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Review article

Structure and interactions in chitosan hydrogels formed by complexation or aggregation for biomedical applications

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Abstract

The aim of this review was to provide a detailed overview of physical chitosan hydrogels and related networks formed by aggregation or complexation, which are intended for biomedical applications. The structural basis of these systems is discussed with particular emphasis on the network-forming interactions, the principles governing their formation and their physicochemical properties. An earlier review discussing crosslinked chitosan hydrogels highlighted the potential negative influence on biocompatibility of covalent crosslinkers and emphasised the need for alternative hydrogel systems. A possible means to avoid the use of covalent crosslinkers is to prepare physical chitosan hydrogels by direct interactions between polymeric chains, i.e. by complexation, e.g. polyelectrolyte complexes (PEC) and chitosan/poly (vinyl alcohol) (PVA) complexes, or by aggregation, e.g. grafted chitosan hydrogels. PEC exhibit a higher swelling sensitivity towards pH changes compared to covalently crosslinked chitosan hydrogels, which extends their potential application. Certain complexed polymers, such as glycosaminoglycans, can exhibit interesting intrinsic properties. Since PEC are formed by non-permanent networks, dissolution can occur. Chitosan/PVA complexes represent an interesting alternative for preparing biocompatible drug delivery systems if pH-controlled release is n/ot required. Grafted chitosan hydrogels are more complex to prepare and do not always improve biocompatibility compared to covalently crosslinked hydrogels, but can enhance certain intrinsic properties of chitosan such as bacteriostatic and wound-healing activity.

Keywords: Biomedical applications; Chitosan; Complexation; Grafting; Hydrogels; Review; Structure

1. Introduction

Chitosan is a copolymer of β-[1 → 4]-linked 2-acetamido-2-deoxy-D-glucopyranose and 2-amino-2-deoxy-D-glucopyranose. This polycationic biopolymer is generally obtained by alkaline deacetylation of chitin, which is the main component of the exoskeleton of crustaceans, such as shrimps [1]. The main parameters influencing the characteristics of chitosan are its molecular weight (MW) and its degree of deacetylation (DD), representing the proportion of deacetylated units. These parameters are determined by the conditions selected during preparation but can be further modified at a later stage. For example, the DD can be lowered by reacetylation [2] and the MW can be lowered by acidic depolymerisation [3].

Chitosan is currently receiving a great deal of attention for medical and pharmaceutical applications. The main reasons for this increasing interest are undoubtedly due to its appealing intrinsic properties. Indeed, chitosan is known for its biocompatibility allowing its use in various medical applications such as topical ocular application [4], implantation [5] or injection [6]. Moreover, chitosan is metabolized by certain human enzymes, e.g. lysozyme, and can be considered as biodegradable [7,8]. In addition, it has been reported that chitosan acts as a penetration enhancer by opening epithelial tight-junctions [9,10]. Due to its positive charges at physiological pH, chitosan is also bioadhesive, which increases retention at the site of application [11,12]. Chitosan also promotes wound-healing [13,14] and has bacteriostatic effects [15,16]. Finally, chitosan is abundant

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in nature, and its production is of low cost and is ecologically interesting [17]. In medical and pharmaceutical applications, chitosan is used as a component in hydrogels.

This review is focused on chitosan hydrogels intended for medical or pharmaceutical applications. There are several possible definitions of a hydrogel; we will use the one given by Peppas [18] who defined hydrogels as macromolecular networks swollen in water or biological fluids. Examples of networks related to hydrogels that correspond to this definition will also be introduced. Due to the various possible definitions of a hydrogel, different methods of classification are possible. Based on the definition given here, hydrogels are often divided into three classes depending on the nature of their network, namely entangled networks, covalently crosslinked networks and networks formed by physical interactions. The latter class contains all the intermediary cases situated between the two other classes representing the extremes [19]. However, with respect to chitosan hydrogels, this classification is not entirely suitable. Indeed, there are no strict borders between these classes, but there is a continuum of various gels ranging from entangled chitosan hydrogels to covalently crosslinked chitosan hydrogels. Therefore, we suggest the following modified classification for chitosan hydrogels; i.e. a separation of chemical and physical hydrogels. Chemical hydrogels are formed by irreversible covalent links, as in covalently crosslinked chitosan hydrogels. Physical hydrogels are formed by various reversible links. These can be ionic interactions as in ionically crosslinked hydrogels and polyelectrolyte complexes (PEC), or secondary interactions as in chitosan/poly (vinyl alcohol) (PVA) complexed hydrogels, grafted chitosan hydrogels and entangled hydrogels. The latter are formed by solubilisation of chitosan in an acidic aqueous medium [4,20,21], which is the simplest way to prepare a chitosan hydrogel. We will not further discuss entangled chitosan hydrogels, as their use is limited by their lack of mechanical strength and their tendency to dissolve. Moreover, they do not exhibit characteristics that allow an efficient control of drug delivery or the modification of properties in response to changes in their physicochemical environment, such as pH or temperature.

In a first review, hydrogels formed by the addition of a crosslinker, namely covalently and ionically crosslinked hydrogels were discussed. It was concluded that due to the potential toxicity of free unreacted covalent crosslinkers that required a purification step during the manufacturing of hydrogels, the development of alternative types of hydrogels was desirable [22]. The aim of the present review was to provide an insight into hydrogels formed by direct interaction between polymeric chains without the addition of crosslinkers. These can be hydrogels prepared by complexation with another polymer or by aggregation after chitosan grafting. The review will discuss their structure, the nature of the network-forming interactions, as well as their physicochemical properties. Examples of systems in various stages of development for medical or pharmaceutical applications in humans will be given.

2. Polyelectrolyte complexed hydrogels

Formation of chitosan hydrogels by polyelectrolyte complexation is an interesting alternative to covalently crosslinked hydrogels. PEC are generally biocompatible networks exhibiting interesting swelling characteristics. However, the main drawback of these systems is their preparation (see principles of formation), especially in large-scale processes [23].

PEC are formed by ionic interactions as with ionically crosslinked networks [22]. Consequently, the distinction between these two types of network is faint. Indeed, crosslinking is usually considered as a bridge of MW much smaller than the MW of the chains between two consecutive crosslinks [24]; the MW of a small polymer forming a PEC and of a large molecule involved in ionic crosslinking could converge and be of a similar magnitude. However, the classification of the examples presented in this review is more straightforward. In ionic crosslinking, the entities reacting with chitosan are ions or ionic molecules with a well-defined MW [22]; in contrast, in polyelectrolyte complexation, the entities reacting with chitosan are polymers with a much broader MW distribution.

2.1. Structure and interactions

PEC are formed by reacting two oppositely charged polyelectrolytes in an aqueous solution, as shown by IR spectroscopy [23,25,26]. Such a network is formed by ionic interactions as represented in Fig. 1 and is characterised by a hydrophilic microenvironment with a high water content and electrical charge density. The electrostatic attraction between the cationic amino groups of chitosan and the anionic groups of the other polyelectrolyte is the main interaction leading to the formation of the PEC. It is stronger than most secondary binding interactions [27], such as those, for example, allowing formation of chitosan/PVA complexes or aggregation of grafted chitosan.

Moreover, additional secondary interactions such as those between crystalline domains of xylan [28] or hydrogen [29,30] and amide bonds [31] can occur between chitosan and the additional polymer. Since chitosan has a rigid, stereo-regular structure containing bulky pyranose rings [32], the formation of PEC can induce a conformational change of the other polyelectrolyte, if the latter has a non-rigid structure; e.g. α-keratose [33], poly (acrylic acid) (PAA) [34], xylan [28] or collagen [29]. However, the influence of this change on the hydrogel or polyelectrolyte properties has not yet been studied.

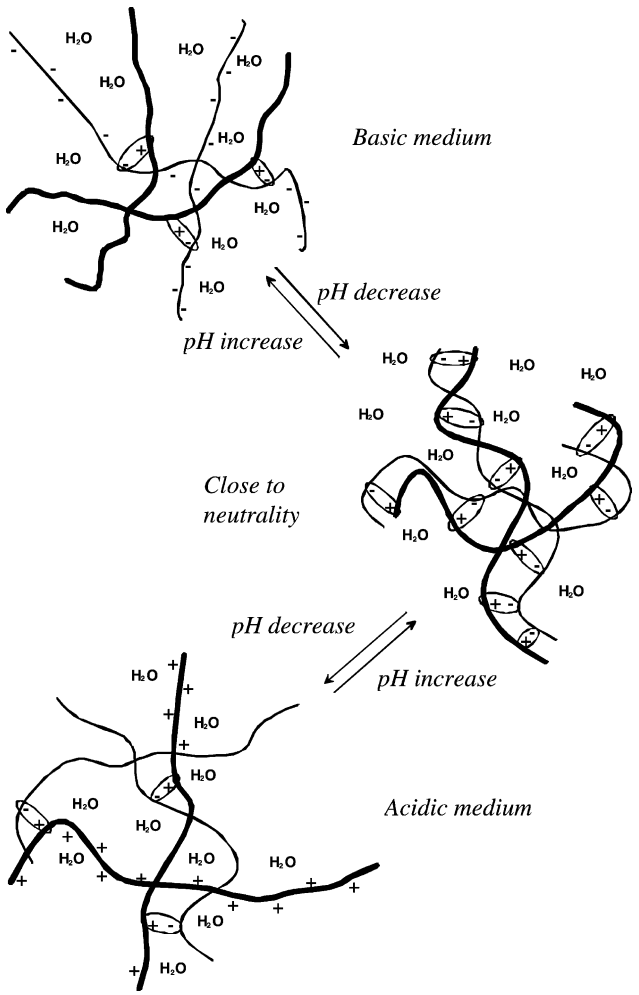

Fig. 1. Structure and pH-sensitive swelling of a polyelectrolyte complex containing chitosan; −, negative charge of the additional polymer; +, positive charge of chitosan; ⬭, ionic interaction; ▬, chitosan; —, additional polymer.

2.2. Principles of formation

The preparation of a PEC requires, besides chitosan, only a polyanionic polymer. No auxiliary molecules such as catalysts or initiators are needed and the reaction is generally performed in aqueous solution, which represents the main advantage over covalently crosslinked networks and thus favours biocompatibility and avoids purification before administration. The most commonly used polyanions are polysaccharides bearing carboxylic groups such as alginate [35], pectin [32] or xanthan [36]. Proteins, such as collagen [37], synthetic polymers, such as PAA [25], or even DNA [38] have also been investigated. Polyanions that form PEC with chitosan are listed in Table 1. PEC can also be formed by positively charged chitosan derivatives, such as glycol-chitosan [39] or *N*-dodecylated chitosan [40]. Moreover, the polyanion can be a chitin derivative bearing negative charges, as phosphated- [41], carboxymethylated- or sulfated-chitin [42]. Finally, PEC could also be formed by complexation between a polyanionic chitosan derivative with a polycation, but to our knowledge no example of such a complex has been reported in the litterature.

In order to form a PEC, both polymers have to be ionised and bear opposite charges. This means that the reaction can only occur at pH values in the vicinity of the pK_a interval of the two polymers (the macro pK_a of chitosan is about 6.5 [27]). During complexation, polyelectrolytes can either coacervate, or form a more or less compact hydrogel. However, if ionic interactions are too strong, precipitation can occur [32], which is quite common as shown in Table 1 and hinders the formation of hydrogels. Precipitation can be avoided if electrostatic attraction is weakened by the addition of salts, such as NaCl. Their presence reduces the attraction between the oppositely charged polyelectrolytes by contributing to the counter-ion environment. Hence, no phase separation occurs, and a viscous and macroscopically homogeneous blend is obtained, which may gel as temperature is lowered [43].

For PEC containing a synthetic polymer, such as PAA, the polymerisation of monomers in an aqueous solution of chitosan offers an additional means to avoid precipitation [27]. However, the addition of auxiliary molecules may modify the biocompatibility. Furthermore, since chitosan serves as a template during polymerisation, it leads to a more crystalline PEC structure [34] the degree of swelling of which is very different to that of PEC prepared by mixing the preformed polymers [25,27].

PEC can be reinforced by additional covalent crosslinking of chitosan. This is possible with chondroitin sulfate [44], collagen [45], PAA [25,46] or xylan [47] and leads to formation of semi-interpenetrating polymer networks (semi-IPN) [22]. However, the addition of covalent crosslinkers may decrease the biocompatibility [22]. PEC can also be reinforced by the addition of ions inducing the formation of ionically crosslinked systems. Ca^{2+} can be added with alginate [30] or pectin [48] and Al^{3+} with carboxymethylcellulose (CMC) sodium salt [23]. These systems are distinct from ionically crosslinked chitosan hydrogels [22], since chitosan is not crosslinked but plays the role of the additional polymer. Nevertheless, chitosan can also be ionically crosslinked, for example, in addition to the formation of a PEC with chondroitin sulfate [22,49].

Just as crosslinking density governs the properties of crosslinked hydrogels, the properties of PEC are mainly determined by the degree of interaction between the polymers. This latter depends essentially on their global charge densities and determines their relative proportion in the PEC. Indeed, the lower the charge density of the polymer, the higher is the polymer proportion in the PEC, since more polymeric chains are required to react with the other polymer. As this proportion and the chemical environment are the main factors influencing swelling, it is possible to modulate the properties of PEC by controlling

Table 1
Polyelectrolytes forming polyelectrolyte complexes with chitosan

Chemical class	Polyelectrolyte	Acidic group	Complex type	Ref.
Polysaccharide	Acacia	$-COO^-$	Precipitate	[60]
	Alginate	$-COO^-$	Hydrogel, microparticles with Ca^{2+}	[35,145]
	κ-Carrageenan	$-OSO^{3-}$	Precipitate, hydrogel with NaCl	[43,146]
	Chondroitin sulfate	$-COO^-$	Hydrogel	[62]
		$-OSO^{3-}$		
	Carboxymethyl-cellulose	$-COO^-$	Precipitate, hydrogel with Al^{3+}	[23,51]
	Chitin derivatives bearing negative charges	$-OSO^{3-}$	Hydrogel, film	[41]
		$-COO^-$		
		$-OPO^{3-}$		
	Dextran sulfate	$-OSO^{3-}$	Precipitate, hydrogel with NaCl	[43,147]
	Gellan gum	$-COO^-$	Spherical droplets	[148]
	Heparin	$-OSO^{3-}$	Precipitate	[149]
	Hyaluronic acid	$-COO^-$	Precipitate	[62]
	Pectine	$-COO^-$	Hydrogel, film, microparticles with Ca^{2+}	[32,143]
	Xanthane	$-COO^-$	Hydrogel	[36,71]
	Xylan	$-COO^-$	Hydrogel, film, microparticles with additional crosslinking	[28,47]
Protein	Collagen	$-COO^-$	Precipitate, film	[37,45,150]
	α-Keratose	$-COO^-$	Precipitate	[33]
Synthetic polymer	PAA	$-COO^-$	Precipitate	[25,26,50,151]
	Polyphosphoric acid	$-OPO^{3-}$	Microparticles	[152]
	Polyphosphate	$-OPO^{3-}$	Microparticles	[153]
	Poly (L-lactide)	$-OPO^{3-}$	Porous matrices	[154]

the complexation reaction [26]. The most important factor that has to be controlled is the pH of the solution, but temperature, ionic strength [50–53] and order of mixing [54] are also important. In addition, there are secondary factors, related to the components that have to be considered, such as flexibility of polymers [26,33], MW and DD of chitosan [32,55–57], the substitution degree of the other polyelectrolyte and the nature of the solvent [32]. PEC formation is influenced by more parameters than the formation of the other networks discussed in this review and in our previous paper [22]. This is one of their main drawbacks and explains the difficulties encountered with large scale processes during PEC preparation [23]. Consequently, it is important to prepare PEC under reproducible conditions, for example, by mixing the polymer solutions at a pH value where complexation does not occur in order to obtain a homogeneous mixture. Subsequently, the pH of the solution is adjusted to the desired value, where the interactions are formed [51]. It is also interesting to be able to follow the complexation process, which can affect polymer solubility, rheology, conductivity and turbidity of the polymer solutions [27] or the pH of the supernatant [58]. It is possible to monitor PEC formation by turbidimetry [52,56,58], rheology [26,59,60], conductimetry [61–63] or pH measurements [61,62,64]. As the monitoring of crosslinking in covalently or ionically crosslinked hydrogels has not been reported in the literature, this is an interesting advantage of PEC hydrogels that may compensate for the difficulties encountered during their preparation.

2.3. Properties and medical applications

As PEC hydrogels are formed by ionic interactions, they exhibit pH-, and to a minor extent, ion-sensitive swelling. In addition, they have a high water content and electrical charge density and allow the diffusion of water and/or drug molecules [65,66]. Moreover, chitosan is known for its biocompatibility and for its ability to promote wound healing [13,14] and both properties are maintained after PEC formation. In addition, depending on the polyanionic polymer used, these systems are generally considered as biodegradable and biocompatible [57,65,67]. Therefore, chitosan hydrogels formed by PEC are well tolerated systems [65,66] and can be used in various applications such as drug delivery systems, in cell culture and enzyme immobilisation or for tissue reconstruction and wound-healing management.

2.3.1. pH-sensitive swelling and drug release

PEC can be used to prepare drug delivery systems, the swelling and release profiles of which can be modulated by appropriate selection of preparation conditions. Essential properties of PEC used in controlled release systems are summarised in Fig. 2. As with ionically crosslinked hydrogels [22], PEC exhibit pH-sensitive swelling not only in acidic but also in basic conditions. As pH changes after administration, the charge balance inside the gel and therefore the degree of interaction between the two polymers is modified and swelling occurs because of the dissociation of

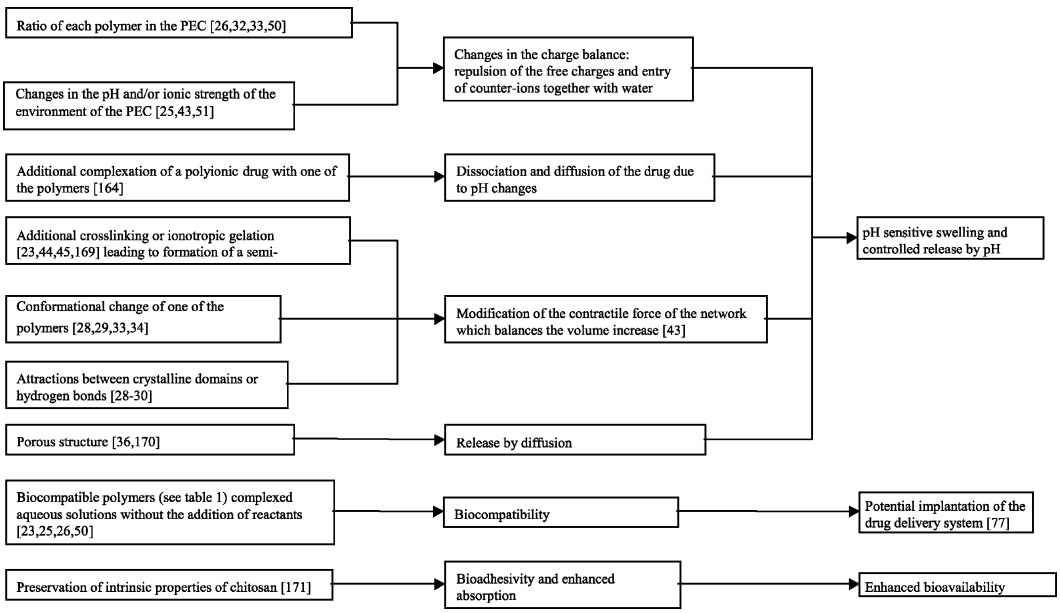

Fig. 2. Essential properties of polyelectrolyte complexes containing chitosan used in controlled release systems [169,170,171].

the complex. In acidic medium, the polyacid is neutralised and due to the free ammonium groups of chitosan, free positive charges appear inside the gel. Their mutual repulsion and the entry of water together with counterions to neutralise these charges cause swelling. However, for prolonged immersion times in water, shrinkage can be observed as a result of the segmental mobility of the polyelectrolyte chains in the swollen state, which allows the completion of the inter-polyelectrolyte reaction [51,68]. In basic medium, the mechanism is the same but swelling is induced by the free negative charges of the polyacid [43,68–70]. Knowing this mechanism, it is logical to admit that swelling is also ionic-sensitive and that the swelling rate, when the pH changes, is controlled by the diffusion of mobile ions and changes in the degree of ionisation [71]. Moreover, osmotic pressure and electrostatic repulsion responsible for swelling are balanced by the contractile force of the network, which depends on elasticity [43]. This latter determines the maximum degree of swelling, which can vary considerably [25,43,69]. As swelling of PEC is influenced by many factors, fine modulation of drug release is possible. If swelling becomes too important, dissolution of the complex can occur at certain pH values if the global charge density of one of the polymers is no longer sufficiently high to ensure complexation [50]. This happens with PEC containing PAA [25], xanthan [69] or xylan [47] but additional covalent crosslinking of chitosan can prevent dissolution [25,47]. However, the introduction of a covalent crosslinker leads to problems regarding biocompatiblity [22] and should be avoided in a PEC whenever possible. Finally, particularly in chitosan/dextran sulfate PEC, release is due to pH-sensitive shrinking [72] instead of swelling. Examples of chitosan PEC as pH-sensitive drug delivery systems are given in Table 2. It should be noted that drug delivery from certain hydrogels has already been tested in vitro, while other hydrogels represent only potential drug delivery systems for the moment.

2.3.2. Cell culture and enzyme immobilisation

Chitosan hydrogels formed by PEC exhibit interesting properties as scaffolds in cell culture and enzyme immobilisation. They can form networks to stabilise cells or enzymes, allowing diffusion of substrates, products and additives for cell culture such as dexamethasone or L-ascorbic acid [42]. The relevant properties for these applications are summarised in Fig. 3. It is possible to further improve the scaffolds by enhancing the swelling capacity, the permeability and the mechanical strength of the PEC via ionic crosslinking [23,73]. Examples of applications are given in Table 2. As no potentially toxic auxiliary molecules or covalent crosslinkers are added to these hydrogels, they surely represent a better medium for cell culture than covalently crosslinked hydrogels, presented in a previous paper [22]. However, their secondary interactions could not completely prevent dissolution and release of incorporated cells in extreme pH conditions.

2.3.3. Tissue reconstruction and wound healing

The glycosaminoglycan (GAG) analogous structure of chitosan is interesting for the preparation of chitosan hydrogels formed by PEC with GAG polyanions found in the cartilage or skin matrix, such as chondroitin sulfate or hyaluronic acid. These hydrogels can be specifically used in cartilage reconstruction and wound-healing. For these particular applications, their intrinsic properties have no equivalent among the other chitosan hydrogels. Indeed, they mimic the GAG-rich extracellular matrix of the articulatory chondrocytes [74]; the cells producing the cartilage matrix

Table 2
Examples of polyelectrolyte complexes containing chitosan and their uses (start)

Polyelectrolyte	Controlled release system	Enzyme and cell support	Tissue reconstruction: bone scaffold and bandage	Various
Alginate	Gel microparticles for the controlled release of nicardipine HCl [73].	Gel microparticles for cell culture or microencapsulation of biochemicals [73].	Sponges impregnated with silver sulfadiazine and dehydroepiandrosterone (DHEA) [35].	Hydrogel for the coating of seeds and food [30]. Membrane for the separation of [methyl tert-butyl ether] and methanol mixtures [155].
Chondroitin sulfate	Hydrogel for the controlled release of paracetamol or prednisolone [156]. Gel beads for the subcutaneous drug delivery of prednisolone [67].	Hydrogel for the engineering of cartilage-like tissue [74].	Carrier gel for the transplant of autologous chondrocytes [74]. Matrix for reconstruction of skin from co-cultured human keratinocytes and fibroblasts on a dermal substrate [44].	n.r.
Carboxymethyl-cellulose	Membranes for the controlled release of drugs or agricultural pesticides [51].	Hydrogel for yeast cell immobilisation in ethanol production [23].	n.r.	n.r.
Chitin				
carboxymethylated	n.r.	Matrix for the culture of human periodontal ligament fibroblasts [42]. Martix for the culture of rat osteoblasts [41].	n.r.	n.r.
phosphated	n.r.	Matrix for the culture of rat osteoblasts [41].	n.r.	n.r.
sulfated	n.r.	Matrix for the culture of human periodontal ligament fibroblasts [42].	n.r.	n.r.
Dextran sulfate	Hydrogel for oral drug delivery [72].	n.r.	Hydrogel for dermal wound healing [157]. Membrane for controlling the proliferation of vascular endothelial and smooth muscle cells [158].	Teat dipping gel solution for lactating animals [159]. Membranes used in hemodialysis [54,160]
Gellan gum	Capsules for the incorporation of anionic drugs [148].	n.r.	n.r.	n.r.
Heparin	n.r.	n.r.	Membrane for dermal wound healing [86].	Teat dipping gel solution for lactating animals [159] Membranes used in hemodialysis [149,160]
Hyaluronic acid	n.r.	n.r.	Film or sponges for wound healing [66].	Recovery of hyaluronic acid after its biotechnological production [161].
Pectine	Gel particles for targeted drug release in colon [48,143].	n.r.	n.r.	n.r.

Xanthane	Cream for the controlled release of vitamins, amino acids, nucleic acids or polypeptides [162]. Hydrogel for zero order release of theophylline or isosorbite dinitrate [163] Neomycin hydrogel for the treatment of infections in ophthalmology or gastroenterology [164] Subcutaneuous implants for the controlled delivery of drugs [65].	Scaffold for immobilisation of enzymes, such as xylanase, lipase, protease and hemicellulase [36].	Bandage formed by impregnation of woven cotton [76].	n.r.
Collagen	Membrane for controlled drug delivery of propranolol [45,150].	Skin analogue for *in vitro* toxicological tests [44]	Wound covering for patients suffering extensive burns [44].	n.r.
Poly (acrylic acid)	Film for the transmucosal drug delivery of triamcinolone acetonide [165].	n.r.	Hydrogel for wound dressing [27].	Complexes for the clarification of beverages [58].
Polyphosphoric acid	Gel beads for the sustained release of 6-mercaptopurine in gastro-intestinal tract [152].	n.r.	n.r.	n.r.
Polyphosphate	Gel beads for controlled release of 6-mercaptopurine [153].	n.r.	n.r.	n.r.

n.r., no available reference.

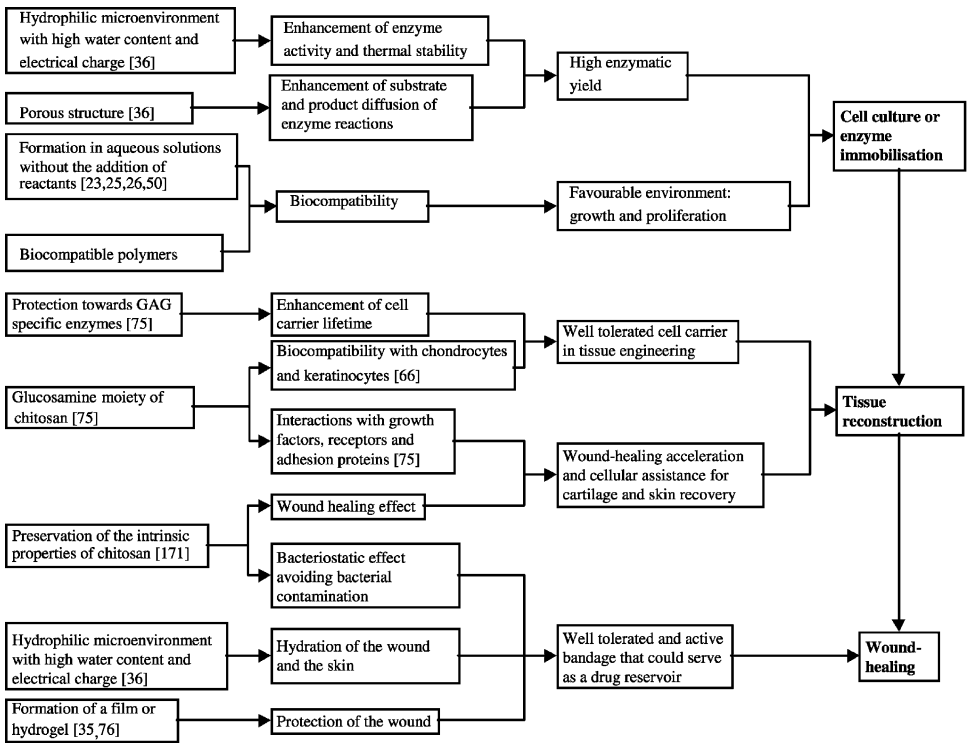

Fig. 3. Essential properties of polyelectrolyte complexes containing chitosan used in enzyme immobilisation, cell support, tissue reconstruction or wound healing.

allow their culture and can be used as a cell-carrier [61,75]. In addition, the GAG incorporated in PEC are protected from their specific hydrolytic enzymes, increasing their lifespan until incorporation in the cartilage matrix after their release, which is ensured by PEC dissolution. During dissolution, chitosan is also released and incorporated into the cartilage matrix, where it may have bioactivities related to its analogous structure, such as specific interactions with growth factors, receptors and adhesion proteins [75]. Therefore, the use of chitosan, in association with cartilage components such as chondroitin sulfate or hyaluronic acid is a logical approach for improving cellular assistance for cartilage recovery [62,66,75]. In vitro tests have shown that these PEC can be used as a carrier material for the transplantation of autologous chondrocytes and/or as a scaffold for the tissue engineering of cartilage-like tissue [74]. Interesting properties of PEC in cartilage reconstruction are summarised in Fig. 3 and examples of applications are given in Table 2.

Chitosan/hyaluronic acid PEC hydrogels allow the culture of another specific cell type, namely keratinocytes, the cells producing the skin matrix. This complex has been shown to be efficient in rats for wound-healing acceleration after skin ablation in the absence of inflammatory reactions and toxicity to the animal [66]. It is also possible to add collagen or chondroitin sulfate to chitosan in order to allow the reconstruction of skin from co-cultured human keratinocytes and fibroblasts [44]. However, although in vitro tests gave interesting results, in vivo tests in mice have shown that chitosan alone was more efficient for cell culture or wound-healing applications compared to PEC with chondroitin sulfate or hyaluronic acid [66]. Consequently, the use of such complexes rather than chitosan alone is still questionable and requires further investigation. PEC containing chitosan and a polyelectrolyte other than a GAG can also be very interesting in wound healing. Indeed, chitosan is known for its bacteriostatic effects [15,16] and for promoting wound-healing [13,14] and these properties are preserved during the preparation of a PEC. Therefore, when combined in a PEC with another biocompatible polymer, it allows the formation of a ‘bandage’ or particles which protect the wound, accelerate healing and prevent bacterial contamination [35,76]. Moreover, it can serve as a drug reservoir [76] and because of the high water content of the system, the skin is well-hydrated. Interesting properties of PEC for wound-healing are summarised in Fig. 3 and examples of use are given in Table 2.

3. Chitosan/poly (vinyl alcohol) complexed hydrogels

The biocompatible chitosan/PVA complex is similar to PEC with respect to its structure, properties and applications but is formed by distinctly different interactions.

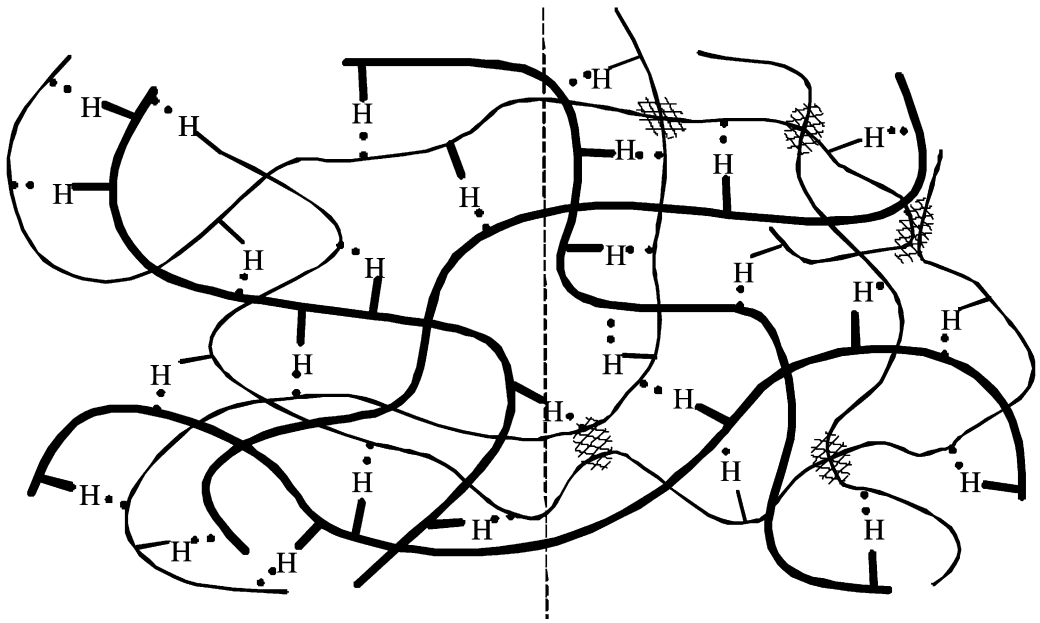

Fig. 4. Structure of a chitosan/poly (vinyl alcohol) (PVA) complexed hydrogel; (left) prepared by the autoclaving method; (right) prepared by the freeze–thaw method; – H◆◆, hydrogen bridge; , crystallite junction zone; ——, chitosan; ▬, PVA.

3.1. Structure and interactions

The structure of chitosan/PVA hydrogels, represented in Fig. 4, can be considered as an intermediary stage between PEC and networks formed by grafted chitosan discussed in the next section. Similarly to PEC, their network is formed by a complex of chitosan and an additional polymer that directly interact together. However, as with grafted chitosan networks, this interaction is via secondary and not via ionic interactions.

Chitosan/PVA complexes can be easily prepared by two methods, namely the autoclaving and the freeze–thaw method. Depending on the method that is used, the structure of the complex is slightly different. As represented in Fig. 4a, the main interactions inside a complex formed by the autoclaving method are hydrogen bonds. These interactions occur between hydroxyl groups of PVA and hydroxyl or amino groups of chitosan [77]. In addition to these interactions, crystallite junction zones between PVA polymeric chains are formed with the freeze–thaw method (Fig. 4b). In pure PVA hydrogels, these zones consist of two to three similar ordered chains with about 42–120 similar segments [78] but the addition of chitosan leads to the formation of a material with a less regular structure [79].

3.2. Principles of formation

In order to prepare a chitosan/PVA complex, only PVA and chitosan are needed. As with PEC, no auxiliary molecules or crosslinkers are required. PVA is a synthetic polymer containing mainly 1,3-glycol units and a low percentage of 1,2-glycol units. Like chitosan, it is non-toxic, biodegradable and highly biocompatible [80,81]. It is one of the most used synthetic polymers available for biomedical applications, for example, as a viscosifier in ophthalmic solutions [82]. Chitosan/PVA hydrogels are composed of well-known biocompatible components and their methods of preparation are easy and ensure their biocompatibility. These are the main advantages of these hydrogels.

Hydrogels prepared by the autoclaving method are formed by simply mixing and autoclaving PVA and chitosan solutions, which produces a highly elastic hydrogel [83]. Hydrogels prepared by the freeze–thaw method are formed by repeated freeze–thaw cycles of a chitosan/PVA aqueous solution [79]. Whatever the method used to prepare a chitosan/PVA complex, the ratio of both polymers influences their degree of interaction and therefore the structure and properties due to the perturbing effect of chitosan on the PVA network [77,79]. Moreover, the gelling process of hydrogels formed by the freeze–thaw method is favoured when the PVA concentration increases and the thawing rate decreases [84].

3.3. Properties and medical applications

The properties and applications of chitosan/PVA complexes are dependent on the method of preparation. Complexes formed by the autoclaving method are easily soluble in acidic conditions [85] and therefore not suitable as drug delivery systems. Complexes formed by the freeze–thaw method are less soluble, although solubilisation can occur via a three-step mechanism, i.e. detachment, diffusion and disentanglement [86]. Complexes formed by the autoclaving method are mainly used

as scaffolds in cell culture and those formed by the freeze–thaw method as drug delivery systems.

3.3.1. Complexes formed by the autoclaving method as scaffolds in cell culture

As chitosan is less hydrophilic than PVA, it is concentrated on the air-surface side of the hydrogel formed by complexation. Distribution on the surface becomes uniform when concentration is increased [77]. Due to the electrostatic interactions formed between the amino groups of chitosan and the cells, this concentration of chitosan on the surface of the hydrogel favours cell attachment [83]. Moreover, the higher the chitosan ratio, the higher the water content, which enhances cell growth rate [80]. Such complexes can be used, for example, in fibroblast cultures, where they have been shown to provide a better scaffold than collagen [80]. Like PEC, chitosan/PVA hydrogels certainly represent a better medium for cell culture than covalently crosslinked hydrogels. However, dissolution and unpredictable release of cells cannot be completely prevented. In addition, due to the required autoclaving procedure during the preparation of the hydrogels, cells cannot be incorporated during their formation. Consequently, cells have to be added afterwards and are concentrated on the surface of the system instead of being dispersed inside its network.

3.3.2. Complexes formed by the freeze–thaw method as drug delivery systems

In a chitosan/PVA complex formed by the freeze–thaw method, the addition of chitosan decreases the PVA degree of cristallinity and perturbs the formation of a regular PVA network [77]. This complex with a less regular structure forms a hydrogel with a high capacity to swell, which is an important property for drug delivery systems [79]. Since there is no pH-sensitive swelling, drug release is diffusion-controlled and modulated by the chitosan ratio [87]. Therefore, chitosan/PVA hydrogels represent an interesting biocompatible alternative to the other types of hydrogels discussed in this review, but are not as versatile. Due to the intrinsic properties of chitosan, its inclusion favours the adhesion of the system at the site of administration and the bioavailability of poorly absorbable drugs is enhanced as shown after oral administration of chitosan/PVA gel-spheres in rats [88]. Such complexes can, for example, be used for the controlled release of growth hormones [87] and for oral administration of theophylline or ampicillin [88].

A minor drawback of chitosan/PVA complexes is that they release a higher amount of PVA compared to pure PVA hydrogels [79]. However, since PVA is biocompatible and biodegradable this should not reduce biocompatibility.

4. Grafted chitosan hydrogels

Chitosan bears two types of reactive groups that can be grafted. First, the free amino groups on deacetylated units and second, the hydroxyl groups on the C_3 and C_6 carbons on acetylated or deacetylated units. Grafting of chitosan allows the formation of functional derivatives by covalent binding of a molecule, the graft, onto the chitosan backbone. The occurrence of grafting has been demonstrated by IR [89–91] and NMR spectroscopy [92–94]. Grafting or functionalising chitosan is a common way to improve chitosan properties such as increasing chelating [95] and complexation properties [96], solubility in water [97,98] or in organic solvents [97,99], bacteriostatic effect [100] or absorption enhancing properties [101,102]. Although the grafting of chitosan modifies its properties, it is possible to maintain some interesting characteristics such as mucoadhesivity [103], biocompatibility [104,105] and biodegradability [106].

Grafting does not always induce the formation of a network. This review deals only with grafting that induces the formation of hydrogels or networks through the development of secondary interactions. In addition, chitosan reacetylation in order to form the so-called chitin gels [107,108] will not be treated here. Reacetylation will not be considered as a particular case of grafting, namely the grafting of acetyl groups, since it does not introduce a new function onto the chitosan backbone, but is a modification of an intrinsic property of chitosan, namely the DD. One can consider the preparation of a hydrogel by chitosan grafting as a two-step procedure. The first step is strictly speaking the grafting of a functional molecule onto the reactive chitosan groups and the second step is the occurrence of interactions leading to formation of a network by aggregation.

4.1. Structure and interactions

Although the nature of interactions leading to the formation of a network can be quite different, the structure of systems containing a grafted chitosan is very similar. As represented in Fig. 5, the network is generally formed by chitosan polymeric chains interacting together via their covalently linked grafted groups, even if interactions between chitosan and grafted groups and/or between two chitosan polymeric chains are possible. Depending on the nature of the graft, the secondary interactions occurring between grafted groups can be hydrogen bonds or hydrophobic interactions. Hydrogen bonds occur, for example, with poly (ethylene glycol) (PEG) as graft [109]. Hydrophobic interactions are responsible for the formation of a network when alkyl chains are grafted to chitosan by an acid [90,110] or an aldehyde [111] group or with 2-hydroxyethylmethacrylate [106], polyacrylic acid [112] or *O*-quinone [113] as grafts. Moreover, these interactions can arise from specific intrinsic properties of the graft. Indeed, functionalisation with Pluronic allows the formation of thermogelling systems [103] due to graft dehydration leading to increased friction and entanglement [114]. Another example is the grafting of poly

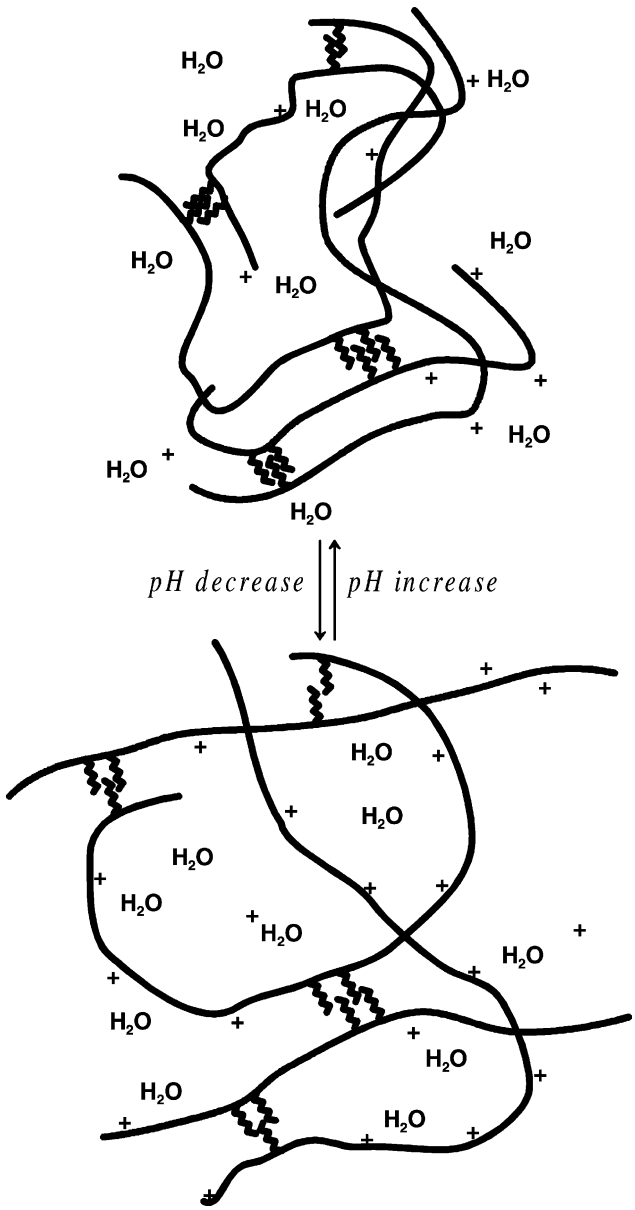

Fig. 5. Structure and pH-sensitive swelling of a system containing a grafted chitosan; ∿∿, graft; +, positive charge of chitosan; ▬, chitosan.

(*N*-isopropylacrylamide). Since it dissolves in water below 32 °C and precipitates above this temperature, it also allows the formation of a temperature-sensitive system [115]. Finally, interactions between chitosan chains can be favoured by grafting, as with carboxymethyl-chitosan, which allows association of ordered chains [116].

4.2. Principles of formation

Preparation of a network with a grafted chitosan obviously requires the use of chitosan and of molecules that will act as grafts. Moreover, auxiliary molecules can be necessary in order to catalyse the grafting reaction.

Grafting is generally performed with molecules possessing one functional group forming covalent bonds with chitosan. These can be, for example, acids [90,117], such as palmitic [110], lactic or glycolic acid [89,118,119], or also aldehydes [105,111,120]. Examples of such molecules are given in Table 3. The use of multifunctional molecules can lead to crosslinking when the concentration of the graft becomes too important, as for ethylenediaminetetraacetic acid (EDTA) which bears four carboxylic groups [121].

Particular attention should be paid to the choice of the potential graft. Indeed, the nature of the grafts determines interactions occurring between polymeric chains and therefore hydrogel properties. Moreover, the solubility of the grafted chitosan depends on the hydrophilicity of the grafted group. Obviously, grafting a hydrophilic group, such as PEG [109] or glycolic acid [118] enhances the water solubility of chitosan and allows minimisation of the required addition of acid for solubilisation. On the other hand, grafting a hydrophobic group, such as an alkyl [93,122], decreases the solubility of chitosan under aqueous acidic conditions and solubilisation could require an increased amount of acid. However, this can be overcome by the addition of a supplementary hydrophilic group, such as a carbohydrate moiety on an alkyl-chitosan [93]. Finally, the nature of the graft determines the type of the grafting reaction, either indirect or direct. Indirect reaction is the most common manner of preparing a grafted chitosan. However, it generally requires the use of auxiliary molecules to allow the reaction. These molecules are generally regarded as toxic and can be found in traces in the hydrogel before administration. These can be catalysts, initiators or organic solvents. Consequently, these hydrogels have no advantage over covalently crosslinked hydrogels with respect to biocompatibility. Examples of catalysts are sodium borohydride [123], 1-ethyl-3-(3-dimethylaminopropyl), carbodiimide [105,124], cetyltrimethylammonium bromide [111], *p*-nitrophenyl [103] or sodium cyanoborohydride, which is the most common catalyst allowing the reduction of iminium ions and the covalent linkage of the amine of chitosan with a carbon of the grafted group [92,122,125]. If the grafted molecule is a synthetic polymer and the polymerisation reaction is performed in the chitosan solution simultaneously to grafting, initiators like ferrous ammonium sulfate or potassium persulfate in the case of PAA grafting [112] or ceric ammonium in the case of poly (*N*-isopropylacrylamide) grafting [115] are required. Finally, a mixture of organic solvents is sometimes needed for the solubilisation of polymers, such as pyridine and chloroform in the case of alkyls [90]. Some indirect reactions are known to avoid the use of toxic auxiliary molecules and therefore favour biocompatibility. Indeed, grafting can be induced by irradiation, such as ^{60}Co gamma-irradiation in the case of graft copolymerisation of 2-hydroxyethyl-methacrylate (HEMA) [106]. However, irradiation is known to produce degradation of chitosan [126] and MW should be checked after such a treatment. Finally, indirect grafting can be performed by enzymes, as with tyrosinase that allows grafting of chitosan with

Table 3
Applications of grafted chitosan networks

Grafted group	Controlled release system	Various
Alkyl	Erodible hydrogel for controlled drug delivery [142].	Foam and emulsion stabilizer [122].
Aldehyde	n.r.	Hydrogel forming a biological adhesive for soft tissues [105].
Palmitic acid	Hydrogel for controlled delivery of rhodamine B [110].	n.r.
Carboxymethyl	Injectable gel for the sustained-release of morphine [104]. Hydrogels for the slow release of gentamycin [129].	Wound-healing agent [166] that can be used in reduction of periodontal pockets in dentistry [123]. Films for the longterm preservation of fruits [129]. Gel or solution to prevent post-surgical peritoneal adhesion [133].
Fructose	n.r.	Scaffold for hepatocyte attachment [132].
2-Hydroxyethyl-methacrylate (HEMA)	Blood compatible film for the pH controlled delivery of glucose [167].	n.r.
Lactic and/or glycolic acid	Hydrogel for drug delivery in the stomach [119].	n.r.
Pluronic	Hydrogel for nasal drug delivery of anti-asthma protein drugs [144]. Bioadhesive hydrogel for drug delivery in the eye, nose or vagina [103].	n.r.
Ethylenediaminetetraacetic acid (EDTA)	Bioadhesive hydrogel for delivery of peptide drugs in the stomach [121].	Constituent of hydro- and hydroalcoholic gels for topical use [124].
PEG	Gel particles for sustained release of insulin [168] or *N*-phenyl-1-naphthylamine [109].	n.r.
PEG + galactosyl	Gel particles acting as gene carriers for hepatocyte targeting [131].	n.r.

n.r., no available reference.

p-cresol [127], hexyloxyphenol [113] or chlorogenic acid [128] via formation of *O*-quinones. Direct grafting does not require the addition of auxiliary molecules and the reaction takes place in common solvents. This should favour the biocompatibility of the system, but is quite rare and only possible by direct reaction of chitosan with some acyl groups [117], lactic and/or glycolic acid [89]. An alternative method is to use a more reactive chitosan derivative instead of chitosan, such as glycol chitosan in the case of direct grafting with palmitoyl groups [110]. Although grafting onto the amino groups of chitosan is generally considered as the main reaction, grafting onto hydroxyl groups cannot be neglected [16,90,129]. Moreover di-substitution is also possible as with *N*,*N*-dicarboxymethyl-chitosan [130]. In both cases, the influence of these side reactions on the properties of the chitosan derivative should be determined. Finally, it is possible to graft different molecules along the same chitosan backbone, as during formation of galactosylated chitosan-PEG [131].

The intermolecular interactions between different polymeric chains induced by grafted groups, can lead to aggregation, and if the density of these interactions is large enough, it gives rise to a polymeric network, such as a hydrogel. Indeed, at low concentration, the behaviour of a grafted chitosan solution is similar to a moderately concentrated polymer solution but there is a sol–gel transition when concentration increases [116].

Further reactions, like covalent crosslinking can also be performed after grafting. For example, after formation of poly (*N*-isopropylacrylamide)- [115], fructose- [132], or *N*,*O*-carboxymethyl-chitosan [133]. Such systems have a structure close to chitosan crosslinked with itself [22].

The properties of the network depend on the interactions occurring between polymeric chains, due to grafting. Therefore, it is important to modulate their density since it allows the modulation of properties, such as swelling and drug release. This density is mainly determined by the nature and number of grafts (degree of substitution) and by the grafted chitosan concentration [120,122,134]. The reaction being direct or indirect, various parameters allow to influence the degree of substitution. These parameters are the ratio of each component [89,105,109], the duration and temperature of the reaction [91,112], the solvent composition [135], the catalyst concentration [112,136], the MW and DD of chitosan [16,136,137] and the nature of the graft [125,138]. One should also consider the homogeneity of the reaction, as it determines the distribution mode of

the grafted groups [112], aggregation being favoured by a homogeneous distribution [139]. If grafting is performed via irradiation, the degree of substitution is also influenced by the dose rate [135].

Besides the degree of substitution, the conditions set during grafting also determine the relative reactivity of chitosan reactive groups, namely amine and hydroxyl groups. For example, by modifying the nature of one of the reactants, one can synthesise either *N*,*O*-carboxymethyl-chitosan or *O*-carboxymethyl-chitosan [16]. In addition, the conditions set during the aggregation of the network influence the density of interactions. There are various parameters influencing aggregation, such as temperature [90,122,134], ionic strength [122,140,141] and pH [111,134] of the reaction medium, which determine the net charge of the system [92,122] and therefore the repulsion between chitosan chains. Moreover, the addition of a surfactant can allow the modulation of properties, since it can induce disruption inside the hydrogel leading to a decrease in viscosity [134].

The discussion above has highlighted the main drawback of grafted chitosan systems. Their formation requires two steps that can be influenced by many factors. Consequently, the formation of these hydrogels can be regarded as quite complex, especially when compared to ionically crosslinked hydrogels [22] or chitosan/PVA hydrogels. Therefore, the monitoring of the grafting reaction and of the aggregation of grafted-chitosan should be further investigated to overcome these problems.

4.3. Properties and medical applications

Since hydrogels containing a grafted chitosan exhibit pH- and ion-sensitive swelling, they can be used as controlled drug delivery systems. Such hydrogels are more complex to prepare relative to others treated in this review. However, grafted chitosan presents interesting properties, for example, in wound-healing, where chitosan derivatives can exhibit enhanced bacteriostatic activity. It should be noted that the biocompatibility of the following examples have not yet been assessed and that due to incoporation of toxic auxiliary molecules, the administration of such systems in humans might be problematic.

4.3.1. pH-sensitive swelling and drug release

As swelling depends on the electrostatic repulsion of the free ammonium groups, leading to chain expansion and eventually increased water uptake by the gels [89], it only occurs in acidic conditions (Fig. 5), which limits their potential applications. Repulsion is determined by the pH and ionic strength of the medium and is balanced by interactions between polymeric chains, modulated by the preparation conditions [109,112,119,122,142]. For PEC systems, dissolution occurs readily [28,75,143]. However, for systems formed by grafting, dissolution is rarely reported [109]. Indeed, interactions induced by grafted chitosans are non-ionic and therefore their density does not vary with changes in the pH of the medium, in contrast to PEC. Nevertheless, since grafted chitosan hydrogels are not formed by a permanent network, one can imagine that these systems would tend to dissolve when swelling reaches a critical level. However, to our knowledge, this aspect has not been studied in detail.

Networks containing a grafted chitosan can be used, for example, for oral [110,119] or nasal [144] administration (Table 3). In addition to their pH-controlled release, hydrogels containing grafted chitosan exhibit some advantages. They can enhance the solubilisation of lipophilic drugs in aqueous conditions. Indeed, grafting of hydrophobic molecules on the hydrophilic backbone of chitosan leads to the formation of amphiphilic polymers, which allows the incorporation of lipophilic drugs inside micelle like structures [109,117]. Moreover, the enhanced chelating properties of a grafted chitosan are useful for oral drug delivery. For example, EDTA grafted onto chitosan is able to chelate ions that are essential for the enzymatic activity of proteases and consequently protects incorporated peptide or protein drugs [121].

4.3.2. Wound-healing management

EDTA grafted onto chitosan increases the antibacterial activity of chitosan by complexing magnesium that under normal circumstances stabilises the outer membrane of gram-negative bacteria [124]. This increase in chitosan antimicrobial activity is also observed with carboxymethyl-chitosan, which makes essential transition metal ions unavailable for bacteria [123] or binds to the negatively charged bacterial surface to disturb the cell membrane [16]. Therefore, these grafted chitosans are used in wound-healing management, such as carboxymethyl-chitosan for the reduction of periodontal pockets in dentistry [123] and chitosan grafted with EDTA as a constituent of hydro- and hydroalcoholic gels for topical use [124] (Table 3).

5. Advantages and disadvantages of chitosan hydrogels formed by complexation or aggregation

Polyelectrolyte complexation occurs under mild reaction conditions, which generally furnish biocompatible PEC systems. PEC hydrogels also exhibit a highly pH-sensitive swelling due to modification of the global charge densities of chitosan and complexed polymer when the pH changes post-administration. Therefore, they can be used for pH-controlled drug delivery not only in acidic but also in basic conditions, though dissolution can occur. Ionically crosslinked chitosan hydrogels exhibit similar characteristics; therefore, the potential applications of the two types of hydrogels are very similar. Although ionically crosslinked hydrogels are extremely simple to prepare, the added polymer in PEC systems can render them preferable for

certain specific applications, e.g. GAG in tissue reconstruction. Consequently, the choice between these two types depends on the application and is a balance between the ease of preparation and the desired properties.

Chitosan/PVA complexes can be prepared with less difficulty than PEC, but not as easily as ionically crosslinked hydrogels. They do not exhibit pH-sensitive swelling and PVA does not have any specific intrinsic property, as some polyelectrolytes do. Therefore, chitosan/PVA complexes have a relatively narrow field of application. However, as the use of PVA in medical and pharmaceutical applications is well documented, chitosan/PVA complexes represent an interesting alternative to PEC and ionically crosslinked hydrogels for the preparation of biocompatible drug delivery systems if a pH-controlled drug delivery is not required.

Grafted chitosan hydrogels do not present significant advantages over covalently crosslinked hydrogels. Most grafted chitosans require the use of potentially toxic auxiliary molecules for their preparation. In addition, these hydrogels only exhibit pH-sensitive swelling in acidic conditions and are, therefore, less versatile as drug delivery systems compared to PEC or ionically crosslinked hydrogels. As their interactions are not ionic, the density of interactions should vary less than in PEC or ionically crosslinked hydrogels and therefore the risk of dissolution should be less marked. However, like other physical hydrogels, they are not formed by a permanent network and therefore cannot really overcome dissolution. Finally, the preparation of grafted chitosan hydrogels is more difficult than the preparation of chemical hydrogels or of any other physical hydrogel. Therefore, grafted chitosans should be considered as a second choice for the preparation of hydrogels, especially for drug delivery systems. Nevertheless, some grafted chitosan hydrogels can be interesting in specific medical or pharmaceutical applications if chitosan is functionalised without auxiliary molecules and if intrinsic properties such as bacteriostatic effect or promotion of wound healing are enhanced by grafting.

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