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Review

Temperature and pH responsive polymers based on chitosan: Applications and new graft copolymerization strategies based on living radical polymerization

A.S. Carreira, F.A.M.M. Gonçalves, P.V. Mendonça, M.H. Gil, J.F.J. Coelho *

Chemical Engineering Department, University of Coimbra, Pólo II, Pinhal de Marrocos, 3030-290 Coimbra, Portugal

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ABSTRACT

The present review aims to highlight the applications of pH and temperature responsive chitosan-based polymers. Such materials are used in the development of several applications, such as drug delivery systems, bioseparation devices, tissue engineering scaffolds, cell culture supports, sensors or actuators systems. Advances in this field are particularly relevant to applications in the areas of regenerative medicine and drug delivery. This review addresses mainly smart polymers, derived from chitosan, including particulate carrier systems, hydrogels and film based materials that are responsive to stimuli such as temperature and pH. A survey of the major contributions is presented. Finally, this review summarizes and analyses recent developments in the field of graft modification of chitosan by living radical polymerization.

1. Introduction

Stimuli–responsive polymers show a sharp transition in physical/chemical properties under a small change in environmental condition(s), such as slight variations in pH, temperature, ionic strength and the concentration of specific substances (Galaev & Mattiasson, 2008). However, the system returns to its initial state when the trigger used is removed. The more important systems and thus the most studied are those sensitive to pH and/or temperature. Polymers that react to pH changes typically have either acidic or basic functional groups. Therefore, the ionization level of the pendant group changes strongly around the pK_a, which results in a modification of the water solubility of the polymer chains (Mano, 2008). The use of temperature and pH stimuli to control the response of a system has been extensively studied in the biomedical field. This is due to the fact that such systems can be controlled and are applicable in both *in vivo* and *in vitro* conditions. With pH responsive polymers, due to the large variations in physiological pH at various body sites, under normal and pathological conditions, they can be used in the tuning the behaviour (Dias, Mano, & Alves, 2008).

1.1. Properties and advantages of chitosan smart polymers

Chitosan is one of the most attractive polymers derived from renewable resources. It possesses remarkable properties, enabling its application in many areas of material science and technology, particularly those related to biomaterials and medical aids. This linear polysaccharide is obtained by extensive deacetylation of chitin. It is mainly composed of two kinds of β (1 → 4) linked structural units *viz*. 2-amino-2-deoxy-D-glucose and *N*-acetyl-2-amino-2-deoxy-D-glucose. The capacity of chitosan to dissolve in dilute aqueous solutions is the commonly accepted criterion that is used to differentiate it from chitin (Belgacem & Gandini, 2008). This weak cationic biopolymer, soluble in water up to pH 6.2, is found in significant quantities in some fungi, such as *Mucor rouxii* (30 per cent) and *Choanephora cucurbitarum* (28 per cent). In these sources, the chitosan is associated with other polysaccharides. Chitosan also has pH-sensitive properties due to the protonation–deprotonation equilibrium of the amino groups, which allowing the fabrication of pH controlled release carriers that are based on chitosan (Chuang, Don, & Chiu, 2009). Natural polymers, such as collagen, alginate, and chitosan carry a number of advantages over the synthetic polymers. One such example is the reduced need for harsh processing conditions (Uchegbu, 2006). The natural polysaccharides are also very abundant and their production is both relatively environmentally safe and of low cost (Peter, 1995). Moreover, regarding controlled delivery drugs (mainly for the retention of the dosage form in the stomach), chitosan has antiulcer and antacid activities that prevent or weaken drug irritation in the stomach (Gupta & Kumar, 2000d).

1.2. Chitosan applications

Chitosan (Fig. 1) is a very useful polymer for biomedical applications due to its biocompatibility, biodegradability and low

* Corresponding author. Tel.: +351 798744; fax: +351 798703.
E-mail addresses: jcoelho@eq.uc.pt, jcoelho3@gmail.com (J.F.J. Coelho).

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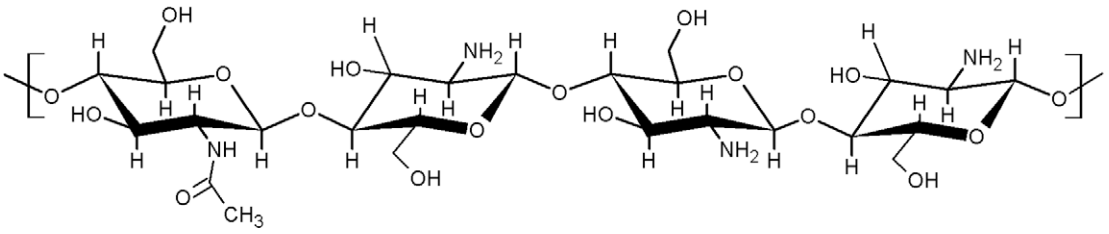

Fig. 1. Chitosan structure.

toxicity (Belgacem & Gandini, 2008). All these important characteristics have led to the development of numerous applications of chitosan and its derivatives not only in biomedicine, like surgical sutures, biodegradable sponges and bandages, matrices in microspheres and/or microcapsules, and the delivery of drugs (glaucoma treatment) (Almeida, Fonseca, Baptista, Leite, & Gil, 2007), but also in orthopedic materials and dentistry (Pedro, Cabral-Albuquerque, Ferreira, & Sarmento, 2009). The great variety of uses of chitosan in the field of biomaterials is due to its excellent properties when interacting with the human body: bioactivity, antimicrobial activity (Joerger, Sabesan, Visioli, Urian, & Joerger, 2009), enzymatic biodegradability, and epithelial permeability that support adhesion and the proliferation of different cell types. Chitosan has been tested in applications such as contact lenses, tissue adhesives, prevention of bacterial adhesion, sutures and others (Aguilar, Elvira, Gallardo, Vázquez, & Román, 2008).

Chitosan has been thoroughly investigated mainly in two biomedical fields. It has been used in the treatment of wounds, ulcers and burns, due to its haemostatic properties and its accelerating wound healing effects. On the other hand, due to its cell affinity and biodegradability, it has been applied in tissue regeneration and restoration, as structural material. Chitosan has been widely used as a matrix in drug-release systems in the form of beads and granules, as promising vehicles for oral drug sustained-release formulations. Also, its properties make it possible to be widely used in combination with other polymers, with the purpose of improving the performance of the material on its application. Polymers that are commonly used with chitosan include poly(acrylonitrile) (PAN) (El-Sherbinyl, Lins, Abdel-Bary, & Harding, 2005; Kim, Shin, Lee, & Kim, 2003), poly(*N*-isopropylacrylamide) (PNIPAAm) (Chen et al., 2007; Guo & Gao, 2007; Shi, Alves, & Mano, 2008), poly(ethylene glycol) (PEG) (Gupta & Kumar, 2001a; Khurma & Nand, 2008), alginates, gelatin (Yao, Xu, Yin, Zhao, & Chen, 1996), among others.

1.3. Chitosan hydrogels and particles

Hydrogels are three dimensional polymer networks that can absorb large amounts of water or biological fluids. They can be divided into three classes, depending on their nature. These classes are: entangled networks, covalently crosslinked networks and networks formed by secondary interactions. The use of hydrogels as biomaterials is receiving a great deal of interest, mainly due to their low toxicity and high biocompatibility, which makes them extremely useful for many applications, mainly regarding the controlled release of bioactive molecules and in tissue engineering. One of the advantages of the hydrogels lies in their sensitivity to some external parameters such as pH, temperature, ionic strength, solvent composition and electrical fields (Khurma & Nand, 2008). Their physicochemical, mechanical and biological properties, as well as new functional properties, can be modulated. In this review, focus is placed on chitosan hydrogels that respond to pH changes and/or temperature changes. Hydrogels with pH-responsive properties are made of polymeric backbones containing ionic pendant groups; these pendant groups can ionize and develop fixed charges on the polymer network, which results in electrostatic repulsive forces, responsible for pH-dependent swelling/shrinkage of the hydrogel. The pendant groups of anionic hydrogels are unionized below and ionized above the pK_a of the polymeric network, which leads to the swelling of the hydrogel at pH above the polymer pK_a, due to the presence of ions; the cationic hydrogels swell at lower pH (Gupta et al., 2002) (Fig. 2).

Among the innumerous factors that influence pH-responsive swelling and drug release, the influence of charge, concentration and pK_a of the polymer and also the properties of the swelling medium, like pH and ionic strength, can be highlighted.

Chitosan hydrogel systems can be divided into three convenient classes, the chemical hydrogels, the physical hydrogels and the crosslinking hydrogels. The systems can be obtained by several mechanisms such as covalent bonding, ionic bonding, hydrogen bonding and also by hydrophobic association. The majority of temperature-sensitive hydrogels exhibit a separation from solution and solidification above a certain temperature, defined as the lower critical solution temperature (LCST). Above the LCST, they turn into a gel, becoming extremely hydrophobic and insoluble. On the other hand, hydrogels that are formed through the cooling of a polymer solution have an upper critical solution temperature (UCST). This type of smart hydrogel, with volume phase transition properties, has been studied mainly for drug delivery. PNIPAAm is one of the more widely used polymers in this respect. The water solubility of PNIPAAm and its copolymers changes radically around the LCSTs, which can be turned to be close to the body temperature. The polymers are soluble in water when the temperature is lower than the LCST, but become insoluble above the LCST. This negative temperature sensitivity of the hydrogel is due to competition between the hydrogen bonding interactions and the hydrophobic interactions. Positive temperature-sensitive hydrogels can be formed by interpenetrating polymer network (IPN) having positive temperature sensitivity. These swell at higher temperatures

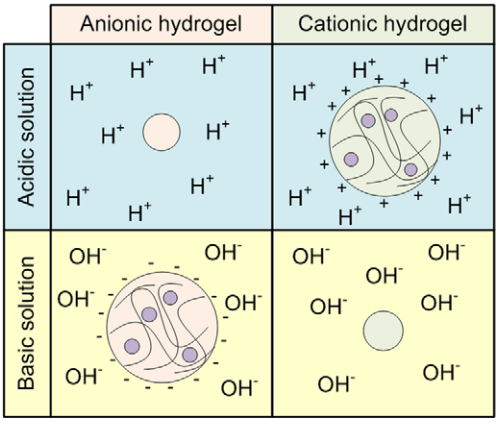

Fig. 2. The pH-responsive swelling of hydrogels adapted from Gupta, Vermani and Garg (2002).

and shrink at lower temperatures. The negative temperature-reversible hydrogels can be tuned to be liquid at room temperature (20–25 °C), undergoing gelation when in contact with body fluids (37 °C), due to the increase in temperature. Hence, polymers having a LCST below human body temperature have potential for injectable applications. There are different strategies to control the drug delivery as we can be seen in Fig. 3.

Fig. 3 presents different strategies to release drug from a thermoresponsive polymeric matrix (Mano, 2008). An hydrophic drug is trapped in a swollen gel and once the temperature decreases below the LCST, the drug is released due to the increase of diffusivity (Fig. 3A). In a second approach, an hydrophobic drug is released from the matrix when temperature is above LCST (Fig. 3B). Finally, the drug is trapped in the polymer swollen matrix due to a dense skin layer made of the thermoresponsive polymer (Fig. 3C).

Particulate carriers offer some unique advantages as delivery, sensing and image enhancement agents. These can be made from various organic materials and inorganic materials. There are several types of carriers, including polymer–drug conjugates, liposomes, micellar delivery systems, dendrimers, whose composition can be manipulated in order to obtain desired stimuli–responsive properties (Ganta, Devalapally, Shahiwala, & Amiji, 2008). These carriers are generally considered for use in the target-specific delivery of drugs and genes to various sites in the body, to improve the therapeutic efficacy, while minimizing undesirable side effects. One advantage of these stimuli-responsive carriers is based on their capacity to respond specifically to a certain pathological trigger. For example, when using temperature-sensitive carriers, these delivery systems will only release the load at a certain temperature, keeping the toxic drug encapsulated in the circulatory system.

2. Chitosan polymers

2.1. Hydrogels based on chitosan

2.1.1. Interpenetrated networks

An IPN (see Fig. 4) is a combination of two crosslinked chemically distinct polymers, that is produced by the synthesis, or crosslinking, of one of the polymers in the immediate presence of the other (El-Sherbinyl et al., 2005). If only one component of the assembly is crosslinked, leaving the other in the linear form, the

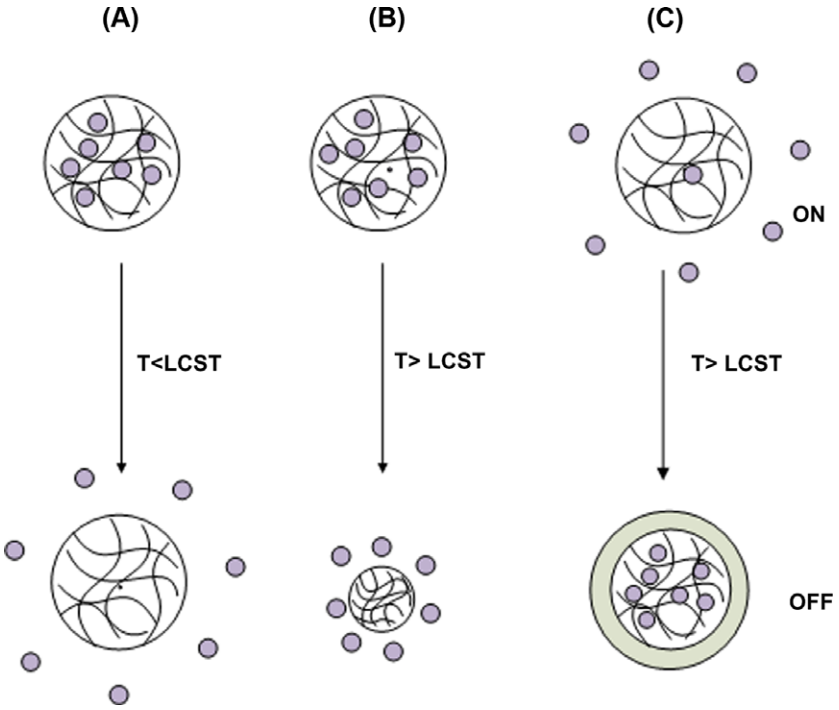

Fig. 3. Modes of drug delivery from temperature-sensitive hydrogels, adapted from Mano (2008).

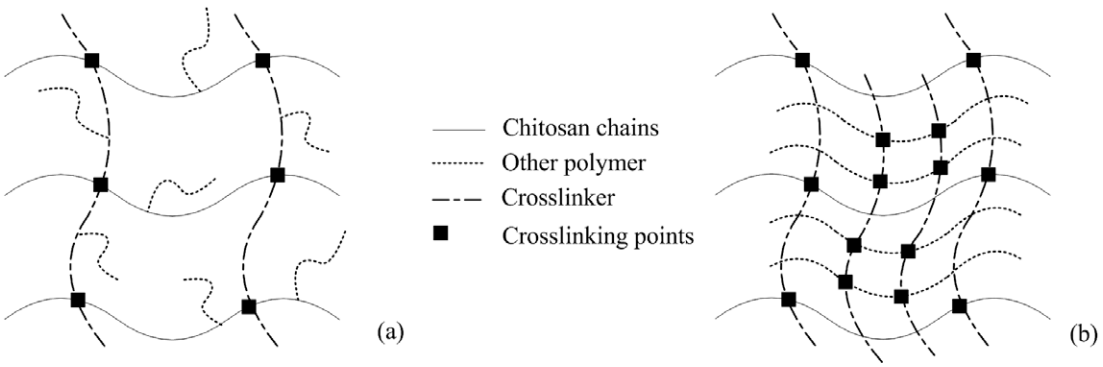

Fig. 4. Generic chitosan semi-IPN (a) and IPN (b).

system is termed a semi-IPN (Prabaharan & Mano, 2006). The resultant polymeric systems are known to change their volume reversibly as a response to some stimuli, including pH and temperature (Kim et al., 2003), making them good candidates for the improvement of the swelling/shrinkage behaviour of materials (Mano, 2008).

With the purpose of mimicking biological systems, Goycoolea et al. (2003) prepared pH and temperature responsive chitosan cylindrical hydrogels by using a combination of chemical and physical crosslinking. The hydrogels were synthesized from alkali chitin, molded in cylinders and crosslinked with glutaraldehyde, in various ratios. It was found that the temperature dependent swelling-contraction behaviour of hydrogels was distinct at pH < 7.6 and pH ⩾ 7.6. At lower pH values, the hydrogels responded positively (swelling) to temperature rises. The opposite behaviour was demonstrated for pH ⩾ 7.6 (Goycoolea et al., 2003). The authors believed that there is a pH value, below 7.6, at which the hydrogel will not respond to temperature changes, due to a match between the hydrophilic forces and the hydrophobic forces.

In order to combine the advantages of synthetic polymers and natural polymers and to overcome its poor reactivity and processability, chitosan has been incorporated into IPN matrices of other polymers (El-Sherbinyl et. al., 2005). Kim and co-workers (2003) prepared semi-IPN with chitosan and PAN, crosslinked with glutaraldehyde, with different contents of chitosan. The authors found that the system was pH responsive and temperature responsive, exhibiting a higher swelling ratio (SR) as the chitosan content increased (SR ∼325% for a weight ratio (chitosan/PAN) = 3/1). Other IPN hydrogels such as (chitosan/poly(*N*-acryloylglycine-chitosan) (El-Sherbinyl et al., 2005), chitosan/PVA (Abdelaal, Abdel-Razik, Abdel-Bary, & El-Sherbinyl, 2007) and PVA/chitosan/PAA (Zhang, Yuan, Wang, & Zhang, 2007) have been produced, using glutaraldehyde as the crosslinking agent. The purpose was to use the products as drug delivery systems. Glutaraldehyde is known to be a toxic agent. Therefore, it must not be used in materials that are to be used in medical applications. This problem has been overcome by Khurma and Nand (2008) and co-workers, who recently synthesized a semi-IPN hydrogel using a naturally occurring non-toxic crosslinking agent, genipin. The system was composed of chitosan and PEG. The pH-responsive behaviour and temperature responsive behaviour was studied at a pH and temperature similar to those found in the human body. The authors found that the hydrogel swelling behaviour was dependent on the pH, temperature and on the PEG content. The swelling ratio increased as the temperature or PEG content was increased and showed the opposite behaviour as the pH was increased (Khurma & Nand, 2008). The differential scanning calorimetry (DSC) tests were able to give information about the states of water in the hydrogels, by analyzing the melting enthalpies of endothermic peaks near 0 °C. The results demonstrated that the equilibrium water content (EWC) increased with the increase in PEG concentration in the gels, suggesting that the addition of PEG enhances the hydrogel's hydrofilicity.

In order to explore new properties of chitosan-based IPNs, carboxymethyl chitosan (CMCS), a natural amphoteric polyelectrolyte derived from chitosan, has been used for biomedical applications such as wound dressings, artificial bone materials and skin, bacteriostatic agents and blood anticoagulants (Guo & Gao, 2007).

Chen and co-workers (2007) developed a novel type of IPN hydrogel membrane based on CMCS and NIPAAm. The chemical crosslinking of PNIPAAm was induced by electron beam irradiation with different doses. The hydrogel exhibited temperature sensitivity and pH-sensitivity. However, it was found that, contrary to other published results (Stile, Burghardt, & Healy, 1999), the introduction of CMCS did not change its LCST, which remained similar to the LCST of PNIPAAm (∼32 °C) (Chen et al., 2007). These results suggest that the interaction of the IPN gives a relatively independent polymer system in which each network may retain its own properties (Chen et al., 2007). Using CMCS, Gou and co-workers synthesized two semi-IPN with PNIPAAm (Guo & Gao, 2007) and poly(2-(dimethylamino) ethyl methacrylate) (PDMAEMA) (Guo, Yuan, Yao, & Gao, 2007), with *N*,*N*′-methylenebisacrylamide (BIS) as the crosslinking agent. All of the obtained semi-IPNs showed negative temperature responsive behaviour but positive pH-responsive behaviour and reversibility. Release experiments with coenzyme A showed that the mechanism was affected by the temperature, the pH and the CMCS content in both semi-IPN systems. In the preparation of these materials, the CMCS is usually dissolved in an aqueous acid that can remain as a residual impurity in the system (Zhou et al., 2008). This can prove to be a drawback in some medical applications. Recently, to overcome this problem, Zhou et al. (2008) prepared a semi-IPN using the water-soluble *N*-carboxyethyl chitosan and poly(2-hydroxyethyl methacrylate) (PHEMA), using UV irradiation. Release experiments, performed with 5-flurouracil, showed that the semi-IPN material was sensitive to pH (greater drug release at low pH values). From a cytotoxicity standpoint, the products were non-toxic. Thus, the synthesized materials are able to be used as drug sustained-release matrix, as it was initially proposed by the authors.

2.1.2. Copolymerization by grafting

Chemical modification of chitosan by grafting vinyl monomer(s), and then crosslinking the material, is another way of improving its performance and enlarging its potential applications (Cai, Zhang, Sun, He, & Zhu, 2005; El-Sherbinyl, Abdel-Bary, & Harding, 2006). For instance, pure chitosan hydrogels provide fast dissolution in the stomach and limited capacity for controlling the release of drugs, a serious drawback in orally administered products (Taleb, 2008).

One of the more intensively investigated monomers is NIPAAm. The polymer has a LCST of around 32 °C, due to its ability to form hydrogels with liquid–gel transition occurring at temperatures that are similar to that of the human body. This characteristic is an advantage in the design of drug delivery systems. This is because the body temperature may change due to fever, local infections or disease (Mano, 2008). Grafting NIPPAm onto chitosan provides an increase in the water content on exposure to aqueous media and improvement of the mechanical properties and the temperature responsive properties (Zhang & Zhong, et al., 2009). Kim and co-workers synthesized a chitosan-*g*-NIPAAm copolymer using Ce(IV) ammonium nitrate as the initiator (Fig. 5). The copolymer was then crosslinked with glutaraldehyde. The efficiency and percentage of copolymerization increased as the monomer concentration (NIPAAm) increased. Such findings have been confirmed by Cai and colleagues in their copolymerization of the same material by gamma-radiation (Cai et al., 2005). The resulting copolymer exhibited pH-responsive behaviour and temperature responsive behaviour, with swelling ratios higher at pH 4 than at pH 7. At 35 °C, above the LCST (∼32 °C), the equilibrium water content was lower in comparison to the one at 25 °C (Kim, Cho, Lee, & Kim, 2000).

Aiming to obtain chitosan-based membranes with dual effects: to accelerate wound healing due to the bioactivities of chitosan and at the same time a drug delivery system, dos Santos and co-authors (2006) prepared different chitosan graft copolymers with HEMA and AA using also Ce (IV) ammonium nitrate as initiator.

The grafting of different amounts of AA and/or HEMA showed a remarkable influence in the swelling degree, cytotoxicity, thrombogenicity, haemolytic activity, and thermal properties (Ferreira, Coelho, dos Santos, Ferreira, & Gil, 2006).

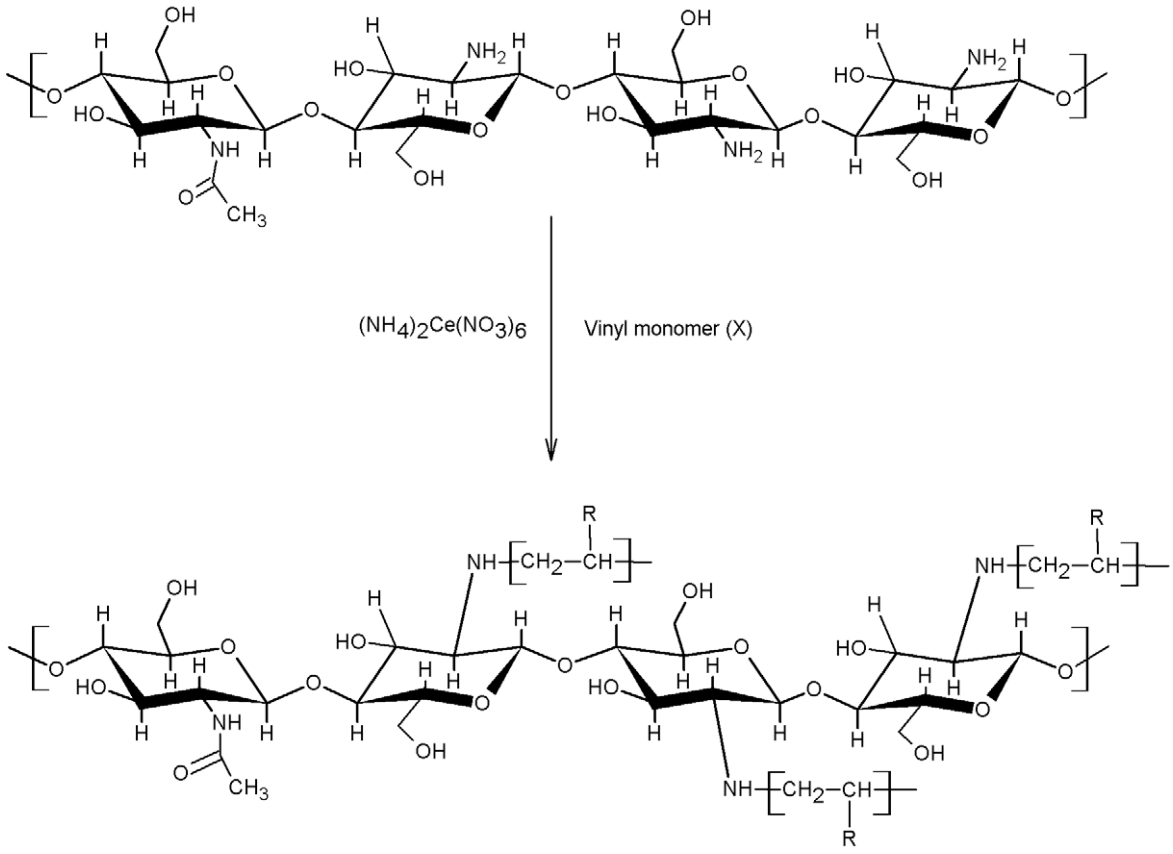

Fig. 5. Schematic representation of graft copolymerization different vinyl monomers (X–AA (dos Santos et al., 2006); X–HEMA (dos Santos et al., 2006); X–NIPAAm (Chung et al., 2005; Lee et al., 2004); X–ethylene glycol diacrylate (EGDA) (El-Sherbinyl et al., 2006)) onto chitosan.

At the same time, El-Sherbinyl and co-workers prepared a chitosan graft poly(ethylene glycol) diacrylate copolymer hydrogel, aiming to improve the hydrophilicity of chitosan, by free radical polymerization, using the same initiator system (El-Sherbinyl et al. 2006). Release tests with 5-Fluorouracil at pH values that were similar to those of gastric (pH 2.1) and intestinal (pH 7.4) fluids were performed at 37 °C. The hydrogels presented negative pH-responsive behaviour and positive temperature responsive behaviour. In drug release tests, the hydrogels were able to deliver greater percentages of drug to provide longer release times, with increase in the poly(ethylene glycol) diacrylate content on the copolymer matrix (El-Sherbinyl et al., 2006).

Chung and colleagues coupled monocarboxy Pluronic® (a temperature-sensitive triblock copolymer of poly(ethylene oxide)/poly(propylene oxide)/poly(ethylene oxide)) and chitosan using 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide and *N*-hydroxysuccinimide as coupling agents (Chung, Bae, Park, Lee, & Park, 2005). FTIR and ^{1}RMN results proved the success of the copolymerization. The resultant material showed thermoreversible behaviour with a sol–gel transition at body temperature (Chung et al., 2005). *In vitro* cell studies, in which these hydrogels were compared with alginate gels, showed the non-toxic and biocompatible character of the material (Chung et al., 2005; Lee, Jung, Park, Park, & Ryu, 2004). These results suggest that the synthesized chitosan-*g*-PNIPAAm and chitosan–pluronic copolymers could be used as injectable materials for cell and/or drug delivery systems. Using the same coupling agents, Chen and co-workers prepared a comb-like polymer using carboxylic acid-ended NIPAAm (synthesized by free radical polymerization using NIPAAm and mercaptoacetic acid in benzene) and chitosan (Chen & Cheng, 2006). The hydrogel obtained (CPN) exhibited superior mechanical strength in comparison to PNIPAAm hydrogels. Culture of chondrocytes and meniscus cells in the hydrogels showed good cell viability, thus proving this material a good candidate for injectable scaffolds for tissue engineering of cartilage and meniscus (Chen & Cheng, 2006).

The same group evaluated the CPN for 3T3 fibroblast cells sheet structures (Chen & Yang, 2008). A hydrogel surface was used for cell attachment and proliferation. The cells exhibited high viability and, after being incubated at 20 °C, they detached, as the hydrogel became dissolute, and showed ability to proliferate in a new culture surface. Similar behaviour to that of the CPN was observed, by the same authors, for a new hydrogel synthesized with CPN and hyaluronic acid (Chen & Cheng, 2008). CPN has been also investigated as a potential platin drug-release system. Using cisplatin and carboplatin as model drugs, Fang, Chen, Leu, and Hu (2008) compared the performance of CPNs (with different chitosan contents) with PNIPAAm hydrogels in drug release. They found that CPN hydrogels promote a superior release to that of PINPAAm. The rate of release was able to be adjusted by controlling the CPN composition, which is an advantage in the design of controlled drug delivery systems (Fang et al., 2008).

Other copolymers based on chitosan and PNIPAAm have been synthesized for use in drug delivery systems. Examples include a chitosan with maleic anhydride (MA) and NIPAAm (Guo, Yuan, & Gao, 2008) and methacrylated chitosan with NIPAAm (Han, Wang, Yang, & Nie, 2009). The copolymers showed pH and temperature responsive behaviour and the ability to release the coenzyme A (Guo et al., 2008), acid orange 8 (AO8) and 5-fluorouracil (Han et al. 2009). Zhang and co-workers have reported a new approach to the creation of temperature responsive hydrogels. These authors prepared a novel copolymer hydrogel based on NIPAAm and a chitosan derivative, CMCS (Zhang & Zhong, et al., 2009).

Copolymerization was initiated using ammonium persulfate. The DSC traces of different hydrogels showed no relevant differences in the transition at 32 °C, which is the LCST of PNIPAAm. Therefore, the authors concluded that CMCS had no impact on the responsive temperature of the hydrogels. However, the copolymers exhibited superior temperature sensitivity and swelling ratio compared to the corresponding PNIPAAm hydrogels. This is due to the higher level of porosity and connectivity in copolymers, observed by Scanning Electron Microscopy (SEM) analysis.

A diblock copolymer based on chitosan and monomethoxy-poly(ethylene glycol) macromere (PEG) was prepared by Ganji and co-workers, using potassium persulfate as the initiator (Ganji & Abdekhodaie, 2008). The resultant hydrogel behaved like a liquid at temperatures below room temperature and like a gel at temperatures close to that of the human body (~36 °C) (Ganji & Abdekhodaie, 2008). The chitosan-*g*-PEG copolymer was considered to have the potential to be applied in injectable biomedical systems.

Copolymers of chitosan and AA produced by gamma-radiation have also been reported. The systems included chitosan grafted with AA and with acrylamide (Taleb, 2008). Systems based on chitosan, grafted with poly(acrylic acid), poly(hydroxy propyl methacrylate) (PHPMA), PVA and gelatin have also been prepared (Sokker, Ghaffar, Gad, & Aly, 2009). These materials exhibited smart behaviour, responding to pH (Taleb, 2008) and to both pH and temperature (Sokker et al., 2009). The results demonstrated the potential of these hydrogels as drug carriers for oral administration PNIPAAm (Sokker et al., 2009).

2.2. Particulate carriers based on chitosan

The use of stimuli–responsive micro/nanocarriers offers interesting opportunities for active agents delivery, in which the delivery system becomes an active participant in the optimization of a therapy (Ganta et al., 2008). These carriers can be manipulated to produce a specific stimuli-response property within several structures. Examples include microparticles with spherical structures (microcapsules and microspheres), microbeads and beads and spherical particles (for large sizes and rigid morphologies) (Kumar, 2000). Nanoparticles can also be in the form of capsules and spheres. Some particles are based on a core and a shell, with different compositions and properties in each layer. These are the core–shell particles. All of these carriers can be based on organic materials and inorganic materials including both non-degradable polymers and biodegradable polymers, lipids self-assembled amphiphilic molecules, dendrimers and metals (Ganta et al., 2008).

Yao and collaborators synthesized a chitosan polyether IPN hydrogel. They studied cimetidine release from the hydrogel and its structural changes with pH (Peng, Yao, Yuan, & Goosen, 1994; Yao et al., 1994). They were one of the earlier groups to study pH-responsive microspheres based on chitosan (Yao et al., 1996). In their study, chitosan/gelatin hybrid polymer network microspheres were synthesized by an inverse emulsion method, with glutaraldehyde as the crosslinker. The analysis of cimetidine release, surface morphology by SEM and FTIR characteristics were also included in the study. The results obtained showed that drug release only occurred in acidic media and that microspheres have potential as carrier for intelligent drug delivery systems.

Gupta and collaborators have made an extensive study using pH-sensitive particles based on chitosan (Gupta & Kumar, 1999, 2000a, 2000b, 2000c, 2001a, 2001b). The group synthesized particles for drug release with chitosan and glycine (Gupta & Kumar, 1999, 2000a, 2000b, 2000c) and with chitosan and PEG (Gupta & Kumar, 2001a, 2001b). All of the particles were prepared identically, with glutaraldehyde as a crosslinker. Isoniazid (Gupta & Kumar, 2001a, 2001b), thyamine hydrochloride (Gupta & Kumar, 1999, 2000b), chlorphenramine maleate (Gupta & Kumar, 2000c) and diclofenac sodium were used as model drugs (Gupta & Kumar, 2000a). The swelling behaviour, solubility, drug loading capacity of the particles and amount of crosslinking agent were analyzed, showing that the degree of swelling is greater in solution at pH 2.0 than at pH 7.4 (Gupta & Kumar, 1999, 2000a, 2000b, 2000c, 2001a, 2001b).

Recently, Choochottiros and colleagues prepared amphiphilic chitosan nanospheres that possessed a responsive performance. These nanoparticles were formed by grafting phthalic anhydride, as a hydrophobic group and PEG, as a hydrophilic group (Choochottiros, Yoksan, & Chirachanchai, 2009). Chitosan nanosphere surfaces, in aqueous solution, are negatively charged. This property promotes a specific behavioural type to these nanospheres, i.e. good solution dispersion from neutral to high pH values and significant precipitation at low pH. The aim of the work by Choochottiros et al. (2009) was to incorporate a hydrophilic drug, lidocaine. The size distributions and morphologies were studied. They also determined the effect of the deacetylation percentage on degree of phthaloylation and mPEGylation. For larger nanospheres the amount of drug loading was higher (Choochottiros et al., 2009).

Similar nanoparticles were synthesized by Chuang et al. (2009). Here, AA was copolymerized with NIPAAm in a chitosan solution. These nanoparticles were prepared using surfactant-free emulsion polymerization with the encapsulation of doxycycline hyclate (Chuang et al., 2009). In this work the structure, particles size, morphology, surface charge, responsive properties and *in vitro* drug release behaviour of nanoparticles, synthesized with amounts of the copolymer compounds, were studied. The release profile of doxycycline hyclate from the drug-loaded nanoparticles at two different pH values (7.0 and 2.0) was distinct, showing faster release at the higher pH (Chuang et al., 2009).

Chitosan presents an opposite behaviour when applied as oral drug administration or drug for intestinal release. The particles must hold the drug while in the stomach (acidic pH) and release it once in the intestine (neutral pH) (Fig. 6). For this reason, chitosan needs to be associated with other reagents that present different properties.

The oral route is the most suitable method of drug administration. However, some active agents are susceptible to enzymatic degradation. Examples include peptides and proteins. Several methods have been employed to improve the bioavailability of orally administered drugs. Sajeesh and Sharma (2005) synthesized pH responsive PMA–chitosan–PEG nanoparticles. The goal of this study was to develop a candidate for oral peptide delivery. Nanoparticles were prepared by free radical polymerization of methacrylic acid in the presence of chitosan and PEG. Encapsulated insulin and bovine serum albumin were used as model drugs. The particles morphology was observed by SEM and by transmission electron microscopy (TEM). The results showed that they have an irregular and aggregated morphology, however these aggregates have less than 1 μm. At a low pH (1–2) these materials shrank and the encapsulated drug was poorly released, and protected from the acidic environment of the stomach. Although, at neutral pH (6–7), an environment that is characteristic of the small intestine, the matrix swells and the drug is released. The preliminary results by Sajeesh and Sharma (2005) suggest that these particles might be a good candidate for oral peptide delivery. However, to the establishment of the efficiency of the system are necessary *in vivo* studies on animal models (Sajeesh & Sharma, 2005).

More recently, in the field of stimuli-responsive particles for oral drug delivery, several studies have been published. For example, Cui et al. (2009) prepared carboxylated chitosan-grafted poly(methyl methacrylate) (PMMA) nanoparticles for use in insulin delivery.

Alginate/chitosan/starch microcapsules containing encapsulated oyster peptides were synthesized by Zhang, Liu, Wu, & Chen

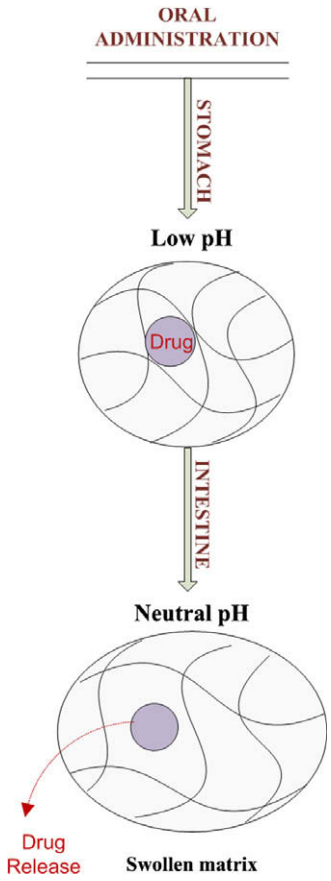

Fig. 6. Drug oral administration and respective drug release in the intestine.

(2009). These microcapsules were synthesized by external internal emulsion gelation method. They were characterized by FTIR, morphology observation (optical microscope) and swelling studies. The oyster peptides encapsulation efficiency and *in vitro* release behaviour was also determined. As an example, for external gelation the cumulative peptide release at pH value of 1.5 was lower than 10% (the peptide was retained), at pH 7.5 it increased to about 80% (the starch blend matrix swells) and at pH 8.6 the release was greatly faster. The results showed that the microcapsules may possibly be a suitable carrier system for protein or peptides delivery in the intestine and that the release of internal gelation process was faster than the external.

Yu et al. (2009) developed microparticles based on chitosan, alginates and pectin. The model drug used was bovine serum albumin. In order to oppose the pH induced behaviour of chitosan, the alginate was used. An alginate with carboxyl groups shrinks at low pH but dissolves at high pH. Therefore, the solubility of chitosan is reduced by the alginate network, under low pH condition. The dissolution of alginates is reduced by chitosan, at high pH. Results show that drug release at pH 1.2 and 5.0 was slow. The release at pH 7.4 was much faster. The authors concluded that the microparticles have a high pH sensitivity and can be potentially used for site-specific protein drug delivery, through oral administration.

It is also possible to prepare particles based in chitosan, alginates and PNIPAAm in order to obtain pH-responsive properties and temperature responsive properties. Shi et al. (2008) prepared chitosan coated alginate particles containing PNIPAAm and indomethacin as the encapsulated drug. These systems can be applied for targeted release to solid tumors in the intestinal track. They bypass the gastric fluids and go to the intestinal fluids, after oral administration, and become subjected to local hyperthermia, starting the drug release. FTIR, SEM, DSC determination of indomethacin encapsulation efficiency and swelling and *in vitro* release studies were used to study the characteristics of these particles. In this study, the LCST determined was about 31 °C and the higher swelling degree was at pH 7.4 (Shi et al., 2008).

In recent years many advances have been observed concerning pH-responsive polymeric micro/nanocarriers for anticancer drugs delivery systems (Fig. 7). Tumor cells have a more acidic pH value (6.5) than that of healthy cells and blood (7.4) at 37 °C (Ganta et al., 2008). Thus, it is possible to manipulate carriers that can make use of these pH differences, allowing the delivery of an encapsulated load to occur specifically in selected extracellular sites or intracellular sites (Ganta et al., 2008).

However, very few systems with particles based on chitosan have been developed. Li et al. (2008) demonstrated, *in vitro*, that the pH responsive characteristics of PNIPAAm/chitosan nanoparticles can be used in the targeting of tumor cells with Camptothecin. The drug-loaded nanoparticles were most sensitive to the tumor cells pH when the ratio between the NIPAAm and chitosan was 4:1 (w/w). These authors have applied the same copolymer for the drug Paclitaxel studies (Li, Wu, Zhang, Gu, & Yang, 2009). The results showed that loaded nanoparticles exhibited a pH response to tumor pH. The study was performed on mice and the treatment demonstrated a significant tumor regression (with complete tumor regression for more than 50% of the mice). Li et al. (2009) considered that these nanoparticles have potential as anticarcinogen carriers.

Yuan, Venkatasubramanian, Hein, and Misra (2008) have prepared a carrier with a magnetite core (Fe_3O_4), conjugated with a drug via a acid-labile hydrazone-bond and encapsulated with the temperature responsive smart polymer, chitosan-*g*-NIPAM-*co*-*N*,*N*-dimethylacrylamide). These polymers presented a LCST of 38 °C. The drug release was faster at pH 5.3. Zhang, Mardyani, Chan, and Kumacheva (2006) developed a pH-sensitive drug delivery system by crosslinking a chitosan derivative *N*-[(2-hydroxy-3-trimethylammonium)propyl] chitosan chloride (HTCC) with sodium tripolyphosphate (TPP). The microgels were loaded with methotrexate disodium (MTX), a cytotoxic drug for cancer treatment, and conjugated with the targeting biomolecule apo-transferrin. The chitosan-based microgels were able to trigger an effective release of MTX in low pH medium in the tumorigenic cells, showing a clear response to the surrounding pH.

A responsive core–shell copolymer latex was synthesized by Lin, Chiu, and Lee (2005), using the method of soapless dispersion copolymerization. The synthesis consisted of two steps, the preparation of the core, poly(chitosan-*co*-NIPAAm); and the shell, MA-*co*-MMA. The copolymers showed potential to be used as targeting drug carriers, as the authors were able to perform caffeine release tests at 37 °C and pH 7.4 and conjugate a protein (bovine serum albumin) on the surface of those at 37 and 25 °C. The impact of different ratios of chitosan/NIPAAm and weight of the crosslinking agent used in the synthesis were investigated on several properties of the particles, such as swelling, zeta potential, average diameter and specific surface area. The increase of the weight ratio of chitosan/NIPAM showed a decrease in the size of the core–shell particles and an increase of swelling ratio of the samples. The authors noted that in terms of swelling behaviour and zeta potential, the obtained values were more influenced by chitosan or MMA depending on the pH tested.

Leung, Zhu, Harris, and Li (2004, 2005) proposed a new method to prepare smart microgels that consists in temperature responsive cores and pH-responsive shells. The microgels were synthesized by the aqueous graft copolymerization of NIPAAm from two water-soluble polymers containing amino groups: poly(ethyleneimine) (PEI) and chitosan. The PNIPAAm crosslinked with BIS and the reaction was promoted without surfactant. The volume phase

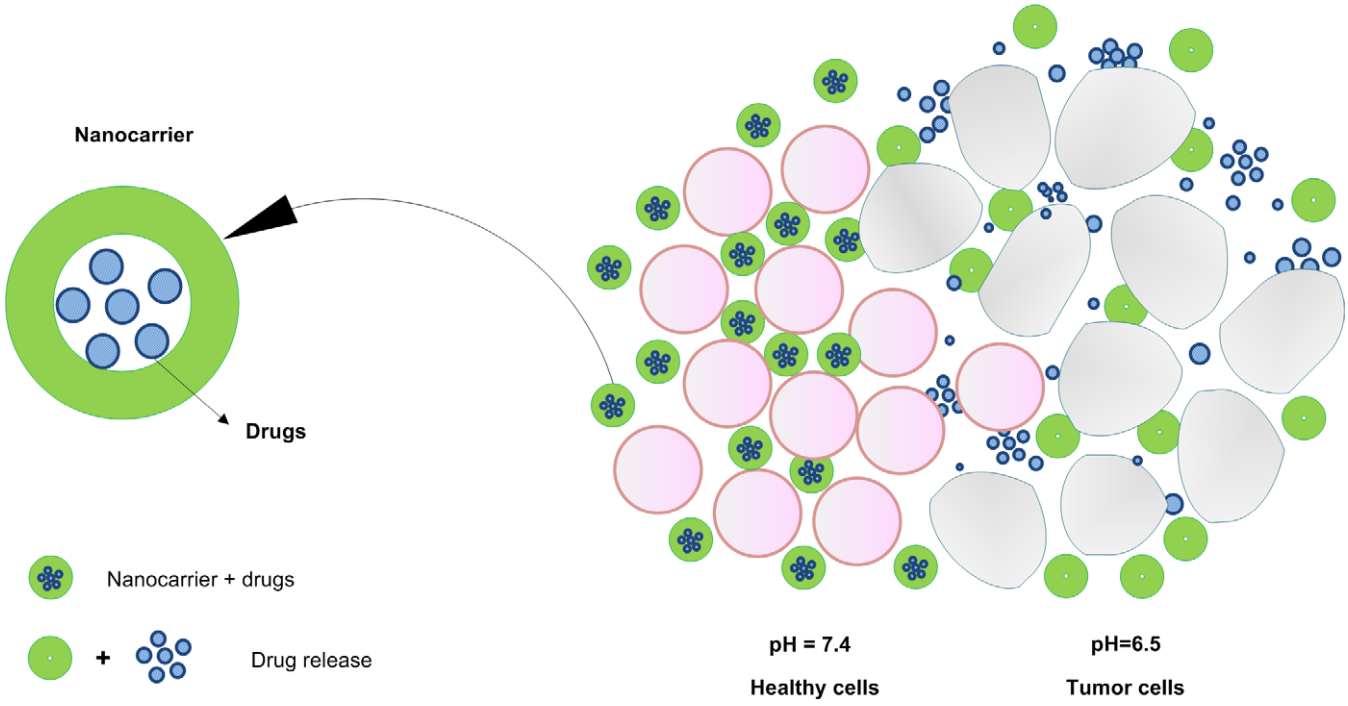

Fig. 7. Carriers for anticancer drugs delivery systems.

transition temperature, VPTT, of the core–shell microgels in water was determined by measuring the transmittance of this dispersion for different temperatures. It was observed that the VPTT of the PNIPAM/chitosan microgels, between pH 4 and 10, was 32.9–33.5 °C. The variation of hydrodynamic sizes of the core–shell microgels, as a function of the pH value, was determined by dynamic light scattering. The results showed that by decreasing the pH of the PNIPAM/chitosan microgel dispersion from pH 7 to 3, the hydrodynamic size of the core–shell increased considerably. These results revealed that the cores were temperature responsive and the shells were pH-responsive. The authors referred to several advantages of their study, such as the variety of synthetic polymers and biopolymers that can be used as pH responsive components. They suggest that core–shell microgels can be produced in the absence of surfactant.

Kaminski, Zazakowny, Szczubialka, and Nowakowska (2008) prepared chitosan microspheres for heparin removal. Heparin is a mixture of highly sulphated glycosaminoglycan polysaccharides with a great anticoagulant activity. This drug is frequently used, but presents several side effects. Thus, after heparin has exerted its activity, it is often required to remove it from the blood. Microspheres were synthesized by crosslinking chitosan with genipin by inverse emulsion polymerization. These authors observed that for pH values below 6.5, the microspheres swelled considerably. At pH values above 6.5 shrank by a small extent. At pH values that are close to the blood value, chitosan microspheres can be optimized by cationic modification with ethylammonium chloride. These genipin-crosslinked chitosan microspheres can potentially be applied to heparin removal in biomedical applications, the main advantage being the use of a synthesis process involving only inexpensive and non-toxic reagents (Kaminski et al., 2008).

2.3. Graft controlled modification of chitosan

Great progress has been made for the last decade on the development of controlled/living radical polymerization methods (Braunecker & Matyjaszewski, 2007; Cunningham, 2008; Hadjichristidis, Iatrou, Pitsikalis, & Mays, 2006; Matyjaszewski & Tsarevsky, 2009; Qiu, Charleux, & Matyjaszewski, 2001). The most widely used CLRP methods are the nitroxide mediated polymerization (NMP), atom transfer radical polymerization (ATRP) and degenerative based methods such as reversible addition fragmentation transfer (RAFT) and iodine transfer (IT) (Fig. 8). These methods are applicable to a wide range of monomers, solvents and end functionalities. The CLRP allow the synthesis of polymers with predetermined molecular weight, narrow molecular weight distribution, chains end functionality, topology, complex architecture and composition (Percec, Popov, Ramirez-Castillo, Coelho, & Hinojosa-Falcon, 2004). The different strategies exploit the equilibria between growing radicals and dormant species and minimize the proportion of terminated chains in radical polymerization.

The application of these techniques on the graft controlled polymerization of natural polymers, like chitosan, could open a new door to synthesize a wide variety of molecular structures, affording the precise synthesis of tailor made hybrid materials based on natural polymers. It will be possible to develop new materials to mimic the complexity of natural structures made by the conjunction of different natural and synthetic polymers, by designing new molecular architectures with controlled topologies and graft controlled segments. In spite of being widely used, the application of CLRP techniques to the modification of chitosan is seldom used.

ATRP have shown to be the most robust to control the polymerization of acrylates, methacrylates, acrylates and acrylonitrile (Matyjaszewski & Tsarevsky, 2009). This method is typically initiated by an alkyl halide and catalyzed by a transition metal complex. The equilibrium between dormant and active radical species is established between a lower oxidation state transition metal complex and its higher oxidation state. The ATRP as the other CLRP methods do not require stringent experimental conditions as in the ionic based methods.

There are three main strategies available for the preparation of graft polymers: "grafting through" – involves the polymerization of macromonomers; "grafting from" – consists in the polymerization side chains from polymeric macroinitiator ; and "grafting onto" –

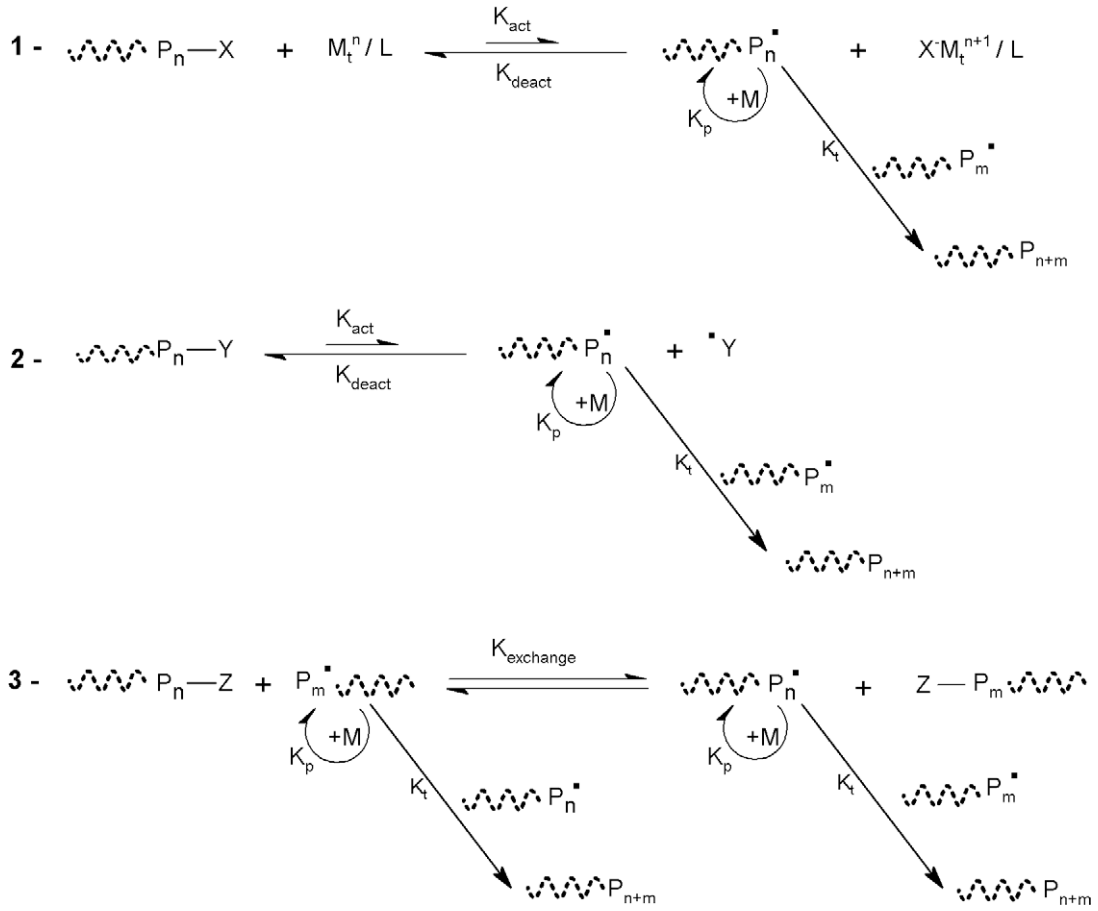

Fig. 8. General schemes of the most used CLRP methods: (1) ATRP; (2) NMP and (3) RAFT.

the addition of side chains to a polymer backbone (Sheiko, Sumerlin, & Matyjaszewski, 2008) (Fig. 9). The different strategies allow control over different structural parameters such as: grafting density, grafting composition, molecular weight of the side chains and the polymer backbone. The control of these parameters constitutes a challenging task due, to steric hindrance at the backbone caused by dense grafting. For each strategy, different polymerization techniques have been employed. To the best of our knowledge and under the scope of this review, the "grafting from" and "grafting to" were the only strategies used so far. The "grafting-from" starts with the preparation of the polymeric macroinitiator with a predetermined number of initiation sites (halogen groups in the ATRP and RAFT agents in the RAFT approach), that will be used to initiate the polymerization.

El Tahlawy & Hudson (2003) prepared chitosan macroinitiators by acetylation of chitosan with 2-bromo-isobutyryl bromide in the presence of pyridine as a base. The chitosan macroinitiator was used to polymerize a methoxy-poly(ethylene glycol)methacrylate (MeO(PEG)MA) monomer using Cu(I)Br/bipyridyl complex under heterogeneous aqueous conditions at 25 °C. The kinetics studies revealed a first order polymerization and polydispersities around 1.5. Using a similar approach, Li, Bai, and Liu (2005) proposed the controlled synthesis of chitosan beads grafted with polyacrylamide via surface-initiated ATRP (SI-ATRP). The bromide-end groups were immobilized on the surfaces of chitosan beads through reaction of $-NH_2$ or –OH groups 2-bromo-isobuturyl bromide using triethylamine as trapping agent in dry tetrahydrofuran (THF) (Fig. 10).

The chitosan beads grafted with polyacrylamide were only characterized by FTIR and X-ray photoelectron spectroscopy (XPS) and no information about the kinetics of graft copolymerization was provided to support the living features of this system. According to the authors, the graft copolymers present interesting properties for adsorption of mercury from aqueous solutions, when compared with chitosan beads.

Liu and Su (2006) proposed the preparation of polystyrene grafted chitosan particles by SI-ATRP from bromoacetyl chitosan. The amidation of the amino groups present in the chitosan was accomplished by immersion of chitosan powder in a solution containing bromoacetylbromide, triethylamine and THF. The SI-ATRP was carried out in toluene by using a catalytic system of CuBr/1,10-phenanthroline. The authors suggested the success of the controlled/"living" polymerization was based only on the linear conversion of styrene and percentage of grafting, which seems clearly insufficient.

Lindqvist and Malmström proposed the graft copolymerization of methyl acrylate and styrene on chitosan films by ATRP (Lindqvist & Malmstrom, 2006). The immobilization of bromide moisture on the film surface was carried out by immersing the films in a solution containing 2-bromo isobuturyl bromide, triethylamine, a catalytic amount of 2-dimethyl aminopyridine in THF. The authors studied the controlled grafting of methyl acrylate in ethyl acetate by using a catalytic system of Cu(I)Br/Me_6TREN in presence and absence of sacrificial initiator (ethyl-2-bromoisobutyrate) at room temperature (Fig. 11). In the absence of sacrificial initiator, a small amount of Cu(II)Br_2 was used to achieve a better control over the polymerization. The same study was undertaken for styrene in the presence and absence of sacrificial initiator (1-phenyl ethyl bromide).

The use of a sacrificial initiator allows a better control of the polymerization since it is known that the amount of initiating sites

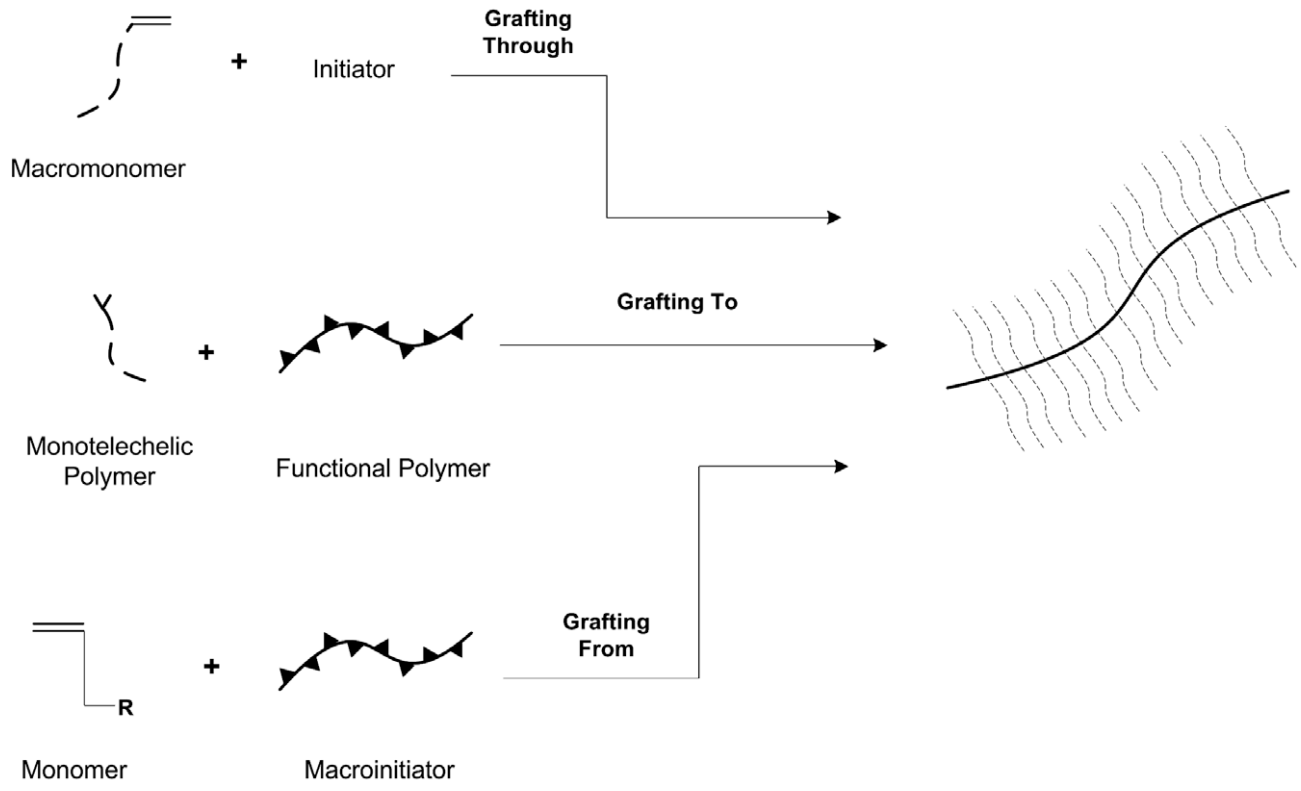

Fig. 9. Different strategies for preparing graft copolymers by using CLRP methods.

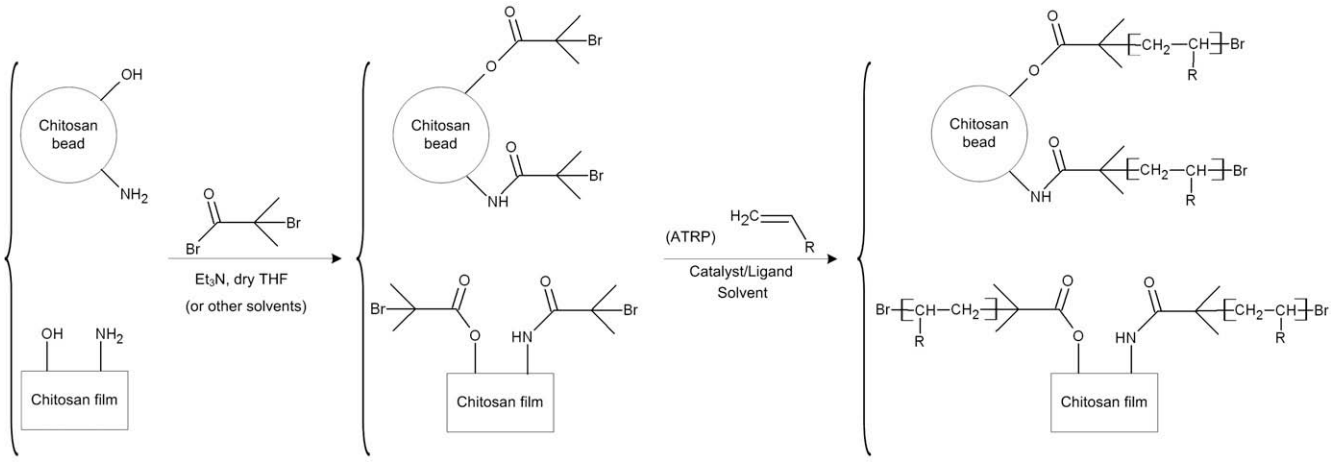

Fig. 10. General scheme used to prepare chitosan macroinitiator for ATRP.

on the surface of chitosan films is negligible compared with the amount of sacrificial initiator. Thus, it is possible to gain control over the degree of polymerization by adjusting the ratio of monomer/initiator. However, since the sacrificial initiator also initiates the polymerization, a bulk polymer is formed at the same time to surface grafting. Therefore, it is necessary to remove the bulk polymer from the surface, which can result in a tedious and troublesome process. The results suggested that polymerizations were controlled and the chain ends remained active for a long period of time.

Recently, Munro, Hanton, Moratti, and Robinson (2009) compared the synthesis of graft copolymers of chitosan-*g*-poly(oligoethylene glycol methacrylate) (POEGMA) via two different synthetic routes: "grafting-from" and "grafting-to". The authors succeeded in the preparation of graft copolymer by both routes, showing that PEOGMA has significant effect on the conformation and hydrogen bonding of the chitosan polymers, leading to hydrodynamic volumes of copolymers lower than chitosan. The grafting-from approach led to the preparation of graft copolymers containing unbound oligomers even after extensive washing procedures. The graft-copolymers prepared by "grafting-to" were found to display significant changes, even with a low amount of grafted polymer incorporated in the copolymers. Tang, Zhang, Zhu, Cheng, and Zhu (2009) proposed the graft copolymerization of poly(methyl methacrylate) (PMMA) and amphiphilic block copolymer PMMA-b-P(PEGMA) on the surface of chitosan nanospheres, The proposed method used iron (III)-mediated ATRP with activators generated by electron transfer (AGET). This work explores the use of iron (III)-mediated catalytic system, instead of copper(I)/copper (II) salts, due to the possible toxic effects of copper

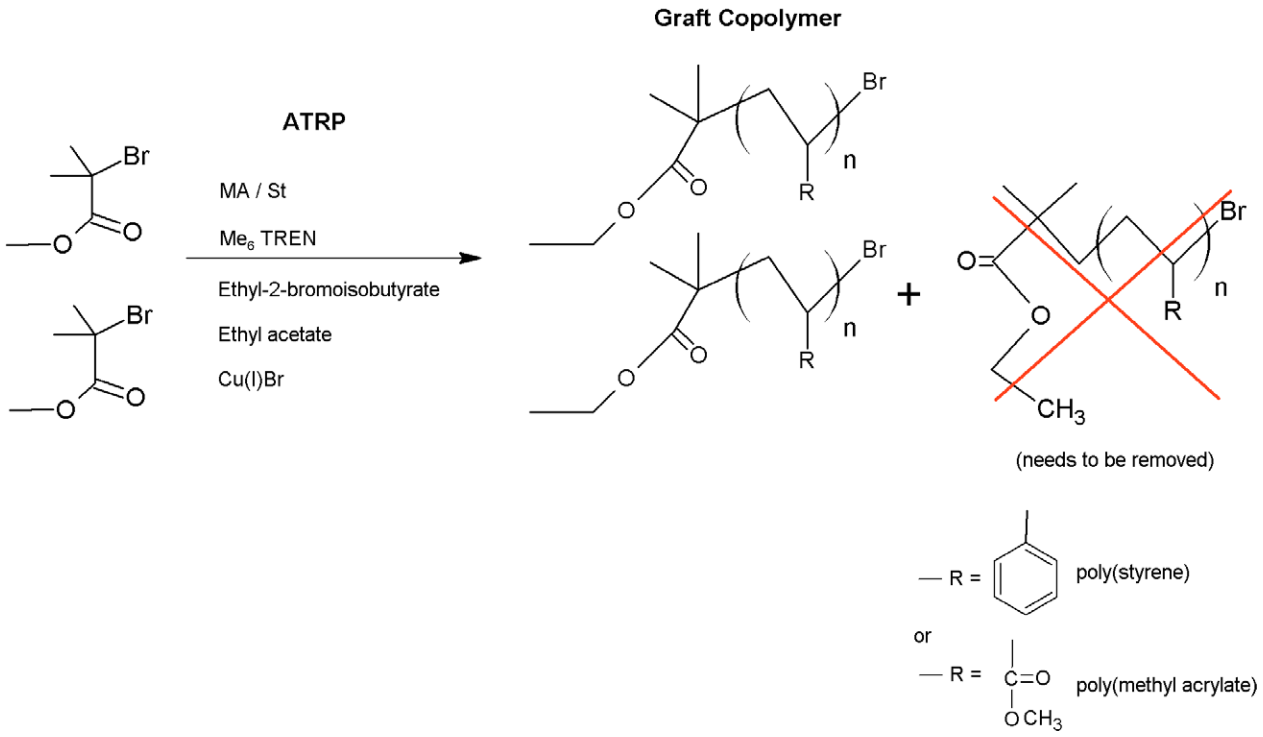

Fig. 11. ATRP of MA and St catalyzed by Cu(I)Br/Me_6TREN in ethyl acetate from chitosan macroinitiators.

to human health, when the materials are intended to be used for *in vivo* biomedical applications. The use of AGET initiating method can overcome an important drawback of ATRP, since transition metal compound in lower oxidation can be easily oxidized leading to an uncontrolled polymerization.

The chitosan nanoparticles were firstly prepared in a water/oil emulsion using glutaraldehyde as crossing agent (Tang et al., 2009). In a further step, the bromide-moistures were immobilized on the chitosan nanospheres using a similar process described in Fig. 10, in the presence of dimethyl formamide (DMF), triethylamine and 2-bromoisbobutyryl bromide. The graft copolymerization was carried out by using $FeCl_3{\cdot}6H_2O$, triphenylphosphine as ligand and ascorbic acid as reducing agent (Tang et al., 2009). The ethyl 2-bromoisobutyrate was used as sacrificial initiator (Tang et al., 2009). The kinetic studies revelead a linear first order kinetics and a linear increase of the molecular weight with the conversion. The active chains ends were successfully reinitiated by using the macroinitiators of chitosan-*g*-PMMA nanospheres for the polymerization of fresh monomers.

Regarding other widely used CLRP methods, RAFT and NMP, the available reports in the literature are, to the best of our knowledge, only three, all from the same laboratory. As for the RAFT approach, Zhu and co-authors proposed the synthesis of chitosan-*g*-PNIPAM (Tang, Hua, Cheng, Jiang, & Zhu, 2008) and chitosan-*g*-PAA (Hua, Tang, Cheng, Deng, & Zhu, 2008) using the *S*-1-dodecyl-*S*′-(α,α′-dimethyl-α″-acetic acid) trithiocarbonate as RAFT agent. Chitosan-RAFT agents was synthesized in dry DMF by reacting *N*-phthaloylchitosan with *S*-1-dodecyl-*S*′-(α,α'-dimethyl-α″-acetic acid) trithiocarbonate in the presence of 1,3-dicyclohexylcarbodiimide and 4-(*N*,*N*-dimethylamino)pyridine. Both graft copolymers (chitosan-*g*-PINPAM and chitosan-*g*-PAA) were carried out in dry DMF, using azobisisobutyronitrile (AIBN) as initiator using reactions temperatures ranging from 60 °C (Tang et al., 2008) to 80 °C (Hua et al., 2008), leading to polymers with living features.

Finally, the controlled graft modification of chitosan with polystyrene was achieved by using NMP method (Hua, Deng, Tang, Cheng, & Zhu, 2006). Chitosan-4-hydroxy-2,2,6,6-tetramethyl piperidine-1-oxyl (4-hydroxy-TEMPO) macroinitiator was obtained from the ^{60}Co- γ irradiation of *N*-phthaloylchitosan and 4-hydroxy-TEMPO in DMF. The graft copolymerization was carried out in dry DMF at 110 °C. The results indicate that the graft copolymerization was well controlled by the NMP method.

The literature available dealing with the controlled/living graft copolymerization of chitosan is very scarce, even so, it is expectable that in a near future it will be possible to establish a comprehensive understanding of the structure-properties correlation of the chitosan-graft controlled copolymers based on a rational design of the macromolecules structures.

3. Conclusions and outlook

Recently, the use of temperature and pH responsive polymers based on chitosan has shown a considerable growth, mainly in drug delivery systems. This review deals with their applicability as smart delivery systems, in tissue engineering and in regenerative medicine. Chitosan is an appropriate material for such applications, due to its biodegradability, biocompatibility and low toxicity. Chitosan is also extensively used with other polymers and copolymers, providing improvements in the performance of the materials. This is a field with plenty of options. Thus, many materials based on chitosan are being developed. The combination of the grafting approach as a tool to prepare materials that present different properties from both polymers, with the precise control over the graft polymer is a powerful strategy for the development of natural based polymer with tailor made properties. The work will inevitably lead to a greater understanding of the structure–property relationship involvement natural based polymers, as a tool for the preparation of new materials. Some problems remain to be addressed, such as: the possibility to prepare graft-copolymers with precise molecular weight and molecular weight distribution in the absence of homopolymers. However, it is expectable that in the next few years the improvement of the graft copolymerization of natural polymers techniques based on the CLRP takes place, leading to the preparation of smart polymers based on chitosan.

Grafted copolymerization by CLRP methods of monomers like NIPAM and AA among others will enable the preparation of the a variety of responsive graft copolymers with precise structure, molecular design to afford materials composed of tailored chitosan and synthetic polymers.

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