Focus on the structure and stimuli-sensitive drug delivery system applications of cross-linked ionic polysaccharides

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Abstract

The review analyses the state of the art of cross-linked ionic polysaccharides as components of drug delivery systems which can regulate drug release as a function of changes in pH, ion nature and concentration, electric and magnetic field intensity, light wavelength, temperature, redox potential, and certain molecules. Polysaccharides are gaining increasing attention as components of stimuli-responsive drug delivery systems particularly since they can be obtained in a well characterized and reproducible way from the natural sources. Ionic polysaccharides can be readily cross-linked to render hydrogel networks sensitive to a variety of internal and external variables, and thus suitable for switching drug release on-off through diverse mechanisms. Hybrids, composites and grafted polymers can reinforce the responsiveness and widen the range of stimuli to which polysaccharide-based systems can respond.

Keywords: ionic polysaccharides, cross-linked, chitosan, carboxymethyl cellulose, heparin, stimuli sensitive, drug delivery system

Introduction

It has been long time since natural polysaccharides, owing to their structural diversities and properties, have contributed immensely to the medical field. The developing nations have adopted the use of polysaccharides replacing the use of costly items widely in advanced diagnosis of a disease. Now-a-days, every effort is being made to convert the discarded wastes into useful materials from renewable sources with added properties. We are focusing here largely on the applications of polysaccharides family.

Polysaccharides may be isolated and extracted from marine, plant, animal and synthetic sources [1]. The naturally growing seaweeds are a good source of sulfated polysaccharides, as reported in most of the cases [2]. In many cases, higher plants, edible fruit, bark, fungi and bacterial sources are reported to contain polysaccharides, which was extracted using standard procedures and cheap solvents, thereby discarding the other by-products in the process. After the extraction process is over, chemical profiling, Smidth degradation and linakge pattern determination is mostly done to establish the structure of the extracted polysaccharides from the natural or synthetic sources [3]. The proposed structure is thereafter confirmed from IR, NMR and GC-MS spectroscopic studies, compared to standard monosaccharides [4]. In fact, these polysaccharides may also be synthesized of diverse architecture with desired molecular weight and functional group. It is due to the coupling of the organic chemistry with the polymer science that has led to the formation of several new materials [5].

The polysaccharides and its composite materials were used in the aquatic feeds and agricultural by-products in the last two decades. With passing time, gradually the synthetic materials have been replaced by these composite polysaccharide materials synthesized with the help of pharmaceutical technology. There has been an increase in the search of new materials using biomedical and pharmaceutical technology produced from daily household waste materials which in turn would additionally reduce the large accumulation of unutilized waste. The polysaccharides are also found bio-compatible due to their similarity in structure in many components in plants, animals and human systems. In food processing industries, there have been reports where the polysaccharides were used as thickeners, gelling agents, binders, etc. in syrups, jellies and other edible foods. Similarly, using the same functionalities and pharmaceutical technologies polysaccharides composites finds potential applications in various solid-liquid formulations as fillers, binders and thickeners for specific drug delivery systems [6].

The term polysaccharides refers to carbohydrate molecules and consists of repeating units of either same or different monosaccharides. Due to the presence of a number of functional reactive groups, variation in molecular weight (MW) and sugar composition, the polysaccharides may have different structures with a wide diversity and property. Now, they may also be classified as polyelectrolytes and non-polyelectrolytes,

which are further, divided into negatively charged polysaccharides (pectin, alginate, etc.) and positively charged polysaccharides (chitosan, etc.). Polysaccharides may be chemically and biochemically modified into its derivatives due to the different functional groups in their chains. These are naturally available, in plenty highly safe, stable, non-toxic, low-cost in processing, bio-degradable and hydrophilic mainly due to the presence of polar functional groups in their structures. It is mainly due to these properties that helps polysaccharides and their derivatives to function as stimuli sensitive and nanoparticle drug delivery system [7].

The aim of this review is to highlight the chemical profiling of the structures of cross-linked ionic polysaccharides and its application towards nanoparticle and stimuli responsive drug delivery systems. In the later part, this review also provides a detailed outline of the possibilities of ionic polysaccharides functioning as drug delivery systems to different environmental stimuli such as temperature, pH, electric field, ionic strength, etc. with a target to provide a functional group based selection criteria for selection of a particular class of polysaccharides for a suitable diagnosis. In this way, different polysaccharides may be assigned for use in specific drug delivery systems.

Ionic polysaccharides

This type of polysaccharides is in ionic state either in their original form or may be induced in a charged state through insertion with such moieties. If uncharged polysaccharide is subjected to a substitution reaction with some charged moieties or by grafting of ionic polysaccharides with suitable ions, ionic polysaccharides may be obtained (**Table-1**). A well known commonly available cationic polysaccharides is chitosan, synthesized by partial alkaline deacetylation of chitin. The structure of chitosan is linear with random distribution of D-glucosamine and N-acetyl-D-glucosamine. The amine groups is responsible for its pH responsiveness and affinity towards oppositely charged drugs and reactivity towards cross-linking or grafting modification.

The food industries have predominantly been employing anionic polysaccharides derived from seaweeds (alginate, agar, carrageens), plant cell walls (pectin) and exudates (gum arabic) as thickening or gelling agents. It is clear from the case of alginate acquired from brown seaweeds is a linear polysaccharide made of β-D-mannuronopyranosyl (M)

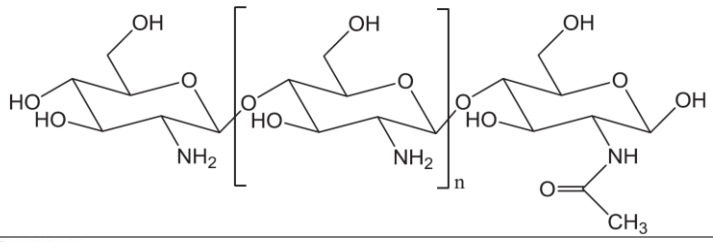
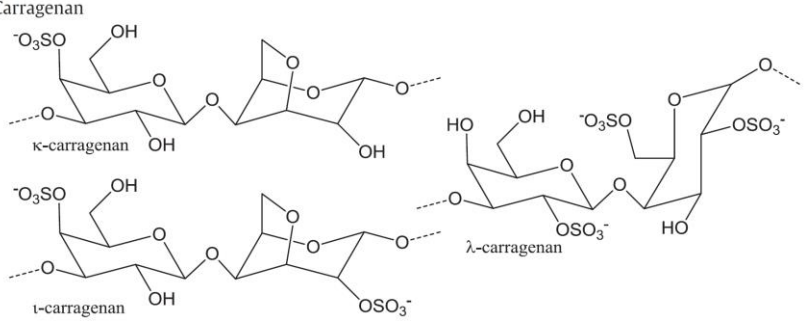
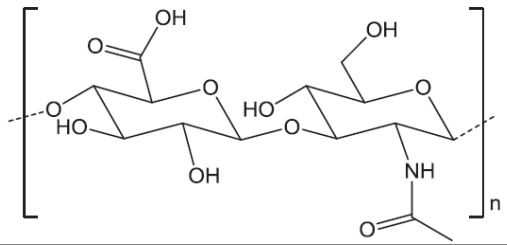
Polysaccharides	Cross-linker	Network
Neutral	Neutral	**Neutral**
Neutral	Ionic	**Ionic mono-functional**
Ionic	Neutral	**Ionic mono-functional**
Ionic	Ionic	**Ionic bi-multifunctional**

Table-1: Generation of neutral/ionic polysaccharides framework

and α-L-guluronopyranosyl (G) blocks. The different relative position of the carboxylic acid group in M and G blocks is the reason why their ratio and distribution influence the sensitiveness of alginate to pH and calcium ions [8]. In biomedical application alginate provides a wide range of probabilities of derivatization thus improving its performance [9]. The cell walls of some species of red algae or seaweeds yield agar (or agar-agar) which is widely used as an element of foods and microbial cultures, due to its performance as thickener and stabilizer. Two unbranched polysaccharides: agaropectin and agarose, which share the same galactose-based backbone heterogeneously mix to produce agar. Agarose has neutral charge and possesses extended chains while agaropectin is ponderously altered with acidic side-groups, such as sulfate and pyruvate [10]. Recently the use of agar as a component of DDSs is mostly limited to physical blendswith other polysaccharides [11-14]. Some other branched hetero-polysaccharides bearing carboxylic acid groups are pectin and gum arabic. Pectin is a polysaccharide found in the cell wall of plants in the form of free acid, simple salt, ester or amidated polysaccharide and is made up of poly (D-galacturonic acid) linked by α-(1,4) bonds and a wide variety of neutral sugars such as rhamnose, arabinose, etc. A natural polysaccharide obtained from the exudates of Acacia trees is gum arabic. Its main chain consists of 1,3-linked β-D-galactopyranosyl units with other carbohydrates such as arabinose, glucuronic acid and rhamnose.

From animal sources, the ionic polysaccharides in the form of hyaluronic acid, heparin and chondroitin sulfate are mainly obtained. Hyaluronic acid is a linear structure of glycosamino glycane. It is linked by α-(1→4) and β-(1→3) bonds alternately of the disaccharide D-glucuronic acid and N-acetylglucosamine respectively. Heparin has the

highest negative charge density of any known biological molecule and is a highly sulfated glycosaminoglycan. It finds applications as an injectable anticoagulant, and off late it is gaining interest as component of growth factor delivery systems [15,16]. Another linear polysaccharide is chondroitin sulfate which is based on the (1→3)-β-N-acetyl-D-galactosamine and (1→4)-β-glucuronic acid presenting sulfates, hydroxyl and carboxylic acid functionalities. Now, the polysaccharides obtained from microorganisms, xanthan gum, gellan gum and scleroglucan are the most important which is widely used in drug delivery. The exo-polysaccharide produced by *Xanthomonas campestris*, Xanthan gum, has a β-(1,4)-D-glucose backbone, and each alternate glucose unit has a side chain consisting of β-D-mannose-(1,4)-β-D-glucuronic acid-(1,2)-α-D-mannose **(Table 2)**.

Structure	Sources
Chitosan 	Exoskeleton of crustacean and insects or cell walls of bacteria and fungi
Carragenan 	Red seaweeds of genus Rhodophyceae
Hyaluronic acid 	Extracellular matrix of higher animals

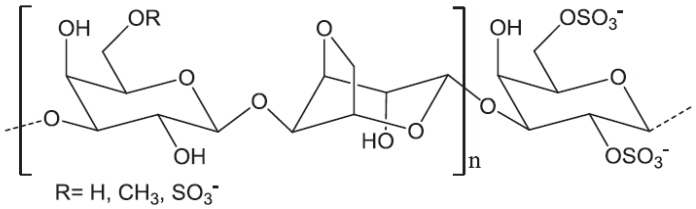
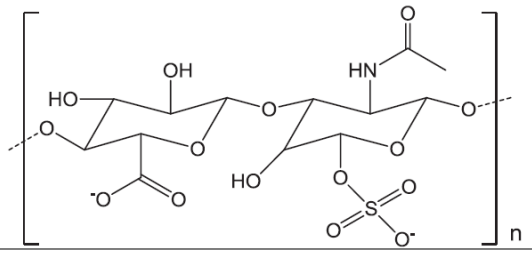
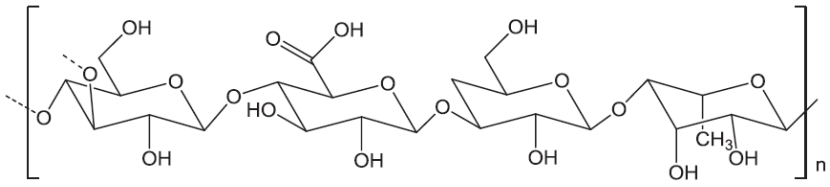
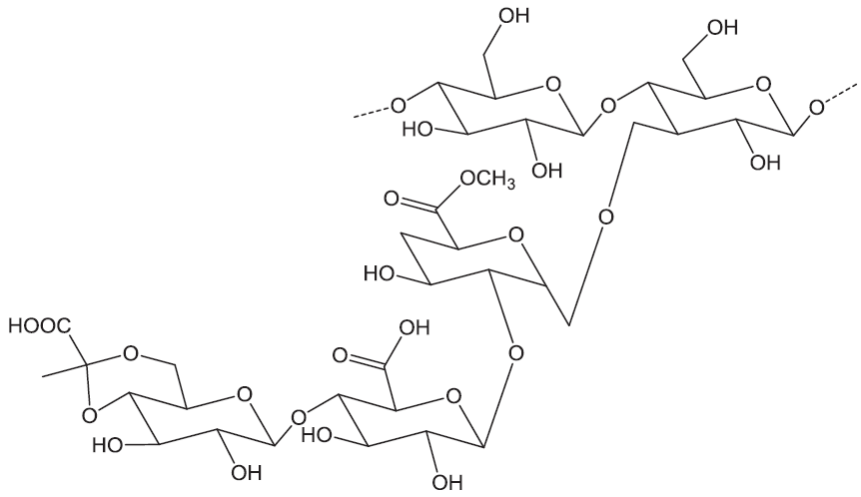
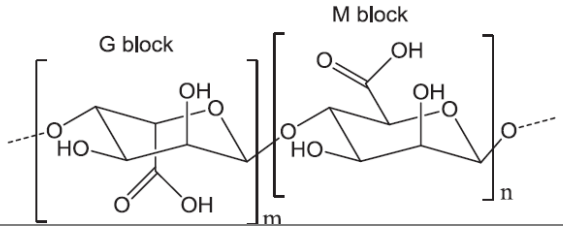
Agar 	Seaweeds of genus Gelidium, *Euchema, Gracilaria* & others
Chondroitin sulfate 	Animals & humans
Gellan gum 	Extracellular secretion of *Pseudomonas elodea*
Xanthan gum 	Microbial exopolysaccacharide of *Xanthomonas campestris*
Alginate 	Marine brown seaweeds and microorganisms

Table-2: Structure and sources of ionic polysaccharides for drug delivery

Ionically cross-linked polysaccharide nanoparticles

Among all other other crosslinking techniques, we will focus here on ionic crosslinking due to the fact that it has more advantages owing to its mild preparation conditions and simple procedures. The low molecular weight of polyanions and polycations acts as ionic crosslinkers for charged polysaccharides. The most abundantly used polyanion crosslinker is tripolyphosphate (TPP) till date and the TPP crosslinked chitosan nanoparticles were also reported [17,18]. TPP is non-toxic and can form a gel by ionic interaction between positively charged amino groups of chitosan and negatively charged counterions of TPP [19]. Its use in various drugs and macromolecules delivery is one of the most important application of the chitosan based nanoparticles. Recently, water-soluble chitosan derivatives were also found ionically crosslinked to prepare nanoparticles. In addition, N-trimethyl chitosan nanoparticles by ionic crosslinking of N-trimethyl chitosan with TPP was prepared and their potential as a carrier system for the nasal delivery of proteins, ovalbumin was evaluated by Amidi et al. [20]. From the findings, the nanoparticles were observed to be of average size ~ 350 nm and a positive zeta potential. They even showed a loading efficiency ~ 95% and a loading capacity ~ 50% (w/w). The report by Shi et al., which synthesized carboxymethyl chitosan nanoparticles ~ 200–300 nm in a narrow distribution through ionic gelification with Ca^{+2} ion and its potential of the nanoparticles as carriers for anticancer drug, doxorubicin was evaluated. The utility of Ca-crosslinked negatively charged polysaccharide nanoparticles as drug carriers have been recently found. Even some polysaccharides with -COOH functional groups on molecular chains can be crosslinked by Ca^{+2} to form nanoparticles.

In another work, carboxymethyl cellulose was used to complex chitosan to generate stable cationic nanoparticles and investigated the topical application of these nanoparticles containing plasmid DNA as a potential approach to genetic immunization as reported by Cui et al. [21] **(Figure 1)**. Plasmid DNA was coated on pre-formed cationic chitosan/carboxymethyl cellulose nanoparticles. Chen et al. also developed chitosan/dextran sulfate nanoparticle delivery system by using a coacervation process [22]. The study investigated the effect of the weight ratio of the two polymers on particle size, surface charge, entrapment efficiency and release characteristics of anti-angiogenesis peptide. The ionotropic pre-gelation of alginate with calcium chloride

followed by complexation between alginate and chitosan was used to prepare insulin loaded nanoparticles as reported by Sarmento et al. [23]. The same group probed the structural integrity of insulin after being entrapped into chitosan/alginate nanoparticles [24]. The results confirmed that no significant conformational changes of insulin occurred in terms of α-helix and β-sheet content. In a similar way, quaternized chitosan/alginate nanoparticles in neutral condition was designed for the oral delivery of protein by Li et al. [25].

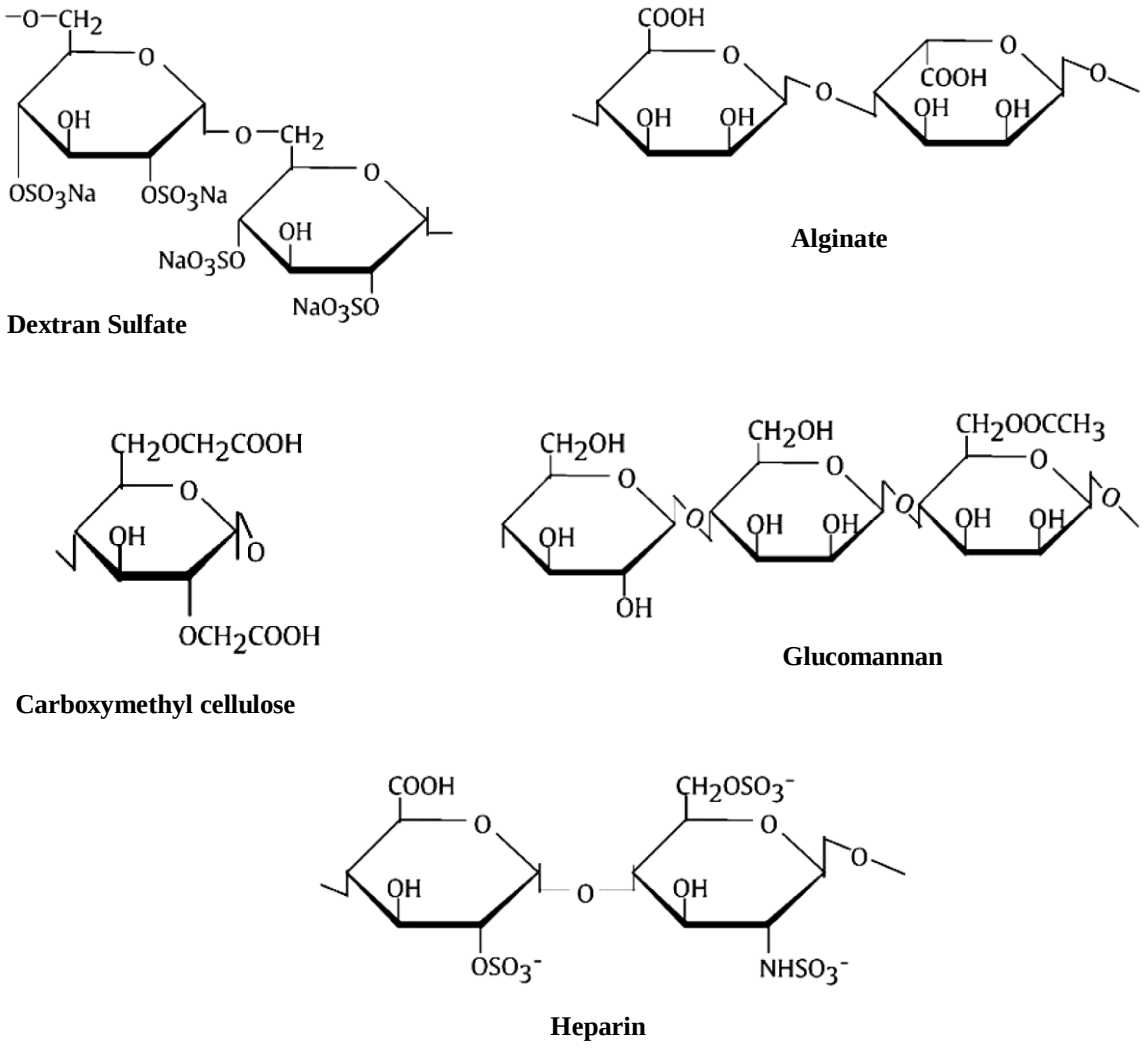


Figure 1: Scheme of chemical structures showing negative polymers complexed with chitosan.

Applications towards stimuli-sensitive drug delivery systems

Temperature sensitive network system

Ionic polysaccharides in general show very limited temperature sensitiveness. The gellan and xanthan gums undergo transitions between an ordered helix structures at low temperature to a disordered coil state at high temperature [26]. Such a transition is seen as a marked decrease in the apparent viscosity of the system, which is totally reversible without thermal hysteresis [27]. To furnish other ionic polysaccharide networks with temperature responsiveness, they have to be merged with other non-ionic polysaccharides such as non-ionic cellulose ethers [28–30] or grafted with synthetic moieties [31] and reinforce their temperature-sensitiveness. Importantly, the higher the cross-linking density of chitosan, the lower the temperature-responsiveness of the poly-(N-isopropyl acrylamide), PNIPAAm network, due to the fact that chitosan disrupts the PNIPAAm domains, which cannot cooperatively participate in the phase transition [32] **(Figure 2)**.

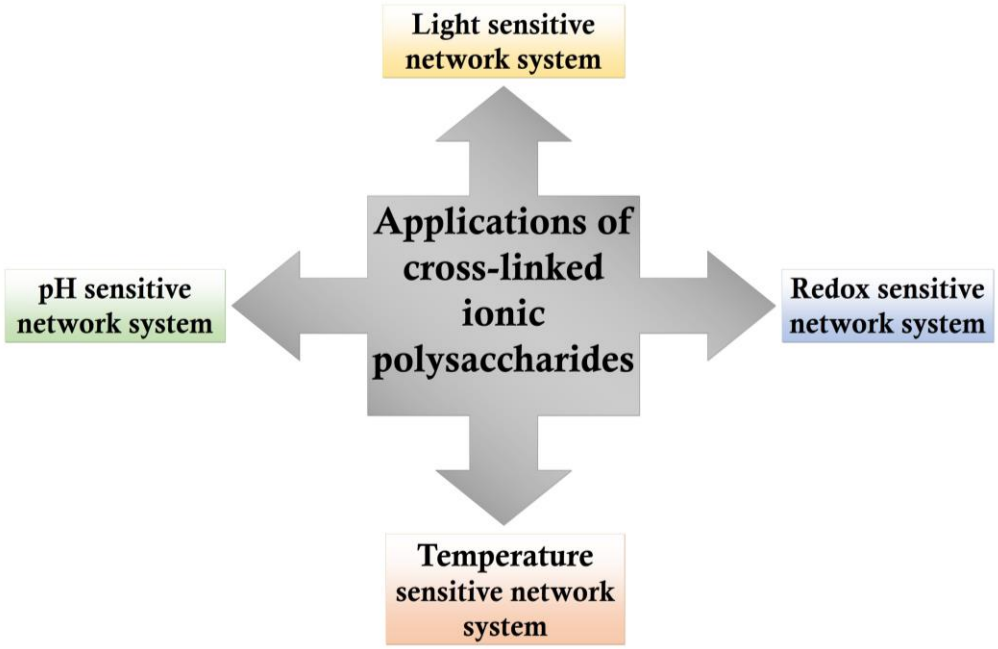


Figure 2: Applications of cross-linked ionic polysaccharides towards stimuli-sensitive drug delivery systems.

The hydrogels sensitive to temperature, in addition to pH and ionic strength, have been also obtained combining PNIPAAm with carboxymethylcellulose [33], gellan gum [34,35], xanthan gum [36], dextran [37] or alginate [38–41].

Calcium alginate/PNIPAAm bead composites with a bio-mineralized layer obtained by reaction between Ca^{2+} and HPO_4^{2-} have shown the interest of the inorganic compound to decrease the permeability of the beads [42]. In a recent study beads of polyelectrolyte complexes (obtained combining alginate, poly-(acrylic acid) and chitosan) with a bio-mineralized layer were grafted at the pores with PNIPAAm.

In another work, chitosan-based temperature responsive system is formed by glycol chitosan and benzaldehyde capped poly (ethylene oxide)-poly (propylene oxide)-poly (ethylene oxide) (GC/OHC-PEO-PPO-PEO-CHO). These materials form gels in aqueous solution instantly by means of imine linkages between the amino and the benzaldehyde groups [43,44]. Further, the hydrogels were loaded with hydrophilic doxorubicin hydrochloride and hydrophobic prednisone [43]. As environmental pH decreased, the cleavage of the benzoic–imine bonds (stable at physiological pH, but labile in acidic medium) occurred, leading to a smooth drug release.

Light sensitive network system

It has been established that polysaccharides may be supplied with light-sensitiveness by grafting method of responsive moieties. The poly-(ethylene glycol), PEG-anthracene monomer was grafted along alginate or hyaluronic backbones to produce hydrogels that change their properties depending on UV light exposure, after crosslinking with adipic dihydrazide [45,46]. On exposure of these hydrogels to the light of wavelength > 300 nm, anthracene dimerized leading to an increase in the cross-linking density. The effects of this photo-responsiveness on the release of small (Coomassie Blue) and large (myoglobin) molecules was evaluated. The slowdown of the release of small molecules and complete cease of the release of protein was due to the irradiation > 300 nm [45].

pH sensitive network system

It has been detected that chitosan network shows a typical pH-sensitive swelling, being swollen in acid medium and shrunk in neutral and alkaline medium. Thus, non-interacting drugs are released faster in pH of acidic medium [47]. These networks is prepared as bulk monoliths, and also as micro- or nano-gels in one or two steps [48]. Chitosan films have been also obtained by crosslinking with different multivalent phosphates, namely pyrophosphate (Pyro) and tripolyphosphate (TPP) [49].

In general, the combination of chitosan with neutral hydrophilic polymers enhances the responsiveness to pH [50]. Films based on blends of chitosan and polyethylene glycol (PEG) can be obtained by a casting/solvent evaporation method that promotes intermolecular hydrogen bonding [51]. The hydrogen bonds are broken in media of pH acid or with a high content in ions, resulting in a faster release of the drug loaded. Another interesting approach to the synthesis of chitosan hydrogels consists in using polysaccharides as macromolecular crosslinking agents. Carboxymethyl chitosan (CM-chitosan) is an amphoteric variety of chitosan with good biocompatibility. Glutaraldehyde cross-linked networks of CM-chitosan showed typical amphoteric character, shrinking at the isoelectric point (pH 2–4) and swelling as the pH shifts from the isoelectric point. As a consequence, protein loaded hydrogels showed faster release in higher pH buffer than in lower pH solution [52]. The anionic groups of sodium carboxymethyl cellulose (CMC) make it suitable as component of hydrogels and interpenetrating networks (IPNs) that shrink at acid pH and swell at neutral-alkaline conditions, particularly when the ionic strength of the medium is low [53].

Alginate typically forms hydrogels by means of Ca^{2+}, which positions in the interstices between G blocks, leading to an ordered conformational structure called “egg-box” array [54]. Monovalent ions (Na, K) do not render gels, but other di- and tri-valent ions (Ba, Sr, Al) are also suitable cross linkers [55, 56]. Such a peculiarity has been exploited to prepare in situ gelling formulations for ophthalmic drug delivery. The concentration of calcium ions in the tear fluid is sufficient to induce the gelling [57, 58]. Pre-cross-linked calcium alginate beads have been intensively explored for site-specific oral delivery. The drug release capacity from such hydrogels cross-linked with Ca^{2+} depends on the solubility of the drug and the pH of the medium.

Redox responsive network system

The responsiveness of the ionic polysaccharides as oxidant and reducing agents has played a major role in a way to attain feed-back regulated release or very precise site-specific delivery. The hyaluronic acid networks chemically cross-linked have been found to degrade *in vitro* by hydroxyl radicals produced by the reaction of H_2O_2 and $FeSO_4$, and *in vivo* in response to inflammation [59]. Networks of carboxymethyl chitosan and poly-(γ-glutamic acid) cross-linked with genipin have been shown to undergo conformational changes and enhanced drug release in the presence of gluconic acid, a product of glucose oxidation [60]. The use of polysaccharides with disulfide bonds is an interesting approach for the intracellular controlled release of drugs which proceeds with cleavage to thiol groups by glutathione in the cells [61]. Furthermore, the glutathione concentration is more in tumor tissues than in the healthy ones. To utilize these physiological differences, the synthesis of 6-mercaptopurine-modified carboxymethyl chitosan has been reported, using a disulfide linker, with self-assembly properties. This modified system showed pH- and glutathione-dependent release of 6-mercaptopurine [62].

Conclusion

As reviewed above, it may easily be inferred that ionic polysaccharides are inherently furnished with pH- and redox sensitiveness and these are suitable to substitute synthetic polymers in the design of stimuli-responsive drug delivery systems. Cationic and anionic polysaccharides both are found to change physiologically depending upon the pH of the medium. The ions regulate the swelling degree through osmotic effects thereby increasing the cross-linking density, thus tuning the release rate, which is very important. Besides, the affinity-controlled mechanisms may occur between the polysaccharides and oppositely charged drug molecules by means of ionic interactions. In that case, the pH-induced swelling might not play any role in the control of drug release. These features make polysaccharides suitable for site-specific oral delivery. Electric field may regulate the release of ionic drugs from topically applied or subcutaneously implanted polysaccharide gels, although composites with inorganic materials are required to endure on–off cycles. The pH/ion/electrical field responsiveness can be strengthened by grafting of ionic polymers. Grafting of suitable moieties and interpenetration with

other polymer networks are being explored to widen the sensitiveness to other stimuli such as light, temperature or redox conditions. Although hyaluronic acid or chitosan has shown certain sensitiveness to hydroxyl radicals or other products of oxidation reactions, grafting or crosslinking with molecules bearing sulfide bonds is particularly attractive for intracellular release and colonic delivery. Overall, there is plenty of information on old-known compatible multifunctional ionic polysaccharides, the recent advances in isolation, characterization and polymer chemistry are notably widening their scope of applications. Further knowledge about the biocompatibility and *in vivo* fate when repeatedly administered via parenteral route will establish the most adequate polysaccharides for each particular purpose in the near future. Cross-linked polysaccharides from these natural polymers are already in the road to offer novel and precise applications as drug delivery system.

Conflict of Interest

The authors declare that they have no conflict of interest for publishing this work in any publishing house.

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