# Characterization and Properties of Some Polysaccharides Used as Biomaterials

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**Summary:** This paper described three natural or pseudo natural polysaccharides often used as biomaterials. Their development opens a large field of applications taking advantage of their specific properties (renewable, biodegradable, biologically active). They are good thickeners and/or gelling polymers in determined thermodynamic conditions. As many polysaccharides they are rich in –OH groups allowing stabilization of H bonds network and giving good film forming properties. The polysaccharides described in this paper are water soluble and have a net charge which allows to form electrostatic complexes which are much developed for biomedical or pharmaceutical applications.

**Keywords:** alginate; biomaterials; chitosan; hyaluronan; physical gel

## Introduction

Polysaccharides are usually obtained by biosynthesis in plants including algae or animals; some microbial polysaccharides are also now developed such as bacterial hyaluronan (HA), gellan or xanthan.[1,2] Few of these polysaccharides are considered as good basis for biomedical applications with alginates, HA or chitosan. Some of them also have recognized biological activity as described for chitosan or HA.[3] The large amount of polysaccharides with different chemical structures and physical properties constitutes a source of materials for more applications in the future especially in the domain of biomaterials for tissue engineering, drug vehicle, viscosupplémentation.... In this paper, we intend to describe the main characteristics and applications of few natural or pseudo natural polysaccharides.

### A. Origin and Original Properties of Polysaccharides

In this paper, we will focus on the water-soluble polysaccharides which are characterized by some general properties; the main sources and types of natural polysaccharides are mentioned in Table 1. Considering the polysaccharides used in the biomedical and pharmaceutical domains, it is necessary to present few polysaccharides from animals, plants (especially algae) and bacterial sources.

Red seaweeds contains agar, carrageenans and few other polysaccharides included under the category of sulfated polysaccharides. Brown algae produce alginates, a carboxylic polymer, initially under different ionic forms.

The three main commercial seaweed polysaccharides are agars, alginates and carrageenans. They are used mainly for their thickening and gelling properties depending on the thermodynamic conditions and on their molecular structures; this behaviour will be discussed in the following.

The physical properties of alginates depend on the chemical structure (M/G ratio of mannuronic/guluronic acids) but also on the microstructure such as the distribution and length of M and G groups blocks; the techniques for characterisation are discussed later. An ionic selectivity exists among divalent counterions (such as $Ca^{+2}$ or $Ba^{+2}$) and will be presented in relation with the mechanism of gelation.

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***Table 1.***
Main sources of polysaccharides for biomedical applications.

| Sources of polymers | Polysaccharides produced |
|---|---|
| Plants: | |
| Algae | Alginates, carrageenans, agarose |
| Tubers | Konjac gum |
| Seeds | Galactomannans (tara, carob, locust bean, guar) |
| Primary cell walls | Pectins |
| Animals: | |
| Crustaceous | Chitin and derivative chitosan |
| Umbilical rod, vitreous humor and roster combs | Hyaluronan |
| Bacteria | Xanthan, hyaluronan, gellan, succinoglycan |

Agarose forms strong thermoreversible gels based on cooperative H-bond interactions between double helices as well as κ and ι-carrageenans, which are sulfated galactans; all of them are (AB) alternating copolymers and when 3,6-anhydrogalactose is present, gelling properties are preponderant; they are good thickener or gelling additives. In this case also, the ionic selectivity in the presence of monovalent counterions is important to control the physical properties and especially the gelation. These polymers are widely used in many applications. The encapsulation of cells is one of the important use.[4]

Plants also produced interesting polysaccharides from which pectins which are found in the middle lamella and primary cell walls of plant tissues. They are linear polymers made of blocks of (1-4)-α-D-galacturonic (with different yields in methoxyl ester, DE) and ''hairy'' rhamnogalacturonan regions representing on the average 15 weight percent.[5] When the DE is low, the physical properties of pectins and especially gelation proceeds by the same mechanism as alginates in the presence of divalent cations.

Seeds produce galactomannan, a linear neutral polysaccharide, having a mannan (M) backbone and galactose units (G) as side groups; the physical properties depend on the M/G ratio, the larger solubility corresponding to the lower M/G ratio.[6] It forms well known molecular complexes with xanthan whose stability depends on the G content in galactomannan and xanthan acetyl content.[7]

Glucomannans (GM) are extracted from tubers or roots ; the most studied is konjac glucomannan obtained from the tubers of the amorphophallus konjac plants.[8] The linear chain is made of mannose and glucose in a heterogeous arrangement; it has few acetyl groups. When treated by NaOH, the acetyl groups are taken off and glucomannans forms strong gels.[9] Therefore, konjac is promising in varied fields, such as food industry, medicine industry, chemistry industry, oil industry, printing and dyeing industry, building materials, agriculture, environmental protection. It has a synergic effect with kappa carrageenan, xanthan gum and locust bean gum. GM is an effective cholesterol-lowering dietary adjunct.[10]

Chitin is the second most important natural polymer in the world. The main sources exploited are two marine crustaceans, shrimps and crabs in which chitin exists as a semi-crystalline polymer.[3] Chitosan is the most important derivative of chitin; it is prepared by partial deacetylation of chitin in strong alkaline conditions. Characterization and properties will be described later.

Bacterial polysaccharides are now more and more developed on industrial level. The first one was xanthan which is produced on large scale and which is a very good thickener now accepted for food applications.[11] It adopts a helical conformation giving to xanthan a ligh persistence length (a large stiffness); it forms complex with galacto- or glucomannans which were used for some drug release or food applications.[7] Hyaluronan was first extrated from bovine vitreous humor, roster combs or umbilical cords but now, for medical applications, it is mainly produced by *Streptococcus zooepidemicus* bacteria.[12]

On a general point of view, polysaccharides are rich in –OH functional groups allowing good solvation in aqueous medium and possible chemical modifications (esterification or etherification); they are renewable, often biocompatible and biodegradable. Due to their stereoregularity, and to the presence of –OH groups, H bond networks are formed which stabilize intra and inter-polymeric chain interactions; they induce the characteristic semi-rigid behaviour of these polysaccharides in well defined thermodynamic conditions (usually at temperature lower than 40 °C) as well as good film forming properties.[13] Polysaccharides may be neutral polymers as cellulose, amylose, and galactomannans or charged; carboxylic or sulfate groups are present in hyaluronan and alginate or carrageenan respectively; amino groups are naturally present in the only pseudo natural cationic polymer, chitosan. Electrostatic character favours the dissolution in aqueous solutions.

## B. Chitosan

### Structure and Characterization

Chitin, poly (β-(1 → 4)-*N*-acetyl-D-glucosamine), is treated under heterogeneous conditions to give chitosan; these conditions imply an irregular distribution of the remaining acetyl groups along the chains. Chitosan has to be considered as a statistic copolymer made of N-acetyl-D- glucosamine (GlcNAc) and D-glucosamine (GlcN) repeat units (Figure 1).

Chitosan is much easier to process than chitin (which has a very low solubility); it is used to prepare hydrogels, films, fibers or sponges from neutralization of its aqueous acidic solution: most of the materials are used in the biomedical domain where biocompatibility is essential. Many chitosan hydrogels are obtained by treatment with multivalent anions as described with glycerol-phosphate,[14] oxalic acid[15,16] as well as tripolyphosphate.[17]

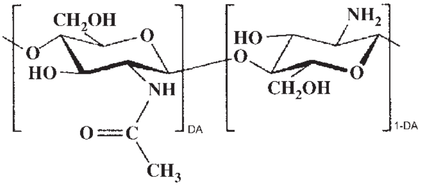

**Figure 1.**
Schematic chemical structure of chitin (when DA = 1) and chitosan (when DA < 0.5).

When the degree of deacetylation of chitin is larger than a value around 50% (depending on the origin of the polymer and on the distribution of the acetyl groups along the chains), it becomes soluble in aqueous acidic medium. The solubilization occurs by protonation of the $-NH_2$ function on the C-2 position of the D-glucosamine repeat unit with an intrinsic pK, $pK_0 = 6$; it is the only pseudo natural cationic polymer.[3,18]

The characterization of a chitosan sample requires the determination of its average degree of acetylation (DA) and the distribution of molecular weights. Different techniques were proposed to get DA: potentiometric titration,[19] IR,[20] $^1$H liquid state and solid state $^{13}$C NMR and $^{13}$C and $^{15}$N solid state NMR (in the whole range of acetyl content from 0 to 100%).[21] An other important characteristic of chitosan is the distribution of acetyl groups along the chain which may modify solubility from one to the other sample.

The second important set of characteristics of these polymers is the molecular weight distribution, the average molecular weights and the related intrinsic viscosity. The first difficulty encountered for this purpose concerns the solubility of the samples; we have proposed to use 0.3 M acetic acid/ 0.2 M sodium acetate (pH = 4.5) as solvent since we had no evidence for aggregation in this mixture. The absolute M values were obtained from steric exclusion chromatography (SEC) equipped with on line viscometer, differential refractometer and multiangle laser light scattering detector; this work allows to determine the Mark-Houwink parameters in the relationship:

$$[\eta](\mathrm{mL/g}) = \mathrm{K\,M^a} \qquad (1)$$

relating the molecular weight to the intrinsic viscosity. The $K$ and $a$ parameters are found to be $\mathrm{K} = 0.079$, $\mathrm{a} = 0.796$ for a $\mathrm{DA} < 0.03$ and $\mathrm{K} = 0.074$, $\mathrm{a} = 0.8$ for a $\mathrm{DA} \sim 0.12$. This analysis involved first the determination of the increment of refractive index dn/dc in the 0.3 M acetic acid/0.2 M sodium acetate solvent; a value 0.190 was adopted.[22]

As many stereoregular polymers, polysaccharides behave as semi-rigid polymers analyzed using the worm-like chain model.[23] The local stiffness of the polymer in this model is characterized by a persistence length Lp. Concerning chitosan with different DA, it was shown from steric exclusion chromatography using the three detectors on line, that $\mathrm{Lp} \sim 11$ nm, Lp being nearly constant for $0 < \mathrm{DA} < 25\%$ when the electrostatic repulsions are screened. Up to 60% acetylation, the stiffness of chitosan is not much influenced by the degree of acetylation and goes to 15 nm.[22] The influence of the substitution has to be related to the stability of the intra chains H-bonds shown for chitin and chitosan from molecular modeling; the small variation of the persistence length with DA is in direct relation with that obtained for the Mark Houwink parameters. This semi rigid character of chitosan and chitin was also investigated by molecular modeling[24] and the persistence length predicted was compared with experimental results obtained by SEC. From this analysis, chitosan, free of acetyl groups, has an intrinsic persistence length $\mathrm{Lp} = 9$ nm; Lp increases when DA increases up to $\mathrm{Lp} = 12.5$ nm for $\mathrm{DA} = 60\%$ then remains constant up to pure chitin at 25 °C.

### Electrostatic Complexes

Many electrostatic polyelectrolyte complexes (PEC) between chitosan and synthetic or natural polymers are cited in the literature: e.g. xanthan, carrageenan, alginate, pectin, heparin, hyaluronan (HA).[25–29] Especially with alginate or HA and HA, a pH-dependent complex is formed, whose stability depends on the ionic strength. At stoichiometric ratio, the complex is usually insoluble and allows to obtain fibers, films or capsules; the alginate/chitosan complex is the more often investigated polyelectrolyte complex especially for biomedical applications. The main applications of these electrostatic complexes are antithrombogenic materials, controlled release systems, encapsulation of drugs, immobilization of enzymes and cells, and gene carriers.

### Chitosan Blends and Composites

Blends and composites including chitin or chitosan have been prepared especially by Hirano.[30] Different systems are proposed in the literature; as example, a new fiber, the Crabyon® fiber, is made of a blend of chitosan and viscose. The advantage of chitosan in such materials is not only its biodegradability and its antibacterial activity, but also the hydrophilicity introduced by addition of the polar groups able to form secondary interactions (–OH and $-NH_2$ groups involved in H bonds with other polymers). The most promising developments at present are in pharmaceutical and biological areas, and at a lower level in cosmetics.

The biodegradability and antibacterial properties of chitosan are recognized to favour their use as hydrogels in tissue engineering (cartilage, bone, nerve tissues) or resorbable suture.[31–33] At last, their positive ionic charge allows interaction with proteins, giving chitosan good mucoadhesive properties.[34]

### Chemical Modifications

Specific chemical modification on the amino group in C-2 position in aqueous medium allows to get reproducible chitosan derivatives. This approach was developed in our group in the last years. Cyclodextrins were grafted with success (up to around 10% of the available $-NH_2$); this modification should allow encapsulation of hydrophobic small molecules.[35] This derivative was tested for its mucoadhesive properties.[36] Alkyl chains with different chain lengths were also grafted by reductive amination to obtain amphiphilic polymers with very interesting thickening or gelling behaviour.[37] The properties result of a balance between electrostatic repulsions and hydrophobic attractions, the alkyl chains forming hydrophobic crosslink points up to gelation. Very recently, a thermoassociative system was prepared with grafted chains of short poly (propylene oxide-co-ethylene oxide) (POEP); the LCST of this small polymer is around 32 °C.[38]

These few examples show that many different derivatives (with a low degree of substitution (DS < 10%)) can be produced from the natural chitosan (or chitin) backbone to extend the domains of applications in the aqueous solutions and to preserve the original characters of chitosan.

## C. Hyaluronan

### Structure and Characterization

Hyaluronan (also called hyaluronic acid or hyaluronate) is a glycosaminoglycan distributed widely throughout connective, epithelial, and neural tissues. HA is the only nonsulfated glycosaminoglycan found in the extracellular matrix. Commercial HA is extracted from rooster combs; it is also produced by bacteria of *Streptococcus* species.[12] Hyaluronan is a linear (AB) copolymer based on β-(1-4)-D-glucuronic acid (GlcA) and β-(1-3)- N acetyl-D-glucosamine (GlcNAc) repeat unit; it is also an anionic polyelectrolyte (Figure 2).

An important characteristic for a polymer is its molecular weight (M) and molecular weight distribution related to its dimensions in solution (i.e., the radius of gyration Rg) and to its hydrodynamic volume. Analysis of the chromatogram obtained by steric exclusion chromatography (SEC) gives the M distribution and the different average molecular weights (Mn, Mw, etc.) (88). A dn/dc value equal to 0.153 was adopted. The parameters K and a are known as the Mark-Houwink parameters in the relation [1] and directly determined; in the range $4 \times 10^5 < M < 1.5 \times 10^6$ and in the SEC conditions adopted (0.1 M $NaNO_3$, 30 °C), one gets respectively $K = 0.0336$ and $a = 0.79$. From our SEC experimental results, it also comes $Lp \approx 80–90$ Å at 25 °C.[39,40]

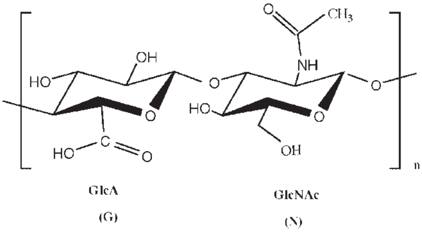

***Figure 2.***
Repeat unit of hyaluronan in the acidic form.

The persistence length was predicted by molecular modeling separately as soon as the exact chemical structure is known.[41] The HA molecule has a regular structure based on (AB) linear copolymer which may be modeled in the absence of long range electrostatic interactions. From modeling the persistence length $Lp = 7.5$ nm in good agreement with the experimental determination.

One characteristic of a polyacid is its intrinsic pK value, $pKo = 2.9 \pm 0.1$ as usually found on polycarboxylic acid in the absence of the specific interaction involving the carboxylic groups.[12] A specific behaviour of HA was obtained when the viscosity of a hyaluronan solution is plotted as a function of the pH.[42] When the pH of the solution is decreased by progressive addition of HCl, a gel-like behaviour ($G' > G''$) is observed around $pH = 2.5$ from rheology in a large range of frequencies; this physical gel is related to the decrease of the carboxylate dissociation, favouring intermolecular interactions and H bond network formation (Figure 3)

This gel in acidic conditions was previously discussed by Balazs[43,44] and tested as film for medical applications.[45]

### Rheological Behaviour

Many applications of HA often involve its rheological behaviour. For this purpose, it is important to determine the viscosity of the solution ($\eta$) and especially how a polymer increases the viscosity of the solvent ($\eta_s$); the problem is then to relate ($\eta - \eta_s$), with the polymer concentration C, its

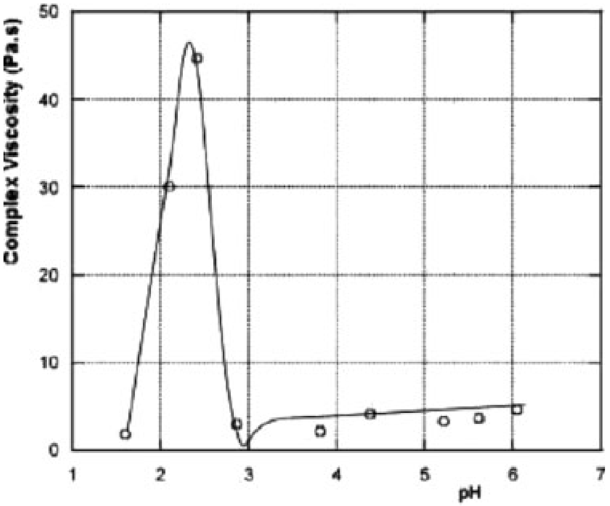

***Figure 3.***
Influence of the pH on the viscosity of HA solution.[42]

molecular weight in dependence with the ionic concentration and temperature. Flow experiments which allow the determination of the viscosity dependence with the shear rate (η(γ) curves) as a function of temperature, hyaluronan and salt concentrations (γ is the shear rate). A master curve is obtained at zero shear rate, for ηsp as a function of C[η] for different samples having different molar masses and at different polymer concetnrations as given by the general development:

$$(\eta\,\mathrm{sp})_0 = \mathrm{C}[\eta] \times \{1 + \mathrm{k}_1(\mathrm{C}[\eta]) + \mathrm{k}_2(\mathrm{C}[\eta])^2 + \mathrm{k}_3([\eta])^3\} \qquad (2)$$

with $k_1 = 0.4$; $k_2 = k_1^2/2!$; $k_3 = k_1^3/3!$.[46]

In this relation $k_1$ equals the Huggins constant ($k_1 = 0.4$ in good solvent). The overlap parameter C[η] takes into account the molecular weight, the polymer concentration but also the expansion of the chains. The dilute regime corresponds to $C[\eta] < 1$; for $C[\eta] > 1$, we enter in the semi dilute regime and progressively entanglements appear.

In dynamic experiments, in more concentrated solutions, the two components of the relaxation modulus are determined: in phase with the strain, G′(ω), the storage modulus, related to the elastic behavior and out of phase related to the viscous behavior G″(ω), the loss modulus. From these two values, one can calculate the complex dynamic viscosity:

$$|\eta*|(\omega) = (G'^2 + G''^2)^{1/2}\omega^{-1} \qquad (3)$$

An excess of NaCl (0.1 M NaCl) is usually added to screen the electrostatic long range interactions which play a role on the dimensions of the chains but also on interchain interactions.

The cross-over of G′ and G″ characterized by $\omega_p$ determines two domains: at low frequency, $G'' > G'$ is typical of solution behavior and over $\omega_p G' > G''$ when the elastic character of the temporary network becomes significant. From these curves, it is important to mention that a master curve can be obtained from a horizontal and a vertical translation of G′(ω) and G″(ω) curves.[12]

Hyaluronan is naturally found in many tissues of the body such as skin, cartilage, and the vitreous humor. The first hyaluronan biomedical product, Healon, was developed in the 1970s and 1980s and is approved for use in ophthalmic surgery (i.e. corneal transplantation, cataract surgery, glaucoma surgery and retinal attachment surgery). Hyaluronan is also used to treat osteoarthritis of the knee. Such treatments are administered as a course of injections into the knee joint and are believed to supplement the viscosity of the joint fluid thereby lubricating the joint, cushioning the joint and producing an analgesic effect. It has also been suggested that hyaluronan has positive biochemical effects on cartilage cells. Two different types of polymeric systems are now on the market: loosely crosslinked HA mixed with linear HA (Synvisc ®) or linear HA such as Arthrum® which have very different rheological behaviour. Based on the rheological behaviour, it is recommended to have the crossover of $G'$ and $G''$ at a frequency lower than that for walk ($0.5\ \text{cycle} \cdot s^{-1}$) and run ($2.5\ \text{cycle} \cdot s^{-1}$) and a similar behaviour as synovial fluid.[47–49] Due to its high biocompatibility and its common presence in the extracellular matrix of tissues, hyaluronan is gaining popularity as a biomaterialscaffold in tissue engineering research. Hyaluronan may also be used postoperatively to induce tissue healing; it is used against postsurgical adhesion.[50,51] Current models of wound healing propose that larger polymers of hyaluronic acid appear in the early stages of healing to physically make room for white blood cells, which mediate the immune response. Modified HA and/or mixtures of polymers can also be used for the same type of applications taking advantage of biocompatibility and biodegradability of HA. Inclusion of HA as a vehicule excipient is interesting in dermal delivery and localization of drug.

As mentioned previously, electrostatic complexes are formed with chitosan; it is the most investigated system for drug release at present time. Some other blends are also proposed.[52]

### Chemical Modification

Different chemical modifications are published; they concern modification on the carboxylic site and on –OH groups mainly. They allow to modify the behaviour of HA in aqueous solution or they involve a chemical crosslinkage to stabilize the materials and produce films or particles. To increase the rheological moduli, and avoid the presence of non bioerodible fraction of material, it was proposed to prepare a physical gelling system for viscosupplementation. Alkyl C-10 and C-12 chains were grafted with success on carboxylic positions in HA to enhance the rheological performances.[53]

## D. Alginates

Alginates located in the intercellular substance in brown algae are linear block copolymers composed of 1,4-linked β-D-mannuronic acid (M) with $^4C_1$ ring conformation and α-L-guluronic acid (G) with $^1C_4$ conformation in varying proportion and in the pyranosic conformation. G-blocks of more than 6-10 residues each form stable crosslinked junctions (and gels) with divalent counterions (Ca,Ba,Sr…) but not with Mg. At low pH, alginates form acidic gels stabilized by H bonds.

NMR is the most powerful technique to characterize the chemical composition and the microstructure of the alginate. Purified alginates isolated under the sodium salt form were characterized by size exclusion chromatography (SEC). Purified alginates under its sodium form are also characterized by their intrinsic viscosity using the Mark Houwink relation [1] with $K = 2 \times 10^{-3}$ and $a = 0.97$ in 0.1 M NaCl solvent at 25 °C.[54]

The worm-like chain model was applied to analyze the behavior of polysaccharides and characterize their local stiffness by the intrinsic persistence length Lp; for alginates rich in G units, Lp was found equal 9 nm while for a sample rich in M units, Lp = 4 nm.[55]

Alginates are able to form gels in the presence of divalent counterions such as Ba, Sr based on a specific cooperative Ca interaction forms on G blocks which is the basis of the junction zones and the crosslink of these ionic networks.

The most important technical properties of alginates are their thickening character, their ionic exchange properties, and their gel forming ability in presence of multivalent counterions.

The polyelectrolyte behaviour (anionic polymer) is the usual one; the viscosity of solution is nearly constant between pH 6 and 8 but, in moderate concentration, it increases below pH 4.5 and reaches a maximum around 3–3.5 and then decreases; alginic acid form gels resulting from H bond attraction over dominating the electrostatic repulsions as it was observed for hyaluronan. The intrinsic pK for alginic acid is around 3 as found for many polyuronic acids.

Because of the linear structure, and high molecular weight, alginates form strong films and good fibers in the solid state; alginates are food additives for thickening soups and jellies. They are used as anti-acid preparation such as Gaviscon ®, in mold making material in dentistry (in the presence of slow release calcium salt to control the delay of gelation), pharmaceutical or textile applications. Alginates can be processed under fibers or films and are commercialized as hemostatic material but also wound dressing. As example, AlgiDERM® or Sorbsan ® are calcium alginate dressings, made of sterile purified alginate fibers. Calcium gels are often used under bead form as a immobilization matrix for animal cells or plant protoplast.[56,57]

The polyelectrolyte complexes are often used in biomedical applications as mentioned before: chitosan-alginate beads were evaluated as an immobilization matrix for lipase-catalyzed hydrolysis of olive oil. The complex is also convenient in tissue engineering and wound dressing.[58–60]

## Conclusion

This paper describes the methods for characterization adopted for alginate, hyaluronan and chitosan, three interesting polysaccharides which are involved at time in many applications especially in the biomedical and pharmaceutical domains. They are more and more often proposed as biomaterials with original properties in comparison with many other systems. These polymers are renewable materials from bacterial, algae and/or animal sources respectively. They are biocompatible, biodegradable and, they often have biological activity.

We have described the main properties of these polysaccharides.

Particularly interesting systems find applications when they are combined in an electrostatic complex where they are strongly associated in dependence on the pH and ionic concentration.

Advantage of these polysaccharides is that they can be used in solution, or as hydrogels, films, fibers, sponges, capsules, powders.

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