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REVIEW

Applications of biopolymers I: chitosan

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Abstract Chitosan is prepared from chitin, the second most abundant natural polymer in the world. It is primarily composed of glucosamine and *N*-acetyl glucosamine residues with a 1,4-β-linkage. It can be obtained by deacetylation of chitin, which is produced from shells of crustaceans, insects, and other sources. Chitosan is a nontoxic, biodegradable, and biocompatible natural polymer and can be used in a wide range of applications such as in the areas of biomedicine, membranes, drug delivery systems, hydrogels, water treatment, food packaging, etc. In this paper, some novel applications of this biopolymer in different fields are reviewed.

Keywords Chitosan · Biopolymer · Application · Biodegradable · Glucosamine

Introduction

Polysaccharides are widely distributed in nature. These materials are important in different fields since they possess unique structures and characteristics that are different from those of typical synthetic polymers. Among the many kinds of polysaccharides, cellulose and chitin are the most important biomass resources. Cellulose is synthesized in plants, whereas chitin is obtained from lower animals. They are the most abundant organic compounds on Earth. Chitin is structurally similar to cellulose, with acetamide groups at the C-2 positions in place of hydroxyl.

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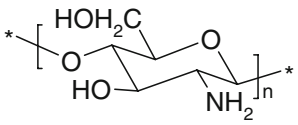
Chitin, poly(β-(1→4)-*N*-acetyl-D-glucosamine), is synthesized by an enormous number of living organisms and, depending on its source, occurs as two allomorphs, namely the α and β forms. It is extracted from crustaceans by acid treatment to dissolve calcium carbonate followed by alkaline extraction to solubilize proteins. In addition, a decolorization step is often added to remove leftover pigments and obtain a colorless product.

Chitosan, the most important derivative of chitin, can be obtained by deacetylation of chitin under alkaline conditions. When the degree of deacetylation of chitin reaches about 50% (depending on the origin of the polymer), it becomes soluble in aqueous acidic media. The solubilization occurs by protonation of the NH_2 functional group on the C-2 position of the D-glucosamine repeating unit, whereby the polysaccharide is converted to a polyelectrolyte in acidic media.

The presence of NH_2 groups in chitosan is the reason why it exhibits much greater potential compared with chitin for use in different applications. It is a special biopolymer having good properties including biodegradability, biocompatibility, and antibacterial activity so it is interesting as a novel type of functional material. Chitosan is the only pseudonatural cationic polymer and thus has many applications in different fields [1, 2]. The structures of chitosan alone and in comparison with cellulose and chitin are shown in Figs. 1 and 2.

Chitosan is commonly prepared by deacetylation of α-chitin using 40–50% aqueous alkali solution at 100–160°C for a few hours. The resulting chitosan has a degree of deacetylation (DA) up to 0.95. For complete deacetylation, the alkaline treatment can be repeated. Since β-chitin can be deacetylated at a much lower temperature than α-chitin, a reaction near 80 °C is adequate for deacetylation as well as for the suppression of coloration

Fig. 1 Structure of chitosan



processes, giving almost colorless chitosan products [3]. In a review paper [4] several methods which have been developed to determine the degree of DA are described. Infrared (IR) spectroscopy is a relatively quick technique for qualitative evaluation of DA through the determination of absorption ratios.

As previously mentioned, the major procedure for obtaining chitosan is based on the alkaline deacetylation of chitin. Isolation of chitin itself is affected by its source. Generally the raw material is crushed, washed with water or detergent, and cut into small pieces. Abdou [5] and coworkers have studied the extraction of chitin and chitosan from local sources. It has been shown that β-chitin is much more susceptible to deacetylation than the α form. Also, chitin is more thermally stable than chitosan. In other research, the influence of γ-irradiation on the processes involved in chitin and chitosan production from prawn shells has been investigated [6].

Chitosan has a wide range of applications. Some of the potential applications of this biopolymer are in the areas of medicine, drug delivery, water treatment, membranes, hydrogels, adhesives, antioxidants, biosensors, and food packaging. Some of these applications are presented in more detail in the following sections.

Biological adhesive

Chitosan and its derivatives have been used in a wide range of applications. In recent research [7], it has been found that the introducing of azide and lactose moieties into chitosan provides much better water solubility at neutral pH values. This type of chemical has been used as a biological adhesive for soft tissues. It is photo-cross-linkable by applying ultraviolet (UV) light, producing an insoluble hydrogel within 60 s. This material has great potential as a biological adhesive for medical use.

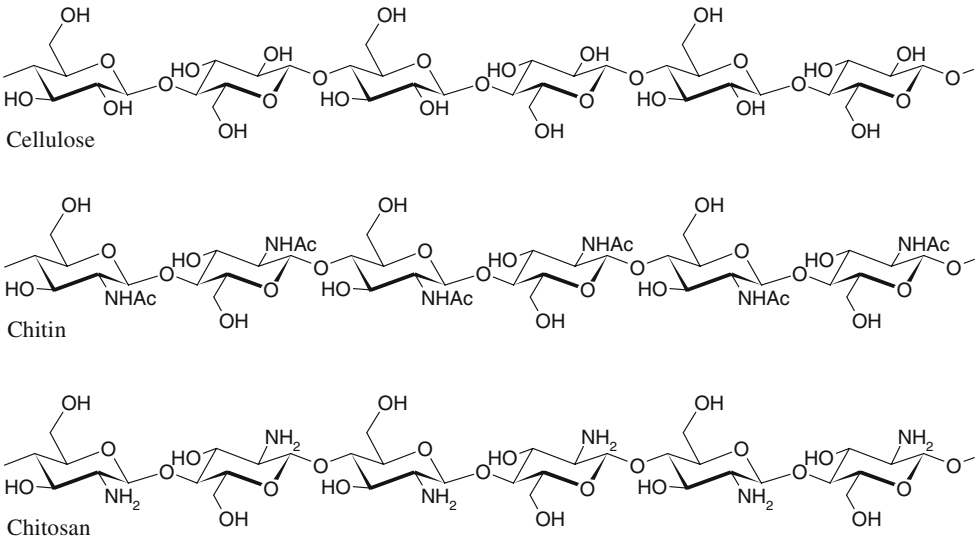
Also, an investigation has been carried out on the application of dilute chitosan solutions gelled by *melB* tyrosinasecatalyzed reaction with 3,4-dihydroxyphenethylamine (dopamine) [8]. The observed adhesive properties appear to be related to the increased viscosity of the modified chitosan material. Adhesive strength increases with increasing molecular mass of the chitosan samples used and their amino group concentration. Therefore, tyrosinase-catalyzed reactions of dopamine can be used to confer water-resistant adhesive properties to dilute chitosan solutions.

Antioxidant

Chitosan has antioxidant properties. Two types of fungal chitosan, B or C, have been prepared by alkaline *N*-deacetylation of crude chitin B or C for different durations of 60, 90, and 120 min [9]. Results show that chitosan has antioxidant activities of 61.6–82.4% at 1 mg cm^{-3} and shows reducing powers of 0.42–0.57 at 10 mg cm^{-3}. Also, no significant difference in antioxidant properties between chitosan B and C has been observed.

In other research [10], antioxidant activity of chitosans with different molecular weights (30, 90, and 120 kDa) in salmon (*Salmo salar*) has been studied. Incorporation of 0.2%, 0.5%, and 1% chitosan, employing 2-thiobarbituric acid-reactive substances (TBARS) and 2,2-diphenyl-1-picrylhydrazyl scavenging assays, shows the antioxidant

Fig. 2 Structures of polysaccharides: cellulose, chitin, and chitosan



properties of chitosan, which cause reduction of lipid oxidation. The results indicate that 30-kDa chitosan, which has high water solubility, may be beneficial as a potential natural antioxidant for stabilizing lipid-containing foods.

Biofilms

Biodegradable flexible composite films from corn starch and chitosan have been synthesized [11]. These films can be described as biofilms with a homogeneous matrix, stable structure, good water barrier, and mechanical properties. Also, novel biodegradable films have been prepared from chitosan and poly(lactic acid) (PLA), by solution mixing and a film-casting method [12]. Chitosan and PLA exhibit interesting qualities in the field of bioactive packaging, due to the antimicrobial properties of chitosan films and the excellent mechanical properties of PLA. These composite films offer a great advantage in preventing surface growth of mycotoxinogen strains; however, the physicochemical properties of such heterogeneous films significantly limit their further usage as packaging materials.

Coatings, biosensors, and surface conditioners

Chitosan may be used as a coating material. Recently, an electrophoretic deposition (EPD) method has been used for the fabrication of nanocomposite silica-chitosan coatings [13]. Good binding and film-forming properties of chitosan result in the formation of relatively thick coatings in the range of up to 100 μm. This process is achieved at room temperature, so the problems related to the high-temperature sintering of ceramic coatings on metallic substrates can be avoided.

Chitosan is also used as a coating material for fruits. The effects of edible chitosan coating on quality and shelf life of mango fruit has been studied [14]. The results show that applying a chitosan coating effectively prolongs the quality and extends the shelf life of fruit.

Chitosan can be used in electrochemistry and biosensors. Natural oligosaccharide-derived ionic liquids have been synthesized from 1-ethyl-3-methylimidazolium hydroxide and carboxymethylated chitosan by acid–base neutralization reaction [15]. The results demonstrate that ionic liquids with low molecular weight have good ionic conductivity and thermal stability.

Also, biocompatible nanocomposites based on clay, chitosan, and gold nanoparticles have been synthesized and used for horseradish peroxidase (HRP) assembly and biosensor fabrication [16].

The optical behavior of chitosan-polyvinyl alcohol blend films has been studied [17]. The opacity of a film indicates its degree of transparency. Chitosan film showed a higher value of opacity (8.11%) while the lowest value was found for polyvinyl alcohol (7.03%). Also, UV–Vis-infrared optical and atomic force microscopy (AFM) study of spin-cast chitosan films has been reported [18]. The purpose of this study was to determine the optical constants of chitosan films prior to chemical modification, over a wide spectral range from the mid-infrared to vacuum-ultraviolet regions.

Chitosan derivatives may be used as pesticides. For example, in new research, 24 types of chitosan derivatives, *N*-alkyl chitosans (NAC), and *N*-benzyl chitosans (NBC) have been synthesized [19] and their insecticidal and fungicidal activities tested. Among them, *N*-(2-chloro-6-fluorobenzyl)chitosan is the most active against the cotton leafworm. These compounds provide the basis for producing novel pesticides based on natural products.

Chitosan is also used as a surface conditioner. Novel chitosan-*N*-dextran graft copolymers with a degree of substitution of chitosan varying from 16% to 60% and carrying 9 or 36 glucopyranose units in the grafted dextran chains have been synthesized. Despite the high density of dextran grafts, chitosan derivatives possess a polyelectrolyte effect and these copolymers can be used as surface conditioners [20].

Biomedical applications

Chitosan and its derivatives are suitable for tissue engineering applications because of their porous structure, gel-forming properties, ease of chemical modification, biodegradability, biocompatibility, antibacterial activity, and high affinity to in vivo macromolecules. It is one of the most important biomaterials in tissue engineering and shows considerably very good physicochemical and biological properties. Various types of chitosan derivatives have been used in skin, bone cartilage, liver, nerve, and blood vessel [21].

Tissue-plasminogen activator (t-PA) encapsulated in poly(lactide-*co*-glycolide) (PLGA) nanoparticles (NPs) with chitosan (Ch) or Ch-GRGD have been fabricated and their drug delivery characteristics examined [22]. The thrombolysis potential of the NPs, including the time for clot lysis and the amount of digested clots, was also determined. The system has been compared with those of free t-PA solution in a clot-occluded tube model. The positive zeta potential of PLGA/Ch shows the presence of Ch on the surface of the PLGA/Ch NPs, and the zeta potential of the PLGA/Ch-GRGD NPs is less than that of PLGA/Ch NPs. Also, PLGA/Ch NPs have the shortest clot lysis time (e.g., 20.7 ± 0.7 min). This may be attributed to a larger amount and faster release of t-PA than others

during the fast release phase. The maximum weight of digested clot is 25.7 ± 1.3 wt% for PLGA/Ch-GRGD NPs with clot lysis time of 28.2 min. Furthermore, t-PA-encapsulated PLGA/Ch-GRGD NPs are associated with faster thrombolysis than those for free t-PA solution. These new NP carriers may benefit many patients and be employed in future clinical studies.

Multiwall carbon nanotubes (MWCNT) scaffolds for tissue engineering purposes have been investigated [23]. These scaffolds are composed of a major fraction of MWCNT (up to 89 wt%) and a minor fraction of chitosan (Ch). They have been employed as biocompatible and biodegradable supports for culture growth and have microchannel porous structure which extends to the whole monolithic dimensions. In vitro tests of cell adhesion and proliferation onto the external surface of MWCNT/Ch scaffolds with a C_2C_{12} cell line (myoblastic mouse cell) have been carried out. Also, the gradual development of the C_2C_{12} cell line in the presence of recombinant human bone morphogenetic protein-2 (rh BMP-2) has been studied, and the ectopic bone formation at muscle tissue evaluated. Under these conditions, scaffolds have cellular behavior, make new natural matrixes, and can be useful for three-dimensional (3D) construction of different tissues.

Recently, graft copolymerization of 2-hydroxyethyl acrylate (HEA) onto chitosan has been reported. In this study, ammonium persulfate was used as an initiator and the reaction was carried out in an aqueous solution. In this way, the water solubility of the modified polymer was observed in comparison with the original chitosan. Scanning electron micrographs show the porous morphology of native chitosan, whereas chitosan grafted onto HEA has fibrous morphology. Graft polymerization of HEA as a hydrophilic monomer can produce multifunctional materials that may be used in medicine and pharmaceutical applications [24].

A type of chitosan derivatives with polypeptide side-chains has been prepared via ring-opening polymerization of N_{ε}-carbobenzyloxy-L-lysine (NCA) and grafting it onto partially deacetylated water-soluble chitosan in water/ethyl acetate. The results show that the membrane surface is smooth and the ε-amino group in the side-chain of L-lysine remains after deprotection, thus chitosan-*graft*-poly(L-lysine) is a kind of polycation. Also, AFM measurement indicates positive charges on the surface of the polymer. It has been shown that the positive charge increases the cell–biomaterial interaction, and this novel material would be a candidate scaffold for cell culture and tissue engineering [25].

In other research [26], the cholesterol-binding capacities of chitosan with different physicochemical properties have been tested in vitro and in vivo. Powdered chitosan facilitates adsorption better than flake chitosan. Chitosan dissolves in the acidic conditions of the stomach, thus the adsorption action may weaken in the body. Also, when the degree of deacetylation and molecular weight are relatively high and the particle size is smaller, the effect is better. Furthermore, electrostatic action, adsorption, and entrapment may occur in the mechanism of the hypocholesterolemic effect of chitosan.

In a new study, electrospun nanofibrous membranes for use as wound dressing have been obtained by electrospinning of chitosan/collagen/polyethylene oxide in aqueous acetic acid solution [27]. It is important to know that collagen and chitosan could not form nanofibers during electrospinning due to their large net charges. Thus, polyethylene oxide was added to decrease the conductivity of the solution. Electrospun fibers were maintained at the nanoscale after cross-linking with glutaraldehyde vapor. After cross-linking, the Young's modulus was increased, but the tensile strength, tensile strain, and water sorption capability decreased. Also, the results indicate that these composite nanofibrous membranes have no cytotoxicity and are beneficial for skin regeneration and wound healing.

Recently, an easy method for synthesis of two-dimensional (2D) Au nanosheets on the basis of chitosan [28] has been reported. These nanosheets exhibit a very broad in-plane plasmon band that extends well into the near-infrared (NIR) region. Also, in other research, Au nanostructures have been synthesized by reduction of $AuCl_4^-$ ions with chitosan [29]. It has been reported that the size and shape, and hence the optical properties, of Au nanostructures could be modulated via cooling treatment. The presence of glutaraldehyde as a cross-linking agent during synthesis accelerates the reduction of the Au precursor and the growth of isotropic Au nanoparticles. Such nanostructures may be useful in biomedical or related applications.

In other research, a perfusion seeding system has been designed to seed dermal fibroblasts onto collagen-chitosan sponges for in vitro dermal regeneration. The effects of perfusion rate on seeding efficiency and cell distribution have been studied. The results show that high seeding efficiencies with uniform cell distributions can be achieved by the perfusion seeding route, which further facilitated cell proliferation and improved the structure of cultured dermal equivalents. These results may be useful for the design of bioreactor systems for in vitro dermal construction [30].

Porous hydroxyapatite/chitosan-alginate composite scaffolds containing various amounts (0, 10, and 30 wt%) of hydroxyapatite have been synthesized by an in situ coprecipitation method [31]. Observations show that the pore structure of the composite scaffolds is similar to that of chitosan-alginate scaffolds, and the morphology of the uniform microstructure is unaffected by the presence of hydroxyapatite. Also, the pore diameter decreases with increasing hydroxyapatite content up to 30 wt%. The

compressive strength of the hydroxyapatite/chitosan-alginate composite scaffolds increases with increasing hydroxyapatite content.

Nanosized particles of calcia-stabilized zirconia (CSZ) have been used as a filler in poly(hydroxyethyl methacrylate-methyl methacrylate), i.e., P(HEMA-MMA), grafted onto chitosan copolymer to prepare a bioactive composite used as a coating on the metallic shaft of hip prostheses. The results show that the grafting percentage of the CSZ-copolymer composite is increased compared with the copolymer as a result of the nanosized filler. Swelling properties decreased for the CSZ-copolymer composite, which proves the stability and lower affinity of this composite for water molecules. Also, in vitro tests demonstrated that the adsorption of calcium ions (Ca^{2+}) and phosphate ions (PO_4^{3-}) on the surface of the composites increased [32].

Chitosan (Ch) particle/carbon nanotube (CNT) composite materials have been synthesized by electrostatic interactions between positively charged Ch particles and negatively charged functionalized CNTs [33]. The size of the Ch particles can be controlled from the nano- to the microscale by using different synthesis methods. On the other hand, functionalized CNTs have been coated onto Ch particles, for which Ch microspheres have been used. These Ch particle/CNT composite materials with different size of Ch particles can be useful for biomedical applications because of the biocompatibility of Ch and the excellent electric, mechanical, and chemical properties of CNTs.

A bioactive hybrid composite of porous chitosan-silicate has been synthesized for tissue engineering applications via a freeze-drying method. The effects of composition and synthesis conditions on pore structure and water uptake have been examined [34]. In this research, solutions derived from chitosan and γ-glycidoxypropyltrimethoxysilane (GPTMS) were frozen at -20 °C and -85 °C before drying to yield 3D porous chitosan–silicate hybrid scaffolds. The results show that the pore size depends little on the GPTMS content. MG63 osteoblastic cells have been cultured up to 7 days on the porous hybrids. The cells adhered to the pore walls and migrated deep into the pore structure.

Three-dimensional porous structures with PVA/bioactive glass and PVA/chitosan/bioactive glass compositions have been synthesized by the sol–gel and foaming method [35]. These organic–inorganic hybrid scaffolds show a hierarchical structure with interconnected macropores (10–500 μm). The results indicate that the increase in the grade of hydrolysis of PVA improves the mechanical properties of the hybrid scaffolds to values suitable for cancellous bone repair. These hybrids have potential for biomedical applications.

Lactose- and heparin-modified chitosan films have been synthesized and their physical and biological properties compared with chitosan, chitosan-*g*-heparin, and chitosan-*g*-lactose films [36]. The results show the chitosan-*g*-lactose/heparin films to have the best biological performance for chondrocyte attachment, proliferation, viability, and glycosaminoglycan (GAG) secretion compared with chitosan, chitosan-*g*-heparin, and chitosan-*g*-lactose films. Chondrocyte aggregates and nodules have been observed on chitosan-*g*-lactose/heparin and chitosan-*g*-lactose films after culturing the cells for 16 h. These results indicate that the incorporation of lactose and heparin into chitosan can increase the cell–biomaterial interaction, and these films are suitable for tissue engineering as a scaffold for chondrogenesis.

Synthesis of gold nanoparticles in alkalic carboxymethylated chitosan solution by UV irradiation has been studied [37]. The results show that the pH, the concentration of $HAuCl_4$, and the irradiation time had obvious influences on the size, amount, and morphology of gold nanoparticles. The size of the prepared gold nanoparticles was 6.2–8.2 nm, which was stable at pH 12.4. This biocompatible gold nanoparticles/carboxymethylated chitosan composite has potential in biomedicine and bioanalytical applications.

Homogeneous chitosan-poly(lactide-*co*-glycolide) (PLGA) composite fibers at a ratio of 50/50 wt% have been prepared from a one-phase mixture of chitosan-PLGA [38]. A cosolvent system including 1,1,1,3,3,3-hexafluoroisopropanol and methylene chloride was used. Thermal studies confirmed the homogeneous microstructure of the chitosan-PLGA, which provided proper degradability as well as improved mechanical properties and hydrophilicity. This novel fibrous matrix might have potential uses in tissue regeneration as a biomaterial scaffold.

Chitosan-*graft*-polyethylenimine copolymer has been prepared by an imine reaction between periodate-oxidized chitosan and polyethylenimine. This copolymer has been successfully evaluated as a new gene carrier. Also, this material has shown good ability to form a complex with DNA, and suitable physicochemical properties for a gene delivery system. The results show that the transfection efficiency of this copolymer is not decreased in the presence of serum, so it has potential as a safe and efficient gene carrier in vitro [39].

In other research, pH-induced monolithic hydrogels were synthesized via uniform neutralization of acidic chitosan solutions with ammonia generated in situ by enzymatic hydrolysis of urea [40]. These gels have a homogeneous 3D coherent structure. The results show that gelation time decreased as temperature increased from 15 °C to 45 °C. This is due to a synergistic effect of increased diffusion of reactants and increased urease

activity (maximum activity at 37 °C). These hydrogels can be considered as injectable gels for tissue engineering. Also, these semiliquid materials can easily be filled and molded to any shape of an in vivo void or defect.

In order to improve the blood compatibility of chitosan, a series of new surfactant polymers have been prepared that can easily modify the surface of hydrophobic biomaterials [41]. These surfactants include a chitosan backbone with hexanal side-chains and polyethylene glycol (PEG) side-chains. The hexanal side-chains facilitate absorption and proper orientation on hydrophobic substrates, and the PEG side-chains provide a steric barrier, avoiding the adsorption of proteins onto the material surfaces. The results show that addition of the chitosan surfactant to a polyethylene (PE) surface improves the blood compatibility of PE. Chitosan surface containing the negatively charged SO_3^- exhibits the greatest improvement in blood compatibility. These materials may be useful for cardiovascular applications.

Some biocompatible materials can support growth and phenotypic expression of osteoblasts and chondrocytes. The search for these materials is a major challenge in the application of tissue engineering techniques for the repair of bone and cartilage defects. The question of whether chitosan can serve as a supporting matrix for connective tissue growth has been answered positively [42]. In this method, an appropriate solution of chitosan was prepared by dissolving chitosan in acetic acid. Then, the chitosan solution was coated onto the surface of coverslips. Osteoblasts and chondrocytes were then seeded at 1×10^5/1 cm^3 into wells containing uncoated and chitosan-coated plastic coverslips. The results show that cells cultured on chitosan-coated surfaces remain viable and maintain spherical morphology more similar to that exhibited by osteoblasts and chondrocytes in vivo. Moreover, chitosan-coated coverslips exhibited higher density of osteoblasts and chondrocytes compared with uncoated coverslips. This may be due to increased affinity of cells for chitosan-coated surfaces.

Antibacterial properties and food packaging

New types of chitosan derivatives with much higher antimicrobial activity have been synthesized [43]. The antimicrobial activities of acetyl, chloroacetyl, and benzoyl thiourea derivatives of chitosan against four bacterial species have been studied. The results show that the antimicrobial activities of the acyl thiourea derivatives are much better than that of the parent chitosan. Also, the antifungal activities of the chloroacetyl thiourea derivatives of chitosan are significantly higher than those of the acetyl and benzoyl thiourea derivatives.

In a new study, chitosan has been blended with nylon-6 by combining solvent evaporation and a phase-inversion technique, followed by chelation with silver ions. Gram-positive and Gram-negative bacteria have been used to study the antibacterial properties of these membranes. The results show that the chitosan/nylon-6 blended membranes with Ag^+ are antibacterial against both Gram-positive and Gram-negative bacteria. By increasing the nylon-6 content, the antibacterial property of the blended membranes is decreased, which may be due to the corresponding decrease of chitosan and Ag^+ content in the blend [44].

In another study, the antimicrobial effect of a commercial chitosan with high deacetylation degree (94%) and low molecular weight on different psychrotrophic spoilage organisms and food pathogens has been quantified. In this research, the influence of different food components (starch, whey protein, and oil) on the antimicrobial effect of chitosan was investigated. Also the effects of chitosan coatings for controlling decay of fruits and vegetables have been studied. The results show that Gram-negative bacteria are very sensitive to the applied chitosan while the sensitivity of Gram-positive bacteria is highly variable [45].

The antimicrobial efficacy of chitosan in lipid emulsions as well as in aqueous solutions has been investigated [46]. This research is aimed at producing a formulation with improved activity in terms of reducing the number of microorganisms. For this purpose, two types of long-chain chitosan have been used with different molecular weights, degree of deacetylation, and viscosity: type I with 8.7×10^4 g mol^{-1}, 92% degree of deacetylation, and viscosity of 14 mPa s; and type II with 5.32×10^5 g mol^{-1}, 73% degree of deacetylation, and viscosity of 461 mPa s. The results showed that chitosan with molecular weight of 8.7×10^4 g mol^{-1}, a high degree of deacetylation, and low viscosity shows higher antimicrobial activity than the others. Also, lipid emulsions containing 0.5% chitosan (type I) conform to the requirements of the preservation efficacy test for topical formulations according to the European pharmacopoeia, while the emulsion without chitosan does not.

Antimicrobial activities of chitosan-zinc (Ch-Zn) complexes with different Zn content have been studied [47]. For this purpose, five Ch−Zn complexes with different Zn content were prepared. The complexes' antimicrobial activities against four Gram-positive bacteria, five Gram-negative bacteria, and two fungi have been studied. The complexes show a wide spectrum of effective antimicrobial activities, which are 2–8 and 4–16 times higher than those of chitosan and zinc sulfate, respectively. Also, the complexes had better antibacterial activity than antifungal activity, and showed very good activity particularly against *E. coli* and *Corynebacteria*, both with a minimum

inhibitory concentration (MIC) value of 0.000313% (Ch–Zn w/v).

In other research, the antifungal efficacy of chitosan and its thiourea derivatives on the growth activities of some sugarbeet pathogens have been studied [48]. The results show that the thiourea chitosan derivatives are much (about 60 times) better fungicidal agents than pure chitosan against most of the fungal strains tested. Also, the molecular weight and degree of deacetylation have an important effect on the growth activities of the pathogens. A higher deacetylation degree causes a stronger effect on growth activities, and lower molecular weight leads to higher activities.

Antibacterial activities of six chitosan and six chitosan oligomers with different molecular weights have been studied against four Gram-negative and seven Gram-positive bacteria [49]. The results indicate higher antibacterial activities for chitosan compared with oligomers. Also, 0.1% chitosan showed stronger bactericidal effects against Gram-positive bacteria than against Gram-negative bacteria. The minimum inhibitory concentration of chitosan varies in the range from 0.05% to >0.1% and depends on the bacteria and the molecular weight of chitosan. The antibacterial activity of chitosan at lower pH was higher, which suggests that addition of chitosan to acidic foods will enhance its effectiveness as a natural preservative.

Sulfanilamide derivatives of chitosan have been synthesized using different molecular weights of chitosan (Ch), carboxymethyl chitosan (CMCh), and chitosan sulfates (ChS) reacted with 4-acetamidobenzenesulfonyl chloride in dimethylsulfoxide solution [50]. The results show that all the prepared sulfanilamide derivatives have an excellent inhibiting effect on the studied fungi. The increased antifungal activities of these derivatives may be attributed to the fact that the sulfanilamide group is grafted onto the chitosan chain and increases the antifungal activity of Ch, ChS, and CMCh. Also, the antifungal activity of the derivatives increases with increasing molecular weight, concentration, and degree of substitution.

Edible antimicrobial films based on yam starch and chitosan have been synthesized [51]. A solution of yam starch (4%) and glycerol (2%) has been gelatinized in a viscoamilograph and chitosan added at concentrations of 1%, 3%, and 5%. Films with and without chitosan have been prepared by the cast method. These films showed a bactericidal effect when tested against *S. enteritidis*. The results indicate that films with 3% and 5% chitosan exhibit similar antimicrobial effects. The film containing 5% chitosan has the highest efficiency for inhibiting growth of *S. enteritidis*, with an effect similar to that of 1% pure chitosan, which suggests a release of 53% of the incorporated chitosan from the film. Also, the films showed good flexibility.

To analyze the antibacterial activities of chitosan, two series of chitosans have been synthesized via γ-irradiation depolymerization and a deacetylation process [52]. Also, several kinds of *N*,*O*-carboxymethylated chitosans and *O*-carboxymethylated chitosans have been prepared to examine the antibacterial activities of chitosan derivatives. The results show that the antibacterial activity of chitosan is influenced by its molecular weight, degree of deacetylation, and concentration in solution, and the pH of the medium. Antibacterial activities have been found to increase in the order: *N*,*O*-carboxymethylated chitosan, chitosan, *O*-carboxymethylated chitosan.

Different weight ratios of collagen/chitosan have been used to immobilize various grafts of acrylic acid on nonwoven polypropylene (PP) fabric, to investigate antibacterial and swelling properties [53]. The results show that the percentage of immobilized collagen/chitosan increased with increasing amount of chitosan in the collagen/chitosan mixture for a given grafting percentage of acrylic acid on PP. The antibacterial properties also significantly increased with increasing amount of chitosan in the mixture. By increasing the amount of chitosan in the collagen/chitosan mixture and the percentage of collagen/chitosan immobilized at the same pH, the water uptake and water diffusion coefficients are decreased.

The effect of chitosan on the growth and production of hemolysin by *Aeromonas hydrophila* and also the effects of temperature, pH, salts, and irradiation on the antibacterial activity of chitosan have been investigated [54]. The results show that 0.04% chitosan is sufficient to prevent the growth of, and inactivate the production of hemolysin by, *A. hydrophila*. The antibacterial activity of chitosan varies with temperature, pH, and some salts. The bactericidal effects of chitosan increased with increasing temperature and decreasing pH. Sodium ions at concentrations of 10 and 25 mM reduce chitosan's activity. Irradiation degrades chitosan and increases its antimicrobial activity.

Solid chitosan acetate has been synthesized by the reaction of chitosan with acetic acid [55]. After characterization of the structure of chitosan acetate, it was found that chitosan acetate with high solubility retains the structure and antibacterial activity of chitosan. Also, the results indicate that chitosan acetate at a concentration of 0.1% (w/v) shows some antibacterial activity but some of the bacteria still grow. At the concentration of 0.15% (w/v), almost no bacteria grew. Chitosan acetate at a concentration of 0.2% (w/v) completely prevented growth of bacteria for the duration of the experiments (48 h).

Starch/chitosan blend films have been prepared by the compression-molding technique in physical gel state [56]. The tensile strength and flexibility of starch films have been improved greatly after incorporation of 20% chitosan. X-ray studies and scanning electron microscope analyses

demonstrate that there is interaction and microphase separation between molecules of starch and chitosan. In order to prepare a kind of antibacterial film, the starch/chitosan blend film was irradiated and the antibacterial activity of the films measured. The results show that there was no obvious change in the structure of the starch/chitosan blend films, but antibacterial activity was induced even when the chitosan content was only 5%, due to degradation of chitosan by the irradiation.

Antimicrobial activity of chitosan and its derivatives against different groups of microorganisms such as bacteria, yeast, and fungi has been reviewed. A review summarizes some of the important developments related to food applications of these materials [57]. There is another review on chitosan-based antimicrobial films used for food packaging applications, which also summarizes formation and antimicrobial activity of chitosan-based films used in food preservation [58].

In another study [59], a mixture of mint extract and chitosan was used as a new preservative for meat and meat products. Mint extract has good antioxidant activity but poor antimicrobial activity, while chitosan alone shows poor antioxidant activity with very good antimicrobial properties. The results indicate that 0.05% chitosan and mint mixture is the minimum inhibitory concentration and is more effective against Gram-positive bacteria. Therefore, use of mint and chitosan in meat products will improve their shelf life and safety.

The inhibitory effect of chitosans with different molecular weight and their complexes with casein on the growth of three milk-fermentative bacteria has been studied [60]. In addition to molecular weight and concentration of the polymer, the concentrations of the bacteria and casein micelles or milk fat are important. The inhibiting effects of chitosan were greatly reversed when the biopolymers were incubated with milk before interaction with bacteria, because casein micelles or milk fat can prevent the inhibitory activity of these biopolymers on bacterial growth.

The effect of preharvest chitosan spraying at low concentration (1 g dm^{-3}) and postharvest chitosan coating with a higher concentration (10 g dm^{-3}) on the quality and physiological response of table grapes has been investigated [61]. The effect was assessed in terms of the decay, weight loss, changes in quality attributes, and the activities of pertinent enzymes during storage at different temperatures. The results show the beneficial effect of chitosan on fruit quality and resistance to decay.

Chitosan glucose complex (CGC), a modified form of chitosan, has been prepared by heating chitosan with glucose [62]. The results show that this complex is a better preservative than chitosan alone. Also, it exhibits excellent antioxidant activity compared with chitosan or glucose alone. The antimicrobial activity of CGC is identical to that of chitosan against the common food spoilage and pathogens. So, it seems to be a natural preservative with both antibacterial and antioxidant activity with potential applications in the food industry.

Antimicrobial paperboard has been fabricated by coating chitosan solution of vinyl acetate–ethylene copolymer on one side of the paper. Its migration and antimicrobial activities have been tested [63]. The results show that migration of the preservative does not depend on the addition of the other antimicrobial agent, and reached about 1% for chitosan. This type of packaging material has potential for use as universal antimicrobial packaging for a variety of foods.

Water treatment

Recently, use of chitosan as an adsorbent has attracted attention in water treatment industries due to its high content of amino and hydroxyl functional groups. This polysaccharide, which is the deacetylated form of chitin, shows high potential for adsorption of dyes, metal ions, and proteins, beside its previously mentioned properties. So it might be a good candidate for removing pollutants from water and wastewater.

In one study [64], the effect of chitosan immobilization of *Scenedesmus* spp. cells on viability, growth, and nitrate and phosphate adsorption has been studied. The results show that a chitosan-bead-immobilized algae system with *Scenedesmus* sp. (strain 1) exhibited greater efficiency for removing phosphate and nitrate from water compared with the conventional free-cell system.

Also, in other research, *N*,*O*-carboxymethylchitosans (*N*,*O*-CMC) with various degrees of substitution (DS) have been synthesized under heterogeneous conditions, by controlling the reaction temperature [65]. This material has more influence on the adsorption capacity of the anionic dye congo red (CR). The results show that *N*,*O*-CMC with DS of 0.35 exhibits much higher adsorption capacity (330.62 mg g^{-1}) for CR compared with chitosan (78.9 mg g^{-1}) under the same adsorption conditions. Also, the adsorption capacity decreased with increasing pH but increased with increasing temperature. *N*,*O*-CMC is beneficial for removing CR from dye-contaminated wastewater.

Chitosan has been used as an adsorbent due to its high contents of amino and hydroxyl functional groups [66]. Since chitosan forms gels below pH 5.5, the use of chitosan as an adsorbent for dye removal will be limited. In new research, cross-linked chitosan has been prepared and stabilized in acid medium. The adsorption of reactive blue 19 dye onto cross-linked chitosan/oil palm ash composite beads has been studied. Maximum adsorption was observed at pH 6 for cross-linked chitosan/oil palm ash

composite beads and was greater than 400 mg g^{-1} at 30 °C. It was found that a decrease in the pH of the solution leads to a significant increase in dye adsorption capacity.

A new spray-dried chelating adsorbent made from chitosan cross-linked with glutaraldehyde has been prepared [67]. In this study sulfoxine was used as a chelating agent and the adsorption of Cd(II) and Zn(II) was investigated. The results show that the complexing agent is microencapsulated on the surface of chitosan and forms a new adsorbent material. The adsorption of Cd^{2+} and Zn^{2+} depends on the pH of the solution, and optimum adsorption was observed at pH 8 in both cases.

Natural organic matter removal from aqueous solutions by a complexation–ultrafiltration process has been studied. In this study, cationic water-soluble polymers such as poly(diallyldimethylammonium chloride) and chitosan have been used [68]. The results show that humic acid rejection on ultrafiltration varied from 98.1% to 99.5% or from 90.5% to 99.2% with the addition of poly(diallyldimethylammonium chloride) or chitosan, when the ratio of humic acid to polymer varied over a range from 1:1 to 1:7. It is possible to achieve a high degree of humic acid removal from aqueous solutions via humic acid binding with water-soluble cationic polymers such as chitosan.

Nanocomposites of Cu_2O and chitosan have been prepared by electrochemical deposition. In this research, needle-shaped cuprous oxide nanocrystallites were combined with chitosan particles by chelation. The results indicate that the best efficiency is obtained when the mass ratio of Cu_2O in Cu_2O/chitosan nanocomposites is 50%. This makes it feasible to eliminate pollutants by visible-light irradiation during treatment of drinking water [69].

Recently, the preparation of polyacrylamide-chitosan (PAA-Ch) and the adsorptive features of chitosan (Ch) and PAA-Ch have been investigated for Pb^{2+}, UO_2^{2+}, and Th^{4+} in terms of dependency on ion concentrations, temperature, and adsorption kinetics [70]. The results show that PAA-Ch has greater adsorption capacity than Ch for all the studied ions; therefore, the sequence is $Th^{4+} > Pb^{2+} > UO_2^{2+}$. The affinity to Ch in PAA was increased for Pb^{2+} and UO_2^{2+} but unchanged for Th^{4+}. Also, Ch and PAA-Ch have the highest affinity to Pb^{2+} in the presence of the other two ions. Consequently, the use of Ch in PAA can increase the efficiency of Ch in separation and removal procedures.

Humic substances are brownish biopolymers and can be found in animals, plants, solids, and water. The yellowish-brown color of natural water is due to humic substances leached out from plants and soil, which can affect water quality. In novel research [71], adsorption of humic acid from aqueous solutions onto irradiation-cross-linked carboxymethylchitosan has been studied. Because of protonation of the amino groups, the cross-linked samples show positive zeta potential below pH 6. The results show that cross-linked CM-chitosan can be applied as an adsorbent for removal of humic acid under acidic pH conditions (pH <6), with optimum pH of 3.5. The protonated amino groups of the cross-linked samples form a surface complex with humic acid, playing an important role in the adsorption process. Therefore the radiation-cross-linked CM-chitosan is beneficial for the separation of humic acid from water.

Selective separation of mercury(II) using magnetic chitosan resin modified by a Schiff base cross-linker has been studied [72]. The results show that interaction between the metal ion and the resin is dependent on the acidity of the medium. Also, the adsorption process is exothermic and follows pseudo-second-order kinetics. At pH 1, Hg^{2+} has been separated from Cu^{2+}, Pb^{2+}, Cd^{2+}, Zn^{2+}, Ca^{2+}, and Mg^{2+}. This resin has shown efficient uptake of Hg(II) compared with commercial resins.

A glycine-modified cross-linked chitosan resin has been synthesized for removal of Au^{3+}, Pt^{4+}, and Pd^{2+} ions [73]. Maximum adsorption occurred at pH 2 for Au^{3+}, Pt^{4+}, and Pd^{2+} ions. The experimental data have been fitted by a Langmuir isotherm model. Based on the Langmuir isotherm, the adsorption capacity is higher for Au^{3+} than for Pt^{4+} and Pd^{2+}. Also, the adsorption–desorption cycle demonstrates that the synthesized resin can be reused up to five times without any change in the amount of adsorption. So, this resin can be introduced for the removal of the mentioned ions from water and wastewater.

In other research, the synthesis of an ion-imprinted chitosan–TiO_2 adsorbent has been discussed [74]. The results show that the strength of the combination between chitosan and TiO_2 is due to not only physical mixing but also hydrogen bonds. This adsorbent can not only adsorb heavy-metal ions but also degrade organic compounds in wastewater. Also, the adsorbent prepared with NaOH solutions shows higher degradability than that obtained from sodium tripolyphosphate solutions. Therefore, the coupling of molecular imprinting technology and photocatalytic degradation suggests its use as a new method for decreasing environmental pollution.

Several treatment methods have been used for the removal of arsenic from water. In new research a biosorbent has been prepared for removing As(III) and As(V) from water by coating chitosan onto ceramic alumina [75]. The results show that this adsorbent has the potential to eliminate arsenic from drinking water and that it has more adsorption capacity for As(V) than for As(III). Sodium hydroxide (0.1 M) has been found to be effective in regenerating the column loaded with arsenic.

A novel cross-linked chitosan resin has been functionalized with a threonine moiety through the extension arm of chloromethyloxirane and its potential assessed for

application in a solid-phase extraction method [76]. The results show good adsorption capability toward Mo, V, and Cu. This method has been successfully applied to the evaluation of Mo, V, and Cu in river and tap water samples.

A new composite chitosan flocculant has been synthesized from chitosan, polyaluminium chloride, and silicate [77]. The percentage removal of organic contaminants, solid substances in suspension, and Al^{3+} in treated water using this composite have been enhanced by 1.8–23.7%, 50%, and 61.2–85.5%, with cost reduction of 7–34%. So, this type of flocculant is more economic than traditional flocculants for water treatment.

In order to study the adsorption properties of some chitosan derivatives, *N*-Schiff base-type chitosan crown ethers (I, III) have been synthesized by the reaction of 4′-formylbenzo-21-crown-7 with chitosan or cross-linked chitosan [78]. Also, *N*-secondary amino-type chitosan crown ether (II) has been obtained by the reaction of *N*-Schiff base-type chitosan crown ether (I) with sodium borohydride. The results show that the adsorption capacity of chitosan crown ethers (I, II, III) for Ag^{+}, Au^{3+}, Pd^{2+}, and Pt^{4+} is much higher than that for Cu^{2+} and Hg^{2+}. Also, chitosan-crown ether II only adsorbs Hg^{2+} and not Cu^{2+} in an aqueous system including Pd^{2+}, Cu^{2+}, and Hg^{2+}.

In another approach, heavy-metal ion sensors using chitosan-capped gold nanoparticles have been reported. The electrostatic attachment of chitosan onto gold nanoparticles has been considered for indicating the concentration of heavy-metal ions (Cu^{2+} and Zn^{2+}) in water [79].

Hydrogels

Hydrogels are three-dimensional networks that swell in water and aqueous solutions. These materials, based on both natural and synthetic polymers, are currently attracting a great deal of interest as bioactive molecules and in tissue engineering. Among natural biopolymers of interest, chitosan stands out due to its unique combination of favorable properties, as mentioned at the beginning of this review. Chitosan hydrogels can be divided into two classes: physical and chemical. Chemical hydrogels are formed by irreversible covalent links, whereas physical hydrogels are formed by various reversible links. For various reasons, physically cross-linked hydrogels have attracted increasing attention as bioactive compounds.

Physically cross-linked chitosan hydrogels have been synthesized by grafting lactic acid (LA) and glycolic acid (GA) [80]. The residual amino groups of chitosan are ionized in acidic buffers, which contributes to the electrostatic repulsion between adjacent ionized residual NH_2 groups of chitosan, leading to chain expansion, and consequently increases the water uptake of the gel. The hydrophobic side-chains aggregate and physical cross-linking is formed. The crystallinity of original chitosan decreases by grafting LA and GA. Also, ΔH_{cryst} and ΔH_{fusion} decrease even below 200 J g^{-1} for chitosan/water systems. This suggests that water molecules crystallize incompletely in these systems.

In a new study, a thermoresponsive hydrogel based on chitosan was evaluated [81], and the thermogelling properties of formulations prepared from autoclaved reacetylated chitosan were assessed. Finally, different storage strategies for phosphate-free thermosetting formulations were tested. The results show that the thermosetting hydrogels are unstable at room temperature as well as during refrigeration. The most convenient and attractive approach certainly consists of a lyophilizate that can be easily handled and stored. This study represents a significant step towards the development of a thermosetting hydrogel based on reacetylated chitosan and a phosphate-free additive.

The temperature-sensitive hydrogel chitosan-glycerophosphate (C/Gp) seeded with human mesenchymal stem cells (MSCs) has been studied [82]. These cells have been cultured for 4 weeks in standard medium. The gene and protein expression profile of the MSCs has been analyzed and compared with that of both nucleus pulposus (NP) cells and articular chondrocytes cultured in C/Gp. The results show that human MSCs cultured in C/Gp gels differentiate and produce a cartilaginous extracellular matrix without the need for exogenous factors. Therefore, MSC-seeded C/Gp gels can be used clinically for regeneration of degenerated human intervertebral disc (IVD).

A series of hydrogels based on chitosan and polyethylene glycol (PEG) with different molecular masses (MW = 300 and 400) have been synthesized by radiation technology [83]. UV radiation shows better results than γ-radiation. Hydrogel characterization demonstrates that the cross-linking of PEG takes place on chitosan chains. The photocopolymerized hydrogels show water absorption of up to 530% after 2 h. So, PEG-chitosan hydrogels may have potential for application in the biomedical field.

In a review article, structure and interactions in covalently and ionically cross-linked chitosan hydrogels for biomedical applications have been critically analyzed [84]. This review focused on chitosan hydrogels intended for medical or pharmaceutical applications. The properties of cross-linked hydrogels depend mainly on the cross-linking density, i.e., the ratio of moles of cross-linking agent to moles of polymer repeating units. The simplest structure of covalently cross-linked chitosan presented in this review is self-cross-linked chitosan. Other structures are the hybrid polymer network, semi-interpenetrating network, and ionic

cross-linking of chitosan. Among these four types of chitosan hydrogels, covalently cross-linked hydrogels are the only systems characterized by a permanent network, due to their irreversible chemical links. Therefore, they exhibit good mechanical properties. Ionically cross-linked chitosan hydrogels exhibit greater swelling with pH changes compared with covalently cross-linked chitosan hydrogels.

Also, in another review the use of chitosan hydrogels as scaffolds for tissue engineering has been studied [85]. In this review three categories of scaffold applications (space-filling agents, bioactive molecule delivery, and cell/tissue delivery) were investigated. These factors are highly dependent on the design of the scaffold. This design depends on both the tissue as well as the environment in which the tissue resides. Pre- and postprocessing methods have been developed to control porosity, improve diffusion, and gently incorporate cells into the scaffold.

Thermo- and pH-sensitive semi-IPN polyampholyte hydrogels have been prepared by using carboxymethyl chitosan and poly(*N*-isopropylacrylamide) with *N*,*N*′-methylenebis(acrylamide) as a cross-linking agent [86]. The results indicate that the semi-IPN hydrogels are pH and temperature responsive and show good reversibility. Coenzyme A (CoA) is widely used in the medical area. It can modulate the metabolism of sugar, fat, and protein in the body. In this study, within 24 h a cumulative release ratio of CoA of 22.6% at pH 2.1 and 89.1% at pH 7.4 at 37 °C was obtained. By increasing the carboxymethyl chitosan content in the hydrogel, the release rate is increased. These semi-IPN hydrogels can be used in pH–temperature oral drug delivery systems.

A full-IPN hydrogel with temperature response has been prepared from chitosan and *N*-isopropylacrylamide in the presence of a suitable cross-linker and an initiator [87]. The swelling behavior of this hydrogel showed better temperature and pH sensitivity, and good reversibility in solution at various temperatures and pH values. This hydrogel can be used as a pH–temperature-responsive oral drug delivery system.

In a new study, chitosan–cellulose hydrogel beads have been synthesized using ethylene glycol diglycidyl ether as a cross-linker for Cu adsorption from aqueous solutions [88]. The results show that both chitosan–cellulose and the cross-linked chitosan–cellulose beads have high adsorption capacities for Cu, although the adsorption is pH dependent (with maximum adsorption at neutral pH) and the cross-linked beads have slightly lower adsorption capacities. Spectroscopic studies have demonstrated that the nitrogen atoms in chitosan are the main binding sites for Cu to form surface complexes during adsorption.

In novel research, *N*-succinyl chitosan/alginate hydrogel beads were prepared by dropping an aqueous solution containing succinyl-chitosan and alginate into a Ca^{2+} solution [89]. The resulting beads were used as a pH-sensitive controlled-release system for delivery of nifedipine. The results show that beads prepared at a ratio of succinyl–chitosan and alginate of 2%:2%, a weight ratio of drug to polymer of 1:4, a volume ratio of alginate/succinyl–chitosan to $CaCl_2$ of 1:4, cross-linking time of 30 min, and $CaCl_2$ concentration of 2% demonstrate the best swelling behavior and excellent pH sensitivity. These beads may be a suitable polymeric carrier for controlled drug delivery in the intestinal tract.

An injectable thermosensitive in situ gelling hydrogel has been prepared [90]. This system consists of a chitosan solution (C) neutralized with β-glycerophosphate (GP) that is liquid at room temperature but gels at body temperature. This thermosensitive hydrogel has been used for sustained release of paclitaxel at tumor resection sites in order to prevent local tumor recurrence. The in vitro release profiles have shown controlled delivery over 1 month. Also, the initial drug loading affected the release. In this study, after 17 days of observation, C/GP hydrogel containing 64 mg cm^{-3} paclitaxel released 32% of its drug load in vitro and the animals that received the formulation intratumorally displayed marked tumor growth inhibition.

Membranes

Chitosan, a natural biopolymer, has been widely used in membrane applications because of its high hydrophilicity, good film-forming character, and excellent chemical resistance properties. Several methods of membrane preparation may be used to improve separation performance, such as surface modification, blending, copolymerization, and grafting of a selective species onto an inert film. Chitosan as a cycloaliphatic biopolymer contains active amino and hydroxyl groups that can be chemically modified by several chemicals. Chitosan-based membranes have been extensively studied not only for dehydration of some organic solvents such as alcohols, tetrahydrofuran (THF), isopropanol, and ethylene glycol, but also for the separation of organic/organic mixtures. It has also been used for pervaporation, ultrafiltration of biomaterials, and protein adsorption/separation.

Modification of chitosan has been achieved by quaternization to increase chitosan hydrophilicity and ionic character [91]. 2-Hydroxypropyltrimethylammonium chloride chitosan (HACC) has been obtained via modification of chitosan with 2,3-epoxypropyltrimethylammonium chloride. This material has been used as a film-forming material for a nanofiltration membrane. A positively charged composite nanofiltration membrane with HACC has been coated on poly(acrylonitrile) (PAN) and cross-linked with

diisocyanate. The effects of curing conditions and temperature of cross-linking on the properties of the resultant HACC/PAN composite nanofiltration have been investigated. The results show that the membrane made from 2 wt% HACC casting solution, cured at 50 °C for 2 h, cross-linked at 50 °C for 9 h with hexamethylene diisocyanate (HDI) and toluene diisocyanate (TDI) [HDI/TDI (w/w) = 0.3 g/0.3 g] in 50 g ethanol, and heat-treated at 50 °C for 20 min has optimum properties. Also, this membrane has potential use for the separation of mono/divalent salts.

In other research, microporous polyamide (nylon) membranes have been activated by bisoxirane and then bonded with chitosan to improve the hydrophilicity and increase reactive sites. These membranes have a large number of reactive groups such as –OH and $–NH_2$ [92]. Polylysine (PLL) as a ligand has been immobilized onto chitosan-coated nylon membranes by 1,1′-carbonyldiimidazole (CDI) activation. CDI has high reactivity with different functional groups, such as amino, carboxyl, and hydroxyl groups. These affinity membranes are useful to adsorb bilirubin from bilirubin-phosphate buffer solutions and bilirubin–albumin solutions. The results show that the mechanism of adsorption is monolayer adsorption, and that the adsorption capacity increases with increasing temperature.

Recently, chitosan-containing PU/poly(*N*-isopropylacrylamide), i.e., poly(NIPAAm), thermosensitive membranes have been synthesized [93]. For this purpose, hydroxyl-terminated polybutadiene (HTPB)-based polyurethane (PU) has been prepared and then the solution of PU was modified with *N*-isopropylacrylamide by UV radiation to obtain thermosensitive membranes (PUNIPAAm). Then, chitosan was impregnated onto the surface of PUNIPAAm and treated by freeze–drying to make chitosan-containing PUNIPAAm. The results show that these membranes have very low cytotoxicity, support the growth of 3T3 fibroblasts, and are antibacterial. Therefore, these materials may be beneficial for wound dressing.

Composite hybrid membranes of chitosan-silica (ChHMs) have been prepared by cross-linking chitosan (Ch) with 3-aminopropyltriethoxysilane [94]. The dynamic characteristics of the Ch membrane and the ChHM have been studied in pervaporation of methanol/dimethyl carbonate (MeOH/DMC) mixtures. The results of PV separation show that the amorphous region of the membranes increases and the contact angle between MeOH and the membrane decreases. The ChHM also exhibits a higher permeation flux than the Ch membrane under the same operating conditions. Repairing of the surface occurs for both the Ch membrane and the ChHM during PV processes, but the time for the surface reconstruction of the ChHM is longer than that of the Ch.

Membranes of H-ZSM-5-filled chitosan have been made by incorporating H-ZSM-5 into chitosan [95]. H-ZSM-5 is a commonly employed kind of hydrophilic zeolite. These membranes have been used for pervaporation/dehydration of aqueous ethanol solution. The results show that hydroxyl groups on H-ZSM-5 can form hydrogen-bonding interaction with the hydroxyl and amino groups of chitosan, which lowers the crystallinity of membranes and consequently improves the interface morphology. Incorporating of H-ZSM-5 into chitosan membranes significantly increases the permeation flux, but the separation factor does not increase for most H-ZSM-5 contents, due to the formation of non-selective voids at the interface.

Recently, another type of composite membranes has been prepared using quaternized chitosan as a selective layer, polyacrylonitrile membrane as a support layer, and an anhydride mixture as a cross-linking reagent [96]. The results show that, by increasing the anhydride mixture concentration, rejection increases, whereas pure water permeability and swelling in water decrease. The order of rejection for various salts followed the decreasing order: $CaCl_2$, $MgCl_2$, NaCl, KCl, $MgSO_4$, Na_2SO_4, K_2SO_4, which indicates the positively charged characteristic of these membranes. These membranes may be useful for reducing the hardness of water.

A bipolar membrane has been synthesized from PVA-GA-Ch/PVA-Fe-SA via a paste method [97]. For this preparation, $FeCl_3$ and glutaraldehyde (GA) were used as cross-linking agents for PVA-sodium alginate (SA) and PVA-chitosan (Ch), respectively. Hydrogen and hydroxide ions were generated by splitting of water at the interface in the electrical field and migrated to cathode and anode. The results show that the swelling level of the bipolar membrane is in the range 25–85%. This prepared bipolar membrane has been used as the separator in the electrolysis cell for electrogeneration of 2,2-dimethyl-3-hydroxypropionic acid.

In a recent study, modified chitosan membranes have been prepared by a solution-casting method [98]. Then, the cell attachment/spreading behavior of L929 mouse fibroblasts on membranes was studied based on physicochemical properties. The physicochemical properties of unmodified and RGDS (tetrapeptide arginine-glycine-aspartic acid-serine)-modified chitosan membranes were investigated by determining surface free energy and interfacial free energy values. The thermodynamic model predicts a negative value for the interfacial free energy of adhesion, which suggests good conditions for cell adhesion. Experiments have been performed in both 10% fetal bovine serum (FBS) containing and serum-free media with unmodified chitosan and RGDS-modified chitosan. Also, the experimental results obtained from cell attachment agree with the theoretical prediction for the free

energy of adhesion except for the cell attachment on a chitosan membrane in serum-free medium.

As we know, hybrid membranes are prepared by incorporating an inorganic filler into a continuous polymer phase. In new research, the effect of zeolites on chitosan/zeolite hybrid membranes for use in direct methanol fuel cells has been studied [99]. A series of zeolites including 3A, 4A, 5A, 13X, mordenite, and HZSM-5 were chosen as the inorganic filler. Various chitosan/zeolite hybrid membranes were made by common solution-casting methods. In this study the effects of zeolite pore size, particle size, hydrophilic/hydrophobic nature, and zeolite content on membrane performance were investigated. The results show that the ratio of Si to Al in the zeolite influenced the free volume of chitosan in the hybrid membranes. The transport of methanol in all membranes was controlled by the diffusivity. The permeability of methanol increased slightly with zeolite pore size, indicating that only a small portion of the penetrant passed through the zeolite pores. Also, the hybrid membranes displayed desirable thermal and mechanical stabilities.

A series of hydrogel membranes with a wide range of water contents (0.9–10 g water/g polymer) have been synthesized from poly(vinyl alcohol), chitosan, carboxymethyl cellulose, alginic acid, and poly(vinylamine) [100]. These membranes have been used for potential CO_2 separation applications. The permeation of CO_2, H_2, He, and N_2 through the membranes at various pressures (200–800 kPa) has been studied. The results show that the gas permeability in water-swollen membranes is lower than the gas permeability in water, and the selectivities of the water-swollen membranes for CO_2/N_2, CO_2/H_2, and CO_2/He are close to the ratios of their permeability in water.

Recently, a chitosan/polyacrylonitrile composite hollow fiber membrane has been fabricated to study the pervaporation performance of aqueous alcohol solution [101]. The chitosan layer of this composite was prepared by dip-coating chitosan solution onto the outer surface of a NaOH-hydrolyzed polyacrylonitrile hollow fiber membrane. The cross-linking agent for chitosan was γ-glycidoxypropyltrimethoxysilane (GPTMS). In this research, a pervaporation performance of 145 g m^{-2} h^{-1} permeation rate and 99.7 wt% water content in the permeate was obtained for 90 wt% aqueous isopropanol solution at 25 °C through a chitosan (1.5 wt%)—GPTMS (2 wt%)/PAN composite hollow fiber membrane. By adding GPTMS to the chitosan solution to synthesize a chitosan-GPTMS/polyacrylonitrile composite hollow fiber membrane, a pervaporation efficiency of 70 wt% aqueous isopropanol solution at 25 °C was maintained after 330 days of operation, while the GPTMS-free chitosan/PAN composite hollow fiber membrane lost its pervaporation efficiency after only 8 days of performance.

In a new study, *N*-methylene phosphonic chitosan (PC) and quaternized (QC) silica composite charged ultrafilter membranes have been prepared by acid-catalyzed sol–gel methods in aqueous media and gelated in methanol [102]. The results show that the introduction of highly acidic $-PO_3H_2$ or basic $-N^+(CH_3)_3$ groups causes the formation of cross-linking or covalent bonding with silica and polyvinyl alcohol. These membranes have been used for separation of proteins from their mixture under coupled driving forces (pressure and electric gradient). This process has potential for use as a modified biopolymer membrane for improving product purity and filtration velocity.

Other research investigated the diffusive permeability coefficients of 15 metal ions (alkali, alkali earth, and transition) through three types of membranes [103]. These membranes were classified as: untreated chitosan membrane, four chitosan membranes cross-linked with glutaraldehyde at concentrations of 0.01%, 0.1%, 0.5%, and 1% then coated with bovine serum albumin (BSA), and a commercial cuprophan membrane (regenerated cellulose) for comparison. The coefficients were compared with those of nonelectrolytes in terms of capillary pore and free volume models of solute diffusion through water-swollen gel membranes. The permeability coefficients of the metal ions deviate from the models; however, for cuprophan the coefficients obey the models. The smallest deviation was reported for the alkali-metal ions ($Li < Na < NH_4 < K$) and the largest for the transition-metal ions ($Cu < Ni < Zn < Mn < Pb < Co < Cd < Ag$). The alkali-earth metal ions ($Mg < Ca < Ba$) were found to be intermediate. The best separation of metal ions from the nonelectrolytes was achieved with the BSA-coated chitosan membrane pretreated with 0.1% glutaraldehyde. These membranes would be beneficial for biological systems.

Sodium alginate and chitosan have been used for preparation of enantioselective membranes [104]. These materials have a high content of chiral active sites and very good hydrophilicity. It is important to mention that this high content of chiral active sites is crucial for the formation of a chiral environment in the membranes, which is critical for the separation of optical isomers. Since optical isomers show exactly the same chemical structure under achiral conditions, they cannot be distinguished. The membranes mentioned above are applied for optical resolution of α-amino acids, especially tryptophan and tyrosine. On increasing the degree of cross-linking, these membranes achieve better enantioselectivity via increasing the interaction between the functional groups of the chiral environment of the membrane and the penetrating optical isomers.

In other research, an ion-exchange membrane matrix has been prepared by grafting of poly(methacrylic acid) and poly(MAA) onto epichlorohydrin cross-linked chitosan

membranes [105]. These membranes were synthesized in two steps: in the first step, chitosan membranes were made by phase inversion and then epichlorohydrin was used as a cross-linking agent to increase its chemical stability in acidic media. In the second step, graft copolymerization of methacrylic acid onto the chitosan membranes was initiated by ammonium persulfate under a nitrogen atmosphere. The results show that the maximum trypsin adsorption onto chitosan-*g*-poly(MAA) membrane was 92.86 mg cm^{-3} at pH 7. The trypsin adsorption capacity of the chitosan-*g*-poly(MAA) membranes decreased with increasing ionic strength. These membranes may be useful as an ion-exchange adsorbent for the separation of basic proteins from biological fluids.

Trimesoyl chloride (TMC) cross-linked chitosan membranes have been prepared for CO_2/N_2 separation and pervaporation/dehydration of isopropanol [106]. The degree of cross-linking was controlled by reaction time. In this research, the membrane with a higher degree of cross-linking exhibited a higher degree of swelling in water, and the degree of swelling decreased after the gas separation and pervaporation. The relaxation of the segmental chains in the chitosan matrix and the packing properties of the matrix were influenced by TMC moieties, which led to changes in pervaporation flux and selectivity.

Membranes with different thicknesses and contents of chitosan and hydroxyethyl methacrylate (HEMA) have been prepared by γ-irradiation from a ^{60}Co source [107]. Antibiotic-release experiments have been performed before or after irradiation over amoxicillin-loaded chitosan/HEMA membranes in physiological saline solution using UV–Vis spectrometry for detection. The results show that the amount of drug released is dependent on the membrane network cross-linking, which may be related to composition, radiation, and thickness of membrane.

Hydrogel membranes have been prepared from chitosan-alginate by a homogenizing interpolyelectrolyte complex method [108]. The results show that chitosan with a low pH value reacts more completely with alginate than chitosan with a high pH value; consequently a ladder-type chitosan–alginate polyelectrolyte complex (PEC) membrane with high stability is prepared. In contrast, alginate with high pH value is suitable to react with chitosan for preparation of a stable ladder-type chitosan-alginate PEC membrane. Ionic cross-linking is helpful to increase the stability and decrease the solubility of chitosan–alginate PEC membranes in physiological buffer saline solution.

Hybrid nanofibrous membranes of poly(lactic-*co*-glycolic acid) (PLGA) and chitosan at different amounts (32.3%, 62.7%, and 86.5%) have been fabricated by using a specially designed electrospinning setup consisting of two sets of separate syringe pumps and power supplies, which could be termed dual-source dual-power electrospinning [109]. By using this setup, PLGA and chitosan nanofibers have been electrospun separately and simultaneously and deposited onto a rotating drum to prepare a multicomponent nanofibrous membrane. Because of the large amounts of hydrophilic chitosan (32.3–86.5%) in the membranes, the hybrid PLGA/chitosan membranes exhibited high water uptake as well as stable mechanical properties. These membranes may be used for skin tissue engineering.

Ionic conductivity and tensile properties of some modified chitosans such as hydroxyethyl chitosan and hydroxypropyl chitosan membranes have been studied. These membranes were synthesized via reaction of alkali-chitosan with 2-chloroethanol and propylene epoxide [110]. The results show that the crystallinity of the modified chitosan membranes decreased gradually with increasing degree of substitution of hydroxyl groups, which led to a gradual increase in the swelling index. Membranes with a higher degree of substitution exhibited an increase in ionic conductivity of about one order of magnitude. Also, these modified membranes did not exhibit a significant change in tensile strength or elongation at breakage. The ionic conductivity of the modified membranes was affected by the degree of deacetylation and should not be too high.

Drug delivery systems

In recent years, significant effort has been devoted to the development of biodegradable materials for drug delivery systems. Among the various biodegradable polymers used for the development of controlled-release formulations, chitosan has been reported to be advantageous since it is a natural, nontoxic, biocompatible product with the potential for biodegradability.

A type of amphiphilic derivatives of chitosan has been synthesized by incorporating (2-hydroxypropyl-3-butoxy)propyl into succinyl–chitosan [111]. The results show that, by increasing the concentration, the surface tension decreases and aggregates form in solution. These chitosan derivatives may be used for controlled release of hydrophobic drugs.

An inexpensive and simple method for the preparation of chitosan microspheres for controlled release of an insoluble drug has been reported [112]. Different methods and various process conditions such as the rate of stirring, the concentration of cross-linking agent, and the drug-to-polymer ratio have been applied in order to optimize the process variables size distribution, microsphere size, degree of swelling, drug entrapment efficiency, and release rates. The results show that the microsphere size/size

distributions increased with decreasing stirring rates as well as glutaraldehyde concentration in the suspension medium. Also, by decreasing the concentration of cross-linker, the swelling ratio increases and the entrapment efficiency decreases. Using this approach, in vitro drug release has been controlled and increased up to 10 h.

Three chitosan derivatives of *N*-carboxymethyl, *N*-carboxybutyl, and *N*-succinyl have been impregnated with flurbiprofen and timolol maleate using the supercritical solvent impregnation (SSI) technique in order to develop hydrogel-type ophthalmic drug release systems [113]. The results show that, for *N*-carboxymethyl chitosan, the predominant effects in the impregnation process seem to be the solubility of drugs in CO_2 and in CO_2 + EtOH mixtures, as well as the swelling and plasticizing effect of CO_2 and ethanol on the polymer. So, the SSI method proved to be a more efficient and tunable impregnation process compared with traditional impregnation of drugs. These *N*-chitosan-derivative-based ophthalmic drug delivery systems can be easily and efficiently used for patients.

Self-aggregated nanoparticles from methoxypoly(ethylene glycol)-grafted chitosan (mPEG-*g*-Ch) have been synthesized by a formaldehyde linking method [114]. These nanoparticles were chosen as a carrier for poorly water-soluble methotrexate (MTx), an anticancer drug. MTx is a folate antimetabolite and has been used in the treatment of various malignancies. However, it may cause adverse effects such as bone marrow suppression, acute, chronic hepatotoxicity, interstitial pneumonitis, and chronic interstitial obstructive pulmonary disease. So, it is necessary to reduce its toxicity and side-effects. Therefore, mPEG-*g*-Ch self-aggregated nanoparticles have been used as a carrier for MTx to sustain its release, prolong its circulation time, increase its therapeutic index, and decrease its toxic effects. The obtained results show continuous release of more than 50% MTx in 48 h from MPEG-*g*-Ch.

In a new study, dry powders were prepared by spray-drying of aqueous ethanol solution of chitosan (drug release modifier), leucine (aerosolization enhancer), and terbutaline sulfate (model drug) [115]. The influence of chitosan molecular weight on the drug release profile has been studied by using low, medium, and high molecular weights of chitosan. The results show that higher-molecular-weight chitosan powders exhibit poorer aerosolization characteristics but substantially longer dissolution profiles, compared with lower-molecular-weight chitosan formulations. Drug release from the chitosan powders follows square-root-time kinetics and is dependent on the molecular weight of the chitosan. Also, by selecting an appropriate molecular weight of chitosan, it is possible to control the rate of drug release.

In other research, development of time-, enzyme-, and pH-controlled colonic drug delivery using spray-dried chitosan acetate (ChA) and hydroxypropyl methylcellulose (HPMC) has been studied for 5-aminosalicylic acid tablets [116]. The results show that a new combination coating material of HPMC and ChA has potential for use in colonic drug delivery. The system is enzyme controlled due to the degradation of ChA by the colonic enzyme. Also, chitosan acetate may interact with the acidic drug but has no effect on the drug release in this system.

Preparation of enteric-coated chitosan-prednisolone conjugate microspheres (Ch-SP-MS) and in vitro evaluation of their potential as a colonic delivery system have been studied [117]. Sonication was utilized to prepare finer Ch-SP-MS and the addition ratio of eudragit was reduced to obtain eudragit-coated Ch-SP-MS with higher drug content. The results show that eudragit coating can protect Ch-SP-MS morphology at gastric pH of 1.2 and allow almost complete regeneration of Ch-SP-MS at intestinal pH of 6.8 a few hours after exposure at this pH. Drug release is also suppressed at gastric pH and raised at intestinal pH.

Recently, water-soluble *N*-(γ-bromopropanoyl amino acid)–chitosan derivatives have been synthesized [118]. Compared with the parent unmodified chitosan, the four peptide-chitosans have higher thermosensitivity, porosity, and water-holding capacity, and these effects increase with the hydrophilicity of the peptide ligands. These new peptide–chitosans possess physicochemical properties that make them useful for drug delivery systems.

The feasibility of efficiently encapsulating acetaminophen into chitosan-tripolyphosphate (Ch-TPP) microspheres of controlled size through a novel process based on the spray-drying method has been studied [119]. In this method, Ch-TPP microspheres are spherical and have smooth surface and high encapsulation efficiency. The results show that the surface morphology of the spray-dried Ch-TPP microspheres and the release rate of the drug can be controlled by varying process parameters used during the preparation of the Ch-TPP microspheres. Most importantly, the drug release rate is controlled by the Ch-TPP matrix density and thus by the degree of swelling of the hydrogel matrix.

Poly(*N*-isopropylacrylamide)-chitosan (NIPAAm-Ch) cross-linked copolymer particles have been synthesized as a drug delivery system via soapless emulsion copolymerization of *N*-isopropylacrylamide and chitosan [120]. An anionic initiator (ammonium persulfate) and a cationic initiator [2,2′-azobis(2-methylpropionamidine) dihydrochloride, AIBA] were used in this synthesis. The results show that the copolymer particles obtained from AIBA show the morphology of a core-shell structure and did not show lower critical solution temperature (LCST). Regardless of the kind of initiator used to synthesize the copolymer, the increase of the pH value decreases the

swelling ratio of the copolymer. Also, the increase of the Ch/NIPAAm weight ratio or the cross-linking agent decreases the amount of caffeine (as a model drug) loading. The pore size and swelling ratio of the copolymer disk controlled the caffeine release from the Ch/NIPAAm copolymer.

Carboxymethyl chitosan (CM-Ch) nanoparticles have been prepared by gelification with calcium ions [121]. These particles have been used as colloidal carriers for the delivery of doxorubicin (DOX), a commonly used cationic anticancer drug. The results show that the degree of substitution (DS) and molecular weight of CM-Ch are important for DOX delivery. CM-Ch with higher MW and DS enhances DOX loading to 4.2% and shows lower release over the test period in vitro. Also, the DOX release rate is hindered by CM-Ch with high MW and DS.

Nanocomposite films from chitosan/organic rectorite (chitosan/OREC) have been synthesized by a casting solvent-evaporation method [122]. Addition of OREC to pure chitosan film enhances many of the properties related to the amount and interlayer distance of the layered silicate in the chitosan/OREC. In vitro drug controlled-release studies show a slower and more continuous release for the nanocomposite films in comparison with pure chitosan film, and the drug delivery cumulative release is proportional to the amount and interlayer distance of OREC.

Conclusions

Chitosan is a natural, biodegradable, biocompatible polymer with antibacterial activity and has a wide range of applications in different fields such as membranes, medicine, drug delivery, hydrogels, water treatment, adhesives, food packaging, fuel cells, and surface conditioner. It is the most important derivative of chitin, the second most abundant natural polymer in the world. The presence of NH_2 groups in chitosan explains its greater potential than chitin for use in different applications. Since it has both hydroxyl and amino groups, it can be modified chemically into many forms and can participate in different types of chemical reactions.

Chitosan and its derivatives are suitable for tissue engineering applications. Various type of chitosan derivatives have been used in skin, bone cartilage, liver, nerve, and blood vessels. This material is also a good candidate for use as a carrier of drugs for controlled release and other pharmaceutical applications.

This polysaccharide has shown high potential for absorption of dyes, metal ions, and proteins. So, it might also be a good candidate for removing pollutants from water and wastewater.

References

1. Kurita K (2001) Prog Polym Sci 26:1921
2. Rinaudo M (2006) Prog Polym Sci 31:603
3. Kurita K, Tomita K, Tada T, Ishii S, Nishimura S, Shimoda K (1993) J Polym Sci, Part A: Polym Chem 31:485
4. Kasaai MR (2008) Carbohydr Polym 71:497
5. Abdou ES, Nagy KSA, Elsabee MZ (2008) Bioresour Technol 99:1359
6. Mahlous M, Tahtat D, Benamer S, Khodja AN (2007) Nuclear Inst Meth Phys Res B 265:414
7. Ono K, Saito Y, Yura H, Ishikawa K, Kurita A, Akaike T, Ishihara M (2000) J Biomed Mater Res 49:289
8. Yamada K, Aoki T, Ikeda N, Hirata M, Hata Y, Higashida K, Nakamura Y (2008) J Appl Polym Sci 107:2723
9. Yen MT, Tseng YH, Li RC, Mau JL (2007) LWT Food Sci Technol 40:255
10. Kim KW, Thomas RL (2007) Food Chem 101:308
11. Garcia MA, Pinotti A, Zaritzky NE (2006) Starch 58:453
12. Sebastien F, Stephane G, Copinet A, Coma V (2006) Carbohydr Polym 65:185
13. Grandfield K, Zhitomirsky I (2008) Mater Charact 59:61
14. Chien PJ, Sheu F, Yang FH (2007) J Food Eng 78:225
15. Huang L, Zhai M, Peng J, Xu L, Li J, Wei G (2008) Carbohydr Polym 71:690
16. Zhao X, Mai Z, Kang X, Zou X (2008) Biosens Bioelectron 23:1032
17. Srinivasa PC, Ramesh MN, Kumar KR, Tharanathan RN (2003) Carbohydr Polym 53:431
18. Nosal WH, Thompson DW, Yan L, Sarkar S, Subramanian A, Woolam JA (2005) Colloids Surf B 43:131
19. Rabea EI, Badawy M, Rogge TM, Stevens CV, Höfte M, Steurbaut W, Smagghe G (2005) Pest Manag Sci 61:951
20. Janciauskaite U, Rakutyte V, Miskinis J, Makuska R (2008) React Funct Polym 68:787
21. Kim IY, Seo SJ, Moon HS, Yoo MK, Park IY, Kim BC, Cho CS (2008) Biotechnol Adv 26:1
22. Chung TW, Wang SS, Tsai WJ (2008) Biomaterials 29:228
23. Abarrategi A, Gutierrez MC, Vicente CM, Hortiguela MJ, Ramos V, Lopez-Lacomba JL, Ferrer ML, Monte F (2008) Biomaterials 29:94
24. Mun GA, Nurkeeva ZS, Dergunov SA, Nam IK, Maimako TP, Shaikhutdinov EM, Lee SC, Park K (2008) React Funct Polym 68:389
25. Chi P, Wang J, Liu C (2008) Mater Lett 62:147
26. Liu J, Zhang J, Xia W (2008) Food Chem 107:419
27. Chen JP, Chang GY, Chen JK (2008) Colloids Surf A: Physicochem Eng Aspects 313–314:183
28. Wei DW, Qian WP, Shi Y, Ding SH, Xia Y (2007) Carbohydr Res 342:2494
29. Wei DW, Qian W, Shi Y, Ding S, Xia Y (2008) Carbohydr Res 343:512
30. Ding CM, Zhou Y, He YN, Tan WS (2008) Process Biochem 43:287
31. Jin HH, Lee CH, Lee WK, Lee JK, Park HC, Yoon SY (2008) Mater Lett 62:1630
32. Mohameh KR, El-Meliegy E (2008) Ceramics Inter 34:285
33. Baek SH, Kim B, Suh KD (2008) Colloids Surf A: Physicochem Eng Aspects 316:292
34. Shirosaki Y, Okayama T, Tsuru K, Hayakawa S, Osaka A (2008) Chem Eng J 137:122
35. Mansur HS, Costa HS (2008) Chem Eng J 137:72
36. Tan H, Lao L, Wu J, Gong Y, Gao C (2008) Polym Adv Technol 19:15

37. Huang L, Zhai M, Peng J, Xu L, Li J, Wei G (2007) J Colloid Interface Sci 316:398
38. Shim IK, Lee SY, Park YJ, Lee MC, Lee SH, Lee JY, Lee SJ (2008) J Biomed Mater Res 84A:247
39. Jiang HL, Kim YK, Arote R, Nah JW, Cho MH, Choi YJ, Akaike T, Cho CS (2007) J Controlled Release 117:273
40. Chenite A, Gori S, Shive M, Desrosiers E, Buschmann MD (2006) Carbohydr Polym 64:419
41. Sagnella S, Mai-Ngam K (2005) Colloids Surf B 42:147
42. Lahiji A, Sohrabi A, Hungerford DS, Frondoza CG (2000) J Biomed Mater Res 51:586
43. Zhong Z, Xing R, Liu S, Wang L, Cai S, Li P (2008) Carbohydr Res 343:566
44. Ma Y, Zhou T, Zhao C (2008) Carbohydr Res 343:230
45. Devliegher F, Vermeulen A, Debevere J (2004) Food Microbiol 21:703
46. Jumaa M, Furkert FH, Muller BW (2002) Eur J Pharm Biopharm 53:115
47. Wang X, Du Y, Liu H (2004) Carbohydr Polym 56:21
48. Eweis M, Elkholy SS, Elsabee MZ (2006) Int J Biol Macromol 38:1
49. No HK, Park NY, Lee SH, Meyers SP (2002) Int J Food Microbiol 74:65
50. Zhong Z, Chen R, Xing R, Chen X, Liu S, Guo Z, Ji X, Wang L, Li P (2007) Carbohydr Res 342:2390
51. Durango AM, Soares NFF, Benevides S, Teixeira J, Carvalho M, Wobeto C, Andrade NJ (2006) Packag Technol Sci 19:55
52. Liu XF, Guan YL, Yang DZ, Li Z, Yao KD (2001) J Appl Polym Sci 79:1324
53. Wang C, Chen C (2005) J Appl Polym Sci 98:391
54. Shaban MA, Swailam TMH, Swailam HMH (2002) Nahrung/Food 46:337
55. Li Y, Chen XG, Liu N, Liu CS, Liu CG, Meng XH, Yu LJ, Kenedy JF (2007) Carbohydr Polym 67:227
56. Zhai M, Zhao L, Yoshii F, Kume T (2004) Carbohydr Polym 57:83
57. Shahidi F, Arachchi JKV, Jeon YJ (1999) Trends Food Sci Technol 10:37
58. Tripathi S, Mehrotra GK, Dutta PK (2008) e-Polymers 093:1
59. Kanatt SR, Chander R, Sharma A (2008) Food Chem 107:845
60. Ausar SF, Passalacqua N, Castagna LF, Bianco ID, Beltramo DM (2002) Int Dairy J 12:899
61. Meng X, Li B, Liu J, Tian S (2008) Food Chem 106:501
62. Kanatt SR, Chander R, Sharma A (2008) Food Chem 106:521
63. Lee CH, An DS, Park HJ, Lee DS (2003) Packag Technol Sci 16:99
64. Fierro S, Sanchez-Saavedra MP, Copalcua C (2008) Bioresour Technol 99:1274
65. Wang L, Wang A (2008) Bioresour Technol 99:1403
66. Hasan M, Ahmad AL, Hameed BH (2008) Chem Eng J 136:164
67. Vitali L, Laranjeira MCM, Goncalves NS, Favere VT (2008) Int J Biol Macromol 42:152
68. Siyanytsya V, Kochkodan V, Goncharuk V (2008) Desalination 223:91
69. Chen JY, Zhou PJ, Li JL, Wang Y (2008) Carbohydr Polym 72:128
70. Akkaya R, Ulusoy U (2008) J Haz Mater 151:380
71. Zhao L, Luo F, Wasikiewicz JM, Mitomo H, Nagasawa N, Yagi T, Tamada M, Yoshii F (2008) Bioresour Technol 99:1911
72. Donia AM, Atia AA, Elwakeel KZ (2008) J Haz Mater 151:372
73. Ramesh A, Hasegawa H, Sugimoto W, Maki T, Ueda K (2008) Bioresour Technol 99:3801
74. Li Q, Su H, Tan T (2008) Biochem Eng J 38:212
75. Boddu VM, Abburi K, Talbott JL, Smith ED, Haasch R (2008) Water Res 42:633
76. Hakim L, Sabarudin A, Oshita K, Oshima M, Motomizu S (2008) Talanta 74:977
77. Zeng D, Wu J, Kennedy JF (2008) Carbohydr Polym 71:135
78. Tang XH, Tan SY, Wang YT (2002) J Appl Polym Sci 83:1886
79. Sugunan A, Thanachayanont C, Dutta J, Hilborn JG (2005) Sci Technol Adv Mater 6:335
80. Qu X, Wirsen A, Albertsson AC (2000) Polymer 41:4589
81. Schuetz YB, Gurny R, Jordan O (2008) Eur J Pharm Biopharm 68:19
82. Richardson SM, Hughes N, Hunt JA, Freemont AJ, Hoyland JA (2008) Biomaterials 29:85
83. Felinto MCFC, Parra DF, Silva CC, Angerami J, Oliveira MJA, Lugao AB (2007) Nuclear Inst Meth Phys Res B 265:418
84. Berger J, Reist M, Mayer JM, Felt O, Peppas NA, Gurny R (2004) Eur J Pharm Biopharm 57:19
85. Drury JL, Mooney DJ (2003) Biomaterials 24:4337
86. Guo BL, Gao QY (2007) Carbohydr Res 342:2416
87. Wang M, Fang Y, Hu D (2001) React Funct Polym 48:215
88. Li N, Bai R (2005) Sep Pur Technol 42:237
89. Dai YN, Li P, Zhang JP, Wang AQ, Wei Q (2008) Biopharm Drug Dispos 29:173
90. Ruel-Gariepy E, Shive M, Bichara A, Berrada M, Garrec D, Chenite A, Leroux J (2004) Eur J Pharm Biopharm 57:53
91. Huang R, Chen G, Sun M, Hu Y, Gao C (2006) J Membr Sci 286:237
92. Shi W, Zhang F, Zhang G (2005) J Chromatogr B 819:301
93. Yang JM, Yang SJ, Lin HT, Wu TH, Chen HJ (2008) Mater Sci Eng C 28:150
94. Chen JH, Liu QL, Fang J, Zhu AM, Zhang QG (2007) J Colloid Interface Sci 316:580
95. Sun H, Lu L, Chen X, Jiang Z (2008) Sep Pur Technol 58:429
96. Huang R, Chen G, Sun M, Gao C (2008) Sep Pur Technol 58:393
97. Xu CX, Chen RY, Zheng X, Chen X, Chen Z (2008) J Membr Sci 307:218
98. Karakecili AG, Gumusderelioglu M (2008) Colloids Surf B 61:216
99. Wang J, Zheng X, Wu H, Zheng B, Jiang Z, Hao X, Wang B (2008) J Power Sour 178:9
100. Liu L, Chakma A, Feng X (2008) J Membr Sci 310:66
101. Tsai HA, Chen WH, Kuo CY, Lee KR, Lai JY (2008) J Membr Sci 309:146
102. Saxena A, Tripathi BP, Shahi VK (2008) J Coll Interface Sci 319:252
103. Krajewska B (2001) React Funct Polym 47:37
104. Kim JH, Kim JH, Jegal J, Lee KH (2003) J Membr Sci 213:273
105. Bayramoglu G, Erdogan H, Arica MY (2008) J Appl Polym Sci 108:456
106. Xiao S, Feng X, Huang RYM (2007) J Membr Sci 306:36
107. Casimiro MH, Gil MH, Leal JP (2007) Nuclear Inst Meth Phys Res B 265:406
108. Yu SH, Mi FL, Wu YB, Peng CK, Shyu SS (2005) J Appl Polym Sci 98:538
109. Duan B, Wu L, Yuan X, Hu Z, Li X, Zhang Y, Yao K, Wang M (2007) J Biomed Mater Res 83A:868
110. Wan Y, Creber KAM, Peppley B, Bui VT (2004) J Appl Polym Sci, Part B: Polym Phys 42:1379
111. Sui W, Wang Y, Dong S, Chen Y (2008) Colloids Surf A: Physicochem Eng Aspects 316:171
112. Kulkarni PV, Keshavayya J, Kulkarni VH (2007) Polym Adv Technol 18:814
113. Braga MEM, Pato MTV, Costa Silva HSR, Ferreira EI, Gil MH, Duarte CMM, Sousa HC (2008) J Sup Fluids 44:245
114. Yang X, Zhang Q, Wang Y, Chen H, Zhang H, Gao F, Liu L (2008) Colloids Surf B 61:125

115. Learoyd TP, Burrows JL, French E, Seville PC (2008) Eur J Pharm Biopharm 68:224
116. Nunthanid J, Huanbutta K, Luangtana-anan M, Sriamornsak P, Limmatvapirat S, Puttipipatkhachorn S (2008) Eur J Pharm Biopharm 68:253
117. Oosegi T, Onishi H, Machida Y (2008) Eur J Pharm Biopharm 68:260
118. Gomes P, Gomes CAR, Batista MKS, Pinto LF, Silva PAF (2008) Carbohydr Polym 71:54
119. Desai KGH, Park HJ (2005) Drug Dev Res 64:114
120. Lee CF, Wen CJ, Lin CL, Chiu WY (2004) J Appl Polym Sci, Part A: Polym Chem 42:3029
121. Shi X, Du Y, Yang J, Zhang B, Sun L (2006) J Appl Polym Sci 100:4689
122. Wang X, Du Y, Luo J, Lin B, Kennedy JF (2007) Carbohydr Polym 69:41