IJNRD.ORG ISSN : 2456-4184
INTERNATIONAL JOURNAL OF NOVEL RESEARCH AND DEVELOPMENT (IJNRD) | IJNRD.ORG
An International Open Access, Peer-reviewed, Refereed Journal

CHITOSAN POLYMER AS A SOURCE OF DRUG SOLUBILITY AND ANTICANCER ACTIVITY

Sayali S. Davang[1], Rutuja R. Shah[2]
Department of Pharmaceutics, Anandi Pharmacy college Kalambe tarf kale, Pin-416205, Maharashtra, India

Abstract:
Chitosan is a natural polysaccharide polymer derived from deacytalation of chitin. Chitosan is a multipurpose biomaterial shows effect against several types of cancer. The biological properties of chitosan are mainly depending on their solubility in water and other solvents. The involvement of chitosan with its amino, acetamino and hydroxyl group can give derivatives of increased solubility and noticeable anticancer activity. It is being used in targeting drug delivery to treat cancer.
In this review, we are going to discuss how chitosan increase poor solubility and boost polymer properties. We focus on some of the main biological properties of chitosan and its anticancer activity.

Keywords: Chitosan, chitin, Derivatives, Solubility, Anticancer.

1) Introduction:

Polymers are macromolecules composed of repeating structural units of monomers connected by covalent chemical bonds and this process is known as polymerization. There are many types of polymers including natural and synthetic moiety. Natural polymers such as proteins (collagen, silk and keratin), carbohydrates (starch, glycogen) are widely used materials for conventional and novel dosage forms. These materials are chemically inert, nontoxic, less expensive, biodegradable, eco-friendly and widely available. The development of new applications for Chitosan and its derivative is mainly due to the fact that these are renewable source of natural biodegradable polymers and also due to chitin and its derivative are the most abundant natural polymers. [3]
Due to their attractive abilities to improve the pharmacokinetics and pharmacodynamics of small drug, protein, and enzyme molecules, macromolecular polysaccharides have been receiving significant attention. Polysaccharide polymers demonstrated very efficient attachments of bioactive therapeutic agents, which leads to an increase in the duration of activity. The bioactive agents can bind covalently to polysaccharide backbone structures. Chitosan has received a significant attention for several decades due to their unique biological activities. This review aims to supply the recent information about the competitive biological activities of chitosan and its derivatives for medical and pharmaceutical applications. Among many biological activities of chitosan and its derivatives discovered so far, antimicrobial, antioxidant, anticancer, and anti-inflammatory activities were described with recently published outcomes. [7]
As the precursor of chitosan, chitin is the most widely occurring biopolymer in nature after cellulose: it can be found in a range of eukaryotic species such as crustacea, insects and fungi.

Organism		Chitin content (%)
Crustacea	Crab[a]	72.1
	Shrimp[a]	69.1
	Lobster[a]	69.8
Insects	True Fly[a]	54.8
	Sulphur butterfly[a]	64.0
Fungi	*Aspergillus niger*[b]	42.0
	Mucor rouxii	44.5

Table 1 Principle sources of chitin [11]

An organic weight of cuticle; [b]dry weight of the cell wall.

Chitin is a polymer of *N*-Acetyl-D-glucosamine, and when it is subject to deacetylation and the repeating units in the polymer are predominantly without the acetyl functional group, i.e. as β-1,4-D-glucosamine, the polymer is known as chitosan. The mole fraction of the *N*-acetylated repeating units is defined as the degree of acetylation (DA), while the percentage of the repeating units of β-1,4-D-glucosamine in the polysaccharides is defined as the degree of deacetylation (DD). Hence DA = 100% - DD as illustrated in Figure 1. Although current publications have no consensus regarding the cut-off of DD values between chitin and chitosan, it is usually between 40% and 75%, and most commercial chitosan have DD values between 70% - 90%.

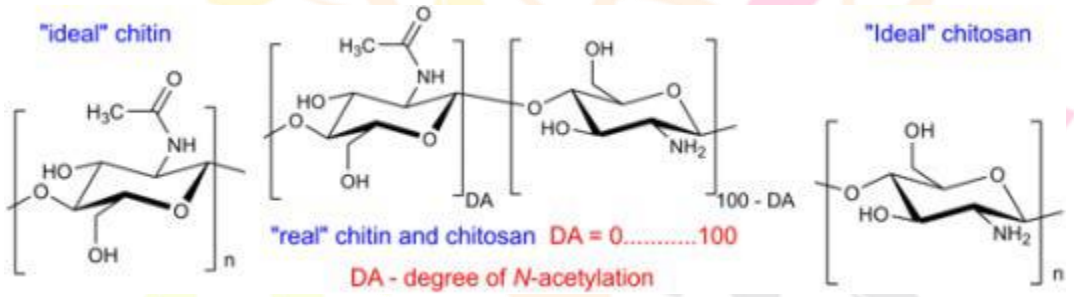

Figure 1. **The relationship between DA and DD. The repeating unit on the left is *N*-Acetyl-D-glucosamine, while the one on the right is β-1,4-D-glucosamine. This figure is from an open access journal without copyright restriction for reuse, modification or republication.**

DD is a critical parameter of chitosan; as prior research has reported that chitosan with a higher DD demonstrates stronger biological effects as well as increased water solubility. This is because a higher DD indicates a higher concentration of amino groups in the molecule, and the protonation of the -NH2 functional group is vital for manifesting chitosan's biological effects and water solubility. Besides DD, molecular weight (MW) is another essential parameter which influences the bioactivity of chitosan. Like DA, lower MW chitosan usually shows more significant bioactivities than higher MW chitosan. Previous studies have described different MW cut-off values to distinguish between high, medium, and low MW chitosan, and chitosan oligosaccharide. Nonetheless, irrespective of what the actual MW cut-off values are, the bioactivity of chitosan is usually found to be stronger when MW is lower (e.g. < 20 kDa) than higher (e.g. > 120 kDa). [1]

2) Manufacture of Chitosan:

Chitosan is commercially produced in different parts of the world (Japan, North America, Poland, Italy, Russia, Norway and India) on a large scale. The raw material for chitin generally consists of crustacean shells. Even though more species are used, chitin itself never seems to alter in terms of chemical composition. However, adjustments during processing may be needed to make the end product of consistent quality. Generally, shells from crab and shrimps are utilized including those of Dungeness crab (*Cancer magister*), the King crab (*Paralithodes camschatica*) and the Pacific Shrimp (*Pandalus borealis*) [12]. Principle sources of chitin are summarized in Table 1 [11]. The basic process for the manufacture of chitosan involves the removal of proteins and minerals such as calcium carbonate and calcium phosphate, by treatment with alkali and acid, respectively. Before the treatment, the shells are ground to make them more accessible, and after the completion of the manufacturing procedure chitin is dried so that it can be stored as a stable intermediate for deacetylation to chitosan at a later stage. The process of deacetylation is achieved by treating chitin with

a strong solution of sodium hydroxide at an elevated temperature [12,13]. A flow diagram depicting the manufacture of chitosan is given in Figure 2.[9]

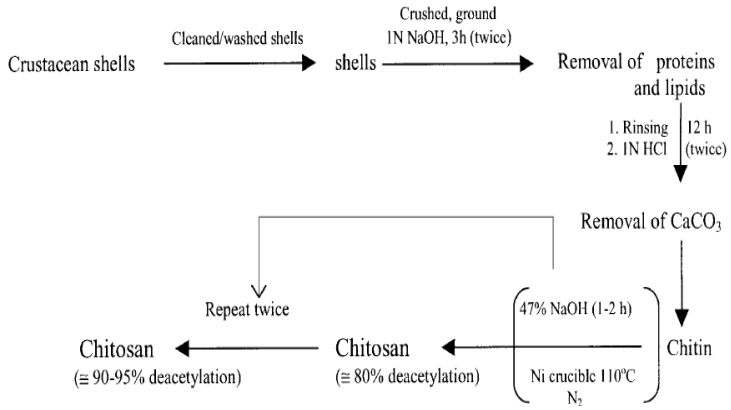

Figure 2: A flow diagram depicting the manufacture of chitosan

3) Physicochemical Properties:

The term chitosan describes a series of chitosan polymers with different molecular weight, viscosity and degree of deacetylation (40±98%). It is a linear polyamine with a number of amino groups that are readily available for chemical reaction and salt formation with acids. Important characteristics of chitosan are its molecular weight, viscosity, degree of deacetylation [14,15,16], crystallinity index, number of monomeric units (n), water retention value, pKa and energy of hydration [17]. Chitosan has a high charge density, adheres to negatively charged surfaces and chelates metal ions. Dueto high molecular weight and a linear unbranched structure, chitosan is an excellent viscosity enhancing agent in an acidic environment. It behaves as a pseudoplastic material exhibiting a decrease in viscosity with increasing rates of shear. The viscosity of chitosan solution increases with an increase in chitosan concentration, decrease in temperature and with increasing degree of deacetylation. The viscosity for 1% chitosan (w}v) in various organic acids at different concentrations [12] is given in Table 2.[18,19]

Properties		
Physical:	Particle size	30l m
	Density	1.35±1.40 g} cc
	pH	6.5±7.5
	Solubility	Insoluble in water but soluble in acids
Chemical:	Cationic polyamine	High charge density at pH!6.5
		Adheres to negatively charged surfaces
		Forms gels with poly anions
	High molecular weight, linear polyelectrolyte	Viscosity ± high to low
	Chelates certain transitional metals	
	Amiable to chemical modifications	Reactive hydroxyl}amino groups

Table 3 Physicochemical properties of chitosan [18,19]

1. Solubility

Chitosan is produced by deacetylation of chitin; in this process, some N-acetylglucosamine moieties are converted into glucosamine units. The presence of large amounts of protonated -NH2 groups on the chitosan structure accounts for its solubility in acid aqueous media since its pKa value is approximately 6.5. When around 50% of all amino groups are protonated, chitosan becomes soluble. Chitosan solubility depends on different factors such as polymer molecular weight, degree of acetylation, pH, temperature, and polymer

crystallinity. Homogeneous deacetylation (alkali treatment, 0ºC) of chitin permits the production of polymers soluble in aqueous acetic acid solutions with DD as low as 28%, with this value never being reached under heterogeneous deacetylation (alkali treatment, high temperatures). Moreover, with a DD of 49%, the samples are soluble in water. This behavior is explained by the fact that homogeneous deacetylation leads to an increase in the number of glucosamine units and a modification in the crystalline structure of the polymer. Depending on polymer DD, these modifications range from a reduction in crystal size and crystal perfection to the presence of a new crystal structure close to β-chitin. Studied the role of crystallinity and inter- or intramolecular forces on chitosan solubility; in this work, a parent chitosan sample was half re-acetylated with anhydride acetic or fully N-deacetylated under homogeneous conditions. After reacetylation, the solubility of the polymer was expanded until pH 7.4, while a slight reduction in the solubility range of the fully deacetylated chitosan was determined. The lower solubility was explained due to the increase in the polymer crystallinity after deacetylation, which offsets the effect of the increase in glucosamine moieties. On the contrary, a reduction in the crystallinity was observed in the half-acetylated sample. The use of hydrogen bond disruptors such as urea or guanidine hydrochloride also alters the solubility window of chitosan. In fact, by a combination of chemical and physical disruption of the hydrogen bonds, broad solubility is achieved.

2. Viscosity

The viscosity of polymers is a parameter of great interest from the technological point of view since highly viscous solutions are difficult to manage. Moreover, viscometer is a powerful tool for determining chitosan's molecular weight, as it is a simple and rapid method even though it is not an absolute method, therefore requiring the determination of constants that are specific to the solvent. The average molecular weight is determined by the Mark–Houwink–Sakurada equation, which relates this parameter with the intrinsic viscosity:

$$\eta = KM_v^{\alpha}$$

Where, K and α are constants that must be determined experimentally. Several values of K and βhave been reported depending on the solvent composition, pH, and ionic strength. Chitosan viscosity depends on the molecular weight of the polymer and deacetylation degree and decreases as the molecular weight of chitosan is reduced. In fact, viscosity can be used to determine the stability of the polymer in solution, as a reduction is observed during polymer storage due to polymer degradation. Shear viscosity increases with chitosan deacetylation degree. The shear viscosity at the same rate was studied in two samples with different deacetylation degrees (91% vs. 75%) and represented versus intrinsic viscosity; it was reported that shear viscosity was larger for those samples with the highest deacetylation degree; when the curves were evaluated, straight lines were observed in both chitosan samples This is explained due to the nature of chitosan, as this polymer is a cationic polyelectrolyte because of the amine protonation in acidic media. Therefore, the higher the DD, the larger chain expansion is expected, as more glucosamine units are found in the polymer chain, leading to a greater charge density in this sample. In order to modulate chitosan viscosity, the addition of different co-solvents has been evaluated; in this sense, studied the effect of the addition of isopropanol and ethanol to a chitosan solution in 1% acetic acid, reporting that the presence of the co-solvents decreased the intrinsic viscosity of the polymer. [10]

4) Mechanism of Anticancer

1. Anticancer Activity

The general cancer treatments performed clinically using chemotherapy, radiotherapy, and surgery have considerably extended the life expectancy of patients. Many current anticancer drugs have non ideal pharmacological properties such as low aqueous solubility, irritating nature, lack of stability, rapid metabolism, and nonselective drug distribution, and they can cause several adverse consequences, including suboptimal therapeutic activity, dose-limiting side effects, and poor-patient quality of life [20, 21]. Thus, many scientists are inspired to search for more effective and harmless medication for cancer-suffering patients. Chitosan and its derivatives are considered the potential anticancer polysaccharide naturally obtained. Many efforts on searching an efficient Anticancer agent from natural products lead an increasing interest in polysaccharides. Zong et al. published a review article about the anticancer activity of polysaccharides from fungi, plants, algae, animals, and bacteria [22]. They resumed the inhibition mechanism of tumor growth by polysaccharides as the following:

(i) Prevention of tumor genesis by oral consumption of active preparations
(ii) Direct anticancer activity, such as the induction of tumor cell apoptosis

(iii) Immunopotentiation activity in combination with chemotherapy
(iv) Inhibition of tumor metastasis

An intrinsic antitumor activity of chitosan and its derivatives with low MW was verified through in vitro and in vivo experiments [23]. Along with antimicrobial and antioxidant activities, the DDA and MW of chitosan and its derivatives are also the major factors deciding antitumor activity. The effects of the DDA and MW of chitosan oligomers on antitumor activity in vitro were investigated by Park et al. [24]. The lower MW and higher DDA (higher solubility) are promising factors for the development of antitumor agents derived from chitosan in in vitro tests with Human PC3 (prostate cancer cell), A549 (carcinomic human alveolar basal epithelial cell), and HepG2 (hepatocellular carcinoma cell). Azuma and his colleagues well reviewed about the antitumor activity of COS in vivo and in vitro cell models showing an effectiveness on tumor growing, reduction of the number of metastatic colonies, suppressing cancer cell growing, and enhancement of acquired immunity [25]. COS has comparatively short chain length and readily soluble in water. Jeon and Kim examined the antitumor activity of COS with different molecular weight against S180 (sarcoma 180 solid) and U14 (uterine cervix carcinoma number 14) tumor cell bearing mice [26]. The results proved that the antitumor activity was clearly dependent on MW and the range of MW 1.5 to 5.5 kDa effectively inhibited the growth of both tumor cells S180 and U14 in the mice. At the same time, the mice survived more days without weight loss. In several studies, nanoparticles prepared with chitosan showed direct inhibition activity to the proliferation of human tumor cell by inducing apoptosis and growth suppression without signs of neurological toxicity or weight loss proving the safeness of chitosan nanoparticles in the mouse model [27,28]. Xu et al. described that the antitumor activity of chitosan nanoparticles might be related to antiangiogenic activity that is correlated with vascular endothelial growth factor receptor (VEGFR2) production and subsequent blockage of vascular endothelial growth factor- (VEGF-) induced endothelial cell activation [109]. The stearic acid-g-chitosan oligosaccharide (CSO-SA) micelles were studied for antitumor drug or gene delivery carriers [29,30]. Hydrophobic drug, podophyllotoxin, was successfully loaded in the CSO-SA micelles demonstrating a sustained release and in vitro anticancer effects for suppressing against human breast carcinoma (MCF-7) cells, human lung cancer cells (A549), and human hematoma cell line (Bel-7402) [29]. Polyethylenimine-conjugated stearic acid-g-chitosan showed good DNA-binding capacity (formation of gene delivery complex) with effectively suppressing the tumor (above 60% tumor inhibition) without systematic toxicity [30]. There are also many other studies about the chitosan and chemically/physically modified chitosan or chitosan derivatives for various types of cancer treatment in vivo and in vitro. Most of the studies commonly demonstrated that chitosan involved ant carcinogenic tools that are very efficient on the inhibition of cell proliferation, inducing apoptosis, cell viability, reduction of tumor size, cell targeting, less side effect, and low toxicity.

2. Activity of Chitosan

a) Permeation Enhancing Mechanism.

Amino group in chitosan leads to protonation in acidic to neutral medium. The positive charge developed in this cationic polysaccharide (pKa~6.5) makes it water soluble and bio adhesive to bind with and enhance permeation through negatively charged surfaces such as mucosal and basement membranes. Consequently, chitosan facilitates oral bioavailability of polar drugs and their transportation through epithelial surfaces. Due to its biocompatibility and nontoxicity, chitosan finds applications in pharmaceutical and commercial fields like in the preparation of binder in wet granulation, tablets with slow release of drugs, drug carrier in micro particle system, disintegrate, hydrogels, site specific drug delivery, and carrier of vaccine delivery and gene therapy. Its ant metastatic activity both *in vitro* and *in vivo* has been reported due to its permeation enhancing mechanism. It has been found that the treatment of MDA-MB-231 human breast carcinoma cells with increasing concentration of chitosan inhibited the migration of these cells through amatrigel coated membrane because this combination of chitosan and carcinoma cell lines lowered the activity and amount of MMP9 protein and this antimetastatic behavior increased with increase in concentration of chitosan [31]

b) Antiangiogenic Mechanism.

Chitosan can exhibit antitumor effect by antiangiogenic mechanism. This process interferes with mutual regulation of proangiogenic and antiangiogenic factors under the pathological conditions [34]. Y. Xu and coworkers (2009) showed that chitosan nanoparticles (CNP) could inhibit the growth of human hepatocellular carcinoma through a mechanism of CNP-mediated inhibition of tumor angiogenesis that was associated to impaired levels of vascular endothelial growth factor receptor 2 (VEGFR2) [35].

c) Sustained Release Mechanism.

A mechanism of anticancer functionality of chitosan is related to its capacity to increase the bio distribution level and accumulation of drug in tumor cells. Zhang et al. [36] through pharmacokinetic study *in vivo* have shown that mifepristone (MIF) loaded chitosan nanoparticles (MCNS) ensure controlled drug delivery in a sustained release manner and enhance the oral bioavailability and anticancer activity of the drug [36].

d) Immunoenhancement Mechanism.

It was also shown that the tumor growth inhibitory mechanism of chitosan involved enhancement of immunological system consisting of tumoricidal immunocytes as cytotoxic lymphocytes natural killer cells as observed in sarcoma 180 bearing mice. Antitumor activity of oligo chitosan was suggested to have been related to activation of intestinal immune functions due to enhancement of NK activity in intraepithelial lymphocytes (IELs) or splenic lymphocytes. Microcrystalline chitosan has been found to inhibit cell viability on HT29 colon carcinoma cell line and suppress the tumor growth in HepG2 bearing severe combined immune deficient (SCID) mice. Applications of native chitosan are limited by its higher molecular weight that results in low solubility in nonacidic aqueous media. So, to be absorbed in human body it is converted into low molecular weight COS. Cellulose treated chitosan forms water soluble oligosaccharide product with low molecular weight due to enzymatic hydrolysis followed by degradation of the chain without any modification in chemical structure of the residues. Such water-soluble product has been found to inhibit the growth of tumor cell. Tokoro et al. suggested that the mechanism of such tumor growth inhibitory effect of hexa-N-acetylchitohexaose and chitohexaose is associated with higher production of interleukin I and interleukin II to bring about the maturation of splenic T- lymphocytes and killer Tcells. Seo et al. showed that the antitumor activity of low molecular weight chitosan was due to activation of murine peritoneal macrophages to kill the tumor cells in the presence of IFN-γ. Immunoenhancing molecular mechanisms of COS could precede either with direct killing of pathogenic microorganisms or tumor cells because of an immune response or with enhancement of cytotoxic activity to inhibit the production of tumor cells by activation of T-cells and NK-cells with the help of IL-1 and TNF-α cytokines. Synergistic effects shown by TNF α are critical to bring about the proliferation of Th1 cells together with IL-1 and IL-2 *in vitr*. So, the innate immune responses shown by COS are associated with up regulation of IL-1, TNF-α, and IFN-γ to increase the immune functions of lymphocytes. The antitumor effect of chitosan has also been shown to be due to its antioxidant profile improvement pathway.

References:

1. Mr Shijie Kou (Gabriel), Dr Linda Peters,Associate Professor Michael Mucalo Chitosan: A review of sources and preparation methods.
2. M. N. V. Ravi Kumar*, R. A. A. Muzzarelli, C. Muzzarelli,H. Sashiwa, and A. J. Domb Chitosan Chemistry and Pharmaceutical Perspectives
3. *Vipin Bansal, Pramod Kumar Sharma, Nitin Sharma, Om Prakash Pal and Rishabha Malviya* Applications of Chitosan and Chitosan Derivatives in Drug Delivery
4. Hari Sharan Adhikari 1 and Paras Nath Yadav 2 *Review Article* Anticancer Activity of Chitosan, Chitosan Derivatives, and Their Mechanism of Action
5. Ling Yien Ing,1 NoraziahMohamad Zin,2 Atif Sarwar,1 and Haliza Katas1 *Research Article* Antifungal Activity of Chitosan Nanoparticles and Correlation with Their Physical Properties
6. Yuniardini S. Wimardhani1,2), Dewi F. Suniarti3), Hans J. Freisleben4),Septelia I. Wanandi5), Nurjati C. Siregar6), and Masa-Aki Ikeda7) Chitosan exerts anticancer activity through induction of apoptosis and cell cycle arrest in oral cancer cells.
7. Suyeon Kim Review Article Competitive Biological Activities of Chitosan and Its Derivatives: Antimicrobial, Antioxidant, Anticancer, and Anti-Inflammatory Activities
8. Wenshui Xia a,b,*, Ping Liu c, Jiali Zhang a,d, Jie Chen a Biological activities of chitosan and chitooligosaccharides
9. K. Singla and M. Chawla Chitosan: some pharmaceutical and biological aspects an update
10. Inmaculada Aranaz 1,2 , Andrés R. Alcántara1 , Maria Concepción Civera 1 , Concepción Arias 1, Begoña Elorza 1, Angeles Heras Caballero 1,2 and Niuris Acosta 1,2,* Review Chitosan: An Overview of Its Properties and Applications
11. Felt, O., Buri, P., Gurny, R. (1998) Chitosan:Aunique polysaccharide for drug delivery. *Drug Dev. Ind. Pharm*. 24: 979±993
12. Skaugrud, O. (1991) Chitosan: New biopolymer for cosmetics and drugs. *Drug Cosmetic Ind*. 148: 24±29

13. Ilango, R., Jayakar, B., Kavimani, S. (1998) Chitosan as a new pharmaceutical excipient. *The Eastern Pharmacist* 41: 47±49
14. Bodek, K. H. (1994) Potentiometric method for determination of the degree of acetylation of chitosan. In: Karnicki, Z. S., Pajak, A. W., Breziski, M. M., Bylowsky, P. J. (eds) *Chitin World*. Bremerhaven,Germany, Wirtschaftsverlag, pp 456±461
15. Ferreira, M. C., Marvao, M. R., Duarte, M. L., Domard, A., Nunes, T., Feio, G. (1994a) Chitosan degree of acetylation: Comparison of two spectroscopic methods (13CCP}MASNMRand dispersive IR) In: Karnicki, Z. S., Brzeski,M. M., Bykowski, P. J., Wojtasz-Pajak, A. (eds) *Chitin World*. Wirtschaftverlag NW-Verlag fur neue, Wissenschaft, GmbH, Germany, pp 476±479
16. Ferreira, M. C., Marvao, M. R., Duarte, M. L., Nunes, T. (1994b) Optimisation of the measuring of chitin} chitosan degree of acetylation by FT-IR spectroscopy. In: Karnicki, Z. S., Brzeski, M. M., Bykowski, P. J., Wojtasz-Pajak, A. (eds) *Chitin World*. Wirtschaftverlag NW-Verlag fur neue, Wissenschaft, GmbH, Germany, pp 480±488
17. Kas, H. S. (1997) Chitosan: Properties, preparation and application to microparticulate systems. *J. Microencap*. 14: 689±711
18. Knapczyk, J., Krowcynscki, L., Krzek, J., Brzeski, M., Nurnberg, E.,Schenk, D., Struszczyk, H. (1989) Requirements of chitosan for pharmaceutical and biomedical applications. In: Skjak-Braek, G., Anthonsen, T., Sanford, P. (eds) *Chitin and Chitosan ± Sources*, *Chemistry*, *Biochemistry*, *Physical Properties and Applications*.Elsevier, London, pp 757±764
19. Sanford, P. A. (1990) Chitosan and alginate: New forms of commercial interest. *Am. Chem. Soc. Div. Polym. Chem.* 31: 628
20. Aljawish, I. Chevalot, B. Piffaut et al., "Functionalization of chitosan by laccase-catalyzed oxidation of ferulic acid and ethyl ferulate under heterogeneous reaction conditions," Carbohydrate Polymers, vol. 87, no. 1, pp. 537–544, 2012.
21. F. Salehi, H. Behboudi, G. Kavoosi, and S. K. Ardestani, "Chitosan promotes ROS-mediated apoptosis and S phase cell cycle arrest in triple-negative breast cancer cells: evidence for intercalative interaction with genomic DNA," RSC Advances, vol. 7, no. 68, pp. 43141–43150, 2017.
22. A. Zong, H. Cao, and F. Wang, "Anticancer polysaccharides from natural resources: a review of recent research," Carbohydrate Polymers, vol. 90, no. 4, pp. 1395–1410, 2012.
23. B. Sarmento and J. Neves, Eds.T. Cunha, B. Teixeira, B. Santos, M. Almeida, G. Dias, and J. Neves, "Chitosanbased systems for biopharmaceuticals: delivery, targeting and polymer therapeutics," in Chapter 5. Biological and Pharmacological Activity of Chitosan and Derivatives, B. Sarmento and J. Neves, Eds., Wiley, 2012.
24. J. K. Park, M. J. Chung, H. N. Choi, and Y. I. Park, "Effects of the molecular weight and the degree of deacetylation of chitosan oligosaccharides on antitumor activity," International Journal of Molecular Sciences, vol. 12, no. 1, pp. 266–277, 2011.
25. K. Azuma, T. Osaki, S. Minami, and Y. Okamoto, "Anticancer and anti-inflammatory properties of chitin and chitosan oligosaccharides," Journal of Functional Biomaterials, vol. 6 no. 1, pp. 33–49, 2015.
26. Y. Jeon and S.-K. Kim, "Antitumor activity of chitosan oligosaccharides produced in ultrafiltration membrane reactor system," Journal of Microbiology and Biotechnology, vol. 12, pp. 503–507, 2002.
27. Y. Xu, Z. Wen, and Z. Xu, "Chitosan nanoparticles inhibit the growth of human hepatocellular carcinoma xenografts through an antiangiogenic mechanism," Anticancer Research, vol. 30, pp. 5103–5110, 2010.
28. Qi, Z. Xu, X. Jiang, Y. Li, and M. Wang, "Cytotoxic activities of chitosan nanoparticles and copper-loaded nanoparticles," Bioorganic & Medicinal Chemistry Letters, vol. 15, no. 5, pp. 1397–1399, 2005.
29. X. Huang, X. Huang, X. H. Jiang et al., "In vitro antitumour activity of stearic acid-g-chitosan oligosaccharide polymeric micelles loading podophyllotoxin," Journal of Microencapsulation, vol. 29, no. 1, pp. 1–8, 2012.
30. F. Q. Hu, W. W. Chen, M. D. Zhao, H. Yuan, and Y. Z. Du, "Effective antitumor gene therapy delivered by polyethylenimine-conjugated stearic acid-g-chitosan oligosaccharide micelles," Gene Therapy, vol. 20, no. 6, pp. 597–606, 2013.
31. Y. N. Gavhane, A. S. Gurav, and A. V. Yadav, "Chitosan and ItsApplications: A Review of Literature," *International journal of research in pharmaceutical and biomedical sciences*, vol. 4, no. 1, pp. 312–331, 2013.

32. M.Thanou, J. C. Verhoef, andH. E. Junginger, "Chitosan and its derivatives as intestinal absorption enhancers," *Advanced Drug Delivery Reviews*, vol. 50, no. 1, pp. S91–S101, 2001.
33. K.-S.Namand Y.-H. Shon, "Suppression of metastasis of human breast cancer cells by chitosan oligosaccharides," *Journal of Microbiology and Biotechnology*, vol. 19, no. 6, pp. 629–633, 2009.
34. Z. Jiang, B. Han, H. Li, Y. Yang, and W. Liu, "Carboxymethyl chitosan represses tumor angiogenesis in vitro and in vivo," *Carbohydrate Polymers*, vol. 129, pp. 1–8, 2015.
35. Y. Xu, Z. Wen, and Z. Xu, "Chitosan nanoparticles inhibit the growth of human hepatocellular carcinoma xenografts through an antiangiogenicmechanism," *Anticancer Research*, vol. 29, no. 12, pp. 5103–5109, 2009.
36. H. Zhang, F. Wu, Y. Li et al., "Chitosan-based nanoparticles for improved anticancer efficacy and bioavailability of mifepristone," *Beilstein Journal of Nanotechnology*, vol. 7, pp. 1861–1870, 2016.