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Chitin and chitosan: Properties and applications

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Abstract

Chitin is the second most important natural polymer in the world. The main sources exploited are two marine crustaceans, shrimp and crabs. Our objective is to appraise the state of the art concerning this polysaccharide: its morphology in the native solid state, methods of identification and characterization and chemical modifications, as well as the difficulties in utilizing and processing it for selected applications. We note the important work of P. Austin, S. Tokura and S. Hirano, who have contributed to the applications development of chitin, especially in fiber form. Then, we discuss chitosan, the most important derivative of chitin, outlining the best techniques to characterize it and the main problems encountered in its utilization. Chitosan, which is soluble in acidic aqueous media, is used in many applications (food, cosmetics, biomedical and pharmaceutical applications). We briefly describe the chemical modifications of chitosan—an area in which a variety of syntheses have been proposed tentatively, but are not yet developed on an industrial scale. This review emphasizes recent papers on the high value-added applications of these materials in medicine and cosmetics.
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Contents

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1. Introduction

Chitin, poly (β-(1→4)-N-acetyl-D-glucosamine), is a natural polysaccharide of major importance, first identified in 1884 (Fig. 1). This biopolymer is synthesized by an enormous number of living organisms; and considering the amount of chitin produced annually in the world, it is the most abundant polymer after cellulose. Chitin occurs in nature as ordered crystalline microfibrils forming structural components in the exoskeleton of arthropods or in the cell walls of fungi and yeast. It is also produced by a number of other living organisms in the lower plant and animal kingdoms, serving in many functions where reinforcement and strength are required.
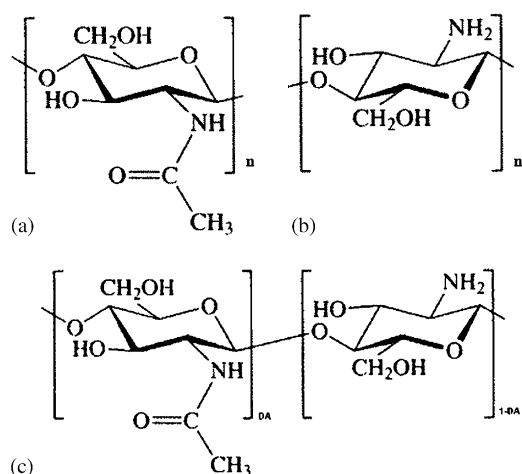

Fig. 1. Chemical structure (a) of chitin poly(*N*-acetyl-β-D-glucosamine) and (b) of chitosan (poly(D-glucosamine) repeat units. (c) Structure of partially acetylated chitosan, a copolymer characterized by its average degree of acetylation DA.

Despite the widespread occurrence of chitin, up to now the main commercial sources of chitin have been crab and shrimp shells. In industrial processing, chitin is extracted from crustaceans by acid treatment to dissolve calcium carbonate followed by alkaline extraction to solubilize proteins. In addition a decolorization step is often added to remove leftover pigments and obtain a colorless product. These treatments must be adapted to each chitin source, owing to differences in the ultrastructure of the initial materials (the extraction and pre-treatment of chitin are not described in this paper). The resulting chitin needs to be graded in terms of purity and color since residual protein and pigment can cause problems for further utilization, especially for biomedical products. By partial deacetylation under alkaline conditions, one obtains chitosan, which is the most important chitin derivative in terms of applications.

This review aims to present state-of-the-art knowledge of the morphology of chitin and chitosan and to indicate the best methods for characterization in solution or solid state. The last decade of development will be discussed, as well as recent chemical modifications solution the uses of chitin to be expanded.

2. Chitin

2.1. Chitin structure in the solid state

Depending on its source, chitin occurs as two allomorphs, namely the α and β forms [1,2], which can be differentiated by infrared and solid-state NMR spectroscopy together with X-ray diffraction. A third allomorph γ-chitin has also been described [1,3], but from a detailed analysis, it seems that it is

just a variant of the α family [4]. α-Chitin is by far the most abundant; it occurs in fungal and yeast cell walls, in krill, in lobster and crab tendons and shells, and in shrimp shells, as well as in insect cuticle. It is also found in or produced by various marine living organisms. In this respect, one can cite the harpoons of cone snails [5], the oral grasping spine of *Sagitta* [6–8] and the filaments ejected by the seaweed *Phaeocystis* [9], etc. These exotic α-chitins have proved particularly interesting for structural studies since, in comparison with the abundant arthropod chitin, some of them present remarkably high crystallinity [10] together with high purity (they are synthesized in the absence of pigment, protein, or calcite). In addition to the native chitin, α-chitin systematically results from recrystallization from solution [11,12], in vitro biosynthesis [13,14] or enzymatic polymerization [15].

The rarer β-chitin is found in association with proteins in squid pens [1,3] and in the tubes synthesized by pogonophoran and vestimetiferan worms [16,17]. It occurs also in aphrodite chaetae [18] as well as in the lorica built by some seaweeds or protozoa [19,20]. A particularly pure form of β-chitin is found in the monocrystalline spines excreted by the diatom *Thalassiosira fluviatilis* [20–22]. As of today, it has not been possible to obtain β-chitin either from solution or by in vitro biosynthesis.

2.1.1. Crystallography of chitin

The crystallography of chitin has been investigated for a long time [23–26]. Examples of diffraction diagrams are shown in Figs. 2 and 3. At first glance the powder X-ray diagrams of chitins from shrimp shell (α-chitin) and anhydrous squid pen (β-chitin) appear nearly the same, but in a refined analysis, they can be differentiated in two ways: (i) a
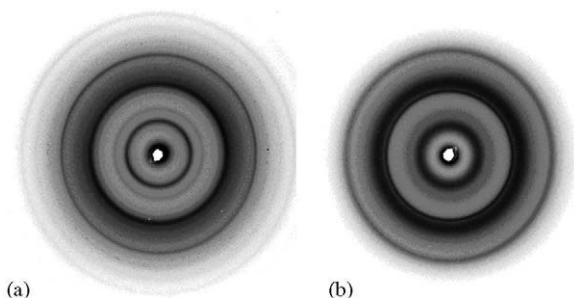

Fig. 2. X-ray powder diffraction diagrams (a) of α-chitin from purified shrimp cuticle and (b) of β-chitin from dried purified squid pen.
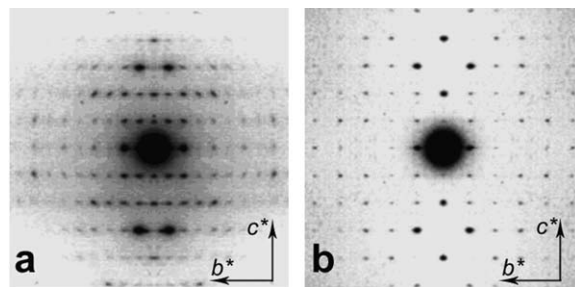

Fig. 3. Electron diffraction patterns of highly crystalline chitin: (a) b^*c^* projection of α-chitin recorded from a fragment of grasping spine of the arrow worm *Sagitta*; (b) b^*c^* projection of dried β-chitin recorded from a microfibril from the tube of the vestimentiferan worm *Tevnia jerichonana*.

strong diffraction ring, often quoted as the α-chitin signature is found at 0.338 nm (Fig. 2a) whereas a similar ring occurs at 0.324 nm in β-chitin; (ii) an inner ring at 0.918 nm in β-chitin is sensitive to hydration, moving to 1.16 nm in the presence of liquid water, whereas a similar strong inner ring at 0.943 nm in α-chitin is insensitive to hydration.

Further information on the crystalline structure of α- and β-chitin is obtained by analysis of electron diffraction patterns of highly crystalline samples. Examples are shown in Fig. 3, where 3a is taken on a fragment of a *Sagitta* grasping spine and 3b on a microfibril extracted from a tube synthesized by a vestimentiferan worm *Tevnia jerichonana*. These two patterns, corresponding to b^*c^* projections, indicate clearly that along the b^* direction, the cell parameter of α-chitin is close to twice that of β-chitin, whereas the c^* parameter is the same in both patterns. In addition the a^*c^* projections (not shown) of α- and β-chitin are nearly identical in both allomorphs. These observations are consistent with the currently accepted crystalline parameters and symmetry elements of α- chitin and anhydrous β-chitin (Table 1). The crystallographic parameters of α and β-chitin reveal that there are two antiparallel molecules per unit cell in α-chitin, whereas only one is present in β-chitin, which consists therefore of a parallel arrangement. Despite this difference, it appears that the N-acetyl glycosyl moiety is the independent crystallographic unit in both allomorphs.

The proposed crystal structures of α- and β-chitin are represented in Figs. 4 and 5. In both structures, the chitin chains are organized in sheets where they are tightly held by a number of intra-sheet hydrogen bonds. This tight network, dominated by the rather strong C–O···NH hydrogen bonds, maintains the

Table 1
Crystallographic parameters of α- and β-chitins

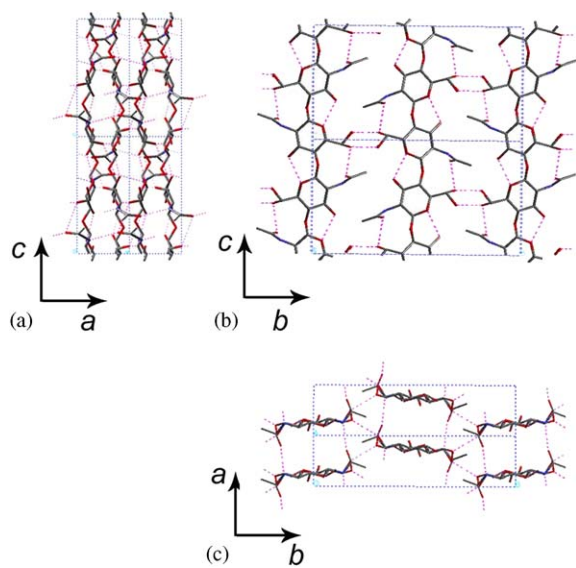|	a (nm)	b (nm)	c (nm)	γ (°)	Space group	Ref.
α-Chitin	0.474	1.886	1.032	90	$P2_12_12_1$	[25]
anhydrous β-chitin	0.485	0.926	1.038	97.5	$P2_1$	[26]

Fig. 4. Structure of α-chitin: (a) *ac* projection; (b) *bc* projection; (c) *ab* projection. The structure contains a statistical mixture of 2 conformations of the –CH₂OH groups [26].

Fig. 5. Structure of anhydrous β-chitin: (a) *ac* projection; (b) *bc* projection; (c) *ab* projection. The set of coordinates defined in Ref. [25] could not be used due to an error in the definition of the *N*-acetyl moiety. Instead coordinates provided by Y. Noishiki, Y. Nishiyama and M. Wada in a private communication were used to draw the molecular structure of β-chitin.

chains at a distance of about 0.47 nm (Figs. 4a, 4c, 5a and 5c) along the *a* parameter of the unit cell. In α-chitin, there are also some inter-sheet hydrogen bonds along the *b* parameter of the unit cell, involving association of the hydroxymethyl groups of adjacent chains. Such a feature is not found in the structure of β-chitin, which is therefore more susceptible than α-chitin to intra-crystalline swelling. The current model for the crystalline structure of α-chitin indicates that the inter-sheet hydrogen bonds are distributed in two sets (Fig. 4b) with half occupancy in each set [26]. It is not clear whether this feature is general for all α-chitin samples or specific to lobster tendon chitin, which was used in the structure determination. In this respect, the observation of diffraction patterns of various α-chitin samples indicates some discrepancy in their diffraction patterns. In particular the X-ray pattern of lobster tendon chitin presents a marked 001 diffraction spot [26], which is absent in the more crystalline *Sagitta* chitin [7,8,10]. Therefore, it

appears that more work is required to resolve these ambiguities about the crystal structure of α-chitin. In contrast, the structure of anhydrous β-chitin appears to be well established. However, the crystal structure of the β-chitin hydrate remains to be refined, as some uncertainty exists, even as to its unit cell parameters [17,27].

2.1.2. Reversible and irreversible intra-crystalline swelling of chitin

As mentioned above, no inter-sheet hydrogen bond is found in the crystal structure of β-chitin, whereas the sheets themselves are tightly bound by a number of intra-sheet hydrogen bonds. This remarkable feature explains why a number of polar guest molecules, ranging from water to alcohol and amines, can readily penetrate the crystal lattice of β-chitin without disturbing the sheet organization

and the crystallinity of the samples. This swelling is quite rapid: it was found that the highly crystalline chitin from pogonophore tubes could be swollen in water in about a minute [28]. Once a guest has penetrated the crystalline lattice of β-chitin, it can be displaced by another one of a different chemical family to produce a wide distribution of crystalline β-chitin complexes. Essentially, during swelling the *b* parameters of the β-chitin unit cell expand laterally whereas *a* and *c* remain constant. The incorporation of the swelling agent within the crystalline lattice is thus indicated by the position of the 010 diffraction spot. Table 2 lists the variation of the position of this spot with respect to a selection of representative guests. This intra-crystalline swelling is reversible, as in all these cases removal of the guest molecule allows the structure to revert to its original state of anhydrous β-chitin, though with some loss of crystallinity.

The inter-sheet swelling of α-chitin crystals is more specific. Whereas water and alcohols cannot penetrate the crystalline lattice of α-chitin, stronger swelling agents such as aliphatic diamines have been shown to intercalate into the crystalline lattice to form highly crystalline complexes [32]. As in β-chitin, the guest molecules are incorporated between the chitin sheets of α-chitin and accordingly, the *b* cell parameter expands, whereas the *a* and *c* parameters remain essentially constant. The inter-sheet parameter expansion, which is about the same in both α- and β-chitin, increases linearly with

Table 2
Variation of the 010 diffraction spot of β-chitin with incorporation of various guest molecules

Guest	Position of the 010 diffraction spot (nm)	Ref.
No guest	0.917	[25]
Water	1.16[a]	[25]
Methanol	1.30	[29]
n-butanol	1.55	[29]
n-octanol	1.97	[29]
n-hexylamine	1.81	[30]
Ethylenediamine (type I)	1.18	[30]
Ethylenediamine (type II)	1.45	[30]
Acrylamide	1.33	[31]
p-aminobenzoic acid	1.31	[31]
D-glucose	1.27	[31]

[a]This value corresponds to β-chitin dihydrate. Under reduced hydration conditions the β-chitin monohydrate is obtained, for which the 010 diffraction spot is at 1.04 nm [31].

the number of carbon atoms in a diamine guest: an expansion of 0.7 nm being observed, for instance, in the case of the C7 diamine [32].

Whereas the intra-crystalline swelling of β-chitin in water, alcohols or amines is reversible, its swelling in relatively strong acid media, namely concentrated nitric acid or 6–8 M HCl, leads irreversibly α-chitin [18,33]. During this swelling, not only the inter-sheet, but also the intra-sheet hydrogen bonds are broken [34] and the crystalline state appears to be completely lost [35]. Nevertheless the crystallinity is restored, as α-form crystals, upon removal of the acid. In the case of oriented material, such as squid pen chitin, the $\beta \rightarrow \alpha$ conversion is also marked by a substantial shrinkage of the structure [33]. To account for this shrinkage and the solid-state $\beta \rightarrow \alpha$ conversion, a chain folding mechanism has tentatively been proposed [33]. Other possibilities involving the interdigitation of β-chitin microfibrils of opposite polarities can also be envisaged. At the ultrastructural level, it was found that substantial hydrolysis followed by partial dissolution occurred during the acid treatment. When a subsequent washing step was applied, the shortest hydrolyzed chains were found to recrystallize by epitaxy on the underlying unhydrolyzed chitin chains, leading to a shish-kebab morphology [35]. Thus, the conversion did not occur at a single crystal level, but some or all β-chitin crystals were destroyed during the acid swelling and new crystals of α-chitin were produced during recrystallization. The irreversibility of the $\beta \rightarrow \alpha$ conversion indicates that α-chitin is thermodynamically more stable than β-chitin. This stability is confirmed by the fact that α-chitin is always obtained in recrystallization from solution.

2.1.3. Infrared spectroscopy of chitin

A number of studies have dealt with the description and interpretation of the infrared spectra of chitin [36–41]. Spectra of α- and β-chitin samples shown in Fig. 6 are typical of polysaccharides; because of the high crystallinity of the samples, they display a series of very sharp absorption bands. The $C=O$ stretching region of the amide moiety, between 1600 and 1500 cm^{-1}, is quite interesting as it yields different signatures for α-chitin and β-chitin. For α-chitin, the amide I band is split at 1656 and 1621 cm^{-1}, whereas it is unique, at 1626 cm^{-1} for β-chitin. In contrast, the amide II band is unique in both chitin allomorphs: at 1556 cm^{-1} for α-chitin and 1560 cm^{-1} for β-chitin. The occurrence of two amide I bands for α-chitin
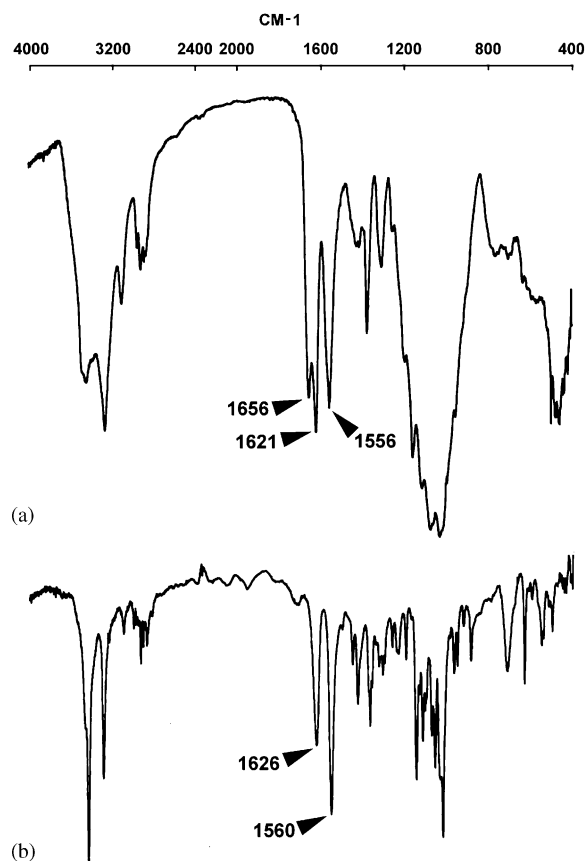
Fig. 6. FTIR spectra of chitin: (a) for single crystals of α-chitin; (b) for deproteinized dried β-chitin from the tube of *Tevnia jerichonana*.

its inter-sheet hydrogen bonding does not allow us to give a definitive explanation for this band.

2.1.4. ^{13}C CP-MAS solid state spectroscopy

A number of ^{13}C solid-state NMR spectra of α- and β-chitin have been published [40,43–45], the most crystalline samples yielding the best resolved spectra. Examples of such spectra are shown in Fig. 7, and a list of their corresponding chemical shifts is presented in Table 3. When recorded at 7.05 T, each spectrum consists of 6 single-line signals and 2 doublets at C-2 and C=O, but these doublets are in fact singlets that are split by the effect of the ^{14}N quadrupole coupling [44]. The splitting disappears if the spectra are acquired at higher field strength and, on the other hand, becomes broader at lower field strength. In accounting for this phenomenon, there are therefore only 8 signals for the 8 carbon atoms of α- and β-chitins. Thus, in both allomorphs, the *N*-acetyl D-glucosamine moiety can be considered as the magnetic independent residue, in full agreement with the crystal structure of α- and β-chitin where this residue is also the crystallographic independent unit. In looking at the data in Table 3, we see that the spectra of α- and β-chitin are nearly the same, and it is not easy to differentiate them by solid-state ^{13}C NMR. Nevertheless, the relaxation time of C-6

has been the subject of debate. The band at 1656 cm^{-1}, which occurs at similar wavelengths in polyamides and proteins, is commonly assigned to stretching of the C=O group hydrogen bonded to N–H of the neighboring intra-sheet chain. Regarding the 1621 cm^{-1} band , which is not present in polyamides and proteins, its occurrence may indicate a specific hydrogen bond of C=O with the hydroxymethyl group of the next chitin residue of the same chain [41]. This hypothesis is reinforced by the presence of only one band in this region for *N*-acetyl D-glucosamine [37,42]. Also, in α-chitin, the band at 1621 cm^{-1} is modified in deuterated water, whereas the band at 1656 cm^{-1} remains nearly unaffected [40]. Other possibilities may also be considered, as the band at 1621 cm^{-1} could be either a combination band or due to an enol form of the amide moiety [37]. The lack of a more precise definition of the molecular structure of α-chitin and
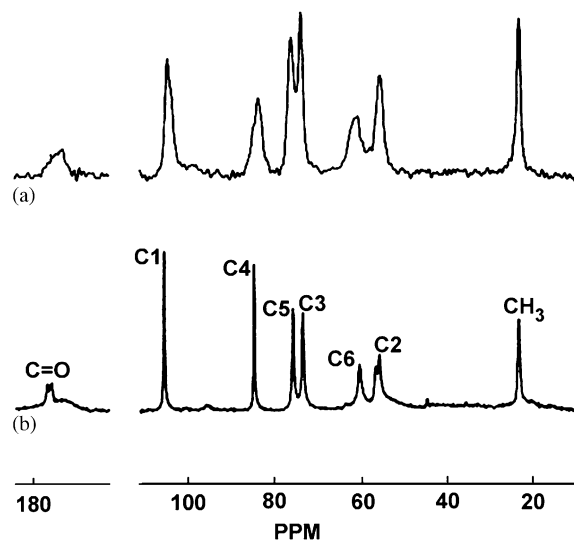

Fig. 7. ^{13}C CP/MAS solid state spectra of (a) α-chitin from deproteinized lobster tendon; (b) β-chitin from dried deproteinized tube of *Tevnia jerichonana*. Reprinted with permission from Macromolecules 1990; 23: 3576–3583. Copyright 2006, American Chemical Society.

Table 3
Chemical shifts of solid chitin [44]

	Anhydrous β-chitin from diatom spines (ppm)	α-chitin from lobster tendon (ppm)
C1	105.4	104.6
C2[a]	55.3	55.6
	73.1	
C3	73.1	73.7
C4	84.5	
C5	75.5	83.6
C6	59.9	61.1
C=O[a]	175.6	173.0
	176.4	
CH$_3$	22.8	23.1

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[a]The splitting for C-2 and C=O is due to the ^{14}N quadrupole coupling.

Table 4
Sources of chitin and chitosan [46]

Sea animals	Insects	Microorganisms
Annelida	Scorpions	Green algae
Mollusca	Spiders	Yeast (β-type)
Coelenterata	Brachiopods	Fungi (cell walls)
Crustaceans:	Ants	Mycelia Penicillium
Lobster	Cockroaches	Brown algae
Crab	Beetles	Spores
Shrimp		Chytridiaceae
Prawn		Ascomydes
Krill		Blastocladiaceae

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in crab shell α-chitin is found to be much shorter than that of the other carbons of this chitin and also shorter than for C-6 of anhydrous β-chitin [44]. A possible explanation may be related to the specificity of the split hydrogen bonds linking the hydroxymethyl groups of the α-chitin molecules from adjacent sheets. A refinement of the crystalline and molecular structure of α-chitin should help in understanding not only this hydrogen bonding situation but should also give a clue for the short relaxation time of C-6. It also remains to be seen whether this fast relaxation is specific for crab shell chitin or is general for all crystalline α-chitins.

2.2. Solubility of chitin and chain characterization

Chitin occurs naturally partially deacetylated (with a low content of glucosamine units), depending on the source (Table 4) [46]; nevertheless, both α and β forms are insoluble in all the usual solvents, despite natural variations in crystallinity. The insolubility is a major problem that confronts the development of processing and uses of chitin. An important mechanism previously mentioned is that a solid-state transformation of β-chitin into α-chitin occurs by treatment with strong aqueous HCl (over 7 M) and washing with water [35]. In addition, β-chitin is more reactive than the α form, an important property in regard to enzymatic and chemical transformations of chitin [47].

Because of the solubility problem, only limited information is available on the physical properties of chitin in solution. The first well-developed study was by Austin [48], who introduced the solubility parameters for chitin in various solvents. He obtained a complex between chitin and LiCl (which is coordinated with the acetyl carbonyl group). The complex is soluble in dimethylacetamide and in N-methyl-2-pyrrolidone. We recall that the same solvents and, especially, LiCl/DMAc mixtures, are also solvents for cellulose, another $\beta(1\rightarrow4)$ glucan [49]. In addition, Austin also used formic, dichloroacetic and trichloroacetic acids for dissolution of chitin chains.

Experimental values of parameters K and a relating intrinsic viscosity $[\eta]$ and molecular weight M for chitin in several solvents according to the well-known Mark–Houwink equation

$$[\eta] = KM^a \qquad (1)$$

are given in Table 5. Molecular weights were determined by light scattering using the dn/dc values mentioned in the table.

For a long time the most widely used solvent for chitin was a DMAc/LiCl mixture, though CaCl$_2$·2H$_2$O-saturated methanol was also employed, as well as hexafluoroisopropyl alcohol and hexafluoracetone sesquihydrate [50]. Vincendon [53] dissolved chitin in concentrated phosphoric acid at room temperature. In this solvent, decreases of the viscosity and of the molar mass were observed with time with no change in the degree of acetylation. The same author also dissolved chitin in a fresh saturated solution of lithium thiocyanate and got the NMR spectra at 90 °C [54]. A few papers discuss preparation of alkali chitin by dissolution of chitin at low temperature in NaOH solution. The chitin is first dispersed in concentrated NaOH and allowed

Table 5
Mark–Houwink parameters of chitin

Solvent	K (mL/g)	a	T (°C)	dn/dc	Ref.
2.77 M NaOH	0.1	0.68	20	0.145	[51a]
DMAc/LiCl 5%	7.6×10^{-3}	0.95	30	0.091	[51b]
DMAc/LiCl 5%	2.4×10^{-1}	0.69	25	0.1	[52]

to stand at 25 °C for 3 h or more; the alkali chitin obtained is dissolved in crushed ice around 0 °C. This procedure allowed the authors to cast transparent chitin film with good mechanical properties [51a,55,56]. The resulting chitin is amorphous and, under some conditions, it can be dissolved in water, while chitosan with a lower degree of acetylation (DA) and ordinary chitin are insoluble. The authors interpreted this phenomenon as related both to the decrease of molecular weight under alkaline conditions and to some deacetylation; they confirmed that to get water solubility, the DA has to be around 50% and, probably, that the acetyl groups must be regularly dispersed along the chain to prevent packing of chains resulting from the disruption of the secondary structure in the strong alkaline medium [56,57].

Recently, an interesting study, utilizing techniques such as rheology, turbidimetry and fluorescence, demonstrated that alkali chitin solubilized in cold (~0 °C) aqueous NaOH (16% w/w) according with the protocol of Sannan et al. [55,56] forms an LCST solution with a critical temperature around 30 °C [58]. A chitin gel, obtained from the solution by washing to extract NaOH, was found to be temperature and pH-sensitive [59]. These authors demonstrated a volume phase transition at ~21 °C as the result of the influence of temperature on polymer–polymer and polymer–water interactions such as hydrogen bonding and hydrophobic interactions. This transition is observed only within a narrow range of pH (7.3–7.6) and modifies the mechanical shear modulus as a function of oscillating variation in temperature.

The rheology of chitin in solution is that of a semi-rigid polysaccharide for which the conformational analysis has been developed in comparison with chitosan; this point will be taken up later in the discussion of the role of the DA on the intrinsic persistence length of the polymer.

2.3. Chitin derivatives

The most important derivative of chitin is chitosan (Fig. 1), obtained by (partial) deacetylation of chitin in the solid state under alkaline conditions (concentrated NaOH) or by enzymatic hydrolysis in the presence of a chitin deacetylase. Because of the semicrystalline morphology of chitin, chitosans obtained by a solid-state reaction have a heterogeneous distribution of acetyl groups along the chains. In addition, it has been demonstrated that β-chitin exhibits much higher reactivity in deacetylation than α-chitin [47]. The influence of this distribution was examined by Aiba [60], who showed that the distribution, random or blockwise, is very important in controlling solution properties. Reacetylation, up to 51%, of a highly deacetylated chitin in the presence of acetic anhydride gives a water soluble derivative, whereas a heterogeneous product obtained by partial deacetylation of chitin is soluble only under acidic conditions, or even insoluble. It was demonstrated from NMR measurements that the distribution of acetyl groups must be random to achieve the higher water solubility around 50% acetylation.

Homogeneously deacetylated samples were obtained recently by alkaline treatment of chitin under dissolved conditions [61]. On the other hand, the reacetylation of a highly deacetylated chitin was done by Maghami and Roberts [62], incidentally providing homogeneous samples for our SEC analysis discussed below. Toffey et al. transformed chitosan films cast from aqueous acetic acid into chitin by heat treatment [63,64]. After chitosan, the most studied derivative of chitin is carboxymethylchitin (CM-chitin), a water-soluble anionic polymer. The carboxymethylation of chitin is done similarly to that of cellulose; chitin is treated with monochloracetic acid in the presence of concentrated sodium hydroxide. The same method can be used for carboxymethylation of chitosan [65]. The method for cellulose derivatization is also used to prepare hydroxypropylchitin, a water-soluble derivative used for artificial lachrymal drops [66,67].

Other derivatives such as fluorinated chitin [68], N- and O-sulfated chitin [65,69,70], (diethylamino)ethylchitin [71], phosphoryl chitin [72], mercaptochitin [73]

and chitin carbamates [74] have been described in the literature. Modification of chitin is also often effected via water soluble derivatives of chitin (mainly CM-chitin). The same type of chemical modifications (etherification and esterification) as for cellulose can be performed on the available C-6 and C-3 –OH groups of chitin [75].

Chitin can be used in blends with natural or synthetic polymers; it can be crosslinked by the agents used for cellulose (epichlorhydrin, glutaraldehyde, etc.) or grafted in the presence of ceric salt [76] or after selective modification [77].

Chitin is partially degraded by acid to obtain series of oligochitins [47,78]. These oligomers, as well as those derived from chitosan, are recognized for their bioactivity: including anti-tumor, bactericidal and fungicidal activity, eliciting chitinase and regulating plant growth. They are used in testing for lysozyme activity. They are also used as active starting blocks to be grafted on protein and lipids to obtain analogs of glycoproteins and glycolipids.

2.4. Applications of chitin

Chitin has low toxicity and is inert in the gastrointestinal tract of mammals; it is biodegradable, owing to the presence of chitinases widely distributed in nature and found in bacteria, fungi and plants, and in the digestive systems of many animals. Chitinases are involved in host defense against bacterial invasion. Lysozymes from egg white, and from fig and papaya plants, degrade chitin and bacterial cell walls. Sashiva et al. [79] showed that a certain degree of deacetylation is necessary to allow hydrolysis of chitin [79].

Chitin has been used to prepare affinity chromatography column to isolate lectins and determine their structure [80]. Chitin and 6-O-carboxymethyl-chitin activate peritoneal macrophages in vivo, suppress the growth of tumor cells in mice, and stimulate nonspecific host resistance against *Escherichia Coli* infection. Chitin also accelerates wound-healing [65b].

Chitin is widely used to immobilize enzymes and whole cells; enzyme immobilization has applications in the food industry, such as clarification of fruit juices and processing of milk when α- and β-amylases or invertase are grafted on chitin [81]. On account of its biodegradability, nontoxicity, physiological inertness, antibacterial properties, hydrophilicity, gel-forming properties and affinity

for proteins, chitin has found applications in many areas other than food such as in biosensors [81].

Chitin-based materials are also used for the treatment of industrial pollutants and adsorbs silver thiosulfate complexes [82a] and actinides [82b].

Chitin can be processed in the form of films and fibers: fibers were first developed by Austin [83] and then by Hirano [84]. The chitin fibers, obtained by wet-spinning of chitin dissolved in a 14% NaOH solution, can also result of blending with cellulose [85] or silk [86]. They are nonallergic, deodorizing, antibacterial and moisture controlling [73]. Regenerated chitin derivative fibers are used as binders in the paper making process; addition of 10% *n*-isobutylchitin fiber improves the breaking strength of paper [87].

However, the main development of chitin film and fiber is in medical and pharmaceutical applications as wound-dressing material [88,89] and controlled drug release [90,91]. Chitin is also used as an excipient and drug carrier in film, gel or powder form for applications involving mucoadhesivity. Another interesting application is in a hydroxyapatite–chitin–chitosan composite bone-filling material, which forms a self-hardening paste for guided tissue regeneration in treatment of periodontal bony defects [92].

Chitin was also *O*-acetylated to prepare gels which are still hydrolyzed by enzyme such as hen-egg white lysozyme [93]. CM-chitin was selectively modified to obtain antitumor drug conjugates [94]. For example, 5-fluorouracil which has marked antitumor activity and the D-glucose analog of muramyl-*L*-alanyl-isoglutamine, responsible for immuno-adjuvant activity were grafted on CM-chitin using a specific spacer and an ester bond.

Chitin oligomers have been claimed as anticancer drugs, and the oligomer with DP = 5 is active in controlling the photosynthesis of maize and soybeans [95].

3. Chitosan

When the degree of deacetylation of chitin reaches about 50% (depending on the origin of the polymer), it becomes soluble in aqueous acidic media and is called chitosan. The solubilization occurs by protonation of the –NH$_2$ function on the C-2 position of the D-glucosamine repeat unit, whereby the polysaccharide is converted to a polyelectrolyte in acidic media. Chitosan is the only pseudonatural cationic polymer and thus, it finds

many applications that follow from its unique character (flocculants for protein recovery, depollution, etc.). Being soluble in aqueous solutions, it is largely used in different applications as solutions, gels, or films and fibers. The first step in characterizing chitosan is to purify the sample: it is dissolved in excess acid and filtered on porous membranes (with different pore diameters down to $0.45 \mu m$). Adjusting the pH of the solution to ca. 7.5 by adding NaOH or NH_4OH causes flocculation due to deprotonation and the insolubility of the polymer at neutral pH. The polymer is then washed with water and dried.

3.1. Chitosan structure and characterization

In the solid state, chitosan is a semicrystalline polymer. Its morphology has been investigated, and many polymorphs are mentioned in the literature. Single crystals of chitosan were obtained using fully deacetylated chitin of low molecular weight [96]. The electron diffraction diagram can be indexed in an orthorhombic unit cell ($P2_12_12_1$) with $a = 0.807$ nm, $b = 0.844$ nm, $c = 1.034$ nm; the unit cell contains two antiparallel chitosan chains, but no water molecules. The influence of experimental conditions on the crystallinity has also been described [97,98].

The main investigations of chitosan concern its preparation with varied molecular weights and DA from chitin, the dependence of its solution properties on the DA, the preparation of derivatives and applications. Sponges, powders and fibers can be obtained by regeneration of chitosan or its derivatives from solutions. These points will be developed in the following discussion.

3.1.1. Solubility of chitosan
A highly deacetylated polymer has been used to explore methods of characterization [99]. The solution properties of a chitosan depend not only on its average DA but also on the distribution of the acetyl groups along the main chain in addition of the molecular weight [57,60,100]. The deacetylation, usually done in the solid state, gives an irregular structure due the semicrystalline character of the initial polymer. Examination of the role of the protonation of chitosan in the presence of acetic acid [101] and hydrochloric acid on solubility[102] showed that the degree of ionization depends on the pH and the pK of the acid. Solubilization of chitosan with a low DA occurs for an average

degree of ionization α of chitosan around 0.5; in HCl, $\alpha = 0.5$ corresponds to a pH of 4.5–5. Solubility also depends on the ionic concentration, and a salting-out effect was observed in excess of HCl (1 M HCl), making it possible to prepare the chlorhydrate form of chitosan. When the chlorhydrate and acetate forms of chitosan are isolated, they are directly soluble in water giving an acidic solution with $pK_0 = 6 \pm 0.1$ [102], in agreement with previous data [103] and corresponding to the extrapolation of pK for a degree of protonation $\alpha = 0$. Thus, chitosan is soluble at pH below 6.

The solubility of chitosan is usually tested in acetic acid by dissolving it in 1% or 0.1 M acetic acid. We demonstrated that the amount of acid needed depends on the quantity of chitosan to be dissolved [101]. The concentration of protons needed is at least equal to the concentration of $-NH_2$ units involved.

In fact, the solubility is a very difficult parameter to control: it is related to the DA, the ionic concentration, the pH, the nature of the acid used for protonation, and the distribution of acetyl groups along the chain, as well as the conditions of isolation and drying of the polysaccharide. It is important also to consider the intra-chain H bonds involving the hydroxyl groups as shown below. The role of the microstructure of the polymer is clearly shown when a fully deacetylated chitin is reacetylated in solution; the critical value of chitosan DA to achieve insolubility in acidic media is then greater than 60%. In addition, solubility at neutral pH has also been claimed for chitosan with DA around 50% [60].

Recently, a water-soluble form of chitosan at neutral pH was obtained in the presence of glycerol 2-phosphate [104–107]. Stable solutions were obtained at pH 7–7.1 and room temperature, but a gel formed on heating to about 40 °C. The sol–gel transition was partially reversible and the gelation temperature depended slightly upon experimental conditions (Figs. 8 and 9).

3.1.2. Degree of acetylation of chitosan and distribution of acetyl groups
The characterization of a chitosan sample requires the determination of its average DA. Various techniques, in addition to potentiometric titration [108], have been proposed, such as IR [42,109–111], elemental analysis, an enzymatic reaction [112], UV [113], ^{1}H liquid-state NMR [114] and solid-state ^{13}C NMR [115–117]. The fraction of $-NH_2$ in the
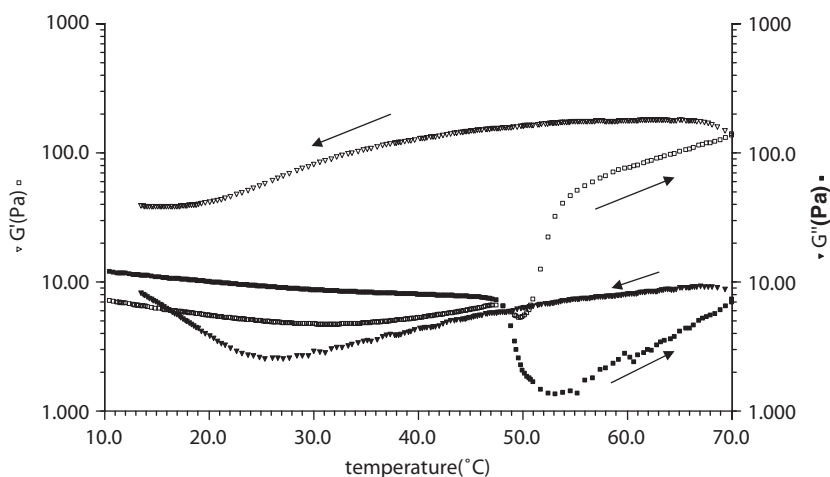
Fig. 8. Dynamic rheology giving the moduli G′ and G″ at 1 Hz frequency as a function of temperature for a chitosan-glycerol-phosphate solution: evidence of thermogelation at pH = 7.19. Polymer concentration 15 g/L. Heating curves: storage modulus G′ (□), loss modulus G″ (■). Cooling curves: G′, (▽), G″ (▼).
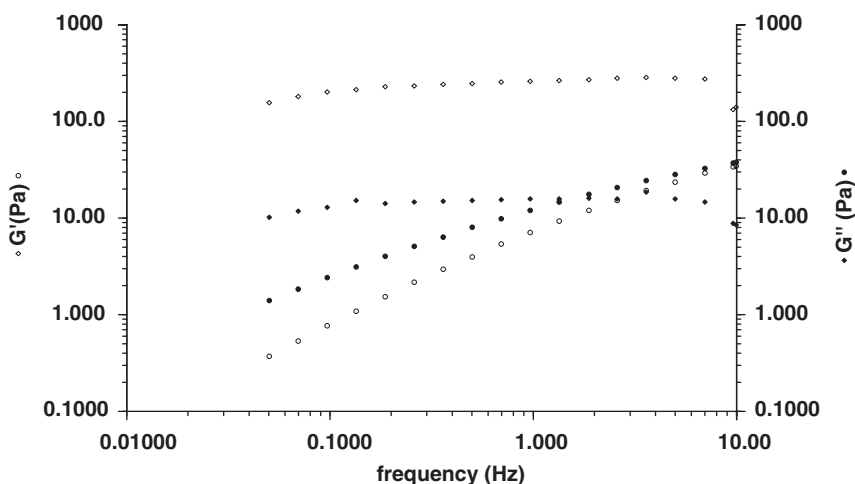

Fig. 9. Dynamic rheological moduli for chitosan-glycerol 2-phosphate at pH = 7.19 at two different temperatures. Polymer concentration 15 g/L. (a) 10 °C: G′ (○), G″ (●) indicate a viscoelastic behaviour. (b) 70 °C: G′ (◇), G″ (◆) indicate a gel-like behaviour.

polymer (which determines the DA) can be obtained by dissolution of neutral chitosan in the presence of a small excess of HCl on the basis of stoichiometry followed by neutralization of the protonated –NH$_2$ groups by NaOH using pH or conductivity measurements. These techniques and the analysis of the data obtained have been previously described [108].

Presently, we consider that ^{1}H NMR is the most convenient technique for measuring the acetyl content of soluble samples. Fig. 10 gives the ^{1}H spectrum obtained for chitosan dissolved in D$_2$O containing DCl (pD ca. 4).The signal at 1.95 ppm allows determination of the acetyl content by reference to the H-1 signal at 4.79 ppm for the D-glucosamine residue and at 4. 50 ppm for the H-1 of the N-acetyl-D-glucosamine unit at 85 °C. ^{13}C and ^{15}N solid state NMR were also tried and discussed recently; these techniques were used over the whole range of acetyl content from 0% to 100%. As an example, the chemical shifts for carbon atoms on 4 samples are given in Table 6: A is an α-chitin, B is a homogeneous reacetylated chitosan and C, D are commercial samples [117]. ^{15}N NMR gives only two signals related to the amino group and to the N-acetylated group (Fig. 11); this technique can be used in the solid state, whatever the DA. [13]C was
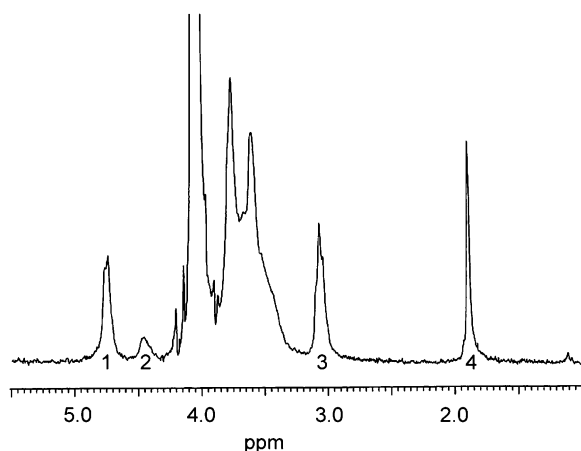
Fig. 10. ^{1}H NMR spectrum of chitosan in D$_2$O, pH~4, $T = 85\,^{\circ}$C, conc. 5 g/L: (1) H-1 of glucosamine units, (2) H-1 of N-acetyl-glucosamine, (3) H-2, (4) protons of the acetyl group of N-acetyl-glucosamine.

Table 6
Chemical shifts of chitin and chitosan obtained by ^{13}CP-MAS. (A) α-chitin, (B) chitosan obtained by partial reacetylation, (C and D) commercial chitosans [117]

Samples	A	B	C	D
C=O	173.8	173.7	173.6	nd
C1	104.1	103.5	104.7	104.7
C4	83.0	82.4	82.4	85.7–81.0
C5	75.7	74.7	75.0	74.1
C3	73.3	74.7	75.0	74.1
C6	60.8	60.3	60.1	60.7–59.6
C2	55.2	56.6	57.6	56.8
−CH$_3$	22.8	23.1	23.2	nd

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also compared with ^{1}H NMR and ^{15}N NMR and good agreement was found over the entire range of DA, whatever the state of the sample (Table 7).

The distribution of acetyl groups along the chain (random or blockwise) may influence the solubility of the polymer and also the inter-chain interactions due to H-bonds and the hydrophobic character of the acetyl group. This distribution was evaluated from ^{13}C NMR measurements [118,119]; diad and triad frequencies were determined for homogeneous and heterogeneous chitosan with different values of DA.

3.1.3. Molecular weight of chitosan

Another important characteristic to consider for these polymers is the molecular weight and its
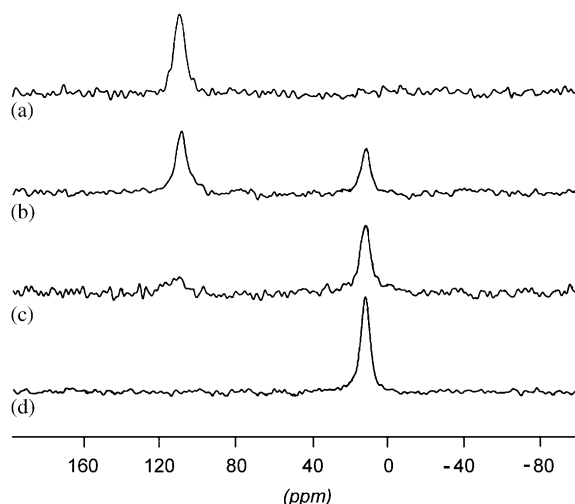

Fig. 11. ^{15}N CP-MAS NMR spectra of (A) α-chitin, (B) homogeneous partially reacetylated chitosan, (C and D) heterogeneous commercial chitosans. Reprinted with permission from Biomacromolecules 2000; 1:746–751.Copyright 2006, American Chemical Society.

Table 7
Degrees of acetylation of chitin and chitosan obtained by liquid state (^{1}H) and solid state (^{13}C and ^{15}N) NMR on the same samples as in Table 6 [117]

Samples	A	B	C	D
DA from ^{1}H NMR (liquid state)	insoluble	0.58	0.21	acetyl traces
DA from ^{13}C NMR (solid state)	0.99	0.61	0.20	0
DA from ^{15}N NMR (solid state)	1	0.63	0.20	0

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distribution. The first difficulty encountered in this respect concerns the solubility of the samples and dissociation of aggregates often present in polysaccharide solutions [120]. As to choice a solvent for chitosan characterization, various systems have been proposed, including an acid at a given concentration for protonation together with a salt to screen the electrostatic interaction.

The solvent is important also when molecular weight has to be calculated from intrinsic viscosity using the Mark–Houwink relation, Eq. (1) above,

with known values of the parameters K and a. One solvent first proposed (0.1 M AcOH/0.2 M NaCl) for molecular weight characterization was shown to promote aggregation and to overestimate the values of molecular weights calculated [121]. Some values of the Mark–Houwink parameters for chitosan solutions are given in Table 8. It was demonstrated that the aggregates perturb not only the molecular weight determination by light scattering but also the viscosity determination. To avoid these artifacts, we then proposed to use 0.3 M acetic acid/0.2 M sodium acetate (pH = 4.5) as a solvent since we had no evidence for aggregation in this mixture [123]. Absolute M values were obtained from size exclusion chromatography (SEC) with on-line viscometer and light scattering detectors to allow determination of the Mark–Houwink parameters, and also the relation between the molecular radius of gyration R_g and molecular weight. This analysis also required determination of the refractive index increment dn/dc (where c is the polymer concentration). More recently, we compared dn/dc values given in the literature with those we determined for samples with various DA values and showed that the DA has a negligible influence on dn/dc in the acetic acid/sodium acetate mixture [124]. We obtained a value of 0.190 ml/g, which is different from values used by some other authors.

The fractionation by SEC on a preparative scale in 0.02 M acetate buffer/0.1 M NaCl (pH = 4.5) was done and discussed by Berth and Dautzenberg [125]. It was applied to chitosans of commercial origin with various DA's obtained by reacetylation following the protocol of Roberts [62,121,126]. On the fractions, static light scattering, using a dn/dc of 0.203 mL/g, and viscosity measurements showed that in the range covered (0.03 < DA < 0.53) the DA had no influence on the properties of the chain. In their paper, the authors also compared their results with all the data previously published in the literature. From this

comparison, they proposed a set of parameters for the dependence of the intrinsic viscosity $[\eta]$ and the rms molecular radius of gyration R_g on molecular weight, valid for all the samples

$$[\eta](\text{mL/g}) = 0.0843 M^{0.92} \tag{2}$$

and

$$R_g(\text{nm}) = 0.075 M^{0.55}. \tag{3}$$

These parameters are in good agreement with the previous results of Rinaudo et al. [123], especially for the Mark–Houwink parameters with $K = 0.082$ mL/g and $a = 0.76$, respectively, when DA = 2%.

In a more recent paper [124], we describe a complete analysis of the molecular weight distribution by SEC using triple detection (viscosity, concentration, molecular weight) on heterogeneous chitosans, obtained from commercial sources after solid-state treatment, and on some homogeneous chitosans with different molecular weights obtained by reacetylation of a highly deacetylated chitosan [121,126]. The DA of these acid-soluble chitosans varied from 0.02 to 0.61. The data confirm the conclusion that the stiffness of the chain is nearly independent of the DA and demonstrate that the various parameters depend only slightly on the DA—a point that will be discussed below in relation to the persistence length.

The relation obtained between R_g and the molecular weight is

$$R_g(\text{nm}) = (0.064 \pm 0.002) M^{0.55 \pm 0.01}. \tag{4}$$

We proposed average values for the Mark–Houwink parameters within *portions* of the total range of DA covered, valid for heterogeneous as well as homogeneous samples (see Table 9) [124]. The relatively high values for the parameter a are in agreement with the semirigid character of this polysaccharide; to validate this conclusion, one

Table 8
Mark–Houwink parameters for chitosan in various solvents

Solvent	K (mL/g)	a	T (°C)	dn/dc	Ref.
0.1 M AcOH/0.2 M NaCl	1.81×10^{-3}	0.93	25		[121]
0.1 M AcOH/0.02 M NaCl	3.04×10^{-3}	1.26	25		[121]
0.2 M AcOH/0.1 M AcONa/4 M urea	8.93×10^{-2}	0.71	25		[122]
0.3 M AcOH/0.2 M AcONa (DA = 0.02)	8.2×10^{-2}	0.76	25	0.163	[123]
0.3 M AcOH/0.2 M AcONa (0 < DA < 0.03)	7.9×10^{-2}	0.796	25	0.190	[124]
0.02 M acetate buffer/0.1 M NaCl	8.43×10^{-2}	0.92	25	0.203	[125]

investigates chitin and chitosan molecular modeling [127] and compares the predictions with the experimental results obtained by SEC. It is important to mention the usual method of preparing chitosans with various molecular weights using nitrous acid in dilute HCl aqueous solution [128,129].

We also investigated the influence of the ionic strength on the Mark–Houwink parameters K and a [123,130,131]. The two series of solvents used were 0.3 M acetic acid/variable Na acetate content and 0.02 M acetate buffer (pH = 4.5) buffer with various concentrations of NaCl, allowing to determine the intrinsic viscosity as a function of the salt concentration; from these experimental values, extrapolation to infinite ionic strength is used to approach the θ-conditions.

3.1.4. Persistence length of chitosan

The dimensions of chitosan chains and their related hydrodynamic volume and viscometric contribution depend on the semi-rigid character of the polysaccharide chains. Since chitosan in an acid medium is a polyelectrolyte, these properties are influenced by the ion concentration. We have discussed this point, citing static and dynamic light scattering experiments in the dilute and semidilute regimes [132,133]. The actual persistence length L_t at a given ion concentration contains an intrinsic contribution L_p and an electrostatic contribution L_e calculated following Odijk's treatment [134]. The worm-like model for a semiflexible chain has been developed by several groups and successfully applied to polysaccharides [123,124,135].

A conformational analysis of chitins with different degrees of deacetylation was recently developed in our group [127]. We concluded that chitin and chitosan are semi-rigid polymers characterized by a persistence length (asymptotic value obtained at high degree of polymerization) that depends moderately on the DA of the molecule (Fig. 12). From this analysis, chitosan without acetyl groups has an intrinsic persistence length $L_p = 9$ nm at 25 °C when the electrostatic repulsions are screened. L_p increases as DA increases up to $L_p = 12.5$ nm for DA = 60%, then remains constant up to pure chitin. The local stiffness is related to the conformation of the molecule, and especially to the intra-chain H bond network formed as shown in Fig. 13. The decrease of the stiffness of chitosan as temperature increases is shown by ^{1}H NMR [136] and follows the prediction from molecular

Table 9
Mark–Houwink parameters for chitosan with different average DA in 0.3 M AcOH/0.2 M AcONa [124]

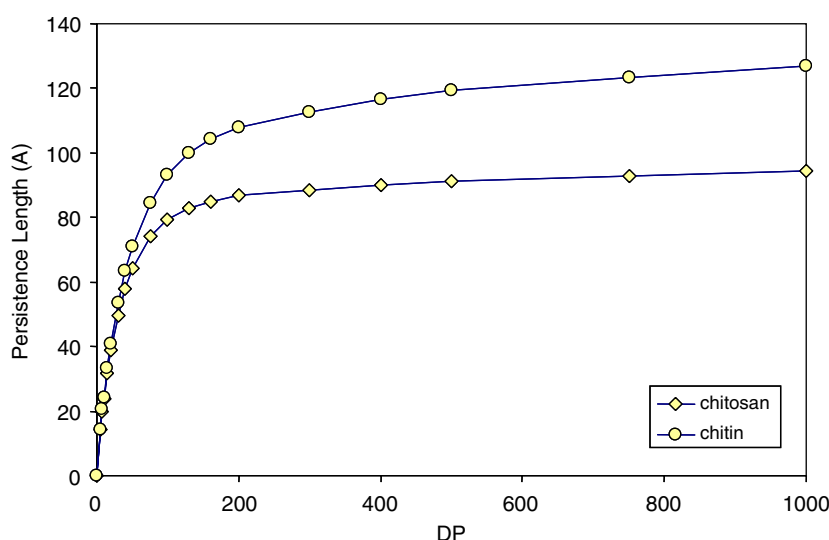| DA (%)	K (mL/g)	a
0–3	0.079	0.79
12	0.074	0.80
22–24	0.070	0.81
40	0.063	0.83
56-61	0.057	0.825

Fig. 12. Persistence length as a function of the degree of polymerization for chitin and chitosan obtained from molecular modelling at 25 °C with a dielectric constant $D = 80$.
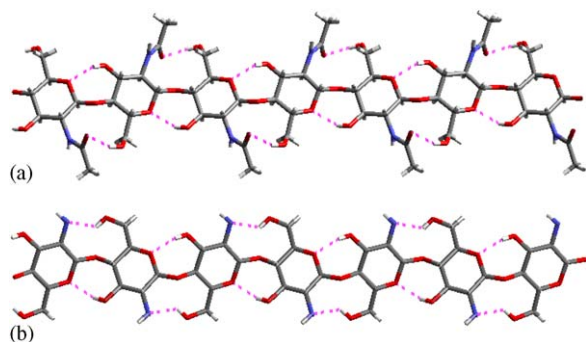
Fig. 13. Molecular modelling: (a) of a chitin chain with two H bonds (1) between—OH 3 and O 5, (2) between—OH 6 and O of C═O; and (b) of a chitosan chain with two H bonds (1) between—OH 3 and O 5, and (2) between—OH 6 and N.

modeling. A critical temperature around 40 °C is found where L_p starts to decrease more rapidly, behavior that is certainly related to the destabilization of H bonds as temperature increases. The difference in L_p values between experiment and prediction is not dramatic for chitosan—and it cannot be directly determined for chitin from experiment because of the low solubility of chitin. It was shown from size exclusion chromatography using three detectors on-line, that L_p is about 11 nm, nearly constant, for $0 < DA < 25\%$. Up to 60% acetylation, the stiffness of chitosan is not much influenced by the DA, rising only to 15 nm. The influence of the substitution has to be related to the stability of the intra-chain H-bonds, as is shown for chitin and chitosan from molecular modeling (see Fig. 13). The small variation of the persistence length with DA is in direct relation with the evolution of the Mark–Houwink parameters in Table 9.

The persistence length has also been determined by several other authors: it was given as $L_p = 4.2$ nm for $DA = 0.15$ [100] from hydrodynamic analysis and the Yamakawa–Fujii approach [137], 8 nm [123] from a combination of SEC experiments and the Odijk treatment[134], then 35 nm for chitin and 22 nm for chitosan ($DA \approx 0.42$) [138], indicating an increase of the chain stiffness as DA increases. A critical ratio of $C_\infty = 9$ was given for $0 < DA < 0.15$ [130]. $C_\infty = \lim C_x = \lim \langle h^2 \rangle / xa^2$ when the number of sugar units (x) goes to infinite; C_x corresponds to the mean-square end-to-end length of the chain normalized by the number x of sugar residues in the chain and a^2, a being the average length between adjacent glycosidic oxygens. The decrease of the stiffness of chitosan chain when the DA decreases has been confirmed and analyzed
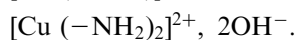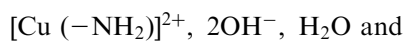
in terms of the destabilization of the local conformation by intra-chain H bonds [139].

The stiffness of the chain plays a large role in the rheological behavior of the molecule but also, even in dilute solution, it affects the existence of inter-chain H-bonds forming multimers that perturb all characterization of these polysaccharides. The aggregation has been discussed recently and its causes have been analyzed; it seems that H-bonds, as well as hydrophobic attractions, have a role, whatever the DA [120].
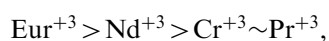
3.2. Complex formation

3.2.1. Complex formation with metals

Chitosan is known to have good complexing ability; the $-NH_2$ groups on the chain are involved in specific interactions with metals. Many papers are concerned with complexation for the recovery of heavy metals from various waste waters [140]. A mechanism for complex formation with copper at pH > 5, was proposed [103] in agreement with X-ray data on chitosan–copper stretched films [141]. Recently, the mechanism of complex formation with copper in dilute solution was re-examined and two different complexes were proposed, depending on the pH and copper content [142]. This chelation depends on the physical state of chitosan (powder, gel, fiber, film). Better chelation is obtained for greater degrees of deacetylation of chitin. Thus chelation is related to the $-NH_2$ content as well as to the $-NH_2$ distribution [143]. It is also related to the DP of oligo-chitosans; the complex starts to form when DP > 6 [144]. The two forms proposed are:

$[Cu(-NH_2)]^{2+}$, $2OH^-$, H_2O and
$[Cu(-NH_2)_2]^{2+}$, $2OH^-$.

The first complex is formed at pH between 5 and 5.8, while the second forms above pH 5.8; the maximum amount of copper fixed is $[Cu]/[-NH_2] = 0.5$ mol/mol.

The nature of the cation is very important in the mechanism of interaction [144]; the affinity of chitosan for cations absorbed on film shows selectivity following the order

$Cu^{+2} \gg Hg^{+2} > Zn^{+2} > Cd^{+2} > Ni^{+2} > Co^{+2} \sim Ca^{+2}$,

$Eur^{+3} > Nd^{+3} > Cr^{+3} \sim Pr^{+3}$,

for divalent and trivalent cations (Fig. 14) used as their chlorides. The effect of the nature of the anion
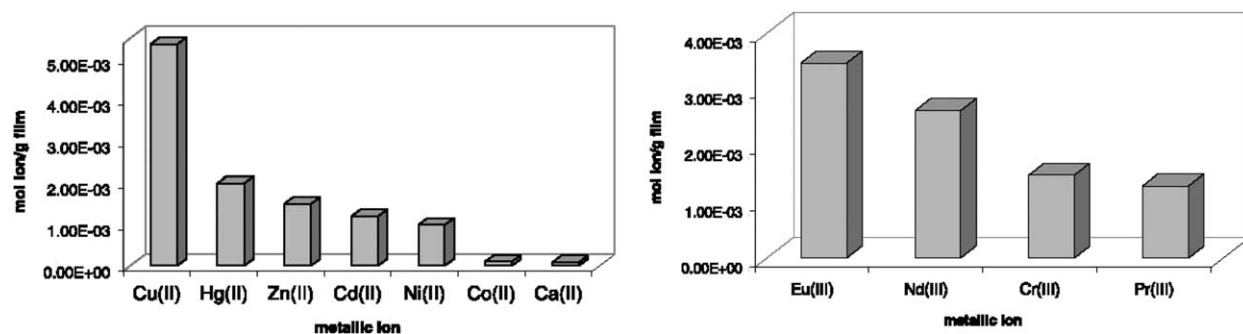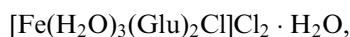
Fig. 14. Ionic selectivity of chitosan: amount (moles) of divalent and trivalent cations fixed per g of film. Reprinted with permission from Eur Polym J. 2002; 38:1523–1530. Copyright 2006, Elsevier.

was separately demonstrated [145]: e.g. sulfate increases the fixation on swollen chitosan beads.

In another study chitosan powder was dispersed in silver nitrate solution or used to fill a column to adsorb mercuric ions from a chloride solution [146]. It was shown that the conditions for using chitosan (50 mesh particles of chitosan or chemically cross-linked beads of chitosan) also play a large role in the adsorption and on the kinetics of retention [147,148].

The complex of chitosan with Fe^{3+} was prepared by mixing chitosan powder in 1.5 M ferric chloride; the solid formed was washed, dried and investigated [149]; these authors obtained an intramolecular water soluble chitosan–Fe(III) complex and determined that one Fe^{3+} is coordinated with two chitosan residues, 3 molecules of water and 1 chloride ion. The general fomula given is

$$[Fe(H_2O)_3(Glu)_2Cl]Cl_2 \cdot H_2O,$$

where Glu represents the glucosamine moiety. In the complex isolated from an aqueous solution of polymer and ferric chloride mixed in stoichiometric proportions, it is concluded that one Fe^{3+} is linked with two $-NH_2$ groups and 4 moles of oxygen from which at least one water molecule, the remaining N and O being part of the two saccharide units of chitosan (Fe^{3+} being hexa or penta coordinated) [150]. In an X-ray study of chitosan–transition metal complexes, Ogawa et al. [151] used tendon chitosan immersed in solutions of various salts. They found the ratio of glucosamine to copper (II) to be 2:1, and the crystal structure of $CuCl_2$/chitosan was different from that in complexes formed with other salts. Derivatives of chitosan have been prepared in efforts to enhance complex formation [152–157]. In one study, the same order of ionic selectivity for divalent cations as given

above [144] was found by calorimetric measurements with *N*-carboxymethylchitosan [157].

3.2.2. Electrostatic complexes

Chitosan, as a polyelectrolyte, is able to form electrostatic complexes under acidic conditions. Two different types of complexes are considered here: electrostatic complexes with an oppositely charged surfactant (SPEC) and polyelectrolyte complexes (PEC).

3.2.2.1. Complexes with surfactants. A general behavior of polyelectrolytes is demonstrated with chitosan and sodium dodecyl sulfate (SDS). An electrostatic complex is formed in the presence of a low DA chitosan involving cooperative stacking of surfactant alkyl chains. Apparently the association forms a micellar system that precipitates out, but for very small amounts of added surfactant, interesting interfacial properties are observed. A critical aggregation concentration (c.a.c.) around 100-fold smaller than the c.m.c. of the surfactant alone is detected by surface tension measurements (Fig. 15) [158,159]. The cooperativity of the observed interaction depends directly on the charge density of the chitosan (in fact, it depends on the distance between two adjacent ionic sites), as is shown for carboxymethylchitin in the presence of tetradecyltrimethylammonium bromide (TTAB) [160].

In addition, a capsule is formed when a chitosan solution is dropped into a SDS surfactant solution; a chitosan gel layer (characterized by an ordered nanostructure) crosslinked by charged surfactant micelles is formed in the interfacial film [161]. Babak et al. [162] showed that this structure can encapsulate enzymes.

This type of electrostatic complex has been examined by calorimetry. The strong affinity and
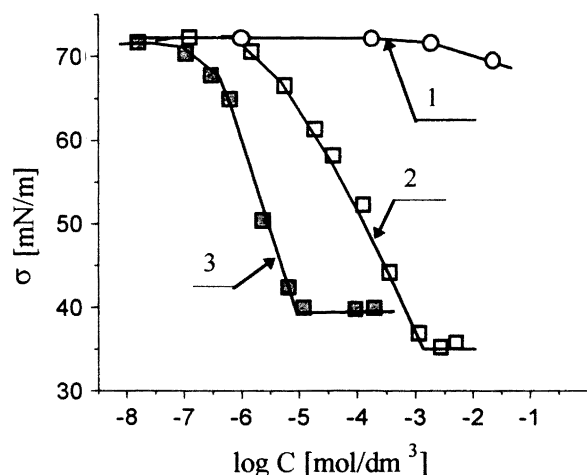
Fig. 15. Electrostatic complex formed between chitosan and an anionic surfactant. Surface tension at air–solution interface as a function of the concentration of (1) cationic chitosan (2) anionic surfactant SDS (3) SDS in chitosan ($c = 2.7 \times 10^{-3}$ monomol/dm^3) /SDS complex in acetate buffer (0.05 M). Reprinted with permission from Colloids and surfaces, A: Physicochem Eng Aspects. 1999; 147: 139–148. Copyright 2006, Elsevier.

its dependence on the excess of external salt confirm the electrostatic mechanism [163–165].

This electrostatic interaction has been compared with covalent analogs obtained by grafting alkyl chains on a chitosan backbone (these derivatives will be described below). The interfacial properties of the chitosan-derived polymer surfactant has relatively low surface tension acitivity but interesting bulk properties. The role of sulfated *N*-acyl chitosan (S–Cn–Chitosan) in a lipid membrane was compared with that of SDS to show that SDS dissociates the membrane, whereas the polymer stabilizes the membrane, and even increases its rigidity, suggesting low toxicity in bioorganisms. In solution, when the alkyl chain in S–Cn–chitosan is longer than 10 units, the polymers form more stable micelles than those formed by the same alkyl chain surfactant alone [166].

Interactions of this kind are relevant to the field of food chemistry, involving specific interactions of chitosans with phospholipids and bile acids [164].

3.2.2.2. Complexes with oppositely charged polymers (proteins, polyanions, DNA). There are no good examples of polymer/polymer complex formation based on chitosan and neutral polymers, although many electrostatic PEC between chitosan and synthetic or natural polymers are cited in the

literature: e.g. polyacrylic acid, sodium salt (PAA), carboxymethylcellulose (CMC) [167,168], xanthan, carrageenan, alginate (extracted from brown algae), pectin, heparin, hyaluronan (HA) [108,169], sulfated cellulose, dextran sulfate, *N*-acylated chitosan/ chondroitin sulfate [170,171]. The electrostatic interaction has been discussed in relation to the stiffness of the backbone and nature of the ionic groups involved. Especially with alginate or HA, a pH-dependent complex is formed, whose stability depends on the ionic strength. The complex formation was investigated in dilute solution by potentiometry following changes in pH and conductivity to determine the fraction of ion pairs ($-COO^{-+}NH_3-$) formed, depending on the experimental conditions [108,172]. The interaction between chitosan and alginate gives an electrostatic complex which so far has been used mostly for biological applications. The complex between DNA and chitosan oligomers (or polymers) is now under investigation in many laboratories. In a recently published investigation of the mechanism and cooperativity of the complexation with chitosan oligomers [173], it was shown that a minimum DP (and charge) around 6–9 is necessary for stability. The stability of this complex is reduced above pH = 7.4, near the physiological pH, a finding that seems highly relevant for gene delivery applications and is interpreted as one reason for the observed high transfection activity of the oligomer-based complex.

The main applications of these electrostatic complexes are antithrombogenic materials, controlled release systems, encapsulation of drugs, immobilization of enzymes and cells, and gene carriers. Some examples will be discussed below where the applications of alginate/chitosan complexes are discussed.

One aspect of these complexes now in development is the preparation, layer-by-layer (successively, one layer of polyanion–one layer of polycation), of polyelectrolyte capsules or films based on charged biocompatible polysaccharides or chitosan/synthetic PEC [169,174,175]. In the case of chitosan capsules [174], PAA is used to form the capsules, then the chitosan is crosslinked and the PAA is redissolved. Such chitosan capsules are more stable than in absence of chemical crosslinking and are pH-sensitive, swelling at low pH and shrinking at high pH. Porous gels (sponges) can be prepared by formation of a calcium alginate gel stabilized by complexation with galactosylated chitosan (a water-soluble derivative) [176]. A complex in the form of

beads was produced by dropwise addition of Na–alginate to a chitosan–CaCl$_2$ solution. These beads differ from Ca–alginate beads in exhibiting maximum swelling at pH 9 [177]. Oligo-chitosans, low molecular weight chitosans, were also complexed with alginates to form capsules with controlled permeability [178,179].

3.3. Chitosan-based materials

Chitosan is used to prepare hydrogels, films, fibers or sponges, as previously mentioned, most of the materials are used in the biomedical domain, for which biocompatibility is essential. Many systems are described in the literature, but we can cite only a few of the most promising. Chitosan is much easier to process than chitin, but the stability of chitosan materials is generally lower, owing to their more hydrophilic character and, especially, pH sensitivity. To control both their mechanical and chemical properties, various techniques are used, as mentioned previously for chitin. Often, the methods are adapted from the cellulose world.

First, chitosan may be crosslinked by reagents such epichlorohydrin [180], diisocyanate [181] or 1, 4-butanediol diglycidyl ether [182]. Specific cross-linking was performed on a blend of starch and chitosan: starch was oxidized to produce a poly-aldehyde that reacts with the –NH$_2$ group of chitosan in the presence of a reducing agent [183]. Many chitosan hydrogels are obtained by treatment with multivalent anions: the case of glycerol-phosphate is mentioned above [104], but oxalic acid has also been used [93b,184] as well as tripolypho-sphate [185,186].

Blends and composites have been prepared especially by Hirano, in the way mentioned previously for chitin [84]. Other systems are proposed in the literature: chitosan/polyamide 6 [187], chitosan/cellulose fibers [188], chitosan/cellu-lose using a common solvent [189], chitosan/polyelthylene glycol [190], chitosan/polyvinylpyrro-lidone and chitosan/polyvinyl alcohol [191]. Re-cently, reinforcement of chitosan film with carbon nanotubes was tested; this composite exhibits a large increase of the tensile modulus with incor-poration of only 0.8% of multiwalled carbon nanotubes [192]. 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. This aspect will be described in the following.

3.4. Chemical modification of chitosan

3.4.1. Modification reactions

Among the many mentions of chitosan deriva-tives in the literature [65,193,194], one can differ-entiate specific reactions involving the –NH$_2$ group at the C-2 position or nonspecific reactions of –OH groups at the C-3 and C-6 positions (especially esterification and etherification) [75]. The –NH$_2$ in the C-2 position is the important point of difference between chitosan and cellulose, where three –OH groups of nearly equal reactivity are available. The main reaction easily performed involving the C-2 position is the quaternization of the amino group or a reaction in which an aldehydic function reacts with –NH$_2$ by reductive amination. This latter reaction can be performed in aqueous solution under very mild conditions to obtain randomly distributed substituents in a controlled amount along the chitosan chain. This method has been proposed to introduce different functional groups on chitosan using acryl reagents in an aqueous medium; introduction of *N*-cyanoethyl groups is said to produce some cross-linking through a reaction between the nitrile group and the amine group [195]. In addition, it is important to note that more regular and reproducible derivatives should be obtained from highly deacetylated chitin [99]—assuring control of the quality of the initial material that is essential before modification, especially when biological applications are to be explored.

3.4.2. Some chitosan derivatives
3.4.2.1. O-and N-Carboxymethlchitosans. Carboxy-methylchitosan (CM-chitosan) is the most fully explored derivative of chitosan; it is an amphoteric polymer, whose solubility depends on pH. Under controlled reaction conditions (with sodium mono-chloracetate in the presence of NaOH), one gets *O*-and *N*-carboxymethylation. The yield of substitu-ents on the three positions was determined by NMR [196]. This reaction extends the range of pH (pH > 7) in which chitosan is water-soluble, but a phase separation due to the balance between positive and negative charges on the polymer was observed at 2.5 < pH < 6.5.

Most interesting is the preparation of *N*-carboxymethylchitosan by reaction with glyoxylic acid in the presence of a reducing agent [196c]. The distribution of monosubstituted (–NH–CH$_2$COOH) and disubstituted (–N (–CH$_2$COOH)$_2$) groups was established by ^{1}H and ^{13}C NMR. Disubstitution is easily obtained, giving an interesting derivative for ion complexation. A specific oxidation of the C-6 position hydroxyl group was realized using the TEMPO reactant on chitin to produce a chitin-based hyaluronic acid analog [197]. This derivative is water soluble in a wide range of pH, but only if it is prepared from a fully acetylated chitin.

3.4.2.2. Chitosan 6-O-sulfate. This derivative is an anticoagulant; it was first prepared as an *O*- sulfated derivative [198,199] and more recently as *N*-sulfated chitosan [200].

3.4.2.3. N-methylene phosphonic chitosans. These interesting anionic derivatives, with some amphoteric character were synthesized under various conditions and proved to have good complexing efficiency for cations such as Ca^{2+}, and those of transition metals (Cu (II), Cd (II), Zn (II) etc.) [201,202]. The complexation provides corrosion protection for metal surfaces [203]. These derivatives were also modified and grafted with alkyl chains to obtain amphiphilic properties that have potential applications in cosmetics [204].

3.4.2.4. Trimethylchitosan ammonium. This cationic derivative, water soluble over all the practical pH range, is obtained by quaternization of chitosan [205] with methyl iodide in sodium hydroxide under controlled conditions, and has been fully characterized by NMR [196c,206]. A large decrease of molecular weight during this reaction is observed under all conditions tested. These polymers show good flocculating properties with kaolin dispersions, suggesting applications to paper making [207]. Other quaternized derivatives have been prepared are claimed to have antistatic properties [208].

3.4.2.5. Carbohydrate branched chitosans. Carbohydrates can be grafted on the chitosan backbone at the C-2 position by reductive alkylation: For that purpose, disaccharides (cellobiose, lactose, etc.) having a reducing end group, are introduced, in the presence of a reductant, on chitosan in the open-chain form [209]. These derivatives are water soluble. Galactosylated chitosan was mentioned

previously [176]. Carbohydrates can also be introduced without ring opening on the C-6 position [210]. These derivatives are important as they are recognized by the corresponding specific lectins and thus could be used for drug targeting [194]. A special case is the grafting of a cyclic oligosaccharide, cyclodextrin, discussed below.

3.4.2.6. Chitosan-grafted copolymers. One of the most explored derivatives is poly(ethylene glycol)-grafted chitosan, which has the advantage of being water soluble, depending on the degree of grafting: higher molecular weight PEG at low DS gives higher solubility than low molecular weight PEG [194]. PEG can be also be introduced by reductive amination of chitosan using PEG-aldehyde [211].

Polypeptides have been grafted by reaction with *N*-carboxyanhydrides of amino acids with the purpose of developing new biomaterials [212], but the degree of polymerization of the grafted chains cited in this work remains low (DP = 5.9–6.6).

3.4.2.7. Alkylated chitosans. Alkylated chitosans are very important as amphiphilic polymers based on polysaccharides. The first derivative having these characteristics was a C-10-alkyl glycoside branched chitosan with a high degree of substitution (DS = 1.5), which gelled when heated over 50 °C [213]. Another approach was used for selective *N*- and *O*-palmitoylation giving a derivative with two or three long alkyl chains per monomeric unit. This reaction involved protection and deprotection of the C-6 position [214].

By using carboxylic anhydrides with different chain lengths on CM-chitosan, highly substituted derivatives with low regularity were obtained. They were insoluble in water and their biodegradability was decreased [215].

Using the reductive amination, a series of amphiphilic derivatives were produced with different chain lengths (C_n from 3 to 14) and controlled DS (usually lower than 10% to maintain water solubility in acidic conditions) [216]. This technique was also used to introduce *n*-lauryl chains [217]. Alkylated chitosans with good solubility in acidic conditions (pH < 6) have a number of very interesting properties. First, they exhibit surface activity and they were compared with corresponding low molecular weight surfactants [158,159,161]; for the same amount of alkyl chains with the same length, they have a relatively low effect on the decrease of the surface tension but they improve much the

stability of the interfacial film [160,218,219]; it was clearly demonstrated that a simple surfactant and modified chitosan have completely different behavior [161b,166]; secondly, they increase considerably the viscosity of aqueous solution due to hydrophobic inter-chain interactions; especially for C-12 chain length and a DS~5%, a physical gel is obtained ; the formation of this gel depends on the pH [220] and on salt concentration [221]. These gels result from a balance between electrostatic repulsions between the positively charged chitosan chains and hydrophobic attraction due to alkyl chains mainly in relation with their length [216]. The hydrophobic domains formed in the systems are important to adsorb hydrophobic molecules such as pyrene (a fluorescent probe used to evidence these domains in dilute solution); these associations can be destroyed reversibly by addition of cyclodextrins which are known to complex the alkyl chains of surfactants [221,222]. It is interesting to mention that alkyl chitosans are compatible with neutral and cationic surfactants; it was demonstrated that cationic surfactant adsorbed on the alkyl chain grafted on chitosan, promotes its solubilization [158].

3.4.2.8. Cyclodextrin-linked chitosans. The cyclic oligosaccharides, namely α-,β-,γ-cyclodextrins (CD), are important because of their ability to encapsulate hydrophobic molecules in their toroidal hydrophobic cavity, whose selectivity depends on the number of glucose units (respectively 6, 7, 8 D-glucose units) [222–224]. For various applications, it is interesting to graft the cyclodextrin on a polymeric backbone such as a biocompatible polysaccharide. A synthesis of α- and β-cyclodextrin-chitosans with relatively high degree of substitution has been described [225]. The authors found that these new derivatives had the ability to differentially recognize and retain certain guest compounds based on their molecular shapes and structures. They proposed to use these polymers as supports for reverse-phase adsorption or as adsorbents in controlled release systems.

A β-cyclodextrin with a specific modification on one of the –OH groups on its small side was grafted to chitosan by reductive amination. At a DS lower than 10%, these derivatives are water soluble in acidic conditions with loose inter-chain interactions [226,227]. The grafted cyclodextrin has the same association constant as the free CD with small hydrophobic molecules such as adamantane [227].

This modified chitosan should be adaptable for drug delivery. When these CD-chitosans were mixed with chitosan grafted with adamantane (AD), the specific recognition led to a self-assembled gel (Fig. 16) [228]. This physical gel is stabilized by specific CD/AD linkages in a dynamic mechanism with a relaxation time depending on polymer concentration, temperature, and the presence of excess free CD or AD [229].

3.5. Applications of chitosan and chitosan derivatives

Table 10 summarizes the main properties of chitosan and potential biomedical and other applications that they imply. The great current interest in medical applications of chitosan and some of its derivatives is readily understood. The cationic character of chitosan is unique: it is the only pseudo-natural cationic polymer. Its film forming properties and biological activity invite new applications. Table 11 recalls the main applications of chitosan. The most important fields where the specificity of chitosan must be recognized are cosmetics (especially for hair care in relation to electrostatic interactions) (Table 12) and the pharmaceutical and biomedical applications on which we focus, which probably offer the greatest promise [230,231].

Drug delivery applications include oral, nasal, parenteral and transdermal administration, implants and gene delivery. The transmucosal administration of drugs has been discussed recently [232].
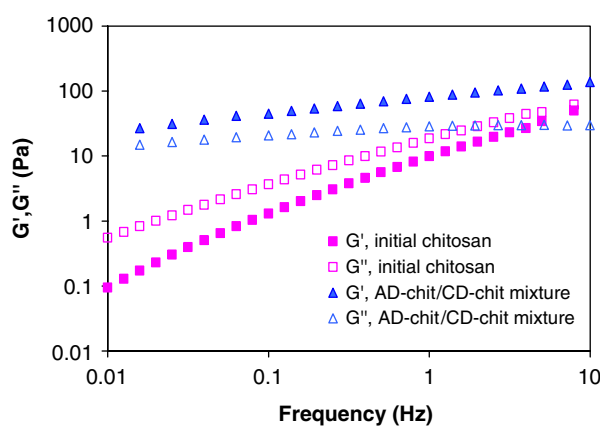

Fig. 16. Rheological behavior (G' and G'' moduli as a function of frequency) for (a) chitosan 30 g/L and (b) 50/50 mixture of CD-chitosan and AD-chitosan at a total polymer concentration 4.43 g/L in 0.3 M AcOH/0.03 M AcONa. Reprinted with permission from J Phys Chem B. 2003; 107:8248–8254. Copyright 2006, American Chemical Society.

Table 10
Principal properties of chitosan in relation to its use in biomedical applications

Potential Biomedical applications	Principal characteristics
Surgical sutures	Biocompatible
Dental implants	Biodegradable
Artificial skin	Renewable
Rebuilding of bone	Film forming
Corneal contact lenses	Hydrating agent
Time release drugs for animals and humans	Nontoxic, biological tolerance
Encapsulating material	Hydrolyzed by lyzosyme
	Wound healing properties
	Efficient against bacteria, viruses, fungi

Table 11
Principal applications for chitosan

Agriculture	Defensive mechanism in plants
	Stimulation of plant growth
	Seed coating, Frost protection
	Time release of fertilizers and nutrients into the soil
Water & waste treatment	Flocculant to clarify water (drinking water, pools)
	Removal of metal ions
	Ecological polymer (eliminate synthetic polymers)
	Reduce odors
Food & beverages	Not digestible by human (dietary fiber)
	Bind lipids (reduce cholesterol)
	Preservative
	Thickener and stabilizer for sauces
	Protective, fungistatic, antibacterial coating for fruit
Cosmetics & toiletries	Maintain skin moisture
	Treat acne
	Improve suppleness of hair
	Reduce static electricity in hair
	Tone skin
	Oral care (toothpaste, chewing gum)
Biopharmaceutics	Immunologic, antitumoral
	Hemostatic and anticoagulant
	Healing, bacteriostatic

Mucoadhesivity of chitosan and cationic derivatives is recognized and has been proved to enhance the adsorption of drugs especially at neutral pH; *N*-trimethyl chitosan chloride interacts with negatively charged cell membranes [233,234].

n-Lauryl-carboxymethylchitosan is an amphiphilic polymer. It forms micelles that solubilize taxol, making it more effective therapeutically, and it is found to be safe in terms of membrane toxicity. This type of derivative is generally useful as a carrier for hydrophobic cancer drugs [235].

Some recent advances in drug release should be mentioned. Films of chitosan incorporating prednisolone, formed by mixing have been tested for this purpose [242]. Chitosan gels and layer-by-layer polyelectrolyte capsules are often used for controlled release of drugs or proteins, as previously noted.

Chitosan and its derivatives have been used for gene transfection: for *N*-alkylated chitosan, it has been shown that transfection efficiency increases upon elongating the alkyl side chains and levels off when the number of carbons in the side chain exceeds 8 [236]. Quaternized chitosan can be used for the same purpose [237].

The physical properties of chitosan recommend it, for use in many types of devices [239]. In addition, proteins and DNA can be assembled with a stimuli-responsive chitosan backbone.

Another point to note is biological activity in regard to agriculture since chitosan exhibits anti-virus and antiphage activities [240]. It inhibits the growth of bacteria and bacterial infection, and stimulates the natural defenses in plants. A mechanism has been proposed via the "octadecanoid pathway" [241].

Alginate/chitosan systems have found applications as wound dressings and in bone tissue engineering [243,244].

An interesting application concerns a self-setting calcium phosphate cement: chitosan glycerophosphate mixed with calcium phosphate and citric acid forms an injectable self-hardening system for bone repair or filling [92,238].

4. Conclusion

In this review we aim to present an overview of the state of art in the knowledge and technical applications of chitin and chitosan. We include an extensive bibliography of recent studies, both basic and applied. Nevertheless, this is an ambitious project; and the very large number of papers published on a wide range of properties and applications forces us to make a selection from the most significant results obtained by the many groups working around the world.

Table 12
Specific characteristics for applications of chitosan in hair care

Properties	Uses
Aqueous solution interacting with negatively charged hair (electrostatic interaction)	Shampoos
Antistatic effect (due to hydrophilic character), maintains moisture in low humidity and hair style in high humidity	Hair tonics
	Rinses
	Permanent wave agents
	Hair colorants
	Lacquers,
	Hair sprays
	Time release delivery (chitosan beads, gels or granules)
Removing sebum and oils from hairs (due to hydrophobic character)	
Antibacterial and antifungal activity	
Thickening polymer	
Role in surfactant stability; stabilize emulsion	
Make hair softer, increase mechanical strength	
Protect elastic film on hairs, increasing their softness.	

Chitin is a natural polymer for which we try to point out some unique features and its potential for useful development. It is important to bear in mind that its insolubility in ordinary solvents, makes chitin difficult to characterize and to process.

Though much work has been done to elucidate the morphology of chitin in the solid state, uncertainties still remain in the published record, and better parameters are still needed on α-chitin and hydrated β-chitin.

Chitin can be transformed and used as fiber, film, sponge or powder. The preparation of derivatives that are soluble, especially in aqueous media, makes it possible to take advantage of the special properties of chitin: this polysaccharide is a film-forming polymer, biodegradable and renewable; it also has antibacterial and fungistatic properties; the semi-rigid character of chitin is valuable for thickening properties but also promotes the inter-chain interactions that cause difficulties in characterization. Due to the diversity of sources of chitin and their state of organization in the solid state, the quality of commercial chitin available is not uniform and causes many difficulties during transformation and chemical modification. This is one of the factors that hinder the development of new uses. In addition, the high cost of extraction and purification of chitin seems to reserve this polymer to high added-value applications. For these reasons, it is assumed that biomedical and pharmaceutical applications are the most promising

domains for development, with cosmetics in second place.

Similar conclusions can be drawn for chitosan, which is the most important derivative of chitin, even though the difficulty of controlling the distribution of the acetyl groups along the backbone makes it difficult to get reproducible initial polymers. Unlike chitin, chitosan is water soluble in acidic media, or under precisely specified conditions at neutral pH, allowing much development in the domains of solutions and hydrogels.

The advantage of chitosan over other polysaccharides (cellulose, starch, galactomannans, etc.) is that its chemical structure allows specific modifications without too many difficulties at the C-2 position, as described in this review. Specific groups can be introduced to design polymers for selected applications.

Finally, the natural biological properties of chitin and chitosan are valuable for both plant and animal applications, and such developments can be considered as valuable extensions of the use of chitin and its derivatives.

Acknowledgments

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