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Influence of the Chemical Structure and Physicochemical Properties of Chitin- and Chitosan-Based Materials on Their Biomedical Activity

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1. Introduction

Chitin and chitosan are an important family of linear polysaccharides consisting of varying amounts of β-(1→4)-linked 2-acetamido-2-deoxy-β-D-glucopyranose (GlcNAc) and 2-amino-2-deoxy-β-D-glucopyranose (GlcN) units (Muzzarelli, 1973; Roberts, 1992). Chitin samples contain a high content of GlcNAc units; hence, they are insoluble in water and common organic solvents. On the other hand, they dissolve only in solvents such as *N*,*N*-dimethylacetamide, hexafluoroacetone or hexafluoro-2-propanol (Pillai et al., 2009; Austin, 1988; Kurita, 2001). When the degree of *N*-acetylation (defined as the average number of *N*-acetyl-D-glucosamine units per 100 monomers expressed as a percentage) is less than 50%, chitin becomes soluble in aqueous acidic solutions (pH < 6.0) and is called chitosan (Pillai et al., 2009). This means that the term "chitosan" represents a group of fully and partially deacetylated chitins, but a rigid nomenclature with respect to the degree of *N*-deacetylation between chitin and chitosan has not been established (Ravi Kumar, 2000). Some authors consider that chitosan is a polysaccharide containing at least 60% GlcN residues (Aiba, 1992). According to the nomenclature proposed by the European Chitin Society (EUCHIS) (Roberts, 2007), chitin and chitosan should be classified on the basis of their insolubility and solubility in 0.1 M acetic acid; the insoluble material is called chitin, whereas the soluble one is chitosan. The structures of "ideal" chitin and "ideal" chitosan, and the "real" structures of these compounds are presented in Figure 1.

Chitin is the second most abundant polysaccharide (next to cellulose) synthesized by a great number of living organisms, serving in many functions where reinforcement and strength are required (Muzzarelli et al., 1986). In nature, chitin is found as structural components in the exoskeleton of arthropods or in the cell walls of fungi and yeast. It is also produced by a number of other living organisms in the lower plant and animal kingdoms. It has been

estimated that 10^{10}–10^{12} tons of chitin are biosynthesized each year (Percot et al., 2003). Unlike chitin, chitosan is produced only by some fungi from the family *Mucoraceae* (Roberts, 1998). Industrially, chitosan is usually produced by de-*N*-acetylation of chitin. The various industrial sources of chitin (α-, β- and γ-chitin) (Roberts, 1992; Tolaimate et al., 2003; Synowiecki & Al-Khateeb, 2003), as well as the processes and conditions under which this polymer is prepared (Al Sagheer, 2009; Manni et al., 2010; Das & Ganesh, 2010; Chaussard & Domard, 2004), cause the physical and chemical properties of chitosan preparations to vary (Rinaudo, 2006; Tolaimate et al., 2003; Domard, 2010).

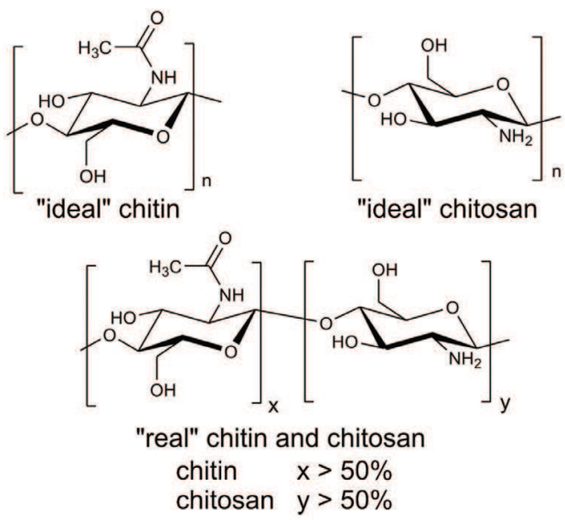

Fig. 1. The chemical structures of chitin and chitosan

Chitin and chitosan have great economic value because of their versatile biological activities such as biocompatibility, biodegradability, non-toxicity and adsorptive abilities, as well as chemical applications, mainly in the medical and pharmaceutical fields (Ravi Kumar, 2000; Rinaudo, 2006; Murugan & Ramakrishna, 2004; Yadav & Bhise, 2004; Aranaz et al., 2009; Ravi Kumar et al. 2004; Di Martino et al., 2005; Krajewska, 2004; Muzzarelli & Muzzarelli, 2005). The biological properties of these compounds depend closely on their physicochemical parameters, especially their solubility in water and other commonly used solvents. Most of the characteristic properties of chitosan are strictly related to its weight-averaged molecular weight (M_W) and the high content of glucosamine residues containing primary amino groups (Aranaz et al., 2009). In comparison to chitosan, chitin, being a highly insoluble and chemically rather unreactive material, has far fewer applications. By chemically modifying the primary amino and free hydroxyl groups of chitin and chitosan, their solubility in water and organic solvents can be improved (e.g. Zhong et al., 2007; Xie et al., 2007; Jeong et al. 2008), thereby increasing their range of biomedical applications (e.g. Kurita, 2001; Rinaudo, 2006; Alves & Mano, 2008).

Chitin/chitosan based materials with different structures show different biological activities, and not all the biological activities were found in one kind of chitin/chitosan material (Xia et al., 2010). Knowledge of the microstructure of chitin and chitosan samples is thus essential for an understanding of the structure–property–activity relationships, and special emphasis in this respect should be placed on the chitin/chitosans used in biomedical applications (Aranaz et al., 2009; Xia et al., 2010; Weinhold et al. 2009; ASTM. F2103-01, 2001;

ASTM. F2260-03, 2003; ASTM. WK965, 2003; Struszczyk & Struszczyk, 2007; Kean & Thanou, 2010).

The aim of this book chapter is to highlight the relationship between the chemical structure and physicochemical properties of chitin- /chitosan-based materials in the context of their biomedical activity. Special emphasis will be placed on the influence of the weight-averaged molecular weight, degree of *N*-acetylation and the types of chemical modification on the biomedical activity of chitin-/chitosan-based materials. A strategy for improving the characterization of chitosan for sustainable biomedical applications will also be presented.

2. Biomedical activity of chitin/chitosan samples differentiated on the basis of weight-averaged molecular weight (M_W)

The range of chitin/chitosan molecular weight is extensive (from several to more than thousands of kDa) and is thus divided into three categories: low-molecular-weight chitosan (LMWC), medium-molecular-weight chitosan (MMWC) and high-molecular-weight chitosan (HMWC) (Harish Prashanth & Tharanathan, 2007; Lin et al., 2009; Sun et al., 2009). As a consequence of increasing M_W, some physicochemical and biological properties of chitin/chitosan and its solutions change, which determines the bioactivity of the material (Table 1).

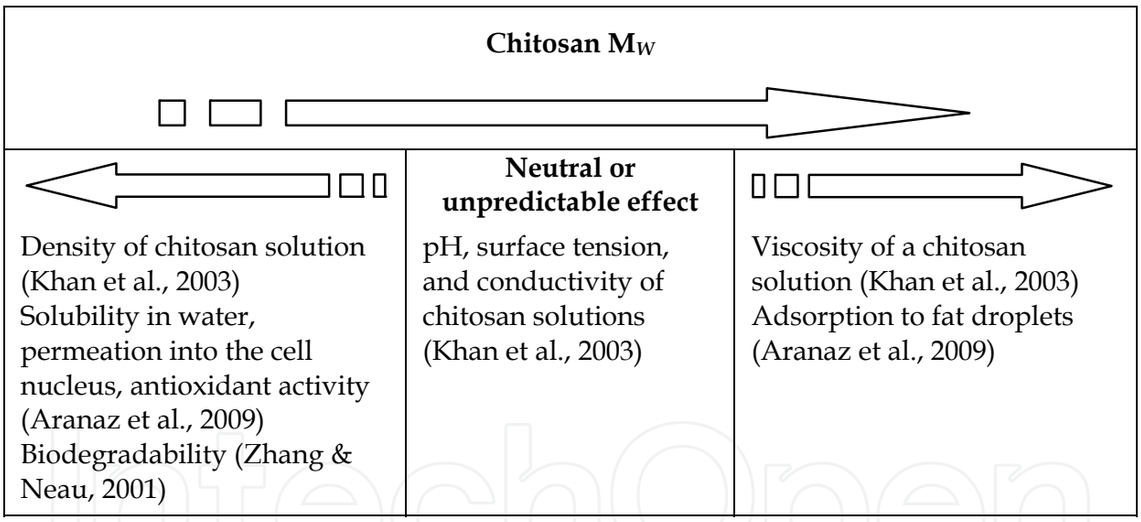

Table 1. The influence of molecular weight on some properties of chitosan and its solutions

Depending on the intended use, specific polymeric or oligomeric forms of chitin/chitosan are required. For some medical and pharmaceutical applications LMWC and chitooligosaccharides (COS) are more suitable. The exact product can be obtained by the enzymatic or chemical hydrolysis of polymer chains (Aranaz et al., 2009; Zhang et al., 2010b; Aam et al., 2010).

2.1 Drug delivery

One of the potential applications of chitin/chitosan products is a drug delivery system (nasal, oral, transdermal, etc.). Depending on the drug and its destination, a chitosan matrix can assume various forms: hydrogels, tablets, microspheres, films, nanoparticles, beads,

granules (Singh & Ray, 2000; Aranaz et al., 2009; Patel, 2006): everything serves a purpose to control drug release. Because they are better soluble in water, maintain a low viscosity with increasing concentration, and are derivatized more easily and faster, chitooligosaccharides appear to be more suitable as an injection material (Boesel et al., 2009). In the literature the expression "water-soluble chitosan" (WSC) is used to describe both LMWC and COS (Chae et al., 2005; Wang et al., 2007). WSC with a molecular weight range between 3.5 and 15.8 kDa was tested as a carrier for protein delivery (Wang et al., 2007). It was found that by increasing M_W, the loading efficiency of a model protein (bovine serum albumin BSA) increased from 8%to 48% for M_W from 3.5 to 6.3 kDa but only by a few percent for M_W = 15.8 kDa. The BSA release rate decreased very quickly up to 6.3 kDa, but then only slightly to 15.8 kDa. The results of this and other studies confirm that WSC nanoparticles are suitable for delivering protein drugs (Wang et al., 2007; Zhang et al., 2010a). There are also several studies relating the M_W effect to the characteristics of chitosan microspheres used as drug carriers (Nair et al., 2009; Sun et al., 2009). The aim of one of them was to test encapsulation with three different chitosans (40 kDa, 480 kDa, 850 kDa) for nasal methotrexate (MTX) administration (Sun et al., 2009). These authors concluded that MMWC microspheres have the best characteristics: the strongest mucoadhesive properties, as well as flowability and drug release in a controlled manner. In the case of LMWC drug release, control and mucoadhesion properties were poor. The disadvantages of HMWC microspheres were poor flowability and a tendency to aggregate. These results are in agreement with Gupta & Jabrail, who found that microspheres containing MMWC showed the best control of drug (nonsteroidal contraceptive - centchroman) release (Gupta & Jabrail, 2007). The effect of molecular weight on drug loading and drug release of drug-loaded chitosan microspheres was investigated using ketoprofen as a model drug (Genta et al., 1998). Comparing chitosans of 70 kDa, 750 kDa, and 2000 kDa, Genta et al. showed that a good ketoprofen content, an encapsulation efficiency independent of the polymer/drug ratio, and good control of drug release were achieved for microspheres composed of HMWC/LMWC (1:2 w/w). The main purpose of another study was to investigate the chitosan M_W effect on the formation and properties of pellets consisting of acetaminophen (model drug), chitosan, sodium alginate, dibasic calcium phosphate dehydrate and microcrystalline cellulose (Charoenthai et al., 2007). The influence of two samples of chitosans with different molecular weights (190 and 490 kDa) was compared. It was demonstrated that the characteristics of pellets, including shape, size, crushing force and speed of drug release, depend closely on chitosan M_W. Charoenthai et al. showed that LMWC had better pellet-forming properties (diameter, sphericity) and guaranteed drug release control. The effect of the molecular weight of four different samples of chitosan (160, 580, 1350, 1930 kDa) on the efficacy of two antibiotics (tobramycin and ofloxacin) used during external ocular infections was investigated over time by Felt et al. (Felt et al., 2001). The antibiotic content in tears was found to increase when HMWC was employed as compared to the controls and samples with a lower molecular weight of chitosan. The antibiotic efficacy of ofloxacin was also higher when HMWC was used, whereas tobramycin was independent of M_W.

The correlation between the molecular weight of chitosan and its interaction with different cell membranes was also tested (Yang et al., 2002; Chae et al. 2005). This information is very useful for the design of new drugs based on chitosan. Chae et al. investigated water soluble chitosan transport through a Caco-2 cell layer (in vitro) and intestinal absorption (in vivo) after the oral administration of fluorescence-labelled samples (Chae et al. 2005). It was

shown that intestinal absorption is molecular-weight-dependent and increases with decreasing chitosan M_W (more than 20 times, comparing WSC samples with molecular weights of 3.8 kDa and 230 kDa). The same tendency was observed with respect to permeation through a Caco-2 cell layer – the lower the M_W, the better the penetration. The interesting problem is the interaction of chitosan with the lipid bilayer. Yang et al. showed that LMWC (4.2 kDa) could destabilize a cell membrane at neutral pH (Yang et al., 2002).
Sometimes in order to increase human immunity, the lactic acid bacteria present in food can be helpful. They should be delivered to the colon, but the problem is not to kill these bacteria in extreme gastric conditions, or during the storage of food in the refrigerator. Microencapsulation offers a solution. Lee et al. investigated the survival of *Lactobacillus bulgaricus* KFRI 673 in alginate microparticles coated with chitosans of different molecular weights (Lee et al., 2004b). The experiment was carried out in simulated gastric and intestinal juices at 37°C, and in skimmed milk at 4° and 22°C. They demonstrated that with increasing chitosan M_W its protective ability towards gastric juices also increases. During storage at 4°C there was no difference between the stabilities of free and microencapsulated bacterial cells, but at 22°C the higher the molecular weight of the chitosan, the better the protection afforded. HMWC can be successfully used as a coating agent for alginate microparticles. Another example of the protection of the intestinal microbial flora is to deliver prebiotic agents. For this purpose chitooligosaccharides are much more useful (Lee et al., 2002). The influence of chitosan molecular weight on the minimum inhibitory concentration (MIC) was tested against bifidobacteria and lactic acid bacteria, and on the cell growth of the same microbes. It was shown that fully deacetyleted COS with a depolimerization degree of DP 2-8 exhibited a stimulatory effect on *Bifidobacterium bifidium* (0.1-0.5%) and *Lactobacillus casei*, and *Lactobacillus brevis* (0.1%), whereas chitosan in polymeric form displayed antimicrobial activity even at a very low concentration (0.078-0.31%).

2.2 Antimicrobial activity

In comparison to the prebiotic activity of chitin/chitosans, their antimicrobial properties are decidedly more often discussed. The antimicrobial activity of chitosans varies depending on their physical properties. These polymers can be used against a broad spectrum of target organisms like bacteria, fungi, viruses or algae (Chirkov, 2002; Rabea et al., 2003; Tikhonov et al., 2006; Visnova & Vavrikova, 2008; Goy et al., 2009). The significant influence of molecular weight on this activity has been demonstrated, but the type of microorganism also plays a significant role. For such investigations *Escherichia coli* (Gram-negative) and *Bacillus cereus* (Gram-positive) are often used (Babiker, 2002; Vishu Kumar et al., 2005; Kittur et al., 2005; Fernandes et al., 2009). Native chitosan, LMWC, COS, and monomers all displayed an inhibitory effect against them. Vishu Kumar et al. demonstrated the better activity of a chito-oligomeric-monomeric mixture (with high DP) compared with native chitosan and monomers (Vishu Kumar et al., 2005). Although *E. coli* is Gram-negative and *B. cereus* is Gram-positive, and the bactericidal mechanism is different in each case, the influence of chitosan molecular weight was similar. This concurred with the results obtained by Kittur et al. (Kittur et al., 2005). They found the COS-monomeric mixture more efficient against both bacteria than native chitosan, and that in the case of *B. cereus* this effect was stronger still. It was interesting in both studies that GlcN showed only slight bacterial growth inhibition, whereas GlcNAc did not display any activity. Among the chitooligomers,

the antibacterial effect increased from dimer to hexamer (Vishu Kumar et al., 2005; Kittur et al., 2005). Fernandes et al. investigated the mechanism of chitosan antibacterial activity against vegetative and resistant forms of *B. cereus* using atomic force microscopy imaging and nanoindentation (Fernandes et al., 2009). They found that HMWC surrounded the bacterial cells. The polymer layer that formed became a mechanical barrier preventing the uptake of nutrients, subsequently leading to the death of the vegetative form, whereas the spores remained unaffected. Chitooligosaccharides caused visible damage to vegetative cells, but were unable to destroy the spores. In another study, gluten peptides were conjugated with HMWC and LMWC, then tested for antibacterial activity against *E. coli* (Babiker, 2002). Bactericidal activity of the gluten peptide-chitosan conjugates was observed when both types of chitosan were used, but the presence of HMWC in a conjugate made this more active, especially at low temperatures. Atomic force spectroscopy was also used to investigate the molecular weight dependency of the antibacterial effect of chitosans, but this time against *Escherichia coli* and *Staphylococcus aureus* (Eaton et al., 2008). These studies also allowed the response strategies used by Gram-negative and Gram-positive bacteria to be compared. In the case of *E. coli* antibacterial activity was better with LMWC than with HMWC. This is the result of the ready penetration of the Gram-negative cell wall by oligomers. Gram-positive bacteria have an intrinsically different cell wall structure. In this case the formation of a chitosan polymer layer preventing nutrient absorption is preferred, and HMWC displayed better antibacterial activity against *S. aureus*. Lin et al. (Lin et al., 2009) reported similar observations: decreasing the chitosan molecular weight was conducive to growth inhibition of *E. coli*, but not of *S. aureus*. They also demonstrated the existence of an intraspecific diversity in sensitivity toward LMWC, which could explain the discrepancies appearing in some studies. They examined the different enzymes used to obtain LMWC, which significantly affects molecular size distribution. Comprehensive studies comparing antibacterial activity against many different microorganisms have also been carried out (Gerasimenko et al., 2004; Park et al., 2004a, Fernandes et al., 2010). Park et al. examined the antibacterial activity of hetero-chitosans (with different degrees of acetylation) and their oligosaccharides (5-10 kDa, 1-5 kDa, and <1 kDa) against three Gram-negative (*Escherichia coli, Salmonella typhimurium, Pseudomonas earuginosa*) and five Gram-positive bacteria (*Bacillus subtilis, Bacillus cereus, Staphylococcus epidermidis, Staphylococcus aureus, Micrococcus luteus*) (Park et al., 2004). Hetero-chitosans inhibited the growth of all bacteria, but the efficiency of this process additionally depended on the microorganism and DA. The inhibitory effect of hetero-COSs was lower in comparison to the activity of hetero-chitosans. The efficiency of inhibition was found to be higher for Gram-positive than for Gram-negative bacteria. The potential antimicrobial activities of chitosans with different molecular weights were tested in the context of their use in textile finishing so as to prevent (or treat) skin disorders (Fernandes et al., 2010). For these investigations six skin-borne miscroorganisms (three Gram-negative bacteria: *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa,* two Gram-positive bacteria: *Staphylococcus aureus*, *Staphylococcus epidermidids*, and the fungus *Candida albicans*), three chitosans (average 628, 591, and 107 kDa), and two mixtures of chitooligosaccharides (<5, <3 kDa) were chosen. The sensitivity of all Gram-negative bacteria increased with decreasing chitosan molecular weight. The opposite effect was observed with the fungus and the Gram-positive bacteria, although in the latter case the trend was not so distinct. The results of many investigations indicate that low molecular weight chitosans could be universal antimicrobial agents against all types of bacteria and fungi (Gerasimenko et al., 2004; Tikhonov et al., 2006).

2.3 Antitumour properties

In some medical applications of chitin/chitosan, as antitumour compounds, for example, their degradation products are preferred, as they have a lower viscosity and a better solubility in water. The antitumour activity of chitin/chitosan is manifested by the stimulation of the immune system (production of lymphokines, including interleukins 1 and 2, stimulation of NK, etc.) (Qin et al., 2002a; Jeon & Kim, 2002; Maeda & Kimura, 2004). Jeon & Kim tested the antitumour activity of three kinds of COSs (high molecular weight ranging from 6.5 to 12 kDa – HMWCOS, medium molecular weight ranging from 1.5 to 5.5 kDa - MMWCOS, and low molecular weight ranging from 0.5 to 1.4 kDa – LMWCOS) against Sarcoma 180 solid (S180) and Uterine cervix carcinoma No. 14 (U14) (Jeon & Kim, 2002). The efficiency of tumour growth inhibition for both types of tumour cells in mice was best in the case of MMWCOS. There are many reports of S180 tumour cells being used for testing the antitumour activity of chitosan (Qin et al., 2002a; Qin et al., 2004; Maeda & Kimura, 2004). Maeda & Kimura investigated the antitumour effect of three water-soluble low molecular weight chitosans (21 kDa, 46 kDa, 130 kDa) and various doses of 650 kDa chitosan in sarcoma 180-bearing mice (Maeda & Kimura, 2004). They found that LMWC (21 and 46 kDa) and also smaller oligosaccharides could activate the intestinal immune system of animals, thus preventing tumour growth. But no antitumour effect was observed after the oral administration of chitosan samples, even of low molecular weight (46 kDa). The same authors confirmed that high molecular weight chitosan (650 kDa) prevents the adverse reactions of some cancer chemotherapeutic drugs. Qin et al. also tested the antitumour activity of LMWC against sarcoma 180, but they came to the opposite conclusions. They noted that oral administration of LMW chitosan decreases the weight of the tumour (Qin et al., 2002a; Qin et al., 2004), although administration by intraperitoneal injection led to a higher inhibitory rate (Qin et al., 2004). It was reported the higher the M_W of LMWC, the better the inhibitory tumour effect (Qin et al., 2002a). The introduction of acidic groups as a result of chitosan oxidation has the opposite effect, and an increase in M_W decreases antitumour activity (Qin et al., 2002b). The influence of LMWC and COS (including the pentamer, hexamer, and higher oligomers) on the growth inhibition of Ehrlich ascites tumour (EAT) cells and tumour-induced neovascularization was investigated (Harish Prashanth & Tharanathan, 2005). Based on experimental results concerning the inhibition of angiogenesis and the induction of apoptosis, it was confirmed that COSs seem to be more potent angioinhibitory and antitumour compounds. Wang et al. reported that chitin oligosaccharides (DP 1-6) also reduced the number of K562 cells (human erythromyeloblastoid leukemia cell line) (Wang et al., 2006).

2.4 Gene delivery

Chitosan possesses many of the properties required of gene therapy material. It is suitable as a base polymer for a nonviral DNA delivery system. It is important to realize that molecular weight is a critical factor in cytotoxicity. Investigations into this problem have shown a reduction in cytotoxic effect with decreasing chitosan molecular weight (Richardson et al., 1999). For studying the relationship between structure and transfection efficiency five chitosans with molecular weights from 31 to 190 kDa were chosen. It was found that gene transfer efficacy was independent of chitosan molecular weight in the range of 31 to 170 kDa (Köping-Höggård et al., 2001). In another work the influence of the degree of polymerization in the 21-88 range (M_W 4.7-33 kDa) on transfection efficiency was tested

(Strand et al., 2010). The maximum level was achieved for DP from 31 to 42. With increasing chain length, gene expression was lower and delayed. Park et al. examined COSs with molecular weights from 3 to 45 kDa (Park et al., 2006). To compare transgene expression they used HEK 293 cells with pEGFP-N1 plasmid encoding green fluorescent protein. The best efficiency was found for COS of molecular weight 9 kDa. There are many reports concerning the design of chitosan/pDNA nanoparticles for gene delivery (Bozkir & Saka, 2004; de la Fuente et al., 2008; Centelles et al., 2008). The aim of Centelles et al. was to investigate the level of transgene expression mediated by three DNA-chitosan nanoparticle formulations differing in chitosan molecular weight (150 kDa, 400 kDa, 600kDa) (Centelles et al. 2008). They did not notice any effect of M_W of chitosan on the long term (105 days) expression. In another study, nanoparticles consisting of chitosan (CS) and hyaluronic acid (HA) were chosen to load with pDNA (de la Fuente et al., 2008). These nanoparticles differed in chitosan and chitooligosaccharide molecular weight (125 kDa, 10-12 kDA respectively), in HA molecular weight, and in HA:CS mass ratio. Taking into account only the influence of chitosan it could be concluded that a lower mass promoted a better transfection efficiency.

The chitosan–based delivery system is not limited to experiments with DNA. It has been used to transfer functionally active siRNA to HeLa cells (Techaarpornkul et al., 2010). For this study human cervical carcinoma cells with stable expression of enhanced green fluorescent protein (EGFP) were prepared. Then the gene-silencing effect of siRNA complexes with chitosans of different molecular weight (20, 45, 200, and 460 kDa) was examined. The best results were obtained with LMWC – 20 kDa. The molecular weight of chitosan did not affect the particle size of the CS/siRNA complex, but higher molecular weights did favour complex formation.

2.5 Tissue engineering

There is also a special place for chitin/chitosan in the field of tissue engineering. Nwe et al. reported that the mechanical properties of chitosan membranes deteriorated when the molecular weight of chitosan increased (New et al., 2009), whereas Hsu et al. maintained that it is precisely the high molecular weight of chitosan that gives the chitosan scaffold its better mechanical strength (Hsu et al., 2004a). The reduced proliferation of human skin fibroblast cells on a collagen/chitosan scaffold with increasing chitosan molecular weight was observed by Tangsadtkakun et al. (Tangsadtkakun et al., 2007), while Howling et al. reported that the M_W of chitin and chitosan samples had no appreciable influence on the proliferation of tissue fibroblasts or on keratinocytes (Howling et al., 2001). The major goal of another study was to measure the effects of chitin/chitosan with different molecular weights on wound healing (Minagawa et al., 2007). They concluded that both chitin and chitosan oligomers and monomers are better for the acceleration of wound healing than the polymeric forms. Additionally, it was found that collagenase activity was greater in the chitosan group (the highest for GlcN), whereas it was lower for chitin and remained at the same level for the whole chitin group. Shelma et al. used chitin and chitosan in their investigations, because both possess wound healing potential (Shelma et al., 2008). They found that introducing chitin nanofibres considerably increased the tensile strength of medium molecular weight (270 kDa) chitosan films, helping this complex to be a better accelerator of wound healing. This stands in agreement with results concerning the tensile strength and arrangement of collagen fibres in the skin, obtained previously by Cho et al.

(Cho et al., 1999). They demonstrated the better efficacy of water-soluble chitin in wound healing in comparison to chitin and chitosan samples.

2.6 The hypocholesterolemic effect

There are also studies concerning the role of chitosan in reducing the total cholesterol level, and the influence of molecular weight on this was tested. The purpose of one such study was to investigate the correlation between feeding chitosan (M_W 120 kDa) and lipid metabolism in hyperlipidemic rats (Xu et al., 2007). The cholesterol-lowering effect of chitosan was noted. The same results were achieved by Osman et al., although they used HMWC (Osman et al., 2010). The hypocholesterolemic effect of chitosan samples on human patients has also been recorded. Jaffer & Sampalis observed a lowering of serum cholesterol concentration after patients were treated with LMWC (40 kDa) (Jaffer & Sampalis, 2007). The results of other experiments did not testify to any statistically significant reductions in cholesterol levels (Tapola et al., 2008). It can be concluded that chitosans with different molecular weights are capable of lowering cholesterol levels, although the mechanisms of this effect are still not clear.

In summary, chitin/chitosan with different molecular weights have numerous potential applications in medicine and pharmacy. Nevertheless, taking into account the properties of chitin/chitosan such as toxicity, water solubility, low viscosity etc., low molecular weight chitosans and chitooligosaccharides would appear to be more useful for the majority of biomedical applications.

3. Biomedical activity of chitin/chitosan samples differentiated with respect to the degree of *N*-acetylation (DA)

As mentioned at the beginning of this review, chitin is insoluble in aqueous solvents whereas chitosan is soluble in acidic or even neutral conditions owing to the free protonable amino groups in the glucosamine residue. The degree of *N*-acetylation is therefore an important characteristic that influences the performance of chitin/chitosan in many of its applications.

The solubility, conformation and dimensions of chitosan chains in aqueous media have been extensively studied as a function of the degree of acetylation (DA) (Berth et al., 1998; Berth & Dautzenberg, 2002; Schatz et al., 2003). Schatz et al. have proposed general laws of chitosan behaviour in aqueous solutions (Schatz et al., 2003; Sorlier et al., 2001; Sorlier et al., 2002):

- at DA below 20%, chitosan exhibits the highest structural charge density. Chitosan displays polyelectrolyte behaviour related to long-distance intra- and intermolecular electrostatic interactions, which are responsible for chain expansion, high solubility and ionic condensation.
- for values of DA 20-50%, hydrophilic and hydrophobic interactions are progressively counterbalanced.
- for DA over 50% electrostatic interactions are essentially short-distance interactions. Then, hydrophobic interactions due to the increase in the acetyl group content become predominant.

It was confirmed that for biomedical applications chitosan with DA 0-30% is the most useful (Sandford, 1984; Aranaz et al., 2009).

The DA values of chitin/chitosan depend directly on the means of deacetylation and the conditions of the process. In general, chitosan is prepared by severe alkaline hydrolysis of

the chitin acetamide groups. Usually, sodium or potassium hydroxides are used at a concentration of 30-50% w/v at ~100°C. Sometimes the deacetylases isolated from certain bacteria and fungi are used. However, the activity of these enzymes is limited by the poor solubility of chitin (Martinou et al., 1998) – this is a major factor limiting its utilization. Most studies and applications have been carried out with chitosan because of its greater versatility and superior biological properties. In addition, Howling et al. demonstrated that highly deacetylated chitosan is biologically more active than chitin and less deacetylated chitosans (Howling et al., 2001). Since the majority of biological properties are related to the cationic behaviour of chitosan (the presence of free amino groups in the polymeric chain), all the below-mentioned properties depend significantly on the DA.

Biodegradability Chitin and chitosan can be degraded by several proteases (lysozyme, papain, pepsin) present in the human body (Vårum et al., 1997). Biodegradation yields non-toxic oligosaccharides that can be assimilated or excreted (Pangburn et al., 1982).

The rate of degradation of chitosan is very important because it affects the properties of the material. For example, scaffold degradation should be compatible with the rate of new tissue formation or be adequate for the controlled release of bioactive molecules. Degradation of chitosan has been shown to increase as the degree of deacetylation decreases (Hirano et al., 1989; Sashiwa et al., 1990; Kurita et al., 2000). Kofuji et al. investigated the enzymatic digestion of various chitosans by observing changes in the viscosity of a chitosan solution in the presence of lysozyme (Kofuji et al., 2005). They concluded that chitosan with a high DA tended to degrade more rapidly. However, the rates of enzymatic degradation of chitosan species differed, even in chitosan species with similar DA.

Aiba et al. and Shigemasa et al. suggested that the differences in degradation are due to variations in the distribution of acetamide groups in the chitosan molecule caused by differences in deacetylation conditions. Moreover, the absence of acetyl groups results in very low rates of enzymatic degradation (Aiba, 1992; Shigemasa et al, 1994). Therefore, it is impossible to estimate the biodegradation rate from the degree of deacetylation alone.

Finally, it should also be mentioned that the lability of chitin towards lysozyme also depends on its crystalline form. Kurita et al. showed that β-chitin was degraded much more readily than α-chitin owing to the weak intermolecular forces in the former (Kurita et al., 2000).

Biocompatibility This property depends on many parameters, although DA seems to be one of the most important. Orally administered chitin/chitosan can be regarded as not bioavailable, because they are not absorbed despite partial enzymatic degradation. The kinetics of enzymatic chitin/chitosan biodegradation, mentioned above, also affects biocompatibility. Very fast rates of degradation produce amino sugars, which can cause an inflammatory response. Therefore, chitosan samples with high DA can induce an acute inflammatory response, whereas samples with low DA can induce only a minimal response.

In the context of biocompatibility, the toxicity of chitin/chitosan should also be mentioned: it seems to depend on the DA. Schipper et al. reported that chitosans with DA lower than 35% displayed low toxicity, whereas DA above 35% caused dose-dependent toxicity (Schipper et al., 1996).

Mucoadhesion Mucus serves to protect epithelial cells in the gastrointestinal and respiratory systems in mammals. It is composed of mucin, which is negatively charged (the presence of sialic acid residues). In the stomach, chitosan is positively charged owing to the acidic condition and interacts with mucin by electrostatic forces.

It has been found that a lower DA leads to an increase in charge density of the molecule and the adhesive properties become more relevant (He et al., 1998). Mao et al. observed a direct correlation between the degree of deacetylation of chitosan and adhesion (Mao et al., 2004) The study revealed that the smaller the increases in DA, the stronger the cell adhesion.
Not only DA, but also the distribution of acetyl groups along the chain (random or blockwise) can affect the biomedical properties of chitin/chitosan as a result of changes in the solubility, inter-chain interactions due to H-bonds and the hydrophobic nature of the acetyl group (Zhang et al., 2010b).
Because of their considerable biocompatibility, quite easily controlled biodegradability and mucoadhesion, chitin and chitosan have been widely employed in drug and gene delivery, wound healing, tissue regeneration and some other biomedical applications.

3.1 Drug delivery

Owing to their controlled biodegradability and good biocompatibility chitin and chitosan are good candidates for drug delivery vehicles. The ability of chitosan to form drug delivery systems is due to its cationic charge and its ability to interact with negatively charged polyanions. Drug delivery systems using chitosan include microspheres, nanoparticles, hydrogels, solutions, films and tablets depend on the delivery route.
The selection of an ideal type of chitosan with certain properties is useful for developing efficient drug delivery systems, prolonging the duration of drug activity, improving therapeutic efficiency and reducing side effects (Aranaz et al., 2009). Kofuji et al. stated that the proper selection of physicochemical properties of chitosan is important for the choice of the appropriate chitosan as a drug delivery vehicle. Among several physicochemical properties, DA seems to be one of the most important (Kofuji et al., 2005).
Gupta & Jabrail observed the influence of DA and cross-linking on the physical characteristics, swelling and release of centchroman-loaded chitosan microspheres (Gupta & Jabrai, 2006). The authors concluded that since the hydrophobicity of chitosan depends on the numbers of *N*-acetyl groups, the loading and release characteristics of chitosan matrices depends closely on DA. Maximum loading capacity was observed at DA 38-52%, whereas very high DA can induce burst release. The degree of cross-linking in chitosans is controlled in a similar way. The larger numbers of free amino groups at lower DA increases the degree of covalent cross-linking. The authors also reported that the size and surface roughness of the microspheres decreased with a reduction in DA. Moreover, a lower DA in chitosan increases the compactness of matrices and its hydrophobicity, thus controlling the degree of swelling and diffusivity of the drug entrapped in chitosan matrices. The value of the diffusion coefficient for centchroman from microspheres decreased on reducing the DA of chitosan (Gupta & Jabrai, 2006).
Chiou et al. studied the influence of different chitosans on the initial burst and drug release from microspheres (Chiou et al., 2001). They observed that chitosans with higher DA reduce the initial burst of drug release more effectively.
Aranaz et al. summarized the relation between DA and the properties of microspheres, one of the most common drug delivery vehicles. A decrease in chitosan DA increases covalent crosslinking and the compactness and hydrophobicity of the matrices, whereas increasing DA reduces the size, surface roughness, swelling, loading capacity and burst release of the microspheres (Aranaz et al., 2009).

Kofuji et al. investigated the relationship between the DA and the ability of chitosan to form spherical gels (Kofuji et al., 2005). Chitosan with low DA was able to form a spherical gel by chelation with metal ions, unlike chitosan with high DA. This ability was explained by the larger numbers of amino groups available in polysaccharides.

3.2 Gene delivery

Discussed compounds forming complexes with DNA play an important role in gene therapy. Mumper et al. proposed a non-viral vector for a gene delivery system (Mumper et al., 1995). As a non-viral vector for gene delivery chitin and chitosan demonstrate some advantages compared to viral vectors. They do not produce endogenous recombination, oncogenic effects or immunological reactions (Ferber, 2001).

The M_W of chitosan and its DA are key parameters in the preparation of chitosan/pDNA complexes. Lavertu et al. studied several combinations of M_W and DA of chitosan. They selected two combinations of high transfection efficiency using a chitosan of 10 kDa and a DA of 8 and 20% (Lavertu et al., 2006).

Kiang et al. observed the effect of the degree of chitosan deacetylation on the efficiency of gene transfection in chitosan/DNA nanoparticles (Kiang et al., 2004). They suggested that the use of chitosan with a DA above 20% might accelerate chitosan degradation and DNA release, since highly deacetylated chitosan (above 80%) releases DNA very slowly.

3.3 Tissue engineering

Chitosan scaffolds are promising materials for the design of tissue engineered systems owing to their low immunogenic activity, controlled biodegradability and porous structure (Ho et al., 2005; Ma et al., 2003; Madihally & Howard 1999). The influence of DA on the structural and biological properties of chitosan scaffolds for cell culture and tissue engineering was studied by Tiğli & Gümüsderelioglu (Tiğli et al., 2007). These authors observed that the mechanical strength of chitosan was better with lower DA and that chitosan with lower DA favoured cell adhesion. They also observed that chitosan scaffolds with DA (15-25%) displayed a more regular structure in comparison to scaffolds with very low DA (<15%). Moreover, the lateral pore connectivity was much lower for chitosans DA 15-25% than for scaffolds with DA <15%. Both observations were very important, because it is very well known that the microstructure of the matrix has an important influence on cell intrusion, proliferation and functioning in tissue engineering.

Freier et al. prepared and characterized chitin and chitosan tubes for nerve regeneration (Freier et al., 2005). The compressive strength of these tubes was found to increase with decreasing of DA.

3.4 Wound healing

Both chitin and chitosan activate the complement system in polymorphonuclear cells, fibroblasts and vascular endothelial cells, thereby contributing to wound healing (Minagawa et al., 2007). Since all of these processes are related to the properties of the matrix (chitin/chitosan), they also depend on the DA (Ueno et al., 2001).

Minagawa et al. studied the influence of chitin and chitosan with different DA, as well as chitin oligomers and monomers (GlcN, GlcNAc) on wound healing (Minagawa et al., 2007). They observed that at lower DA wound break strength was greater and that fibroblasts were more highly activated.

3.5 Antimicrobial activity

Chitin and chitosan have been investigated as antimicrobial materials with respect to a wide range of target organisms like algae, bacteria, yeasts and fungi.

Chitin and chitosan with different DA were analyzed with respect to fungi (*Aspergillus fumigatus, Aspergillus parasiticus, Fusarium oxysporum, Candida albicans*), Gram-positive bacteria (*Staphylococcus aureus, Staphylococcus saprophyticus, Bacillus cereus, Listeria monocytogenes*) and Gram-negative bacteria (*Escherichia coli, Salmonella* Typhimurium, *Pseudomonas aeruginosa, Enterococcus faecalis, Aeromonas hydrophila, Shigella dysenteriae, Vibrio cholerae, Vibrio parahaemolyticus*). In all cases the antimicrobial activity increased with decreasing DA (Andres et al., 2007; Tipparat, H. & Riyaphan, 2008; Tsai et al., 2002).

Park et al. studied the antimicrobial activity of chitosans with different degrees of deacetylation against three Gram-negative bacteria and five Gram-positive bacteria and found that 75% deacetylated chitosan exhibited more effective antimicrobial activity than 90% or 50% deacetylated chitosan (Park et al., 2004a).

Three antibacterial mechanisms have been proposed:

- ionic surface interaction resulting in wall cell leakage;
- mRNA inhibition and protein synthesis via the penetration of chitosan into the nuclei of the microorganisms;
- the formation of an external barrier chelating metals and provoking the suppression of essential nutrients to microbial growth. It is likely that all events occur simultaneously but at different intensities (Goy et al., 2009; Helander et al., 2001; Liu et al., 2001; Roller, & Covill, 1999).

It should be mentioned that some authors have not found a clear relationship between DA and antimicrobial activity. They suggested that the antimicrobial activity of chitosan is dependent on both the chitosan and the microorganism used (Chien & Chou, 2006; Oh et al., 2001).

3.6 Antioxidative activity

Chitosan possesses a significant scavenging capacity against different radical species. Its antioxidant activity depends on the degree of deacetylation (Koryagin et al., 2006). Park et al. studied the abilities of chitosan samples with a DA of 50, 25 and 10% to scavenge 1,1-diphenyl-2-picrylhydrazyl (DPPH), hydroxyl, superoxide and alkyl radicals. The results indicated clearly that the radical scavenging activities of hetero-chitosans depend on their DA. Chitosan with the lowest DA exhibited the best scavenging activity (Park et al., 2004b; Je & Kim, 2006).

A similar study was performed with chitooligosaccharides. The oligosaccharide prepared from chitosan with DA 10% displayed the greatest radical scavenging activity (Je et al., 2004). It was concluded that the free amino groups in chitooligosaccharides can react with free radicals to form stable macromolecule radicals (Xie et al., 2001).

4. Biomedical activity of chitin-/chitosan-based materials differentiated with respect to chemical modification

They are a lot of chemical methods of modification which can generate completely new chitin-based biofunctional materials. The most common one is graft copolymerization, which allows the formation of applicative derivatives by the covalent binding of a molecule, the graft, onto the chitosan backbone (Alves & Mano, 2008). Chitosan can also be derivatized by introducing a small functional group such as alkyl or carboxymethyl into its structure. Other chemical modifications of chitin and chitosan include phosphorylation, combination

of chitosan derivatives with cyclodextrins, and thiolation. In this section the most important applications of such modified chitin and chitosan-based materials in biomedical fields such as drug delivery, tissue engineering and antimicrobial agents will be discussed.

4.1 Drug delivery

Drug delivery has been one of the major applications of chitin and chitosan derivatives in recent years. The main factors that allow the use of chitin and chitosan as drug delivery vehicles is their biodegradability and the fact that they can be easily metabolized by certain human enzymes, especially lysozyme (Muzzarelli, 1997).

There are several chitosan-based drug delivery systems prepared by different methods (Agnihotri et al., 2004). The usual ones are:

- tablets – prepared by coating - sodium diclofenac, pentoxyphylline, salicylic acid;
- capsules – prepared by capsule shell - insulin, 5-aminosalicylic acid;
- microspheres/microparticles -prepared by emulsion cross-linking – progesterone, aspirin, theophylline;
- nanoparticles – prepared by ionic gelation – ricin, bovin serum albumin;
- beads – prepared by coacervation/precipitation – riboflavin, adriamycin;
- films – prepared by solution casting – trypsin, testosterone, beta-oestradiol;
- gel – prepared by cross-linking – hydrocortisone acetate, caffeine, 5-fluorouracil.

In general, drug delivery materials can be supported via various routes, like nasal, ocular, oral, parenteral and transdermal. Some important applications of organ-specific drug delivery using chitosan derivatives are presented below.

4.1.1 Colon-targeted drug delivery

A chitosan-based delivery system can efficiently protect therapeutic agents from the hostile conditions of the upper gastrointestinal tract and release the entrapped agents, especially in the colon, through degradation of the glycosidic linkages of chitosan by colonic microflora (Hejazi & Amiji, 2003). In 1998 Lorenzo-Lamosa et al. designed microencapsulated chitosan microspheres for colonic drug delivery (Lorenzo-Lamosa et.al., 1998). Sodium diclofenac (SD), an anti-inflamantory drug, was efficiently entrapped in chitosan cores and then microencapsulated into EudragitL-100 and Eudragit S-100 to form a multireservoir system. *In vitro* release studies revealed that no SD was released at the gastric pH; when the microsphere reached the colonic environment, however, continuous release was observed for a variable time (8-12 h). Other derivatives – chitosan succinate and chitosan phthalate – were synthesized and assessed as potential matrices for colon-specific orally administered drug delivery applications (Aiedeh & Taha, 1999). These matrices resisted dissolution under acidic conditions. More recently, Jain A. and Jain S.K. reported a nanoparticular system for the colon-specific delivery of 5-fluorouracil (Jain & Jain, 2008). They prepared hyaluronic acid-coupled chitosan nanoparticles bearing 5-fluorouracil using ionotropic gelation. These derivatives showed enhanced cellular uptake by HT-29 colon cancer cells compared to uncoupled nanoparticles. Wang et al. also presented some fresh chitosan derivatives useful as drug delivery materials (Wang et al., 2009), e.g. a polyelectrolyte complex formed by sodium cellulose sulphate and chitosan as a drug carrier for colon-specific delivery.

4.1.2 Mucosal drug delivery

Ocular, nasal, peroral, vaginal and pulmonary mucosal surfaces are receving a great deal of attention as alternative routes of systemic administration. It has been shown in many

publications that *N*-trimethyl chitosan and *N*-carboxymethyl chitosan have a special feature enabling them to adhere to mucosal surfaces, which has made them some of the most useful chitosan derivatives for mucosal drug delivery (Lueben et al., 1996; Thanou et al., 2001; Jayakumar et al., 2006). More recently PEG-g-chitosan nanoparticles have been proposed as promising vehicles for insulin transport through the nasal mucosa (Zhang et al., 2009). Also not long ago, Perioli et al. prepared vaginal mucoadhesive tablets containing the bioadhesive polymers chitosan and polyvinylpyrrolidone in a ratio of 1:1 (Perioli et al., 2009). Several chitosan-based nanosystems, resulting in innovative ocular nanomedicines with a significant impact on clinical practice, have been described by Paolicelli et al. (Paolicelli et al., 2009). In turn, van der Lubben et al. in a review article presented the details of a chitosan-based delivery system for the transmucosal administration of drugs (van der Lubben et al., 2001).

4.1.3 Cancer therapy

The concept of polymer-drug conjugates for delivering hydrophobic, small molecular drugs to their site of action was first propounded by Ringsdorf in 1975 (Ringsdorf, 1975). Based on this strategy several chitosan-anticancer drug conjugates have recently been investigated and successfully applied in cancer therapy. A representative example is doxorubicin-conjugated glycol chitosan (DOX-GC) with a *cis*-aconityl spacer (Son et al., 2003). When these chitosan-based nanoparticles were systemically administered to mice they preferentially accumulated in the tumour tissue – this was ascribed to the EPR (enhanced permeability and retention) effect. *n*-Lauryl-carboxymethylchitosan is another example of a useful carrier for hydrophobic cancer drugs, developed by Miwa et al. (Miwa et al., 1998). These chitosan derivatives have the ability to form micelles that solubilize taxol, making it therapeutically more effective. More recently, Zhang et al. examined another chitosan-based polymeric micelle for taxol delivery in cancer therapy (Zhang et al., 2003). The results show that this new *N*-alkyl-*O*-sulphated chitosan can be effectively used as an potential drug carrier for taxol. Good antitumour activities were also exhibited by *N*-succinyl-chitosan derivatives conjugated with mitomycin C (MMC) (Kato et al., 2004). These chitosan-based conjugates are active against various tumours such as murine leukaemias (L1210 and P338), B16 melanoma, Sarcoma 180 solid tumour, murine liver metastatic tumour (M5076) and murine hepatic cell carcinoma (MH134). Further interesting examples of chitosan-anticancer drug conjugates are discussed in Tan et al. (Tan et al., 2009).

4.1.4 Gene delivery

Chitosan has the ability to interact ionically with negatively charged DNA to form polyelectrolyte complexes, which results in better DNA protection against nuclease degradation (Agnihotri et al. 2004). Several interesting studies describing the potential use of chemically modified chitosan in gene delivery have been reported. Quite a lot of them were focused on alkylated chitosan, mostly because of its high transfection efficiency. Among this class of derivatives dodecyl-chitosan has the most important application in DNA delivery (Liu et al., 2001a). Another example of chitosan-based gene delivery was studied by Mao et al. (Mao et al., 2001). They produced modified chitosan nanospheres with transferrin and poly (ethylene glycol) (PEG) but did not observe any significant enhancement in transfection. PEGylation of chitosan was also used by Zhang et al. to prepare chitosan-DNA complexes conjugated with alpha-methoxy-omega-succinimidyl

poly (ethylene glycol) (Zhang et al., 2007). As a result the gene expression was improved in comparison with the chitosan-DNA complex both *in vitro* and *in vivo*. More recently, Sajomsang et al. examined a methylated chitosan derivative/DNA complex containing different aromatic moieties (Sajomsang et al., 2009a). Of all the derivatives tested, *N*-(4-pyridinylmethyl) chitosan (MPyMeChX) exhibited the highest transfection efficiency in human hepatoma cells (Huh 7 cells). Many more examples of chemically modified chitosan used as potential gene delivery vehicels have recently been reported by Jaykakumar (Jayakumar et al., 2010).

4.1.5 Other ways of delivery

There are many publications showing that chitin and chitosan derivatives could also be used for liver-, kidney- and lung-targeted delivery. For example, Kato et al. evaluated the potential of lactosaminated *N*-succinyl-chitosan (Lac-Suc) as a liver-specific drug carrier (Kato et al., 2001). More recently, Yang et al. prepared polyion complex micelles (PIC micelles), also for liver-targeted delivery (Yang et al., 2009a). These micelles were based on methoxy poly (ethylene glycol)-graft-chitosan and lactose-conjugated poly (ethylene glycol)-graft-chitosan, and was prepared for the delivery of diammonium glycyrrhizinate (DG). The experiments showed that the lactose-conjugated PIC delivered more DG to the liver than conventional PIC micelles. As mentioned above, chitosan derivatives could also be used for lung-targeted delivery. For this purpose Yang et al. prepared chitosan-modified poly (lactic-coglycolic acid) nanoparticles containing paclitaxel (Yang et al., 2009). The results demonstrated that *in vitro* uptake of the nanoparticles by a lung cancer cell line (A549) was significantly increased by chitosan modification.

4.2 Tissue engineering

The present generation of tissue engineering research is based on seeding cells onto a porous biodegradable polymer matrix. The most recently published studies in this field have suggested the use of scaffolds to support and organize damaged tissue. Because of their controlled biodegradability and porous structure, chitosan and its derivatives have been reported as attractive candidates for scaffolding materials. Extensive studies on the use of chitosan-based materials in tissue engineering were described by Kim et al. in their recent review paper (Kim et al., 2008). Here we present just a few examples of the various types of chitosan derivatives modified for these applications. The strategies for using these derivatives in different kinds of organs such as skin, bone cartilage, liver, nerves and blood vessels will also be discussed.

4.2.1 Chitosan derivatives as tissue supporting materials

One of the most important groups of chitosan modification used for these applications involves the specific recognition of cells by sugars. For example, Li et al. reported that chitosan bound to D- and L-fucose showed specific interactions with lectin and cells (Li et al., 2000). Other chitosan derivatives prepared from lactobionic acid and chitosan with different activating agents, such as 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC) and *N*-hydroxysuccinimide (NHS), demonstrated the possibility of a synthetic extracellular matrix for hepatocyte attachment (Park et al., 2003). More recently, Kim et al. prepared mannosylated chitosan with a specific recognition for antigen-presenting cells such as B-cells, dendritic cells and macrophages (Kim et al., 2006).

The devlopment of the chemical grafting of chitosan has produced a series of practically useful tissue supporting materials. For example, chitosan graft-polymerized onto a poly (L-lactide) surface can be used to control the morphology and function of cells (Ding et al., 2004). Prabaharan et al. proposed a novel poly (L-lactide)-chitosan hybrid scaffold as a tissue engineering material that is simultaneously a drug release carrier (Prabaharan et al., 2006). These hybrid foams exhibit a much greater rigidity than pure chitosan foams, indicating that this strategy may allow for the use of chitosan-based structures in tissue engineering applications.

Another approach regarding the chemical modification of chitosan for tissue engineering applications has been to use electrospinning to create polymeric nanofibres. These materials have several useful properties such as a large specific surface area and extensive porosity. Chitin and chitosan nanofibres were produced, for example, by Min et al. (Min et al., 2004). Further investigations found that these chitosan-based nanofibres promoted the adhesion of chondrocyte and osteoblast cells and maintained characteristic cell morphology (Bhattarai et al., 2005).

Tissue	Types of derivatives	References
Skin	chitosan in combination with alginate polyelectrolyte complex chitosan/collagen porous scaffold made by cross-linking with glutaraldehyde	Yan et al., 2000; Ma et al., 2003
Bone	chitosan-calcium phosphate composites such as beta-tricalcium phosphate) hydroxyapatite/chitosan materials	Zhang & Zhang, 2002; Kawakami et al., 1992
Cartilage	chitosan-alginate hyaluronan scaffold attachment with RGD-containing protein porous collagen/chitosan/glycosaminoglycan scaffold loaded with transforming growth factor TGF-β1	Hsu et al., 2004b; Lee et al., 2004a
Liver	chitosan complex with glycosaminoglycans (GAG) chitosan/collagen/heparin matrix	Chupa et al., 2000; Wang et al., 2005
Nerve	hydroxyapatite-coated chitosan tubes with adsorbed laminin-1 and laminin peptides chitosan/gelatin composites	Itoh et al., 2003; Cheng et al., 2003
Blood vessel	heparin-chitosan scaffolds	Kratz et al., 1997

Table 2. Different tissue engineering applications of chitosan derivatives

The combination of chitosan with other materials seems to be a good opportunity for tissue engineering applications. Chung et al. prepared a galactosylated chitosan-based scaffold

combined with alginate to improve mechanical properties and biocompatibility (Chung et al., 2002). Sarasam & Madigally reported the effect of blending chitosan with poly(ε-caprolactone) (Sarasam & Madihally, 2005). Chitosan matrices were also modified by γ-poly (glutamic acid), which enabled the maximum strength to be increased in tissue engineering applications (Hsieh et al., 2005).

4.2.2 Applications of chitosan derivatives for different organs

Various types of chitosan derivatives have been used in different tissue engineering applications, namely, skin, bone, cartilage, liver, nerve and blood vessel tissue. Some of them are summarized in Table 2.

4.3 Antimicrobial agents

The antimicrobial activity of chitin, chitosan and their derivatives against different groups of microorganism, such as bacteria, yeast and fungi is considered to be one of the most important properties in recent years. These activities were first developed by Allan and Hadwiger in 1979, since then many interesting studies have been reported in this field (Allan & Hadwiger, 1979). Several mechanisms have been suggested as the cause of the inhibition of microbial cells by chitosan. One mechanism involves interaction with predominantly anionic components, such as lipopolysaccharides and microorganism surface proteins, resulting in changes in permeability, which causes cell death by inducing leakage of intracellular components (Helander et al., 2001). A second mechanism involves the inhibition of RNA and protein synthesis following permeation of the cell nucleus (Liu et al., 2001b). Yet another option is that chitosan on the surface of the cell can form a polymer membrane, which prevents nutrients from entering the cell (Liu et al., 2004). In this section we focus on the antibacterial and antifungal activities of some chitin and chitosan derivatives.

4.3.1 Antibacterial activity of chitosan derivatives

Many chitosan derivatives inhibit the growth of a wide range of bacteria. For example, Papineau et al. showed that chitosan lactate and chitosan glutamate are antagonistic towards *Escherichia coli* and *Staphylococcus aureus* (Papineau et al., 1991). Quaternary ammonium salts of chitosan, such as *N*,*N*,*N*-trimethylchitosan, *N*-propyl-*N*,*N*-dimethylchitosan and *N*-furfuryl-*N*,*N*-dimethylchitosan, were also shown to be effective in inhibiting the growth and development of *Escherichia coli*, especially in acidic media (Jia et al., 2001). Again, Xie et al. prepared multiple-derivatized chitosan (HPCTS-*g*-MAS) by the etherification of chitosan with propylene epoxide followed by the graft copolymerization of sodium malate and tested its antimicrobial activities against *Staphylococcus aureus* and *Escherichia coli* (Xie et al., 2002). Compared with chitosan, these derivatives exhibited a better inhibitory effect against these two bacterial species. The antibacterial activities of water-soluble *N*-alkylated disaccharide chitosan derivatives against *E. coli* and *S. aureus* were also investigated by Yang et al. (Yang et al., 2005). These bacteria were the most susceptible to cellobiose-chitosan derivatives and maltose-chitosan derivatives. More recently, Sajomsang et al. synthesized 17 derivatives of chitosan consisting of a variety of *N*-aryl substituents bearing either electron-donating or electron-withdrawing groups (Sajomsang et al., 2009b). Each of the derivatives was further quaternized using *N*-(3-chloro-2-hydroxypropyl)

trimethylammonium chloride and studied for their antibacterial activities. All the quaternized derivatives of chitosan displayed antibacterial activity against *E. coli* and *S. aureus*, as observed using the minimum inhibitory concentration (MIC) method.

4.3.2 Antifungal activity of chitosan derivatives

Chitosan is able to induce the *in vitro* growth of a number of fungi except Zygomycytes, which have chitosan as a component of their cell walls (Allan & Hadwiger, 1979). In 1996 Chen et al. reported that chitosan films made in dilute acetic acid solutions are able to inhibit the growth of *Rhodotorula rubra* and *Penicillium notatum* by direct applications of the film to the colony-forming organism (Chen et al., 1996). The antimicrobial activity of chitosan graft copolymers against *Candida albicans*, *Trichophyton rubrum* and *Trichophyton violaceum* was also observed (Jung et al., 1999). It was shown that the number and type of grafted chains, as well as the pH, substantially influenced the activities examined. Very extensive studies investigating the antifungal activity of chitosan derivatives were carried out by Rabea et al. (Rabea et al., 2005). They used a radial hyphal growth bioassay of *B. cinerea* and *P. grisea* to assess the fungicidal activity of 24 new derivatives of chitosan (i.e., *N*-alkyl, *N*-benzylchitosans). The results showed that all the derivatives are better fungicides than native chitosan. *N*-dodecylchitosan, *N*-(*p*-isopropylbenzyl) chitosan and *N*-(2,6-dichlorobenzyl) chitosan were the most active against *B. cinerea*, and *N*-(*m*-nitrobenzyl) chitosan likewise against *P. grisea*. Zhong et al. prepared 12 kinds of new hydroxylbenzenesulphonilanide derivatives of chitosan (CS), carboxymethyl chitosan (CMCS) and chitosan sulphated (CSS) and evaluated their antimicrobial activities against five pathogenic fungi: *P. asparagi*, *A. solani*, *F. oxysporum f. sp. vasinfectum* and *C. gloeosporioides* (Zhong et al., 2009). All these derivatives displayed stronger antifungal properties than the original materials (CS, CSS and CMCS). Palma-Guerrero et al. demonstrated that chitosan can permeabilize the plasma membrane of *Neurospora crassa* and kills the cells in an energy-dependent manner (Vruggink, 1970).

4.4 Other biomedical properties of chitin and chitosan derivatives

Chitin and chitosan derivatives have a great potential to be used in other biomedical applications. For example, chitin and chitosan sulphates display blood anticoagulant activity (Whistler & Kosik, 1971; Horton & Just, 1973). Substitution of a carboxyl group at position 6 in *N*-sulphonated chitosan yields a product with 23 % of the activity of heparin, and its *O*-sulphonated form exhibited 45 % activity *in vitro*. It has also been reported that the anticoagulant activity of sulphonated chitosan increases with rising sulphur content in chitosan. These derivatives could be useful as heparinoids for artificial blood dialysis. Grafted chitosan materials exhibited similar properties (Li et al., 2003). It has been demonstrated that the permeability of chitosan membranes grafted with HEMA can be controlled through plasma-treatment with the potential to be used in dialysis. Another very important biomedical activity of chitosan derivatives was investigated by Sosa et al. (Sosa et al., 1991), who demonstrated that *N*-carboxymethylchitosan *N*,*O*-sulphate inhibits HIV-1 replication and viral binding with CD4. Selective sulphation at *O*-2 and/or *O*-3 affords potent antiretroviral agents showing a much higher inhibitory effect against infection with the AIDS virus than that by the known 6-*O*-sulphated derivative (Nishimura et al., 1998). It was also shown that *N*-hexanoyl and *N*-octanoyl chitosan fibres can be used as haemostatic and anti-thrombogenic materials (Hirano, 1999). More recently, Huang et al. developed a

nanofibrous chitosan/PVA membrane for lipase immobilization (Huang et al. 2007). The results showed that this system can be used for biosensor applications.

5. Suitable strategy for the characterization of chitin-/chitosan-based materials used in biomedical applications

5.1 Standardization of M_W and DA determination

During recent decades of chitosan research, many methods have been proposed to determine the degree of acetylation (DA) and the weight-averaged molecular weight (M_W). Although these are two of the most important parameters of chitosan, no standard technique could be established. This is due mainly to some typical drawbacks of chitosan: poor solubility, polyelectrolyte behaviour and its polymeric character. As a consequence of the lack of a standard technique, many alternative methods have been put forward to determine these parameters. However, their accuracy and reliability are sometimes questionable and often depend on sample composition. DA and M_W values determined by different methods are sometimes scarcely comparable, and interpretation or comparison of these data must be done carefully. To inform the reader about the methods that can be applied, we will briefly discuss and summarize the techniques used and described in the literature.

5.2 M_W determination

In linear polymers the polymer chains rarely have the same degree of polymerization and molar mass, and there is always a distribution around an average value especially in natural polymers (except proteins and DNA). Different average values can be defined depending on the statistical method that is applied. Four values, such as the number-averaged molecular weight M_N, the weight-averaged molecular weight M_W, the viscosity-averaged molecular weight M_V, and the z-averaged molecular weight M_Z are in use; one of these, M_W, is the most important. The polydispersity of a polymer sample is defined as M_W divided by M_N and gives an indication of just how narrow the distribution is. Several techniques have been used over the years, often measuring just one of the values mentioned above. In modern times the most common technique has become size-exclusion chromatography (SEC). Apart from batch methods such as batch viscosity measurements (Nah & Jang, 2002), batch static light-scattering (SLS) measurements (Berth & Dautzenberg, 2002; Cölfen et al., 2001; Lamarque et al., 2005; Pa & Yu, 2001), or batch multi-angle light-scattering (MALS) measurements (Anthonsen et al., 1994; Chen & Tsaih, 1998; Tsaih & Chen, 1997), chromatography has the advantage of determining the polydispersity of the sample. The width of the molecular weight distribution is often the key parameter of a chitosan sample. The higher this distribution, the less conclusive is the observed effect of one sample due to the presence of several thousand different molecular weights at the same time. The reliable determination of the polydispersity of a sample is therefore an important requirement for pharmaceutical and scientific observations.

- SEC-RI (pullulan calibration) (Hasegawa et al., 1994; Knill et al., 2005; Lin & Lin, 2003)
- SEC-LALS (Kubota et al., 2000; Ottøy et al., 1996, 1995)
- SEC-MALS (Tømmeraas et al., 2002; Sorlier et al., 2001; Sorlier, 2002; Berth et al., 1998; Lamarque et al., 2004, 2005; Schatz et al., 2003; Brugnerotto et al., 2001)
- SEC-LALS-Vis (Weinhold et al., 2009)
- SEC-MALS-Vis (Christensen et al., 2008)

Since the use of size-exclusion chromatography is commonly agreed upon, more controversy is present about surrounds the different detectors used online for chromatography. Nowadays, all the old bench methods can be applied to a chromatography setup such as a refractive index detector (RI), viscosity detector (Vis) and light-scattering detectors (single-90°, low-angle and 90° (LALS), or multi-angle with 3, 5, or up to 18 angles (MALS). But using only refractive index or viscosity detectors M_W can only be obtained with reference to some standard samples (often pullulan or dextran). These values differ significantly from the ”real” molecular weight because chitosan coils differ in solution in comparison to pullulan and dextran. Absolute values can be obtained by light-scattering, but sample composition determines the type of light-scattering device to be used. If the sample is below roughly 100 000 g/mol, a single-90° is sufficient to give reliable M_W values. If the the sample weight is much higher, scattered light underlies the angular dependence and a significant decrease of laser intensity is observable at a detection angle of 90°. To overcome this problem laser light is also detected at rather low angles (LALS) (where the decrease becomes negligible) or at many angles (MALS). Additionally, light-scattering detectors can be combined with a viscosity detector to increase information about sample composition as a result of the simultaneous observation of conformation (via the Mark-Houwink plot) and branching behaviour. However, the type of light scattering device to be used in chitosan science (low angle vs. multi angle vs. triple detection) is still a matter of discussion and may be based mainly on the use of different parameters in light-scattering experiments in chitosan research. For a light-scattering experiment, values such as dn/dc (refractive index increment) and R_θ (Rayleigh ratio) must be known for the molecular weight analysis. If only one value is changed, for example, for an interlab comparison, the resulting M_W values will be significantly different even for identical samples.

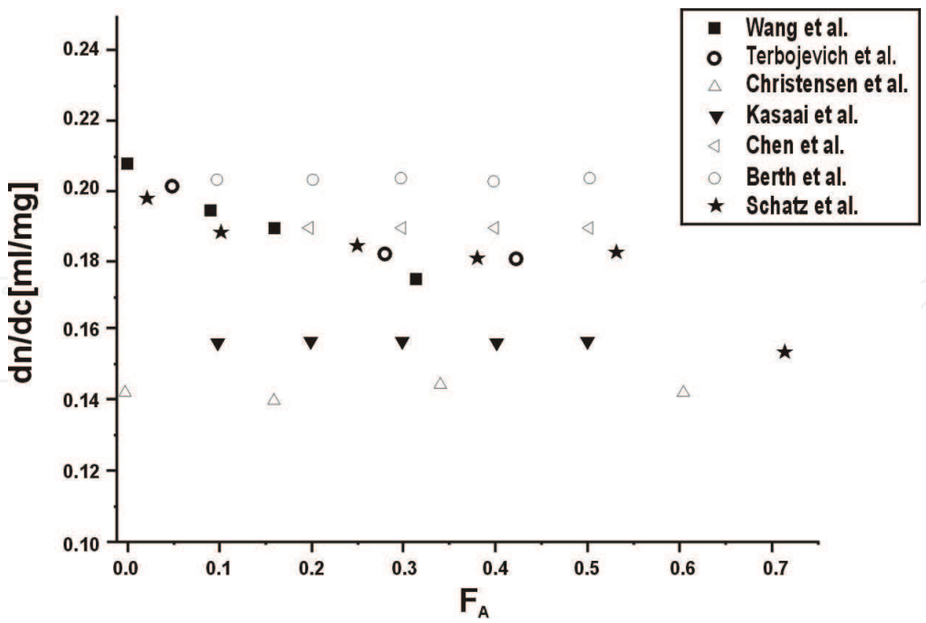

Fig. 2. Variation of the dn/dc values.

In different studies of chitosan different dn/dc values were used. The diagram (Fig. 2) presents values obtained at 436 nm (Wang et al. 1991) and values obtained at 633 nm

(Terbojevich et al., 1991; Christensen et al., 2008; Kasaai et al., 2000; Chen & Tsaih, 1998; Berth et al., 1998; Schatz et al., 2003) for different chitosan preparations with changing FA(DA).

The reason for this change is that dn/dc enters the molecular weight calculation quadratically. Using, for example, dn/dc = 0.142 (Christensen et al., 2008) for calculating the M_W of a high molecular weight chitosan sample, we obtain a molecular weight of 1098 kg/mol. Changing the dn/dc value to 0.208 (Berth et al., 1998) for the same chromatography data, the M_W decreases to 750 kg/mol. The use of published dn/dc values on identical data therefore leads to a drastic change in the molecular weight of up to 32%! Thus, comparison of molecular weight data obtained by light-scattering must include information about the dn/dc value; otherwise, a comparison will never show conformity when two different dn/dc's are used.

A similar effect can be seen by changes of R_θ. To make a light scattering detector into an absolute measurement device the equipment needs to be calibrated once after manufacturing to ensure its accuracy and functionality. This is a typical procedure and is often done by the manufacturer. For this calibration, a pure organic solvent with known Rayleigh ratio, e.g. toluene (Wyatt, 1993; Santos & Castanho, 1996) is used. Nowadays, a R_θ of 1.402 x $10^5 cm^{-1}$ obtained at 90° using 632.8 nm laser light (Kaye & McDaniel, 1974) should be used; this is stipulated by the Federal Institute for Materials Research and Testing (BAM, Bundesanstalt für Materialforschung und -prüfung). However, there are still other values in use such as 1.3522 x $10^{-5} cm^{-1}$ and 1.340 x $10^{-5} cm^{-1}$ (Itakura et al., 2005). Again, to obtain comparable molecular weight data for interlab comparisons, information about the Rayleigh ratio used must be included.

Despite these difficult conditions, it has been shown that a reliable determination of molar masses with acceptable deviations is possible, according to a round-robin test with four different polymer reference materials (Just et al., 2005). In this test, different detector devices were applied (low-angle and multi-angle light scattering detectors), indicating negligible dependencies on different instruments (especially LALS vs. MALS), different cell geometries, or different wavelengths of laser light. Dependencies on dn/dc values, inappropriate solvents and the Rayleigh ratio of toluene $R_{\theta,toluene}$ were excluded as they were prescribed by the regulations. This indicates how difficult it is to initiate a standardization of molecular weight determination of chitosan, even for light-scattering devices, as long as different dn/dc and $R_{\theta,toluene}$ values are being used. But it also shows that standardization in chitosan research might be possible in the future.

5.3 DA determination

For the determination of DA, up to 17 different methods have been applied in the literature. Considering the solubility issue of some chitin/chitosan samples, some of these methods are inapplicable to insoluble samples. In several methods, the samples have to be carefully purified to avoid any interference with the measurements. Moreover, most methods have several drawbacks with respect to analysis time, cost or accuracy.

To present the problem more clearly, we will summarize these methods in two bullet lists. The first one contains the methods that were proposed some time ago but are hardly used regularly as the procedures they require are time-consuming. All the methods have the determination of non-absolute DA values in common. The second bullet list contains the more accurate methods for DA determination; thus they are used more regularly. We will

also indicate whether the method is suitable only for chitosan, highlighted by "soluble material", or whether it is also applicable to chitin, highlighted by "solid material".

- conductometric titration (Raymond et al., 1993) (soluble material)
- UV-VIS (Muzzarelli, 1985) (soluble material)
- circular dichroism (Domard, 1987) (soluble material)
- thermal analysis (Alonso et al., 1983) (solid material)
- enzymatic hydrolysis (Nanjo et al., 1991) (soluble material)
- picric acid assay (Neugebauer et al., 1989) (soluble material)
- pyrolysis GC (Lal & Hayes,1984) (solid material)
- acid hydrolysis GC (Holan et al., 1980) (solid material)
- acid hydrolysis HPLC (Niola et al., 1993) (solid material)
- ninhydrin test (Curotto & Aros, 1993; Prochazkova et al., 1999) (soluble material)
- X-ray deffraction (Zhang et al., 2005) (solid material)

Since chitosan is a natural product it may contain certain residues (protein, alkali, salt, astaxanthin), depending on the quality of the chitin source and the reaction conditions. Problems with sample contamination may arise with several methods. Residual salts may interfere with conductometric titration by influencing the endpoint of the titration and therefore shift the measured DA value. Other residues like proteins display UV activity and may interfere with the UV-VIS and the circular dichroism method. In 2002, the European Pharmacopeia proposed the UV method as a standard method for characterizing chitosan hydrochloride. However, Aiba et al. (Aiba, 1986) found changes in the DA values (30%, strong) in comparison to IR spectroscopy and colloid titration, which reflects the vulnerability of this method. Enzymatic hydrolysis requires non-standard enzymes, the activity of which must be checked beforehand, and the overall procedure is relatively time-consuming. Together with pyrolysis, acid hydrolysis GC and acid hydrolysis HPLC need a 100% rate for the preceding hydrolysis reaction, which cannot be checked easily. Furthermore, the measurement time including sample preparation is very long. The ninhydrin method is non-quantitative and depends on different molecular weights and therefore needs a calibration curve determined by a second method (Prochazkova et al., 1999). The XRD method is affected by the lyophilization of the samples, which changes the original crystalline properties. In summary, the overall accuracy of the above methods may be directly dependent on the purity of the samples investigated, and their applicability is limited. Thus, they do not qualify as standard methods for DA determination.

Let us now focus on the more important methods used for DA determination. To illustrate the impact in the scientific community, the number of citations is shown after each method (determined via Web of Science (ISI Web of Knowledge) 20th July 2010).

- IR spectroscopy (Miya et al., 1980) (solid samples) cited 117 times.
- ^{1}H-NMR (Hirai et al., 1991; Vårum et al., 1991a) (soluble material) cited 176 and 203 times, respectively.
- ^{13}C-NMR (Vårum et al., 1991b) (soluble material) cited 84 times.
- solid state ^{13}C-NMR (Saito et al., 1987; Raymond et al., 1993) (solid samples) cited 76 times.
- ^{15}N-NMR (Heux et al., 2000) (soluble material) cited 49 times.
- colloid titration (Broussignac, 1968) (soluble material) .

The IR spectroscopy method has two main advantages: it is rather quick and can also be used for chitin samples as the measurement requires solid material. However, there are

uncertainties about the best baseline setting and the selection of peaks for analysis (amide, amino, hydroxyl) (de Velde & Kiekens, 2004), which results in different DA values for the same spectrum. This shortcoming of IR spectroscopy is shown up by very highly deacetylated samples, in the spectra of which the amide band is scarcely distinguishable. On the other hand, it gives good results with very slightly deacetylated samples, which cannot be measured by many other methods. A serious problem is that it does not give absolute values and needs to be calibrated with a direct method such as NMR (de Velde & Kiekens, 2004). Given the number of citations, it is obvious that ^{1}H-NMR is the favourite among the scientific community. With up to 200 citations, the leading position of liquid state ^{1}H-NMR is reflected by the best accuracy in combination with very quick results (Fernandez-Megia et al., 2005). Both the Hirai method and the Vårum method yield comparable DA values (deviation no greater than 1 or 2% of the DA value); in addition, the Vårum method is more accurate as it incorporates the H1 and H2 signals. However, these authors prefer Hirai's method because the Vårum method requires measurements at high temperatures and extends the measuring time, whereas the Hirai method can be performed at room temperature. The main advantage of the NMR method is the generation of absolute values through the simultaneous detection of deacetylated and acetylated signals. The ratio of the one to the other reveals the DA value without any calibration or assumptions. In contrast, liquid state ^{1}H-NMR cannot be used for chitin because this technique is limited to soluble material and the equipment is extremely costly. Similar results can be obtained by ^{13}C-NMR, solid state ^{13}C-NMR and ^{15}N-NMR, but these techniques extend the measurement time to more than one hour per sample at the least (depending on the device and conditions) and are, despite the good accuracy, far from becoming standard methods. Exceptionally, solid state ^{13}C-NMR shows greater potential because it covers the range of 0% to 100% deacetylated samples. Nevertheless, NMR has become the most reliable technique for DA determination in recent years (Domard, 2007). It shows good repeatability and reproducibility of results, not only for interoperator comparisons but also for interlab comparisons on different NMR devices (at least, this is our experience). Beyond the scientific research community, the simple colloid titration method enjoys great popularity mainly due to its unbeatable costs. Small chitosan producing facilities typically do not have access to NMR equipment or simply want to avoid their expense. Although it is prone to impurities (alkali residues), it can be performed very quickly in even the simplest lab. Like the above-mentioned methods it does not give absolute values and needs to be calibrated or should first be performed with samples of known DA.

6. Conclusions

Chitin and chitosan are natural aminopolysaccharides with unique structures, multidimensional properties, highly sophisticated functions and wide ranging applications, especially in the biomedical and pharmaceutical fields. Moreover, the chemical modification of these polymers improves their solubility in water or organic solvents, which in turn enhances their biological activities and raises the number of potential biomedical applications. Chitin and chitosan have an intrinsic structural and physicochemical variability because of their natural origin and the manufacturing process. Furthermore, the poor physicochemical characterization of the chitin-/chitosan-based products used in biomedical experiments makes it very difficult to compare results and to establish relationships between the physiological behaviour of these compounds and their properties.

At present, the accurate study of the properties of chitin/chitosan requires the use of well-characterized, high-quality products. Knowledge of the microstructure of these compounds is essential for an understanding of structure–property–activity relationships. On the basis of literature data it has been possible to present in this chapter important information about the influence of the chemical structure and physicochemical properties of chitin-/chitosan-based materials on their biomedical activity. This knowledge is especially important for biotech companies. In each of these uses, it is necessary to control the different parameters which influence the characteristics of chitin/chitosan so as to produce it according to the characteristics desired by companies. The proper adjustment of the process conditions will make it possible to produce chitins very similar to their native form, and to prepare chitosans and their derivatives with well-controlled characteristics. An understanding of these molecular-level details provides insights into the unknown biochemical functions of chitin-/chitosan-based products and helps to accelerate their future applications.

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8. References

Aam, B.B.; Heggset, E.B.; Norberg, A.L.; Sørlie, M.; Vårum, K.M. & Eijsink, V.G.H. (2010). Production of Chitooligosaccharides and Their Potential Applications in Medicine. *Mar. Drugs*, 8, 1482-1517, ISSN 1660-3397

Agnihotri, S.A.; Mallikarjuna, N.N. & Aminabhavi T.M. (2004). Recent advances on chitosan-based micro- and nanoparticles in drug delivery. *J. Control. Release*, 100, 5–28, pISSN 0168-3659

Aiba, S. (1986). Studies on chitosan: 1. Determination of the degree of *N*-acetylation of chitosan by ultraviolet spectrophotometry and gel-permeation chromatography *Int. J. Biol. Macromol.*, 8, 173–176, pISSN 0141-8130

Aiba, S. (1992). Studies on chitosan: 4. Lysozymic hydrolysis of partially *N*-acetylated chitosans. *Int. J. Biol. Macromol.*, 14, 225-228, pISSN 0141-8130

Aiedeh, K. & Taha, M.O. (1999). Synthesis of chitosan succinate and chitosan phthalate and their evaluation as suggested matrices in orally administered, colon-specific drug delivery systems. *Arch. Pharm.*, 332, 103–107, pISSN 0365-6233

Al Sagheer, F.A. Al-Sughayer, M.A. Muslim, S. & Elsabee, M.Z. (2009). Extraction and characterization of chitin and chitosan from marine sources in Arabian Gulf. *Carbohydr. Polym.*, 77, 410–419, ISSN 0144-8617

Allan, C. & Hadwiger, L.A. (1979). The fungicidal effect of chitosan on fungi of varying cell wall composition. *Exp. Mycol.*, 3, 285–287, ISSN 0147-5975

Alonso, I.; Corvas, C.R. & Nieto, J. (1983). Determination of the degree of acetylation of chitin and chitosan by thermal analysis. *J. Therm. Anal. Calorim.*, 28, 189–195, ISSN 1388-6150

Alves, N.M. & Mano, J.F. (2008). Chitosan derivatives obtained by chemical modifications for biomedical and environmental applications. *Int. J. Biol. Macromol.*, 43, 401–414, ISSN 0141-8130

Andres, Y.; Giraud, L.; Gerente, C. & Le Cloirec, P. (2007). *Environ. Techno*l., 28, 1357-1363, pISSN 0959-3330

Anthonsen, M.; Vårum, K.; Hermansson, A.; Smidsrød, O. & Brant, D. (1994). Aggregates in acidic solutions of chitosans detected by static laser light scattering. *Carbohydr. Polym*., 25, 13–23, ISSN 0144-8617

Aranaz, I.; Mengíbar, M.; Harris, R.; Paños, I.; Miralles, B.; Acosta, N.; Galed, G. & Heras Á. (2009). Characterization of Chitin and Chitosan. *Curr. Chem. Biol*., 3, 203–230, pISSN 1872-3136

ASTM. F2103-01 (2001). Standard guide for characterization and testing of chitosan salts as starting materials intended for use in biomedical and tissue-engineered medical product applications

ASTM. F2260-03 (2003). Standard Test Method for Determining Degree of Deacetylation in Chitosan Salts by Proton Nuclear Magnetic Resonance (1H NMR) Spectroscopy

ASTM. WK965 (2003). New Test Method for Determining the Molar Mass of Chitosan and Chitosan Salts by Size Exclusion Chromatography with Multi-angle Light Scattering Detection (SEC-MALS)

Austin, P.R. (1988). Chitin solutions and purification of chitin. *Methods Enzymol*., 161, 403–407, pISSN 0076-6879

Babiker, E.E. (2002). Effect of chitosan conjugation on the functional properties and bactericidal activity of gluten peptides. *Food Chem.*, 79, 367-372, ISSN 0308-8146

Berth, G. & Dautzenberg, H. (2002). The degree of acetylation of chitosans and its effect on the chain conformation in aqueous solution *Carbohydr. Polym.*, 47, 39–51, ISSN 0144-8617

Berth, G.; Dautzenberg H. & Peter, M.G. (1998). Physico-chemical characterization of chitosans varying in degree of acetylation. *Carbohydr. Polym.,* 36, 205-216, ISSN 0144-8617

Bhattarai, N.; Edmondson, D.; Veiseh, O.; Matsen F.A. & Zhang, M. (2005). Electrospun chitosan-based nanofibers and their cellular compatibility. *Biomaterials,* 26, 6176–6184, pISSN 0142-9612

Boesel, L.F.; Reis, R.L. & Román, J.S. (2009). Innovative Approach for Producing Injectable, Biodegradable Materials Using Chitooligosaccharides and Green Chemistry. *Biomacromolecules*, 10, 465-470, pISSN 1525-7797

Bozkir, A. & Saka, O.M. (2004). Chitosan Nanoparticles for Plasmid DANN Delivery: Effect of Chitosan Molecular Structure on Formulation and Release Characteristics. *Drug deliv*., 11, 107-112, pISSN 1071-7544

Broussignac, P. (1968). Haut Polymère Naturel Connu dans I'Industrie: Le Chitosane. *Chim. Ind. Genie Chim*., 99, 1241–1247

Brugnerotto, J.; Desbrières, J.; Heux, L.; Mazeau, K. & Rinaudo, M. (2001). Overview on structural characterization of chitosan molecules in relation with their behavior in solution. *Macromol. Symp.*, 168, 1–20, ISSN 1022-1360

Centelles, M.N; Qian, C.; Campanero, M.A. & Irache, J.M. (2008). New methodologies to characterize the effectiveness of the gene transfer mediated by DNA-chitosan nanoparticles. *Int. J. Nanomedicine*, 3, 451–460, pISSN 1176-9114

Chae, S.Y.; Jang, M.-K. & Nah, J.-W. (2005). Influence of molecular weight on oral absorption of water soluble chitosans. *J. Control. Release*, 102, 383-394, pISSN 0168-3659

Charoenthai, N.; Kleinebudde, P. & Puttipipatkhachorn, S. (2007). Influence of Chitosan Type on the Properties of Extruded Pellets With Low Amount of Microcrystalline Cellulose. *AAPS Pharm. Sci. Tech*., 8, Article 64, ISSN 1530-9932

Chaussard, G. & Domard, A. (2004). New Aspects of the Extraction of Chitin from Squid Pens. *Biomacromolecules*, 5, 559-564, ISSN 1525-7797

Chen, M.C.; Yeh, G.H.C. & Chiang, B.H. (1996). Antimicrobial and physicochemical properties of methylcellulose and chitosan films containing a preservative. *J. Food Process Preserv.,* 20, 379-390, ISSN 01458892

Chen, R. & Tsaih, M. (1998). Effect of temperature on the intrinsic viscosity and conformation of chitosans in dilute HCl solution. *Int. J. Biol. Macromol.*, 23, 135–141, pISSN 0141-8130

Cheng, M.; Deng, J.; Yang, F.; Gong, Y.; Zhao N. & Zhang, X. (2003). Study on physical properties and nerve cell affinity of composite films from chitosan and gelatin solutions. *Biomaterials,* 24, 2871–2880, pISSN 0142-9612

Chien, P. & Chou, C. (2006). Antifungal activity of chitosan and its application to control post-harvest quality and fungal rotting of Tankan citrus fruit (Citrus tankan Hayata). *J. Sci. Food Agric*., 86, 1964-1969, pISSN 0022-5142

Chiou, S.H.; Wu, W.T.; Huang, Y.Y. & Chung, T,W. (2001). Effects of the characteristics of chitosan on controlling drug release of chitosan coated PLLA microspheres. *J. Microencapsul*., 18, 613-625, pISSN 0265-2048

Chirkov, S.N. (2002). The Antiviral Activity of Chitosan (Review). *Appl. Biochem. Microbiol*., 38, 1-8, pISSN 0003-6838

Cho, Y.-W.; Cho, Y.-N.; Chung, S.-H.; Yoo, G. & Ko, S.-W. (1999). Water soluble chitin as a wound healing accelerator. *Biomaterials*, 20, 2139-2145, pISSN 0142-9612

Christensen, B.; Vold, I. & Vårum, K. (2008). Chain stiffness and extension of chitosans and periodate oxidised chitosans studied by size-exclusion chromatography combined with light scattering and viscosity detectors. *Carbohydr. Polym.*, 74, 559–565, ISSN 0144-8617

Chung, T.W.; Yang, J.; Akaike, T.; Cho, K.Y.; Nah, J.W.; Kim, S.I. & Cho, C.S. (2002). Preparation of alginate/galactosylated chitosan scaffold for hepatocyte attachment. *Biomaterials,* 23, 2827–2834, pISSN 0142-9612

Chupa, J.M.; Foster, A.M.; Sumner, S.R.; Madihally, S.V. & Matthew, H.W. (2000). Vascular cell responses to polysaccharide materials: in vitro and in vivo evaluations. *Biomaterials,* 21, 2315–2322, pISSN 0142-9612

Cölfen, H.; Berth, G.& Dautzenberg, H. (2001). Hydrodynamic studies on chitosans in aqueous solution. *Carbohydr. Polym.*, 45, 373–383, ISSN 0144-8617

Curotto, E. & Aros, F. (1993). Quantitative determination of chitosan and the percentage of free amino groups. *Anal. Biochem*., 211, 40–241, pISSN 0003-2697

Das, S. & Ganesh, E.A. (2010). Extraction of Chitin from Trash Crabs (*Podophthalmus vigil*) by an Eccentric Method. *Curr Res. J. Biol. Sci.,* 2, 72-75, pISSN 2041-076X

de la Fuente, M.; Seijo, B. & Alonso, M.J. (2008). Design of novel polysaccharidic nanostructures for gene delivery. *Nanotechnology*, 19, 075105 (9pp) doi:10.1088/0957-4484/19/7/075105 pISSN 0957-4484, ISSN 1361-6528 (online)

De Velde, K.V. & Kiekens, P. (2004). Structure analysis and degree of substitution of chitin, chitosan and dibutyrylchitin by FT-IR spectroscopy and solid state ^{13}C-NMR. *Carbohydr. Polym.*, 58, 409–416, ISSN 0144-8617

Di Martino, A.; Sittinger, M. & Risbud, M.V. (2005). Chitosan: A versatile biopolymer for orthopaedic tissue-engineering, *Biomaterials,* 26, 5983–5990, pISSN 0142-9612

Ding, Z.; Chen, J.; Gao, S.; Chang, J.; Zhang, J. & Kang, E.T. (2004). Immobilization of chitosan onto poly-l-lactic acid film surface by plasma graft polymerization to

control the morphology of fibroblast and liver cells. *Biomaterials,* 25, 1059–1067, pISSN 0142-9612

Domard, A. (2007). Recent concepts regarding the physical chemistry of chitosan and their applications, plenary lecture held on the 8th International Conference of the European Chitin Society, Antalya, Turkey

Domard, A. (2010). A perspective on 30 years research on chitin and chitosan. *Carbohydr. Polym.*, doi:10.1016/j.carbpol.2010.04.083, ISSN 0144-8617

Domard, A.(1987). Determination of *N*-acetyl content in chitosan samples by c.d. measurements. *Int. J. Biol. Macromol.*, 9, 333–336, pISSN 0141-8130

Eaton, P.; Fernandes, J.C.; Pereira, E.; Pintado, M.E. & Malcata, F.X. (2008). Atomic force microscopy study of the antimicrobial effects of chitosans on *Escherichia coli* and *Staphylococcus aureus*. *Ultramicroscopy*, 108, 1128-1134, pISSN 0304-3991

Felt, O.; Baeyens, V.; Buri, P. & Gurny, R. (2001). Delivery of Antibiotics to the Eye Using a Positively Charged Polysaccharide as Vehicle. *AAPS Pharm. Sci*., 3, Article 34, DOI: 10.1208/ps030434, eISSN 1522-1059

Ferber, D. (2001). Gene therapy: safer and virus-free? *Science*, 294, 1638-1642, pISSN 0036-8075

Fernandes, J.C.; Eaton, P.; Gomes, A.M.; Pintado, M.E. & Malcata, F.X. (2009). Study of the antibacterial effects of chitosans on *Bacillus cereus* (and ist spores) by atomic force microscopy imaging and nanoindentation. *Ultramicroscopy*, 109, 854-860, pISSN 0304-3991

Fernandes, J.C.; Tavaria, F.K.; Fonseca, S.C.; Ramos, Ó.S.; Pintado, M.E. & Malcata, F.X. (2010). In Vitro Screening for Antimicrobial Activity of Chitosans and Chitooligosaccharides, Aiming at Potential Uses in Functional Textiles. *J. Microbiol. Biotechnol*., 20, 311–318, pISSN 1017-7825

Fernandez-Megia, E.; Novoa-Carballal, Quiñoá, R.E. & Riguera, R. (2005). Optimal routine conditions for the determination of the degree of acetylation of chitosan by 1H-NMR. *Carbohydr. Polym.*, 61, 55–161, ISSN 0144-8617

Freier, T.; Montenegro, R.; Koh, H..S. & Shoichet, M.S. (2005). Chitin-based tubes for tissue engineering in the nervous system. *Biomaterials*, 26, 4624-32, pISSN 0142-9612

Genta, I.; Perugini, P. & Pavanetto, F. (1998). Different molecular weight chitosan microspheres: influence on drug loading and drug release. *Drug Dev. Ind. Pharm*., 24, 779-784, pISSN 0363-9045

Gerasimenko, D.V.; Avdienko, I.D.; Bannikova, G.E.; Zueva, O.Yu. & Varlamov V.P. (2004). Antibacterial Effects of Water-Soluble Low-Molecular-Weight Chitosans on Different Microorganisms. *Appl. Biochem. Microbiol*., 40, 253-257, pISSN 0003-6838

Goy, R.C.; de Britto, D. & Assis, O.B.G. (2009). A Review of the Antimicrobial Activity of Chitosan. *Polímeros: Ciência e Tecnologia*, 19, 241-247, ISSN 0104-1428

Gupta, K.C. & Jabrail, F.H. (2006). Effects of degree of deacetylation and crosslinking on physical characteristics, swelling and release behavior of chitosan microspheres. *Carbohydr. Polym.*, 66, 43-54, ISSN 0144-8617

Gupta, K.C. & Jabrail, F.H. (2007). Glutaraldehyde cross-linked chitosan microspheres for controlled release of centchroman. *Carbohydr. Res*., 342, 2244-2252, pISSN 0008-6215

Harish Prashanth, K.V. & Tharanathan, R.N. (2005). Depolymerized products of chitosan as potent inhibitors of tumor-induced angiogenesis. *Biochim. Biophys. Acta*, 1722, 22-29, ISSN 0006-3002

Harish Prashanth, K.V. & Tharanathan, R.N. (2007). Chitin/chitosan:modifications and their unlimited application potential – an overview. *Trends Food Sci. Technol*., 18, 117-131, pISSN 0924-2244

Hasegawa, M.; Isogai, A. & Onabe, F. (1994). Molecular mass distribution of chitin and chitosan. *Carbohydr. Res.,* 262, 161–166, pISSN 0008-6215

He, P.; Davis, S.S. & Illum, L. (1998). In vitro evaluation of the mucoadhesive properties of chitosan microspheres. *Int. J. Pharm*., 166, 75-88, pISSN 0378-5173

Hejazi, R.& Amiji, M. (2003). Chitosan-based gastrointestinal delivery systems. *J. Control Release,* 89, 151–165, pISSN 0168-3659

Helander, I.M.; Nurmiaho-Lassila, E.L.; Ahvenainen, R.; Rhoades, J. & Roller, S. (2001). Chitosan disrupts the barrier properties of the outer membrane of gram-negative bacteria. *Int. J. Food Microbiol.,* 71, 235–244, pISSN 0168-1605

Heux, L.; Brugnerotto, J.; Desbrières, J.; Versali, M.F. & Rinaudo, M. (2000). Solid state NMR for determination of degree of acetylation of chitin and chitosan. *Biomacromolecules*, 1, 746–751, pISSN 1525-7797

Hirai, A.; Odani, H. & Nakajima, A. (1991). Determination of degree of deacetylation of chitosan by ^{1}H-NMR spectroscopy. *Polym. Bull*., 26, 87-94, pISSN 0170-0839

Hirano, S. (1999). Chitin and chitosan as novel biotechnological materials. *Polym. Int.*, 48, 732-734, pISSN 0959-8103

Hirano, S.; Tsuchida, H. & Nagao, N. (1989). *N*-acetylation in chitosan and the rate of its enzymic hydrolysis. *Biomaterials*, 10, 574-576, pISSN 0142-9612

Ho, M.H.; Wang, D.M.; Hsieh, H.J.; Liub, H.C.; Hsienc, T. Y. ; Laid, J.Y. & Hou, L.T. (2005) Preparation and characterization of RGD-immobilized chitosan scaffolds. *Biomaterials*, 26, 3197-3206, pISSN 0142-9612

Holan, Z.; Votruba, J. & Vlasalova, V. (1980) New method of chitin determination based on deacylation and gas-liquid chromatographic assay of liberated acetic acid. *J. Chromatogr*., 190, 67-76, pISSN 0021-9673

Horton, D. & Just, E.K. (1973). Preparation from chitin of (1→4)-2-amino-2-deoxy-β-D-glucopyranuronan and its 2-sulfoamino analog having blood-anticoagulant properties. *Carbohydr. Res.,* 28, 173–179, pISSN 0008-6215

Howling, G.I.; Dettmar, P.W.; Goddard, P.A.; Hampson, F.C.; Dornish, M. & Wood, E.J. (2001). The effect of chitin and chitosan on the proliferation of human skin fibroblasts and keratinocytes in vitro. *Biomaterials*, 22, 2959-2966, pISSN 0142-9612

Hsieh, C.Y.; Tsai, S.P.; Wang, D.M.; Chang Y.N. & Hsieh, H.J. (2005). Preparation of gamma-PGA/chitosan composite tissue engineering matrices. *Biomaterials,* 26, 5617–5623, pISSN 0142-9612

Hsu, S.; Whu, S.W.; Tsai, C.-L.; Wu, Y.-H.; Chen, H.-W. & Hsieh, K.-H. (2004a). Chitosan as Scaffold Materials: Effects of Molecular Weight and Degree of Deacetylation. *J. Polym. Res.*, 11, 141-147, ISSN 1022-9760

Hsu, S.H.; Whu, S.W.; Hsieh, S.C.; Tsai, C.L.; Chen D.C. & Tan, T.S. (2004b). Evaluation of chitosan–alginate–hyaluronate complexes modified by an RGD-containing protein as tissue-engineering scaffolds for cartilage regeneration. *Artif. Organs,* 28, 693–703, pISSN 0160-564X

Huang, X.J.; Ge, D. & Zu, C.K. (2007). Preparation and characterization of stable chitosan nanofibrous membrane for lipase immobilization. *Eur. Polym*. J., 43, 3710-3718, ISSN 0014-3057

Itakura, M.; Shimada, K.; Matsuyama, S.; Saito, T. & Kinugasa, S. (2005). A convenient method to determine the rayleigh ratio with uniform polystyrene oligomers. *J. Appl. Polym. Sci.,* 99, 1953–1959, ISSN 1097-4628

Itoh, S.; Yamaguchi, I.; Suzuki, M.; Ichinose, S.; Takakuda, K.; Kobayashi, H. Shinomiya, K. & Tanaka, J. (2003). Hydroxyapatite-coated tendon chitosan tubes with adsorbed laminin peptides facilitate nerve regeneration in vivo. *Brain Res.,* 993, 111–123, pISSN 0006-8993

Jaffer, S. & Sampalis, J.S. (2007). Efficacy and safety of chitosan HEP-40™ in the management of hypercholesterolemia: a randomized, multicenter, placebo-controlled trial. *Altern. Med. Rev*., 12, 265-273, ISSN 1089-5159

Jain, A. & Jain, S.K. (2008). In vitro and cell uptake studies for targeting of ligand anchored nanoparticles for colon tumors. *Eur. J. Pharm. Sci.* , 35, 404–416, pISSN 0928-0987

Jayakumar, R.; Chennazhi, K.P.; Muzzarelli, R.A.A.; Tamura, H.; Nair, S.V. & Selvamurugan, N. (2010). Chitosan conjugated DNA nanoparticles in gene therapy. *Carbohydr. Polym.*, 79, 1–8, ISSN 0144-8617

Jayakumar, R.; Reis, R.L. & Mano, J.F. (2006). Synthesis of *N*-carboxymethyl chitosan beads for controlled drug delivery applications. *Mater. Sci. Forum.,* 514–516, 1015–1019, ISSN 0255-5476

Je, J.Y. & Kim, S.K. (2006). Reactive oxygen species scavenging activity of aminoderivatized chitosan with different degree of deacetylation. *Bioorg. Med. Chem*., 14, 5989-5994, pISSN 0968-0896

Je, J.Y.; Park, P.J. & Kim, S.K. (2004). Free radical scavenging properties of hetero-chitooligosaccharides using an ESR spectroscopy. *Food Chem. Toxicol*., 42, 381-387, pISSN 0278-6915

Jeon, Y.-J. & Kim, S.-K. (2002). Antitumor Activity of Chitosan Oligosaccharides Produced In Ultrafiltration Membrane Reactor System. *J. Microbiol. Biotechnol*., 12, 503-507, pISSN 1017-7825

Jeong, Y.-I.; Kim, D.-G.; Jang, M.-K. & Nah, J.-W. (2008). Preparation and spectroscopic characterization of methoxy poly(ethylene glycol)-grafted water-soluble Chitosan. *Carbohydr. Res*., 343, 282–289, ISSN 0008-6215

Jia, Z.; Shen, D. & Xu, W. (2001). Synthesis and antibacterial activities of quaternary ammonium salt of chitosan. *Carbohydr. Res*., 333, 1–6, ISSN 0008-6215

Jung, B.O.; Kim, C.H.; Choi, K.S.; Lee, Y.M. & Kim, J.J. (1999). Preparation of Amphiphilic Chitosan and Their Antimicrobial Activities. *J. Appl. Polym. Sci.,* 72, 1713–1719, ISSN 1097-4628

Just, U.; Weidner, S.; Kilz, P. & Hofe, T. (2005). Polymer reference materials: Round-robin tests for the determination of molar masses. *Int. J. Polym. Anal. Charact.*, 10, 225–243, pISSN 1023-666X

Kasaai, M.; Arul, J. & Charlet, G. (2000). Intrinsic viscosity-molecular weight relationship for chitosan. *J. Polym. Sci. B Polym. Phys.,* 38, 2591–2598, ISSN 0887-6266

Kato, Y.; Onishi, H. & Machida, Y. (2001). Biological characteristics of lactosaminated N-succinyl-chitosan as a liver-specific drug carrier in mice. *J. Control Release,* 70, 295–307, pISSN 0168-3659

Kato, Y.; Onishi, H. & Machida, Y. (2004). *N*-succinyl-chitosan as a drug carrier: waterinsoluble and water-soluble conjugates. *Biomaterials,* 25, 907–915, pISSN 0142-9612

Kawakami, T.; Antoh, M.; Hasegawa, H.; Yamagishi, T.; Ito M. & Eda, S. (1992). Experimental study on osteoconductive properties of a chitosan-bonded hydroxyapatite self-hardening paste. *Biomaterials,* 13, 759–763, pISSN 0142-9612

Kaye, W. & McDaniel, J. (1974). Low-angle laser light scattering: Rayleigh factors and depolarization ratios. *Appl. Opt.*, 13, 1934–1937, ISSN 0003-6935

Kean, T. & Thanou, M. (2010). Biodegradation, biodistribution and toxicity of chitosan. *Adv Drug Deliv Rev.*, 62, 3–11, ISSN 0169-409X

Khan, T.A. & Peh, K.K. (2003). Influence of chitosan molecular weight on its physical properties. *The International Medical Journal*, 2, 1, ISSN 1823-4631

Kiang, T.; Wen, J.; Lim, H.W.; Leong, K.W. & Kam, K.W. (2004). The effect of the degree of chitosan deacetylation on the efficiency of gene transfection. *Biomaterials*, 25, 5293-301, pISSN 0142-9612

Kim, I.Y.; Seo, S.J.; Moon, H.S.; Yoo, M.K.; Park, I.Y.; Kim, B.C. & Cho, C.S. (2008). Chitosan and its derivatives for tissue engineering applications. *Biotechnol. Adv.,* 26, 1–21, pISSN 0734-9750

Kim, T.H.; Nah, J.W.; Cho, M.H.; Park, T.G. & Cho, C.S. (2006). Receptor-mediated gene delivery into antigen presenting cells using mannosylated chitosan/DNA nanoparticles. *J. Nanosci. Nanotechnol.,* 6, 2796–2803, pISSN 1533-4880

Kittur, F.S.; Vishu Kumar, A.B.; Varadaraj, M.C. & Tharanathan, R.N. (2005). Chitoologosaccharides-preparation with the aid of pectinase isozyme from *Aspergillus niger* and their antibacterial activity. *Carbohydr. Res.*, 340, 1239-1245, pISSN 0008-6215

Knill, C.; Kennedy, J.; Mistry, J.; Miraftab, M.; Smart, G.; Groocock, M. & Williams, H. (2005). Acid hydrolysis of commercial chitosans. *J. Chem. Technol. Biotechnol.,* 80, 1291–1296, ISSN 0268-2575

Kofuji, K.; Qian, C.J.; Nishimura, M.; Sugiyama, I.; Murata, Y. & Kawashima, S. (2005). Relationship between physicochemical characteristics and functional properties of chitosan. *Eur. Polym. J.*, 41, 2784-91, ISSN 0014-3057

Köping-Höggård, M.; Tubulekas, I.; Guan, H.; Edwards, K.; Nilsson, M.; Vårum, K.M.; & Artursson, P. (2001). Chitosan as a nonviral gene delivery system. Structure-property relationships and characteristics compared with polyethylenimine in vitro and after lung administration in vivo. *Gene Ther.*, 8, 1108-1121, pISSN 0969-7128

Koryagin, A.S.; Erofeeva, E.A.; Yakimovich, N.O.; Aleksandrova, E.A.; Smirnova, L.A. & Malkov, A.V. (2006). Analysis of antioxidant properties of chitosan and its oligomers. *Bull. Exp. Biol. Med*., 142, 461-463, ISSN0007-4888

Krajewska, B. (2004). Application of chitin- and chitosan-based materials for enzyme immobilizations: a review, *Enzyme Microb. Technol.,* 35, 126–139, pISSN 0141-0229

Kratz, G.; Arnander, C.; Swedenborg, J.; Back, M.; Falk, C.; Gouda I. & Larm, O. (1997). Heparin–chitosan complexes stimulate wound healing in human skin. *Scand. J. Plast. Reconstr. Surg. Hand Surg.,* 31, 119–123, pISSN 0284-4311

Kubota, N.; Tatsumoto, N.; Sano, T. & Toya, K. (2000). A simple preparation of half *N*-acetylated chitosan highly soluble in water and aqueous organic solvents. *Carbohydr. Res.*, 324, 268–274, pISSN 0008-6215

Kurita, K. (2001). Controlled functionalization of the polysaccharide chitin. *Progr. Polym. Sci.*, 26, 1921–1971, pISSN 0079-6700

Kurita, K.; Kaji, Y.; Mori, T. & Nishiyama, Y. (2000). Enzymatic degradation of [beta]-chitin: susceptibility and the influence of deacetylation. *Carbohydr. Polym.*, 42, 19-21, ISSN 0144-8617

Lal, G. & Hayes, E. (1984). Determination of the amine content of chitosan by pyrolysis-gas chromatography. *J. Anal. Appl. Pyrolysis*, 6, 183–193, ISSN 0165-2370

Lamarque, G.; Cretenet, M.; Viton, C. & Domard, A. (2005). New route of deacetylation of *α*- and *β*-chitins by means of freeze-pump out-thaw cycles. *Biomacromolecules*, 6, 1380–1388, pISSN 1525-7797

Lamarque, G.; Viton, C. & Domard, A. (2004). Comparative study of the second and third heterogeneous deacetylations of *α*-and *β*-chitins in a multi step process. *Biomacromolecules*, 5, 1899–1907, pISSN 1525-7797

Lavertu, M.; Methot, S.; Tran-Khanh, N. & Buschmann, M.D. (2006). High efficiency gene transfer using chitosan/DNA nanoparticles with specific combinations of molecular weight and degree of deacetylation. *Biomaterials*, 27, 4815-4824, pISSN 0142-9612

Lee, H.G.; Park, Y.-S.; Jung, J.-S. & Shin, W.-S. (2002). Chitosan oligosaccharide, dp 2-8, have prebiotic effect on the *Bifidobacterium bifidium* and *Lactobacillus* sp. *Anaerobe*, 8, 319-324, pISSN 1075-9964

Lee, J.E.; Kim, K.E.; Kwon, I.C.; Ahn, H.J.; Lee, S.H.; Cho, H.; Kim, H.J.; Seong, S.C. & Lee, M.C. (2004a). Effects of the controlled-released TGF-beta 1 from chitosan microspheres on chondrocytes cultured in a collagen/chitosan/glycosaminoglycan scaffold. *Biomaterials,* 25, 4163–4173, pISSN 0142-9612

Lee, J.S.; Cha, D.S. & Park, H.J. (2004b). Survival of Freeze-Dried Lactobacillus bulgaricus KFRI 673 in Chitosan-Coated Calcium Alginate Microparticles. *J. Agric. Food Chem*., 52, 7300-7305, pISSN 0021-8561

Li, X.; Tsushima, Y.; Morimoto, M.; Saimoto, H.; Okamoto, Y.; Minami, S. & Shigemasa, Y. (2000). Biological activity of chitosan–sugar hybrids: specific interaction with lectin. *Polym. Adv. Techno*l., 11, 176–179, pISSN1042-7147

Li, Y.; Liu L. & Fang, F. (2003). Plasma-induced grafting of hydroxyethyl methacrylate (HEMA) onto chitosan membranes by a swelling method. *Polym. Int.,* 52, 285–290, pISSN 0959-8103

Lin, C.W. & Lin, J.C. (2003). Characterization and blood coagulation evaluation of the water-soluble chitooligosaccharides prepared by a facile fractionation method. *Biomacromolecules*, 4, 1691–1697, pISSN 1525-7797

Lin, S.-B.; Lin, Y.-C. & Chen, H.-H. (2009). Low molecular weight chitosan prepared with the aid of cellulase, lysozyme and chitinase: Characterisation and antibacterial activity. *Food Chem.,* 116, 47-53, ISSN 0308-8146

Liu, H.; Du, Y.; Yang, J. & Zhu, H. (2004). Structural characterization and antimicrobial activity of chitosan/betain derivative complex. *Carbohydr. Polym.*, 55, 291–297, ISSN 0144-8617

Liu, W.G.; Yao, K.& D. Liu, Q.G. (2001a). Formation of a DNA/*N*-deadecylated chitosan complex and salt-induced gene delivery. *J. Appl. Polym. Sci*., 82, 3391–3395, ISSN 1097-4628

Liu, X.F.; Guan, Y.L.; Yang, D.Z.; Li Z. & Yao, K.D. (2001b). Antimicrobial action of chitosan and carboxymethylated chitosan. *J. Appl. Polym. Sci.,* 79, 1324–1335, ISSN 1097-4628

Lorenzo-Lamosa, M.L.; Remunan-Lopez, C.; Vila-Jato, J.L. & Alonso, M.J. (1998). Design of microencapsulated chitosan microspheres for colonic drug delivery. *J. Control. Release,* 52, 109–118, pISSN 0168-3659

Lueben, H.L.; Leeuw, B.J.D.; Langemeyer, B.W.; Boer, A.G.D.; Verhoef, J.C. & Junginger, H.E. (1996). Mucoadhesive polymers in peroral peptide drug delivery. VI. Carbomer and chitosan improve the intestinal absorption of the peptide drug buserelin in vivo. *Pharm. Res.,* 13, 1668–1672, ISSN 0724-8741

Ma, L.; Gao, C.; Mao, Z.; Zhou, J.; Shen J.; Hu, X. & Han, C. (2003). Collagen/chitosan porous scaffolds with improved biostability for skin tissue engineering. *Biomaterials,* 24, 4833–4841, pISSN 0142-9612

Madihally, S.V. &, Howard, W.T. (1999). Porous chitosan scaffolds for tissue engineering. *Biomaterials*, 20, 1133-1142, pISSN 0142-9612.

Maeda, Y. & Kimura, Y. (2004) Antitumor Effects of Various Low-Molecular-Weight Chitosans Are Due to Increased Natural Killer Activity of Intestinal Intraepithelial Lymphocytes in Sarcoma 180–Bearing Mice. *J. Nutr*., 134, 945-50, pISSN 0022-3166

Manni, L.; Ghorbel-Bellaaj, O.; Jellouli, K.; Younes, I. & Nasri, M. (2010). Extraction and Characterization of Chitin, Chitosan, and Protein Hydrolysates Prepared from Shrimp Waste by Treatment with Crude Protease from Bacillus cereus SV1. *Appl. Biochem. Biotechnol*., 162, 345–357, pISSN 0273-2289

Mao, H.Q.; Roy, K.; Troung-Le, V.L.; Janes, K.A.; Lin, K.Y.; Wang, Y.; August T. & Leong, K.W. (2001). Chitosan-DNA nanoparticles as gene carriers: synthesis, characterization and transfection efficiency. *J. Control. Release,* 70, 399–421, pISSN 0168-3659

Mao, J.S.; Cui, Y.L.; Wang, X.H.; Sun, Y.; Yin, Y.J.; Zhao, H.M. & Yao, K.D. (2004). A preliminary study on chitosan and gelatin polyelectrolyte complex cytocompatibility by cell cycle and apoptosis analysis, *Biomaterials,* 25, 3973-3981, pISSN 0142-9612

Martinou, A.; Bouriotis, V.; Stokke, B.T. & Vårum, K.M. (1998). Mode of action of chitin deacetylase from *Mucor rouxii* on partially *N*-acetylated chitosans. *Carbohydr. Res.*, 311, 71-78, pISSN 0008-6215

Min, B.M.; Lee, S.W.; Lim, J.N.; You, Y.; Lee, T.S.; Kang, P.H. & Park, W.H. (2004). Chitin and chitosan nanofibers: electrospinning of chitin and deacetylation of chitin nanofibers. *Polymer*, 45, 7137–7142, pISSN 0032-3861

Minagawa, T.; Okamura, Y.; Shigemasa, Y.; Minami, S. & Okamoto, Y. (2007). Effects of molecular weight and deacetylation degree of chitin/chitosan on wound healing. *Carbohydr. Polym*., 67, 640-644, ISSN 0144-8617

Miwa, A.; Ishibe, A.; Nakano, M.; Yamahira, T.; Itai, S.; Jinno, S. & Kawahara, H. (1998). Development of novel chitosan derivatives as micellar carriers of taxol. *Pharm Res.*, 15, 1844–1850, ISSN 0724-8741

Miya, M.; Iwamato, R. & Yoshikawa, S. (1980). I.r. spectroscopic determination of CONH content in highly deacylated chitosan. *Int. J. Biol. Macromol.*, 2, 323–324, pISSN 0141-8130

Mumper, R.; Wang, J.; Claspell, J. & Rolland, A.P. (1995). Novel polymeric condensing carriers for gene delivery. *Proc. Int. Symp. Controll. Release Bioact. Mater.,* 22, 178-179

Murugan, R. & Ramakrishna, S. (2004) Bioresorbable composite bone paste using polysaccharide based nanohydroxyapatite. *Biomaterials*, 25, 17, 3829-3835, pISSN 0142-9612

Muzzarelli, R. (1985). Removal of uranium from solutions and brines by a derivative of chitosan and ascorbic acid. *Carbohydr. Polym.*, 5, 85–89, ISSN 0144-8617

Muzzarelli, R.A.A. & Muzzarelli, C. (2005). Chitosan chemistry: Relevance to the biomedical sciences, *Adv. Polym. Sci.*, 186, 151–209, ISSN 0065-3195

Muzzarelli, R.A.A. (1997). Human enzymatic activities related to the therapeutic administration of chitin derivatives. *Cell Mol. Life Sci.,* 53, 131–140, pISSN 1420-682X

Muzzarelli, R.A.A., (Ed) (1973). *Natural Chelating Polymers*, Pergamon Press, New York, NY, USA,. pp. 83

Muzzarelli, R.A.A.; Jeuniaux, C. & Gooday, G.W. (1986). Chitin in nature and technology, Plenum Publishing Corporation, New York

Nah, J.W. & Jang, M.K. (2002). Spectroscopic characterization and preparation of low molecular, water-soluble chitosan with free-amine group by novel method. *J. Polym. Sci. A Polym. Chem.,* 40, 3796–3803, pISSN 0887-624X

Nair, R.; Reddy, B.H; Kumar, C.K.A. & Kumar, K.J. (2009) Application of Chitosan microspheres as drug carriers : A Review. *J. Pharm. Sci. & Res*., 1, 1-12, ISSN 0975-1459

Nanjo, F.; Katsumi, R. & Sakai, K. (1991). Enzymatic method for determination of the degree of deacetylation of chiosan. *Anal. Biochem*., 193, 164–167, pISSN 0003-2697

Neugebauer, W.; Neugebauer, E. & Brezinski, R. (1989). Determination of the degree of *N*-acetylation of chitin-chitosan with picric acid. *Carbohydr. Res.,* 189, 363–367, pISSN 0008-6215

Niola, F.; Basora, N.; Chornet, E. & Vidal, P. (1993). A rapid method for the determination of the degree of *N*-acetylation of chitin-chitosan samples by acid hydrolysis and HPLC. *Carbohydr. Res*., 23, 1–9, pISSN 0008-6215

Nishimura, S.; Kai, H.; Shinada, K.; Yoshida, T.; Tokura, S. & Kurita, K. (1998). Regioselective syntheses of sulfated polysaccharides: specifc anti-HIV-1 activity of novel chitin sulfates. *Carbohydr. Res.,* 306, 427–433, pISSN 0008-6215

Nwe, N.; Furuike, T. & Tamura, H. (2009) The Mechanical and Biological Properties of Chitosan Scaffolds for Tissue Regeneration Templates Are Significantly Enhanced by Chitosan from Gongronella butleri. *Materials*, 2, 374-398, ISSN 1996-1944

Oh, H.; Kim, Y.; Chang, E. & Kim. J. (2001). Antimicrobial Characteristics of Chitosans against Food Spoilage Microorganisms in Liquid Media and Mayonnaise. *Biosci. Biotechnol. Biochem*., 65, 2378-83, pISSN 0916-8451

Osman, M.; Fayed, S.A.; Ghada, I.M. & Romeilah, R.M. (2010). Protective Effects of Chitosan, Ascorbic Acid and *Gymnema Sylvestre* Against Hypercholesterolemia in Male Rats. *Aust. J. Basic Appl. Sci*., 4, 89-98, ISSN 1991-8178

Ottøy, M.; Vårum, K. & Smidsrød, O. (1995). Compositional heterogeneity of heterogeneously deacetylated chitosans. *Carbohydr. Polym*., 29, 17–24, ISSN 0144-8617

Ottøy, M.; Vårum, K.; Christensen, B.; Anthonsen, M. & Smidsrød, O. (1996). Preparative and analytical size-exclusion chromatography of chitosans. *Carbohydr. Polym*., 31, 253–261, ISSN 0144-8617

Pa, J.H. & Yu, T. (2001). Light scattering study of chitosan in acetic acid aqueous solutions. *Macromol. Chem. Phys*., 202, 985–991, ISSN1022-1352

Pangburn, S.H.; Trescony, P.V. & Heller, J. (1982). Lysozyme degradation of partially deacetylated chitin, its films and hydrogels. *Biomaterials*, 3, 105-108, pISSN 0142-9612

Paolicelli, P.; de la Fuente, M.; Sanchez, A.; Seijo, B. & Alonso, M.J. (2009). Chitosan Nanoparticles for Drug Delivery to the Eye. *Expert Opin. Drug Deliv*., 6, 239–253, pISSN 1742-5247

Papineau, A.M.; Hoover, D.G.; Knorr, D. & Farkas, D.F. (1991). Antimicrobial effect of water-soluble chitosans with high hydrostatic pressure. *Food Biotechnol*., 5, 45-47, pISSN 0890-5436

Park, I.K.; Yang, J.; Jeong, H.J.; Bom, H.S.; Harada, I.; Akaike, T.; Kima, S.I. & Cho, C.H. (2003). Galactosylated chitosan as a synthetic extracellular matrix for hepatocytes attachment. *Biomaterials,* 24, 2331–2337, pISSN 0142-9612

Park, J.-K.; Chae, S.J.; Choi, C. & Nah, J.-W. (2006). Modulation of molecular weight, charge ratio, and pH effect properties of high purity chitosan oligosaccharide for Efficient Gene Carrier. *Appl. Chem.*, 10, 53-56

Park, P.J.; Je, J.Y.; Byun, H.G.; Moon, S.H. & Kim, S.K. (2004a). Antimicrobial Activity of Hetero-Chitosans and Their Oligosaccharides with Different Molecular Weights. *J. Microbiol Biotechnol*., 14, 317-23, pISSN 1017-7825

Park, P.J.; Je, J.Y. & Kim, S.K. (2004b). Free radical scavenging activities of differently deacetylated chitosans using an ESR spectrometer. *Carbohydr. Polym*., 55, 17-22, pISSN 0144-8617

Patel, S.S. (2006). Pharmaceutical Significance of Chitosan: A Review, *Pharm. Rev.*, 4, 6, ISSN 1918-5561

Percot, A.; Viton, C. & Domard, A. (2003). Optimization of chitin extraction from shrimp shells. *Biomacromolecules*, 4, 12–18, ISSN 1525-7797

Perioli, L.; Ambrogi, V.; Pagano, C.; Scuota, S. & Rossi, C. (2009). Chitosan as a New Polymer for Metronidazole Mucoadhesive Tablets for Vaginal Administration. *Int. J. Pharm.*, 377, 120–127, pISSN 0378-5173.

Pillai, C.K.S.; Paul, W. & Sharma, C.P. (2009). Chitin and chitosan polymers: Chemistry, solubility and fiber formation. *Prog. Polym. Scie*., 34, 641–678, ISSN 0079-6700

Prabaharan, M.; Rodriguez-Perez, M.A.; de Saja, J.A. & Mano, J.F. (2006). Preparation and Characterization of Poly(L-lactic acid)-Chitosan Hybrid Scaffolds With Drug Release Capability. *J. Biomed. Mater. Res.* Part B. *Appl. Biomaterials*, 81, 427-434, pISSN 0142-9612

Prochazkova, S.; Vårum, K. & Østgaard, K. (1999). Quantitative determination of chitosans by ninhydrin. *Carbohydr. Polym.*, 8, 115–122, ISSN 0144-8617

Qin, C.Q.; Du, Y.M.; Xiao, L.; Gao, X.H.; Zhou, J.L. & Liu, H.L. (2002b). Effect of Molecular Weight and Structure on Antitumor Activity of Oxidized Chitosan. *Wuhan Univ. J. Nat. Sci.*, 7, 231-236, ISSN 1007-1202

Qin, C.Q.; Du, Y.M.; Xiao, L.; Li, Z. & Gao, X.H. (2002a). Enzymic preparation of water-soluble chitosan and their antitumor activity. *Int. J. Biol. Macromol*., 31, 111-117, ISSN 0141-8130

Qin, C.Q.; Zhou, B.; Zeng, L.; Zhang, Z.; Liu, Y.; Du, Y.M. & Xiao, L. (2004). The physicochemical properties and antitumor activity of cellulose-treated chitosan. *Food Chem.*, 84, 107-115, ISSN 0308-8146

Rabea, E.I.; Badawy, M.E.-T.; Stevens, C.V.; Smagghe, G. & Steurbaut, W. (2003). Chitosan as Antimicrobial Agent: Applications and Mode of Action. *Biomacromolecules*, 4, 1457-1465, ISSN 1525-7797

Rabea, E.I.; El Badawy, M.T.; Rogge, T.M.; Stevens, C.V.; Höfte, M.; Steurbaut, W. & Smagghe, G. (2005). Insecticidal and fungicidal activity of new synthesized chitosan derivatives. *Pest Manag. Sci.*, 61, 951–960, pISSN 1526-498X

Ravi Kumar, M.N.V. (2000). A review of chitin and chitosan applications. *React. Funct. Polym.*, 46, 1–27, ISSN 1381-5148

Ravi Kumar, M.N.V.; Muzzarelli, R.A.A.; Muzzarelli, C.; Sashiwa, H A. & Domb, J. (2004). Chitosan Chemistry and Pharmaceutical Perspectives, *Chem. Rev.*, 104, 6017-6084, pISSN 0009-2665

Raymond, L.; Morin, F. & Marchessault, R. (1993). Degree of deacetylation of chitosan using conductometric titration and solid-state NMR. *Carbohydr. Res*., 246, 331–336, pISSN 0008-6215

Richardson, S.C.W.; Kolbe, H.V.J. & Duncan, R. (1999). Potential of low molecular mass chitosan as a DNA delivery system: biocompatibility, body distribution and ability to complex and protect DNA. *Int. J. Pharm*., 178, 231-243, ISSN 0378-5173

Rinaudo, M. (2006). Chitin and chitosan: Properties and application. *Prog. Polym. Sci*., 31, 603–632, ISSN 0079-6700

Ringsdorf, H. (1975). Structure and properties of pharmacologically active polymers. *J. Polym. Sci. Polym. Symp.*, 51, 135–153

Roberts, G.A.F. (1998). *Chitin Chemistry*, 2nd ed. MacMillan, London

Roberts, G.A.F. (1992). *Chitin Chemistry*, 1st ed. MacMillan, London

Roberts, G.A.F. (2007). The Road is long.... *Adv. Chitin Sci*., 10, 3–10, ISBN 978-975-491-250-0

Roller, S. & Covill, N. (1999). The antifungal properties of chitosan in laboratory media and apple juice. *Int. J. Food Microbiol*., 47, 67-77, pISSN 0168-1605

Saito, H.; Tabeta, R. & Ogawa, K. (1987). High-Resolution solid state ^{13}C-NMR-study of chitosan and its salts with acids. *Macromolecules*, 20, 2424–2430, pISSN 0024-9297

Sajomsang, W.; Ruktanonchai, U.; Gonil, P. Mayen, V. & Opanasopit, P. (2009a). Methylated *N*-aryl chitosan derivative/DNA complex nanoparticles for gene delivery: Synthesis and structure–activity relationships. *Carbohydr. Polym.,* 78, 743–752, pISSN 0144-8617

Sajomsang, W; Tantayanon, S; Tangpasuthadol, V. & Daly, W.H. (2009b). Quaternization of N-aryl chitosan derivatives: synthesis, characterization, and antibacterial activity. *Carbohydr. Res*., 344, 2502-2511, pISSN 0008-6215

Sandford, P. (1989). *Chitosan: Commercial uses and potential applications*. In: Skjak-Braek E.; Anthonsen, T.; Standorf, P., Ed. *Chitin and chitosan: Sources chemistry, Biochemistry, Physical properties and Applications*. London, Elsevier Applied Science, pp. 51-69

Santos, N. & Castanho, M. (1996). Teaching light scattering spectroscopy: The dimensions and shape of tobaccomosaicvirus. *Biophys. J*., 71, 1641–1650, pISSN 0006-3495

Sarasam, A. & Madihally, S.V. (2005). Characterization of chitosan–polycaprolactone blends for tissue engineering applications. *Biomaterials,* 26, 5500–5508, pISSN 0142-9612

Sashiwa, H.; Saimoto, H.; Shigemasa, Y.; Ogawa, R. & Tokura, S. (1990). Lysozyme susceptibility of partially deacetylated chitin. *Int. J. Biol. Macromol.,* 12, 295–296, pISSN 0141-8130

Schatz, C.; Viton, C.; Delair, T.; Pichot, C. & Domard, A. (2003). Typical Physicochemical Behaviors of Chitosan in Aqueous Solution. *Biomacromolecules*, 4, 641–648, pISSN 1525-7797

Schipper, N.G.M.; Vårum, K. & Artursson, P. (1996). Chitosans as absorption enhancers for poorly absorbable drugs. 1: influence of molecular weight and degree of acetylation on drug transport across human intestinal epithelial (Caco-2) cells. *Pharm Res*., 13, 1686-1692, ISSN 0724-8741

Shelma, R; Paul, W. & Sharma, C.P. (2008). Chitin Nanofibre Reinforced Thin Chitosan Films for Wound Healing Application. *Trends Biomater. Artif. Organs*, 22, 111–115, pISSN 0391-3988

Shigemasa, Y.; Saito, K.; Sashiwa, H. &, Saimoto, H. (1994). Enzymatic degradation of chitins and partially deacetylated chitins. *Int. J. Biol. Macromol*., 16, 43-9, pISSN 0141-8130

Singh, D.K. & Ray, A.R. (2000) Biomedical Applications of Chitin, Chitosan, and Their Derivatives. *J.M.S.-Rev. Macromol. Chem. Phys*., 40, 69-83, pISSN 1558

Son, Y.J.; Jang, J.S.; Cho, Y.W.; Chung, H.; Park, R.W.; Kwon, I.C.; Kim, I.S.; Park, J.Y.; Seo, S.B.; Park, C.R. & Jeong, S.Y. (2003). Biodistribution and anti-tumor efficacy of

doxorubicin loaded glycol-chitosan nanoaggregates by EPR effect. *J. Control Release,* 91, 135-145, pISSN 0168-3659

Sorlier, P. (2002). Ph.D. thesis, Lyon, France

Sorlier, P.; Denuziere, A.; Viton, C. & Domard, A. (2001). Relation between the Degree of Acetylation and the Electrostatic Properties of Chitin and Chitosan *Biomacromolecules*, 2, 765-772, pISSN 1525-7797

Sorlier, P.; Viton, C. & Domard, A. (2002). Relation between Solution Properties and Degree of Acetylation of Chitosan: Role of Aging. *Biomacromolecules*, 3, 1336-1342, pISSN 1525-7797

Sosa, M.; Fazely, F.; Koch, J.; Vercellotti, S. & Ruprecht, R. (1991). *N*-Carboxymethylchitosan-*N,O*-sulfate as an anti-HIV-1 agent. *Biochem. Biophys. Res. Commun.,* 174, 489–496, pISSN 0006-291X

Strand, S.P.; Lelu, S.; Reitan, N.K.; de Lange Davies, C.; Artursson, P. & Vårum, K.M. (2010). Molecular design of chitosan gene delivery systems with an optimized balance between polyplex stability and polyplex unpacking. *Biomaterials*, 31, 975-987, pISSN 0142-9612

Struszczyk, M.H. & Struszczyk, K.J. (2007). *Medical Application of Chitin and Its Derivatives*; Polish Chitin Society, Monograph XII, 139–147

Sun, Y.; Cui, F.; Shi, K.; Wang, J.; Niu, M. & Ma, R. (2009). The Effect of Chitosan Molecular Weight on the Characteristics of Spray-Dried Methotrexate-Loaded Chitosan Microspheres for Nasal Administration. *Drug Dev. Ind. Pharm*., 35, 379-386, ISSN 0363-9045

Synowiecki, J. & Al-Khateeb, N.A. (2003). Production, Properties, and Some New Applications of Chitin and Its Derivatives. *Crit. Rev. Food Sci. Nutr.*, 43, 145–171, pISSN 1040-8398

Tan, M.L.; Choon, P.F.M. & Dass, C.R. (2009). Cancer, Chitosan Nanoparticles and Catalytic Nucleic Acids. *J. Pharm. Pharmacol*., 61, 3–12, pISSN 0022-3573

Tangsadthakun, C.; Kanokpanont, S.; Sanchavanakit, N.; Pichyangkura, R.; Banaprasert, T.; Tabata, Y. & Damrongsakkul, S. (2007). The influence of molecular weight of chitosan on the physical and biological properties of collagen/chitosan scaffolds. *J. Biomater. Sci. Polym. Ed*., 18, 147-163, pISSN 0920-5063

Tapola, N.S.; Lyyra, M.L.; Kolehmainen, R.M.; Sarkkinen, E.S. & Schauss, A.G. (2008). Safety Aspects and Cholesterol-Lowering Efficacy of Chitosan Tablets. *J. Am. Coll. Nutr.*, 27, 22–30, pISSN 0731-5724

Techaarpornkul, S.; Wongkupasert, S.; Opanasopit, P.; Apirakaramwong, A.; Nunthanid, J. & Ruktanonchai, U. (2010). Chitosan-Mediated siRNA Delivery In Vitro: Effect of Polymer Molecular Weight, Concentration and Salt Forms. *AAPS Pharm. Sci.Tech*., 11, DOI: 10.1208/s12249-009-9355-6, 64-72, ISSN 1530-9932

Terbojevich, M.; Cosani, A.; Conio, G.; Marsano, E. & Bianchi, E. (1991). Chitosan: chain rigidity and mesophase formation. *Carbohydr. Res.*, 209, 251–260, pISSN 0008-6215

Thanou, M.; Nihot, T.; Jansen, M.; Verhoef, J.C. & Junginger, H.E. (2001). Mono-*N*-carboxymethylated chitosan (MCC), a polyampholytic chitosan derivative, enhances the intestinal absorption properties of low molecular weight heparin in rats. *J. Pharm. Sci*., 90, 38–46, pISSN 0022-3549

Tiğli, R.; Karakeçili, A. & Gümüsderelioğlu, M. (2007). In vitro characterization of chitosan scaffolds: influence of composition and deacetylation degree. *J. Mater. Sci. Mater. Med.*, 18, 1665-1674, pISSN 0957-4530

Tikhonov, V.E.; Stepnova, E.A.; Babak, V.G.; Yamskov, I.A.; Palma-Guerrero, J.; Jansson, H.-B.; Lopez-Llorca, L.V.; Salinas, J.; Gerasimenko, D.V.; Avdienko, I.D. & Varlamov, V.P. (2006). Bactericidal and antifungal activities of a low molecular weight chitosans and its *N*-/2(3)-(dodec-2-enyl)succinol/-derivatives. *Carbohydr. Polym*., 64, 66-72, pISSN 0144-8617

Tipparat, H. & Riyaphan, O. (2008). Effect of deacetylation conditions on antimicrobial activity of chitosans prepared from carapace of black tiger shrimp (*Penaeus monodon). Songklanakarin J. Sci. Technol*., 30, Suppl. 1, 1-9

Tolaimate, A.; Desbrieres, J. ; Rhazi, M. & Alagui, A. (2003). Contribution to the preparation of chitins and chitosans with controlled physico-chemical properties, *Polymer,* 44, 7939–7952, pISSN 0032-3861

Tømmeraas, K.; Köping-Höggård, M.; Vårum, K.; Christensen, B.; Artursson, P. & Smidsrød, O. (2002). Preparation and characterization of chitosans with oligosaccharide branches. *Carbohydr. Res*., 337, 2455–2462, pISSN 0008-6215

Tsai, G.J.; Su, W.H.; Chen, H. C. & Pan, C. L. (2002). Antimicrobial activity of shrimp chitin and chitosan from different treatments and applications of fish preservation. *Fish Sci*., 68, 170-177, pISSN 0919-9268

Tsaih, M. & Chen, R. (1997). Effect of molecular weight and urea on the conformation of chitosan molecules in dilute solutions. *Int. J. Biol. Macromol*., 20, 233–240, pISSN 0141-8130

Ueno, H.; Mori, T. & Fujinaga, T. (2001). Topical formulations and wound healing applications of chitosan. *Adv. Drug Deliv. Rev*., 52, 105-115, pISSN 0169-409X

van der Lubben, I.M.; Verhoef, J.C.; Borchard, G. & Junginger, H.E. (2001). Review. Chitosan and its derivatives in mucosal drug and vaccine delivery. *Eur. J. Pharm. Sci.,* 14, 201–207, pISSN 0928-0987

Vårum, K.; Anthonsen, M.; Grasdalen, H. & Smidsrød, O. (1991a). Determination of the degree of *N*-acetylation and the distribution of *N*-acetyl groups in partially *N*-deacetylated chitins (chitosans) by high-field NMR spectroscopy. *Carbohydr. Res*., 211, 17–23, pISSN 0008-6215

Vårum, K.; Anthonsen, M.; Grasdalen, H. & Smidsrød, O. (1991b). ^{13}C-NMR studies of the acetylation sequences in partially *N*-deacetylated chitins (chitosans). *Carbohydr. Res*., 217, 19–27, pISSN 0008-6215

Vårum, K.M.; Myhr, M.M.; Hjerde, R.J.N. & Smidsrød, O. (1997). In vitro degradation rates of partially *N*-acetylated chitosans in human serum. *Carbohydr. Res*., 299, 99-101, pISSN 0008-6215

Vinsova, J. & Vavrikova, E. (2008). Recent Advances in Drugs and Prodrugs Design of Chitosan. *Curr. Pharm. Des.,* 14, 1311-1326, ISSN 1381-6128

Vishu Kumar, A.B.; Varadaraj, M.C.; Gowda, L.R. & Tharanathan, R.N. (2005). Characterization of chito-oligosaccharides prepared by chitosanolysis with the aid of papain and Pronase, and their bactericidal action against *Bacillus cereus* and *Escherichia coli*. *Biochem. J*., 391, 167-175, pISSN 0264-6021

Vruggink, H. (1970). The effect of chitin amendment on actinomycetes in soil and on the infection of potato tubers by *Streptomyces scabies*. *Neth. J. Plant Pathol., 76*., 293–295, ISSN 0929-1873

Wang, M.J.; Xie, Y.L.; Zheng, Q.D. & Yao, S.J. (2009). A Novel Potential Microflora-activated Carrier for a Colon-Specific Drug Delivery System and Its Characteristics. *Ind. Eng. Chem. Res.,* 48, 5276–5284, pISSN 0888-5885

Wang, S.-L.; Lin, T.-Y.; Yen, Y.-H.; Liao, H.-F. & Chen, Y.-J. (2006). Bioconversion of shellfish chitin wastes for the production of *Bacillus subtilis* W-118 chitinase. *Carbohydr. Res.*, 341, 2507–2515, pISSN 0008-6215

Wang, C.; Fu, X. & Yang, L. (2007). Water-soluble chitosan nanoparticles as a novel carrier system for protein delivery. *Chin. Sci. Bull.*, 52, 883-889, ISSN 1001-6538

Wang, W.; Bo, S.; Li, S. & Qin, W. (1991). Determination of the Mark-Houwink equation for chitosans with different degrees of deacetylation. *Int. J. Biol. Macromol.*, 13, 281–285, pISSN 0141-8130

Wang, X.; Yan, Y.; Lin, F.; Xiong, Z.; Wu, R.; Zhang, R. & Lu, Q. (2005). Preparation and characterization of a collagen/chitosan/heparin matrix for an implantable bioartificial liver. *J. Biomater. Sci. Polym. Ed.*, 16, 1063–1080, ISSN 0920-5063

Weinhold, M.X.; Sauvageau, J.C.M.; Keddig, N.; Matzke, M.; Tartsch, B.; Grunwald, I.; Kübel, C.; Jastorff, B. & Thöming, J. (2009). Strategy to improve the characterization of chitosan for sustainable biomedical applications: SAR guided multi-dimensional analysis. *Green Chem.*, 11, 498–509, ISSN 1463-9262

Whistler, R.J. & Kosik, M. (1971). Anticoagulant activity of oxidized and *N*- and *O*-sulfated chitosan. *Arch. Biochem. Biophys.*, 142, 106–110, pISSN 0003-9861

Wyatt, P. (1993). Light scattering and the absolute characterization of macromolecules. *Anal. Chim. Acta*, 272, 1–40, ISSN 0003-2670

Xia, W.; Liu, P.; Zhang, J. & Chen, J. (2010). Biological activities of chitosan and chitooligosaccharides. *Food Hydrocolloids*, doi:10.1016/j.foodhyd.2010.03.003, ISSN 0268-005X

Xie, W.; Xu, P. & Liu, Q. (2001). Antioxidant activity of water-soluble chitosan derivatives *Bioorg. Med. Chem. Lett.*, 11, 1699-1701, pISSN 0960-894X

Xie, W.; Xu, P.; Wang, W. & Liu, Q. (2002). Preparation and antibacterial activity of a water-soluble chitosan derivative. *Carbohydr. Polym.*, 50, 35-40, ISSN 0144-8617

Xie, Y.; Liu, X. & Chen, Q. (2007). Synthesis and characterization of water-soluble chitosan derivate and its antibacterial activity, *Carbohydr. Polym.*, 69, 142–147, ISSN 0144-8617

Xu, G.; Huang, X.; Qiu, L.; Wu, J. & Hu, Y. (2007). Mechanism study of chitosan on lipid metabolism in hyperlipidemic rats. *Asia Pac. J. Clin. Nutr.*, 16, 313-317, pISSN 0964-7058

Yadav, A.V. & Bhise, B.B. (2004). Chitosan a potential biomaterial effective against typhoid. *Curr. Sci.*, 187, 1176-1178, pISSN 0011-3891

Yan, X.L.; Khor, E. & Lim, L.Y. (2000). PEC films prepared from chitosan alginate coacervates. *Chem. Pharm. Bull.*, 48, 941–946, ISSN 0009-2363

Yang, R.; Yang, S.G.; Shim, W.S.; Cui, F.; Cheng, G.; Kim, I.W.; Kim, D.D.; Chung, S.J. & Shim, C.K. (2009b). Lung-specific delivery of paclitaxel by chitosan-modified PLGA nanoparticles via transient formation of microaggregates. *J. Pharm. Sci.*, 98, 970–984, pISSN 0022-3549

Yang, F.; Cui, X. & Yang, X. (2002). Interaction of low-molecular-weight chitosan with mimic membrane studied by electrochemical methods and surface plasmon resonance. *Biophys. Chem.*, 99, 99-106, pISSN 0301-4622

Yang, K.W.; Li, X.R.; Yang, Z.L.; Li, P.Z.; Wang, F. & Liu, Y. (2009a). Novel polyion complex micelles for liver-targeted delivery of diammonium glycyrrhizinate: in vitro and in vivo characterization. *J. Biomed. Mater. Res. A.*, 88, 140–148, pISSN 1549-3296

Yang, T.C.; Chou, C.C. & Li, C.F. (2005). Antibacterial activity of *N*-alkylated disaccharide chitosan derivatives. *Int. J. Food Microbiol.*, 97, 237-245, pISSN 0168-1605

Zhang, C.; Ping, Q.; Zhang, H. & Shen, J. (2003). Preparation of *N*-alkyl-*O*-sulfate chitosan derivatives and micellar solubilization of taxol. *Carbohydr. Polym.*, 54, 137–141, ISSN 0144-8617

Zhang, H. & Neau, S.H. (2001). In vitro degradation of chitosan by a commercial enzyme preparation: effect of molecular weight and degree of deacetylation, *Biomaterials,* 22, 1653-1658, pISSN 0142-9612

Zhang, H.-L.; Wu, S.-H.; Tao, Y.; Zang, L.-Q. & Su, Z.-Q. (2010a). Preparation and Characterization of Water-Soluble Chitosan Nanoparticles as Protein Delivery System. *J. Nanomater.*, Article ID 898910, 5 pages, doi:10.1155/2010/898910, pISSN 1687-4110

Zhang, J.; Xia, W.; Liu, P.; Cheng, Q.; Tahirou, T.; Gu W. & Li, B. (2010b). Chitosan Modification and Pharmaceutical/Biomedical Applications *Mar. Drugs*, 8, 1962-1987, ISSN 1660-3397

Zhang, L.F.; Wang, M.Y.; Kang, X.D.; Boontheung, P.; Li, N.; Nel, A.E. & Loo, J.A. (2009). Oxidative Stress and Asthma: Proteome Analysis of Chitinase-like Proteins and FIZZ1 in Lung Tissue and Bronchoalveolar Lavage Fluid. *J. Proteome Res.,* 8, 1631–1638, pISSN 1535-3893

Zhang, Y. & Zhang, M. (2002). Calcium phosphate/chitosan composite scaffolds for controlled in vitro antibiotic drug release. *J. Biomed. Mater. Res.,* 62, 378–786, pISSN 0021-9304

Zhang, Y.; Chen, J.; Zhang, Y.; Pan, Y.; Zhao J.; Ren L.; Liao, M.: Hu, Z.; Kong, L & Wang, J. (2007). A novel PEGylation of chitosan nanoparticles for gene delivery. *Biotechnol. Appl. Biochem.,* 46, 197–204, pISSN 0885-4513

Zhang, Y.; Xue, C.; Xue, Y.; Gao, R. & Zhang, X. (2005). Determination of the degree of deacetylation of chitin and chitosan by x-ray powder diffraction. *Carbohydr. Res.*, 340, 1914–1917, pISSN 0008-6215

Zhong, Z. ; Chen, R.; Xing, R.; Chen, X.; Liu, S.; Guo, Z.; Ji, X.; Wang, L. & Li, P. (2007). Synthesis and antifungal properties of sulfanilamide derivatives of Chitosan, *Carbohydr. Res.*, 342, 2390–2395, ISSN 0008-6215

Zhong, Z.; Li, P.; Xing, R. & Liu, S. (2009). Antimicrobial activity of hydroxylbenzenesulfonailides derivatives of chitosan, chitosan sulfates and carboxymethyl chitosan. *Int. J. Biol. Macromol.,* 45, 163–168, pISSN 0141-8130

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