A Review: Chitosan nanoparticles and its biological application

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Abstract. Chitosan is a polysaccharide consisting of N-acetyl-D-glucosamine and β-linked D-glucosamine that are distributed uniformly. It is obtained by treating the chitin shells of crustaceans with a basic agent such as NaOH. Due to its capacity to attach to various biomolecules and compounds, it has also been used as a chelating agent. Chitosan and chitin nanofibers can be biodegradable and biocompatible with 50-500 nm diameter. Hence, they can be used as wound healing and hemostatic materials. It is widely used as a biopolymer across fields such as water treatment, food processing, clothing and cosmetics. Therefore, chitosan formulations with such molecules, including films, hydrogels, fibers and emulsions, have been developed in a wide variety of ways and have been used in various fields. In terms of use and distribution, after cellulose, the second largest green biomaterial is known to be chitosan. The synthesis of chitosan nanoparticles through the method of green engineering is the main focus of modern research in nanotechnology. Chitosan nanoparticles are also becoming a cost-effective and an eco-friendly alternative for successful applications in biomedical field. Therefore, this study summarizes the research on chitosan, its derivatives and chitosan nanoparticles, focusing on their synthesis, characterization and medicinal applications.

1. Introduction

The aim of this review is studying the uses of chitosan nanoparticles in different applications. The field of nanotechnology comes from various scientific fields, such as chemical, physical, biological and engineering sciences, where new techniques for controlling single atoms and molecules are being developed. In nanotechnology, nanoparticles are classified as a small object that acts as a whole unit in terms of its potential application and its transport properties.

Chitosan is a biopolymer which can be used for replacing synthetic plastic compounds and they are found in crustacean and insect exoskeletons. Due to their special biodegradable, non-toxic, biocompatible, and antimicrobial nature, chitosan nanoparticles have drawn significant interest in the biomedical field. Several perspectives on antibacterial and antimicrobial impacts and mechanism of action of nanoparticles are reviewed. Chitosan nanoparticles can increase drug permeability through the barrier of blood-brain, so they also can be used as efficient drug transport carriers for the brain [1].

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The linear polysaccharide chitosan is derived from chitin's deacetylation under basic conditions, or by chitin deacetylase enzymatic hydrolysis. Without any harmful effect on the body, chitosan nanoparticles can interact easily with living cells. In this review, a large range of new biomedical technologies involving chitosan are included [2]. Chitosan nanoparticles have a surface which is positively charged and have muco-adhesive properties that can bind to mucus membranes and release the payload of drug in a continuous release manner.

Solid particles or particulate dispersions from 1nm to 1000 nm in size are called as nanoparticles. Chitosan is the most significant derivative of chitin, as shown in Figure 1, formed by extracting the acetate moiety from chitin [3]. Chitosan is a naturally forming biocompatible polymer polysaccharide, highly basic, cationic, mucoadhesive, which is approved by the United States FDA for drug delivery and tissue engineering. Chitin, is naturally found as bound to minerals and proteins that should be separated by acidification and alkalization processes before the preparation of chitosan. Then the purified chitin is transformed to chitosan by N-deacetylation. By opening the tight junctions of epithelium, it works as an enhancer of penetration. Chitosan, both paracellular and transcellular, as seen in Figure 2, facilitates drug delivery. It interacts with the negatively charged mucus, to form a complex through hydrogen or hydrophobic interactions and ionic bonding [1].

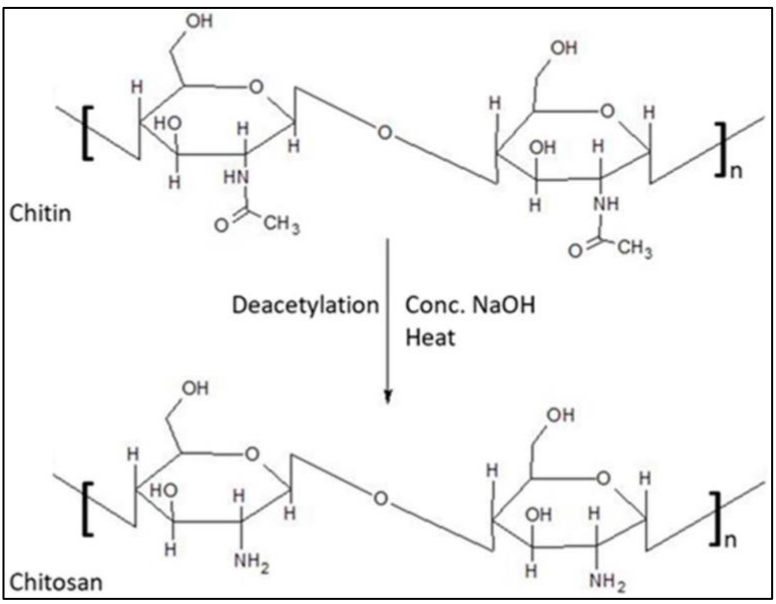

Fig. 1. Chitin Deacetylation to Chitosan [1]

Chitosan nanoparticles can either be synthesized using "top-down" or "bottom-up" methods, or else both procedures can be combined. It is possible to modify its backbone to change properties such as muco-adhesion, solubility and stability [5]. The sites for modification are both the amine and hydroxyl chitosan groups. Although there is a growing interest in research on drug delivery systems based on chitosan nanoparticles, current understanding does not allow a fair consideration of the advantages and disadvantages that will arise from the delivery of drugs using chitosan nanoparticles [4]. Some research indicates that the direct application of N- carboxybutyl chitosan to the infected area tends to aid wound healing and reduce the development of scars following plastic surgery.

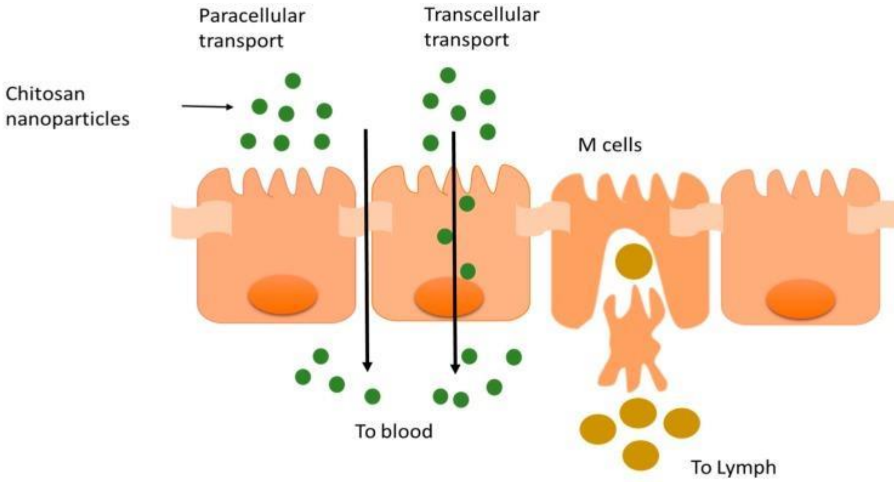

Fig. 2. A paracellular and transcellular transport mechanism via the epithelium of chitosan nanoparticles [1].

2. Chitosan nanoparticles

Chitosan helps in the production of traditional and new delivery of drug systems, which is non-toxic, mucoadhesive, biodegradable and biocompatible [6]. First crustacean's shells are removed, and after that ground into powder, that is further processed for production of chitosan [7]. Their nano-size plays a significant role in consuming it orally by mucosal pathways and parenteral administration. Clinical research suggests that, relative to traditional cancer therapeutic medications, therapeutic nanoparticles can increase efficacy and decrease side effects. Their use as special drug carriers would be a huge value to existing cancer therapeutics. The ability of nanoparticles to protect drugs from degradation, target the drug at the action site and minimize side effects or toxicity is unique. Most of the nanoparticles are insoluble in water but chitosan is one of the polymer soluble in water. This is due to chitosan's ideal properties, such as non-toxic, biodegradable, biocompatible, and economical [6]. Even though structurally, chitosan is similar to cellulose, it is containing acetylamine or free amino groups, in addition to hydroxyl groups, which show quite different properties from cellulose groups [10]. The therapeutic properties of chitosan have also been observed, such as microorganism growth inhibition and pain relief and epidermal cell growth and promotion of hemostasis. Delivery systems based on chitosan varies from micro to nanoparticle films and composites [11].

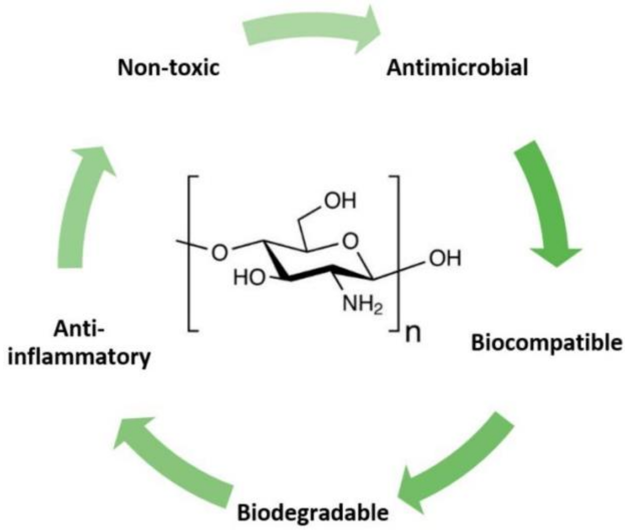

Fig. 3. Chitosan's Biological Properties

2.1. Preparation of Chitosan Nanoparticles

A two-step procedure is involved in the processing of chitosan.

I. Chitin extraction and the removal of $CaCO_3$ from the shells of crustacean using dil HCl and eliminating protein using dil aqueous NaOH.

II. For several hours without oxygen, 40-50 percent aqueous sodium hydroxide at 110°C-115° C is used for chitin deacetylation.

When the deacetylation degree is greater than 50 per cent, then formation of chitosan occurs. The two fundamental parameters which can influence chitosan's functionality and properties are the molecular weight and degree of deacetylation [12].

2.1.1 Ionotropic gelation

Mechanism of chitosan nanoparticle formation is based on the interaction by electrostatic method between chitosan $-NH_2$ group and the polyanion negative charge group, like tripolyphosphate (TPP). This technique in an aqueous condition provides a simple and mild form of preparation of chitosan nanoparticles. With or without the presence of stabilizing agent, dissolve chitosan in acetic acid and add it to chitosan solution after or before the polyanion addition [8].

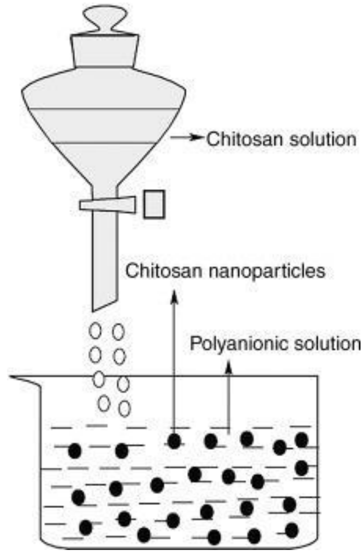

Fig. 4. Preparation by the ionotropic gelation process of chitosan nanoparticles [11]

2.1.2 Microemulsion method

This method is based on the production of chitosan nanoparticles in the reverse micellar droplet aqueous core and then cross-linked with glutaraldehyde. The surfactant which is dissolved in N-hexane is added to chitosan in glutaraldehyde (C5H8O2) and CH3COOH under constant stirring at room temperature kept overnight so the cross- linking process will be completed in which free NH2 group of chitosan is conjugated with C5H8O2. The product obtained by this method are chitosan nanoparticles which are cross-linked and the left-over surfactant which is removed further [9]. A narrow distribution of lesser than 100 nm is given by this technique and the size of particle can be adjusted by altering the volume of glutaraldehyde that changes the cross-linking degree.

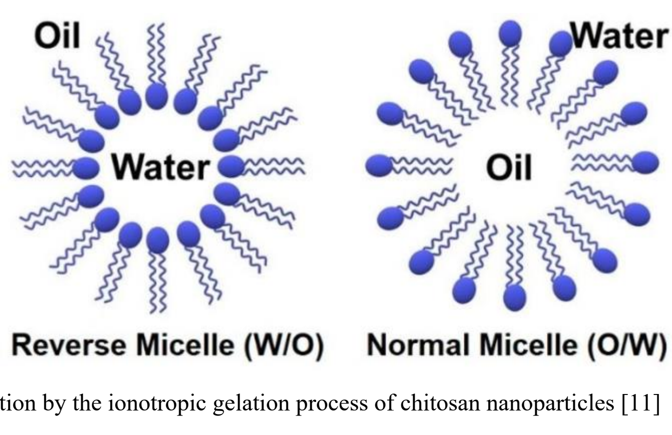

Fig. 5. Preparation by the ionotropic gelation process of chitosan nanoparticles [11]

2.1.3 Sieving method

Chitosan nanoparticles can be produced by cross-linking the chitosan in this technique to get a non-sticky glass hydrogel following by a sieve passing through it. In a 4 percent acetic acid solution, a sufficient amount of chitosan is dissolved to form a dense jelly mass and by adding glutaraldehyde it can be cross-linked. In order to get nanoparticles, the cross-linked non-sticky mass is being passed by means of a sieve with the right mesh size. To eliminate the unreacted excess glutaraldehyde, the nanoparticles is to be washed with 0.1 N sodium hydroxide solutions and dried in an oven at 400 degree Celsius [10]. The choice of one of the techniques depends on factors like the requirement for particle size, the chemical and thermal stability of active agent, the excess toxicity associated to the final product and the stability of the final product. Due to their unique chemical properties, chitosan nanomaterials have drawn considerable interest in a large range of bio-medical fields. Chitosan is an effective biomaterial to create matrixes of extracellular tissue. From the literature review, it has been noticed that the study on chitosan nanoparticulate systems containing various drugs have rapidly increased for various therapeutic applications.

3. Applications

Chitosan has a broad range of characteristics and properties which makes it applicable in many fields. Chitosan is biocompatible, highly flexible, and biodegradable and due to its high-water permeability, film-forming capability, mechanical strength, cost-effectiveness and susceptibility to chemical modifications, it can be used as a stabilizing agent. Chitosan's biological properties which is described in Table 1 permit them to be used for applications in the medical, pharmaceutical, cosmetic, nutritional, food sectors, and in agriculture, tissue engineering and water treatment [15].

3.1. Oral Drug Delivery

Oral delivery of drug is probably one of the most productive way for administration of drug because of the easy administration. Some complications stop the drug from reaching the circulatory system, decreasing bioavailability orally as a result. Such challenges are like varying pH, first-pass liver effect, enzyme presence and the intestinal barrier for adsorption of drugs [12]. In order to overcome the disadvantages of the delivery of oral drugs, nanoparticles are progressively used. There are some benefits to nanoparticles, like small particle size, potentially a modifiable surface and large surface area. When the particle size is small it can increase the drug dissolution rate.

Table 1. Chitosan nanoparticle's application in various fields [15].

Application	Function
As antimicrobial agent	Fungistatic and Bactericidal
In food industry	Food stabilizer, preservative, gelling agent, food additive, controlled moisture, antioxidants release, color stabilization, etc.
In agriculture	Seed coating, fertilizer, etc
In medical applications	Wound healing, clotting agent, tissue engineering, surgical sutures, skin burn, antitumor agent, blood cholesterol control, scaffolds and membranes, etc.

In cosmetics	Hair and skin products
In delivery	Gene delivery, drug delivery, siRNA delivery, oral protein delivery, etc

3.2. Wound Dressing

The wound healing process involves coordinated events in a sequence: hemostasis, swelling, proliferation, migration, and remodeling. For an efficient method of healing, antimicrobial wound dressings can be used [13]. Because of the distinctive properties of chitosan, it is one of the best choice for wound dressing because it has antimicrobial intrinsic properties and it has the ability for the wounded area to deliver extrinsic antimicrobial agents. Without any use of toxic chemicals chitosan can be easily made into hydrogels (they have the capacity to store water and moisten the infected area), sponges, films, and membranes. Antimicrobial wound dressing based on chitosan is possible to manufacture using only natural molecules, all alone, or by adding antibiotics (tetracycline, ciprofloxacin), natural compounds and extracts (honey, Aloe vera) and metallic antimicrobial particles (nAg, nTiO2) [2]. The antibiotics introduced to chitosan can interfere with the metabolic pathways of function of bacterial structure.

3.3. Mucoadhesion

The system of delivery of drug must be mucoadhesive and it releases the drug to the nasal cavity or gastro-intestinal tract or to pulmonary, or urethral sites for local delivery. Mucoadhesive nanoparticles with the capability to decrease the frequency of dose, can bind to the membrane of mucus and release of drug over time. The reversible and temporary the activation by chitosan of tight junctions in between the epithelial cells will achieve the drug absorption into the systemic circulation [14].

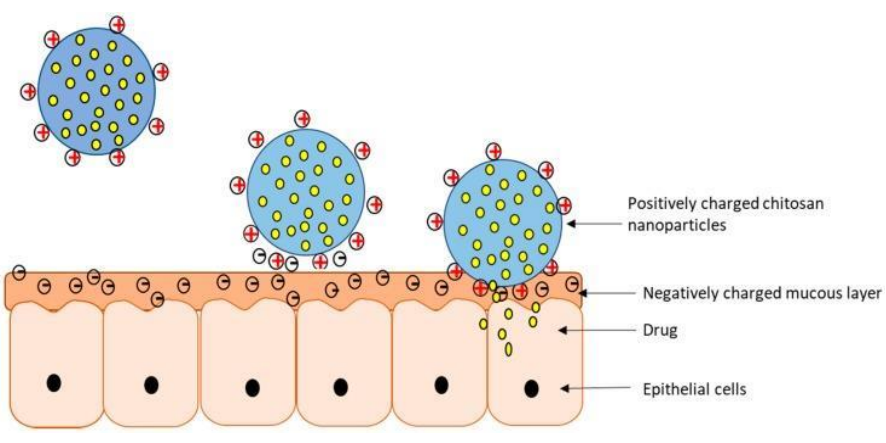

Fig. 6. Interaction between chitosan nanoparticles and mucus layer [2]

By way of electrostatic attraction, chitosan nanoparticles bind to the negatively charged mucus upon entering the mucosal layer and releases the drug over time. Chitosan's mucoadhesive property is due to its high positive charge, and which helps to attach with mucus which is negatively charged. In chitosan nanoparticles, particle size plays a major role, as smaller particles may be more capable of penetrating into the mucous layer [2].

3.4. Antimicrobial Agent in Aquaculture

Farming of aquatic species such as crustaceans, fish, aquatic plants and molluscs is aquaculture. It is one of the food-producing sectors with the fastest growth. The use of large quantities of a wide range of antibiotics means that they remain in the marine environment for extended periods of time. This process has contributed to the production in aquaculture environments of antibiotic- resistant bacteria. An alternative to the need for these antibiotics, chitosan as an antimicrobial agent, may be considered. Chitosan itself has been reported to have antimicrobial activity because of its cationic properties that create a membrane-disrupting effect. Chitosan's antibacterial activity is affected by a variety of factors, including the degree of polymerization of chitosan and many of its other physicochemical properties. To enhance its antibacterial activity, a range of chitosan derivatives with different modifications have been prepared; chitosan nanoparticles show specific physical and chemical characteristics in this way [16]. Chitosan’s antimicrobial activity is suggested to be dependent on different factors, such as deacetylation’s degree, pH, molar weight, pKa, metal cation presence, and microorganism species. To increase the antimicrobial effect, it can be either manufactured in its original form or combined with antimicrobial particles, antibiotics, natural compounds, and extracts.

4. Conclusion

Chitosan is a naturally available linear polysaccharide biopolymer that shows antimicrobial activity against a wide range of fungi and bacteria. [2]. By storing the drug locally, chitosan nanoparticles can effectively distribute medication at specific locations to allow an extended period for drug absorption [1]. Preparation of the chitosan nanoparticles and its use for biomedical field has been summarized in this review. It can be inferred from this study that nanostructured chitosan particles can be used as carriers of bioactive ingredients. They are very guaranteed carriers for drug delivery and they are cell proliferation enhancers because of its biological properties, like biodegradability, non-toxicity, antibacterial ability, and biocompatibility [3]. Chitosan nanoparticles are now being studied and developed further for targeted and controlled drug delivery. Their nano-size, across the cell membrane, promotes drug uptake. While significant improvement has been made in the applications of chitosan NPs as drug carriers, there are some issues that persist to be immediately addressed. Chitosan nanoparticles deliver flexible routes of drug administration thus making them suitable for topical, oral and injectable drug delivery systems. In conclusion, the increased potential of chitosan as drug carriers for commercial use is now greater than ever. In today's clinical applications, chitosan-based nanomaterials are frequently present and are anticipated to have an even larger effect on future biomedical applications [2].

5. Future Prospects

Farming of aquatic species such as crustaceans, fish, aquatic plants and molluscs is aquaculture. It is one of the food-producing sectors with the fastest growth. Due to the characteristic features of chitosan nanoparticles they are one of the most important polysaccharide biomaterial which is being synthesized [15]. Further developments in the usage of chitosan nanoparticles in dermatological applications, targeted parenteral delivery of drug and targeted cancer therapy can be expected in the future. By the emergence of novel strategies to resolve the drawbacks of chitosan through improvised methods, we are expecting further to view more study on chitosan in the future, mainly in the delivery of pulmonary and nasal drugs. We also hope that in future we will also concentrate on toxicity studies of chitosan nanoparticles in humans [1].

In combination with natural extracts and compounds, there are several possibilities for future studies on the use of chitosan. Further modification on wound dressings by chitosan is also necessary. This involve the creation of new smart dressings containing sensors released simultaneously with microbial growth. Another biomedical use is brain drug delivery, where chitosan and its play a major role as nano-biodegradable carriers. Considering the toxicity of some chitosan derivatives, which will serve as material carriers for drug delivery from nose-to-brain, there are still problems to be solved which is also a future concern [2].

In order to increase their efficacy in biomedical applications, recent study is primarily focused on improvisation of the bio - compatibility, stability, and production of new chitosan nanoparticles. These nanoparticles can be incorporated in a dissolved form, like suspensions, films, coatings and hydrogels, taking into account the targeted applications, and have a higher capacity for antitumor, peptide and protein delivery of drug, bone repair, cardiovascular, purification of blood, tissue regeneration and treatment of cancer applications. Although the usage of nanomaterials provides good merits in the biomedical industry, current study on the safety of different nanoparticles in the biomedical field is not enough for their use. Therefore, current knowledge of chitosan-based nanomaterials is not adequately established and there is an urgent need for comprehensive study on the manufacture of chitosan nanoparticles and their properties. In addition, green and environmental synthesis techniques for chitosan and its derivatives should be built for the protection of the environment. Nonetheless, considering the deficiencies, chitosan nanoparticles are taken into consideration to be guaranteed biomedical material [23]. Most of these experiments are still at the level of laboratory, however. Prior to their industrial implementation, additional studies are required. We hope that in the near future, further chitosan-based nano-carriers will be developing and applied in the fields of food engineering and biochemical.

References

1. Mohammed, Munawar A., Jaweria Syeda, Kishor M. Wasan, and Ellen K. Wasan. "An overview of chitosan nanoparticles and its application in non-parenteral drug delivery." Pharmaceutics (2017).
2. Kravanja, Gregor, Mateja Primožič, Željko Knez, and Maja Leitgeb. "Chitosan-based (Nano) materials for novel biomedical applications." (2019).
3. Zhao, Li-Ming, Lu-E. Shi, Zhi-Liang Zhang, Jian-Min Chen, Dong-Dong Shi, Jie Yang, and Zhen-Xing Tang. "Preparation and application of chitosan nanoparticles and nanofibers." Brazilian Journal of Chemical Engineering 28 (2011).
4. Subramani, Parasuraman A., Kalpana Panati, Veeranjaneya R. Lebaka, Dharaneeswara D. Reddy, and Venkata Ramireddy Narala. "Nanostructures for curcumin delivery: possibilities and challenges." In Nano-and Microscale Drug Delivery Systems (2017).
5. Grz ̧abka-Zasadzi ́nska, A.; Amietszajew, T.; Borysiak, S. Thermal and mechanical properties of chitosan nanocomposites with cellulose modified in ionic liquids. (2017).

6. Yateen, S. P.,Saikishore,V., & Srokanth, K. Drug delivery systems using chitosan nanoparticles (2012).
7. Abd Elgadir, M., Md Salim Uddin, Sahena Ferdosh, Aishah Adam, Ahmed Jalal Khan Chowdhury, and Md Zaidul Islam Sarker. "Impact of chitosan composites and chitosan nanoparticle composites on various drug delivery systems: A review." Journal of food and drug analysis (2015).
8. Desai, K. G. (2016). Chitosan nanoparticles prepared by ionotropic gelation: An overview of recent advances. *Critical Reviews™ in Therapeutic Drug Carrier Systems*, *33*(2).
9. López, R. G., Pineda, M. G., Hurtado, G., de León, R. D., Fernández, S., Saade, H., & Bueno, D. (2013). Chitosan-coated magnetic nanoparticles prepared in one step by reverse microemulsion precipitation. *International journal of molecular sciences*, *14*(10), 19636-19650.
10. Alfatama, M., Choukaife, H., Alkhatib, H., Al Rahal, O., & Zin, N. Z. M. (2024). A comprehensive review of oral chitosan drug delivery systems: applications for oral insulin delivery. *Nanotechnology Reviews*, *13*(1), 20230205.
11. Holban, Alina Maria, and Alexandru Mihai Grumezescu. Nanoarchitectonics for Smart Delivery and Drug Targeting. (2016).
12. Guadarrama-Escobar, O. R., Serrano-Castañeda, P., Anguiano-Almazán, E., Vázquez-Durán, A., Peña-Juárez, M. C., Vera-Graziano, R., ... & Escobar-Chávez, J. J. (2023). Chitosan nanoparticles as oral drug carriers. *International journal of molecular sciences*, *24*(5), 4289.
13. Dekoninck, Sophie, and Cédric Blanpain. "Stem cell dynamics, migration and plasticity during wound healing." Nature cell biology (2019).
14. Coutinho, A. J., Lima, S. A. C., Afonso, C. M., & Reis, S. (2020). Mucoadhesive and pH responsive fucoidan-chitosan nanoparticles for the oral delivery of methotrexate. *International journal of biological macromolecules*, *158*, 180-188.
15. Rizeq, Balsam R., Nadin N. Younes, Kashif Rasool, and Gheyath K. Nasrallah. "Synthesis, bioapplications, and toxicity evaluation of chitosan-based nanoparticles." International journal of molecular sciences (2019).
16. Dima, Jimena Bernadette, Cynthia Sequeiros, and Noemi Zaritzky. Chitosan from marine crustaceans: production, characterization and applications. InTech, 2017.