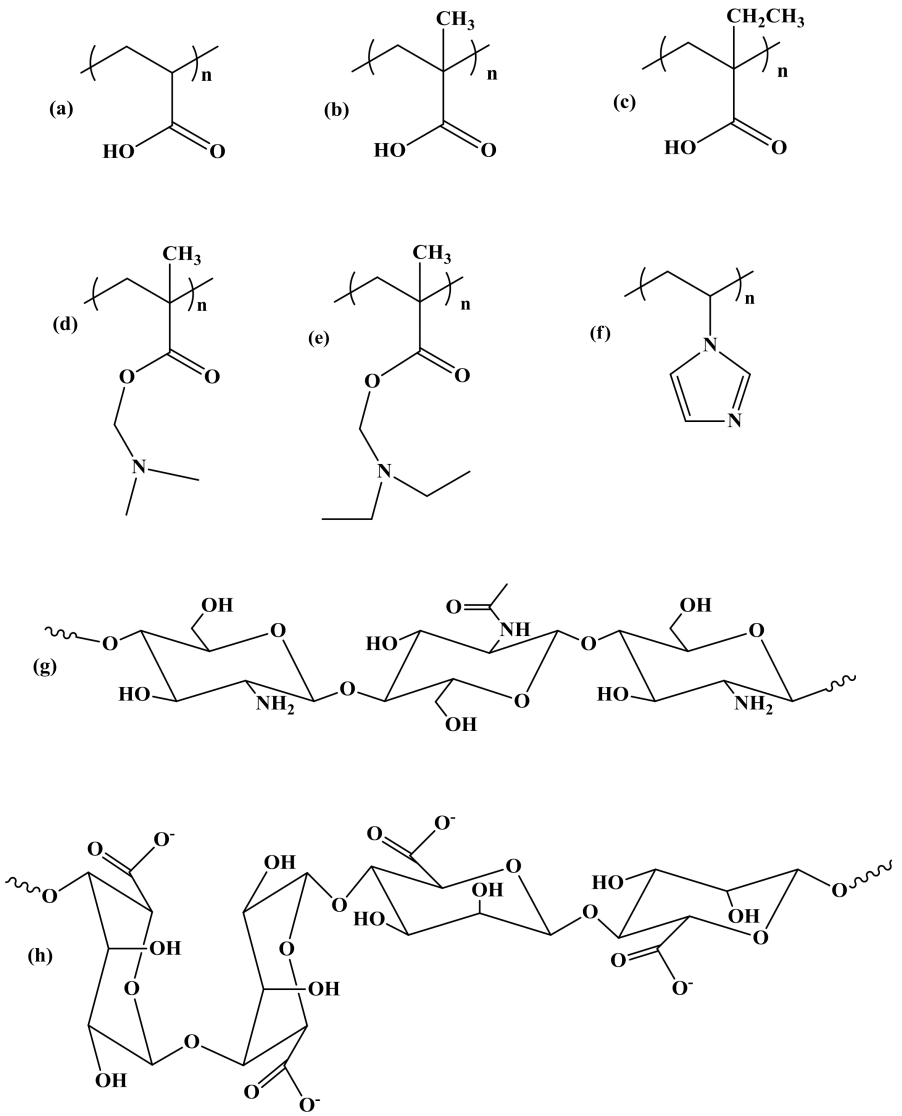

Figure 2. Chemical structure of some pH-responsive polymers: (a) poly (acrylic acid); (b) poly (methacrylic acid); (c) poly (2-ethyl acrylic acid); (d) poly (*N,N*-dimethyl aminoethyl methacrylate); (e) poly (*N,N*-diethyl aminoethyl methacrylate); (f) poly (vinyl imidazole); (g) chitosan; (h) alginate.

PNIPAAM-COOH chains onto chitosan backbone by using 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride/N-hydroxysuccinimide (EDC/NHS) as the condensing agents, in acid medium, at room temperature. The resulting nanoparticles were loaded with curcumin. The *in vitro* drug release was effective only above LCST, which was attributed to higher polymer-polymer interaction than polymer-drug interaction when phase transition was reached. The drug loaded nanoparticles also showed cell uptake, cytocompatibility and specific toxicity on cancer cells, indicating that theses sensitive materials could be effective nanovehicles for controlled curcumin delivery[118].

Some others research groups have been preparing poly (*N*-isopropylacrylamide)-*co*-poly (acrylic acid) (PNIPAM-*co*-PAA) copolymers to be further anchored onto chitosan backbone by coupling reactions[119,120]. Proceeding in this method, PNIPAM-*co*-PAA copolymers were prepared by the redox pair ammonium persulfate/ *N,N,N',N'*-tetramethylethylenediamine (APS/TEMED). Then, the PNIPAM-*co*-PAA side chains were bonded to chitosan by a coupling reaction, with the aid of EDC. The final copolymer was evaluated by means of its ability in removing phenol of aqueous solution. Phenol was oxidized by enzymatic reaction, aiming to produce compounds that could react with the amino groups of the chitosan derivative, forming Schiff bases or Michael-type adducts. Through heating and shaking the solution, the copolymer containing highly concentrated oxidized compounds deposited and agglutinated to a condensed coagulate. By increasing polymer concentration and chitosan content in the copolymer, the removal of phenol and its oxidized compounds was increased[119].

Core-shell nanoparticles, based on PNIPAM-*co*-PAA as core and chitosan as shell were designed, by means of previously copolymerization of *N*-isopropylacrylamide (NIPAM) with

acrylic acid (AA), using sulfate persulfate as initiator and *N,N*-methylenebisacrylamide (MBA) as crosslink agent, at 75 °C in water medium, followed by coupling reaction between PNIPAM-*co*-PAA with chitosan, using EDC as the condensing agent, at 25 °C in aqueous media. The particles size was reduced from 380 to 25 nm as the temperature of the medium increased. While PNIPAM-*co*-PAA did not present thermosensitivity, core-shell smart nanoparticles showed temperature responsiveness and might also be more biocompatible than PNIPAM-*co*-PAA itself due to polysaccharide shell[121].

Chitosan-*g*-PNIPAM smart copolymers have also been prepared through radiation-based methods[80,122-124]. Zhao and collaborators prepared pH and temperature sensitive smart hydrogels by exposing the mixture of allylated chitosan, NIPAM and the photoinitiator, 2,2-dimethoxy-2-phenylacetophenone (DMPA), in acid medium, to UV irradiation[125]. Swelling kinetics was dependent on pH, temperature and composition of the hydrogels. The *in vitro* release of the model drug methyl orange (MO) from the hydrogels was strongly pH dependent, being gradually released at pH 7.4 and rather low released in pH 2.0. This occurred due to the lack of ionic attractive interactions between MO and hydrogels at pH 7.4 and strong ionic attractive interactions between $–SO^{3-}$ groups of MO molecules and $–NH^{3+}$ groups of chitosan at acid medium[125].

Grafting vinyl monomers onto the polysaccharide backbone using free radical polymerization (FRP) is a very common route to obtain chitosan-*g*-poly (*N*-isopropylacrylamide) responsive copolymers. Ceric ammonium nitrate (CAN), 2,2'-azoisobutyronitrile (AIBN) and persulfates (XPS) are some of the most employed initiators, which can be thermally activated, or using a redox initiation system[58,115].

There are many proposals regarding the anchored points for grafted chains when free radicals are used as initiators. When using CAN, for instance, some authors have exhibited a mechanism in which the chitosan units are predominantly oxidized through C_2–C_3 bond cleavage induced by Ce^{+4} ions, producing free-radicals sites onto the polysaccharide[126,127]. Others researchers suggest that the graft copolymerization of chitosan in the presence of CAN occurs onto amino groups of chitosan[78,128,129]. Initiation by persulfate has been also presented as occurring at different sites of chitosan backbone[130-133].

Duan and collaborators prepared chitosan-*g*-PNIPAM nanogels *via* free radical copolymerization at 80 °C, using APS as initiator and MBA as a crosslink agent. They suggested a synthetic route in which sulfate anion radicals, produced by thermal homolytic cleavage of APS, interacted with the hydroxyl groups of the polysaccharide to form alkoxy radicals, which then initiated the graft copolymerization of *N*-isopropylacrylamide (NIPAM) onto the backbone with MBA as a crosslinking agent. The final nanogels were loaded with oridonin (ORI), a powerful anticancer agent in chinese traditional medicine. The *in vitro* tests, performed at 37 °C, demonstrated a much faster drug release at acid condition than in pH 7.4. ORI loaded nanogels also presented better anti-tumor activity under acid media, as showed by both MTT assay and cellular morphological analysis, indicating that these nanogels are good candidates for pH-sensitive drug release of hydrophobic anticancer drugs, such as ORI[134].

More recently, our group evaluated the stability and rheological behavior of suspensions of PNIPAM, chitosan-*g*-PNIPAM and chitosan-*g*-(PNIPAM-*co*-PAA) particles, which were prepared by using potassium persulfate (50 °C) as initiator and MBA as a crosslinking agent. Differences on particle-particle and particle-solvent attractive interactions were obtained by changing the composition of the particles and also pH and temperature environment, demonstrating that the particles stability can be adjusted depending on the desired application. The presence of chitosan onto the chemical network had particular importance on the particles behavior, as even at high pHs, in which chitosan is not protonated, the rigidity of polysaccharide chains helped to control stability of the particles[135].

Great attention has also been paid on controlled/living radical polymerization methods (CLRP), which include, mainly, atom transfer radical polymerization (ATRP), reversible addition fragmentation transfer (RAFT) and stable free radical polymerization (SFRP). The CLRP methods are based on a dynamic equilibrium between active species and dormant species, aiming to minimize the chance of termination reactions during the polymerization by decreasing the concentration of active species and, as a result, being able to produce polymers with precise architectures and compositions[58,136-138].

Chen and collaborators synthesized dual pH and temperature responsive chitosan-*g*-PNIPAM copolymers *via* ATRP. In order to protect the amino groups of chitosan, *N*-phthaloyl chitosan (PHCS) was firstly prepared, followed by the synthesis of the macroinitiator bromoisobutyryl-terminated *N*-phthaloyl chitosan (PHCS-Br), through the reaction of PHCS with 2-bromoisobutyryl bromide, in the presence of triethylamine, in dimethylfomamide (DMF) medium; then, PHCS-*g*-PNIPAM was prepared by combining PHCS-Br macroinitiatior with NIPAM, 2,2'-Bipyridyl and cuprous chloride (CuCl), in DMF at 70 °C and under nitrogen atmosphere; finally, deprotection was made by the reaction of PHCS-g-PNIPAM with hydrazine hydrate in water medium, under nitrogen atmosphere. The LCST of chitosan-*g*-PNIPAM in aqueous solution was 33 °C at pH 6.3 and 35 °C at pH 5.0, which indicated that the thermoresponsive behavior is also pH dependent in these materials, since the lowest pH implies on a more hydrophilic material[139].

5. Conclusions

In this paper, the stimuli-sensitive polymer systems are described, by means of their behavior and applications. The pH-responsive, thermoresponsive and the dual pH and thermoresponsive copolymers were presented, giving focus on the combination of chitosan with poly (*N*-isopropylacrylamide) by graft copolymerization, obtained *via* different routes and their properties. Chitosan-*g*-PNIPAM copolymers are very promising materials especially on biomedical applications, such as in drug delivery systems and tissue engineering. The pH-sensitiveness and biological favorable properties from chitosan associated to the thermosensitive properties from PNIPAM lead to a powerful responsive material that

can be synthesized to a target physical-chemical behavior, as well as with an enhanced therapeutic efficiency and reduced side effects.

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7. References

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