NEW CHITOSAN-BASED CHEMO PHARMACEUTICAL DELIVERY SYSTEMS FOR TUMOR CANCER TREATMENT: SHORT-REVIEW

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ABSTRACT

The aim of this review is to provide an overview of the delivery systems of chitosan-based chemotherapy agents that have been developed for the treatment of tumor cancer. Cancer treatment is a challenge that has always provided opportunities in different areas of study, due to its very complexity. Innovative options to make chemotherapy an effective treatment by targeting drugs to cancer cells through different modifications in delivery systems are being investigated. Chitosan, a biopolymer that is obtained from the partial deacetylation of chitin (the second most abundant biopolymer on earth) and is present in the exoskeleton of crustaceans, some insects, and also in the cell wall of some fungi. Chitosan has specific characteristics of solubility, functional groups in its structure, crosslinking power, affinity with other materials, biocompatibility, biodegradability, muco adhesiveness, provides bioavailability of the chemotherapeutic agent on cancer cells, without harming healthy cells. This document compiles some interesting studies on the use of chitosan in conjugation with other agents and safe materials for use in biomedicine, for the design, characterization, and development of new transport systems for chemotherapeutic agents, increasing the efficacy of this therapy in cancer treatment tumors.

Keywords: Tumor cancer, Chemotherapy agents, Drug delivery, Chitosan, Bioavailability.

1. INTRODUCTION

Currently, the WHO defines cancer as a generic term for a broad group of diseases that can affect any part of the body, also called "tumors or malignant neoplasms" [1]. According to projection data from the GLOBAL CANCER OBSERVATORY (GCO) of WHO, in 2040, about 30.2 million people will suffer from cancer in the world, regardless of gender or age [2], Figure 1.

With this alarming figure, work in the fields that can provide solutions to this problem is imminent. Understanding the whole process of cancer, from the general aspects, biology, causes and prevention, detection and diagnosis, treatment, could help to face this disease of global interest.

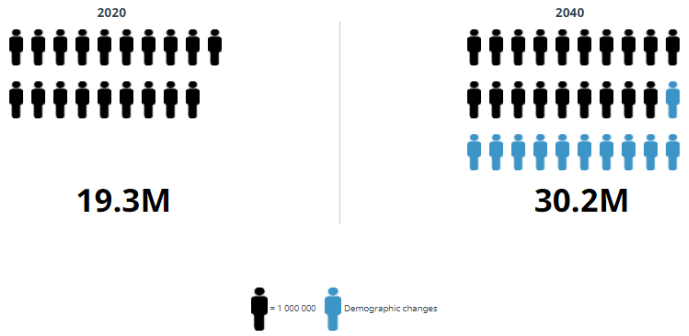

Figure 1. Estimated number of cancer cases for 2040 worldwide. (GCO).

Many cancers form solid tumors, which are masses of malignant tissue. On the other hand, blood cancers, such as leukemia, usually do not form tumors. This difference makes the treatment therapies different.

Tumor cancer can begin in the cells of any organ of the body, the most common being: colon and rectal cancer, endometrial cancer, liver cancer, pancreatic cancer, prostate cancer, lung cancer, kidney cancer, breast cancer, thyroid cancer, bladder cancer, among others, according to the National Cancer Institute of the United States [3]. The travel of these cancer cells from these organs to other parts of the body and contamination of other tissues is called metastasis. To name it, it always refers to the tumor cells in the initial organ; for example breast cancer with lung metastases.

In general, the treatments carried out for the therapy of carcinoma tumors are surgery (recessions of tumors attached to organs or parts of organs and tissues already affected), radiotherapy, chemotherapy, targeted therapy, immunotherapy, hormone therapy, and clinical trials. Recent studies point to the visualization of new alternatives for the treatment of tumor cancer. E. dos Santos et al, in their review, found that some epigenetic modulation actions of the tumor microenvironment in head and neck cancer can control tumor maintenance and metastasis [4]. D. Ribatti et al, investigated the controversial role of mast cells in breast cancer progression and angiogenesis [5]. On the other hand, Y. Deng et al studied the effect of biofilms (bacteria) on tumor progression; in a multi-fluidic model (anti-biofilm-antibiotic-drug) against cancer, to simultaneously eradicate biofilms and tumors [6]. Another interesting study is that of S. Mukhopadhyay et al, who focused on the biochemical aspect of cancer and recent advances in autophagy signaling in the tumor microenvironment, to be used as a possible cancer therapy [7]. New research within *in vivo* studies (rats), carried out in India by N. Subhapradha et al, indicates that chitosan from squid showed antioxidant and antilipidemic properties that can protect liver cells from cancer-produced by N-diethylnitrosamine molecules [8].

While all these new approaches to cancer treatment research are being developed, existing therapies are also being refined to improve their efficacy. One of the therapies considered effective, but not selective, is chemotherapy. Based on drugs to disrupt the formation of cancer cells (destroying them or preventing their multiplication), they enter the bloodstream and travel throughout the body, also affecting rapidly dividing healthy cells, generating systemic toxicity [9]. The mechanism of action of some chemotherapeutic agents is growth factor inhibition, angiogenesis inhibition, DNA synthesis inhibitors, among others. There are several forms of chemotherapy administration: intravenous, oral, injected, intra-arterial, injected into the peritoneum, or topical [9]. However, being unable to differentiate healthy cells from cancerous cells, the chemo pharmaceutical can affect healthy cells and generate unpleasant and often serious side effects in patients. It is well known that the application and dosage of drugs alone or in combination with other drugs depend on the stage of cancer and the patient's health status. In addition, there are generalized side effects for certain drugs, and many times these can vary among patients receiving the same drug.

2. CHITOSAN

Chitosan (Cs) is a linear amino polysaccharide composed of monomeric units of β-D-glucosamine and N-Acetyl D-glucosamine linked by β-bonds (1-4), Figure 2. Derived from the partial deacetylation of chitin [10] present in several natural resources, this biopolymer has attractive properties such as solubility in acidic aqueous media, reactive functional groups for functionalization and crosslinking [11], non-oxidation, biodegradability, muco adhesibility, biocompatibility, and FDA approval for use in wound dressings and dietary products [12], [13].

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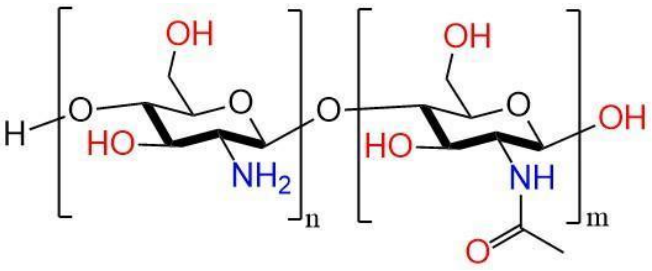

Figure 2. Chitosan structure.

Chitosan is highly appreciated and currently used in several industries such as paper; textile, including chitosan nanoparticles, loaded with textile dyes [14] and the incorporation of chitosan beads for the treatment of textile wastewater [15]; food and beverage, as biopolymers for edible food packaging [16] and antibacterial chitosan composite films with carbon spheres [17]; environmental, in the manufacture of composites with other materials to be used as sensors that detect polluting gases [18]; agriculture, as new antimicrobial agents for sustainable agriculture [19], [20]; water treatment and cosmetics sectors. In addition, thanks to its characteristics, Cs is considered as a biomacromolecule of high value particularly in the biomedical area where it is used to manufacture hemodialysis membranes, biodegradable suture threads, skin and bone substitutes [21] and is widely studied in the areas of drug delivery [22], [23] and transport of chemotherapeutic agents [24], [25].

In the field of drug delivery, various forms of this versatile polymer have been adopted for this purpose: tablets, beads, films, aerogels [26], hydrogels [27], nanofibers [28], nanoparticles, conjugates, and nanocomposites (including other materials and the drug) [29], Figure 3.

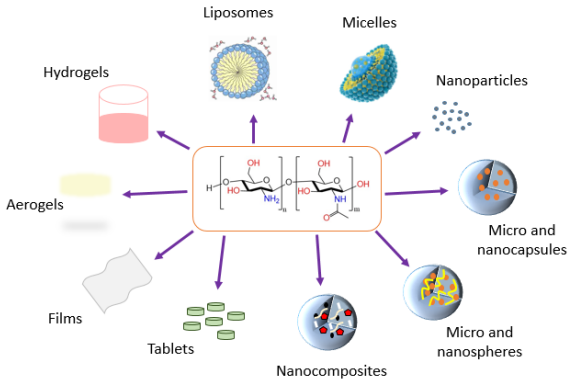

Figure 3. Forms of chitosan-based chemo drug delivery systems.

The most important properties that chitosan provides when included in drug delivery systems is the protection of the physiological environment [30], increases bioavailability, is biocompatible, so it will not generate toxicity [31]. Its metabolism within the biological system will lead to the decomposition into monomeric units easily absorbable and useful to the organism [32] aided by the action of lysozyme (present in the lung, liver, and bloodstream) which causes hydrolytic degradation of the acetylated residue of Cs [33]. The mucoadhesive property allows the delivery system to release the drug by adhering to the mucous membrane when it is administered locally in the gastrointestinal, nasal/ buccal, vaginal, urethral, or pulmonary tract [34]. The reactive functional groups (hydroxyl and amino groups) in their structure can give way to interactions with other biomolecules, materials, and even drugs that make Cs a potent carrier in drug delivery systems. The amino groups (NH_2) have the particularity of being proposed in an acidic medium and Cs become soluble; on the contrary, in a basic medium Cs are insoluble and precipitates, Figures 4 and 5.

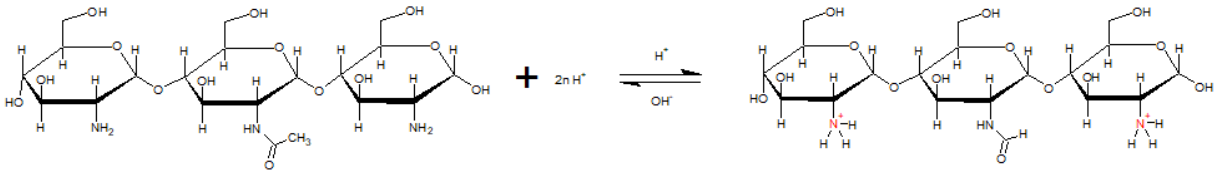

Figure 4. Acid-base equilibrium reaction of chitosan.

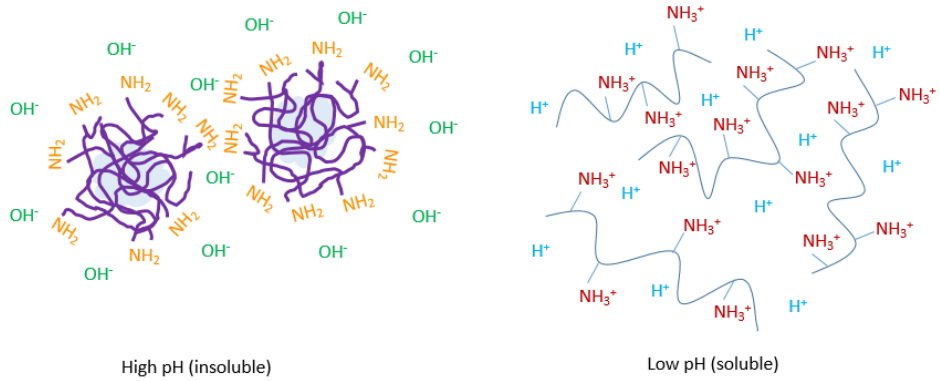

Figure 5. pH-dependent structural change of chitosan.

This property of solubility in acidic media and protonated amino groups provides fluid interaction with biomolecules, molecular drugs, and oppositely charged materials in an aqueous solution. It also influences the disintegration and release of the drug at the target site, taking into account that one of the characteristics of tumor cells is that they change the pH (acidify their microenvironment) in order to survive [35].

On the other hand, the interaction of Cs with molecules that modify it (with the addition of functional groups) and crosslinking agents, result in amphiphilic carriers that also allow the incorporation of drugs with hydrophobic characteristics [36], [37].

The chemistry of the cross-linked chitosan influences the new properties acquired by the system and the subsequent behavior of the system, in terms of the loading capacity of materials or drugs, release profiles, and of course the final degradation or decomposition of the Cs within the biological system. Among the most common crosslinking agents are sodium TPP, Pluronic F-68, and Pluronic F-127. New trials with average results have been carried out with sodium citrate. Glutaraldehyde, now less used in biomedicine because it has been shown to retard the degradation of Cs in biological media, is being implemented in other areas [38]. Crosslinking agents are generally used when the chitosan used is of low molecular weight.

As for the affinity of chitosan with other materials, due to its characteristics, it is not limited to its good interaction with materials and molecules of organic origin [39]. Materials such as silica nanoparticles, magnetic microspheres, and carbon derivatives (carbon nanotubes, nano horns, graphene, and its oxides, fullerenes, carbon dost, and nanodiamonds) have been studied in combination with chitosan for the development of new drug delivery systems and cancer therapy [40]–[42].

3. DRUG DELIVERY SYSTEMS COMPRISED OF CHITOSAN AND OTHER MATERIALS

For several years now, chitosan has been studied for the development of drug delivery systems, including preparation methods, drug loading studies, drug delivery, and kinetics, and modified chitosan [43], [44]. Due to its structural characteristics and under specific conditions, chitosan has dismissed possibilities of being modified with the inclusion of other molecules [45] that react or have some type of interaction with its reactive functional groups.

In addition, depending on the nature of this biopolymer and some of its main characteristics such as molecular weight and degree of deacetylation (%DD), crosslinking agents are used to improve encapsulation conditions, stability, and drug loading percentages. The most used are tripolyphosphate, pluronic F68, glutaraldehyde, and sodium citrate [11], among others. On the other hand, many nanocomposites involving chitosan are generally mixed with other materials to improve drug targeting, sustained loading and release, delivery, target site location, and cellular uptake [46], [47].

With applications in the biomedical area or in many other matrices, the affinity and compatibility that chitosan has with other materials is surprising, allowing the development of new materials that carry all its characteristics and also those resulting from the synergy for the desired purpose, Figure 6. For example, G. Barbosa et al, designed and characterized a composite film of chitosan and zeolite to be used as a dressing [48]. On the other hand, in order to observe the growth and immune response of rainbow trout, A.K. Oushani and coworkers studied the effect of dietary chitosan and clinoptilolite loaded with nano chitosan [49]. As an example, in the controlled delivery of diclofenac sodium, M.V. Dinu et al, performed the synthesis and characterization of a 3D biocomposite cryo gel of chitosan and clinoptilolite [50]. A new dual water-soluble thiolated chitosan/mesoporous calcium carbonate-based dual microsphere was developed by X. Liu et al. in order to obtain controlled dual drug delivery (in this case BSA and Rhodamine B) [51]. Chitosan-coated hollow copper sulfide nanoparticles for photothermal and immunological therapy were developed by L. Guo et al, demonstrating that the photoresponse of inorganic nanoparticles can improve the efficacy of therapy [52]. Some studies involving modified chitosan, chitosan with crosslinking agents, and composites for possible use as chemo pharmaceutical delivery systems can be seen in Table 1.

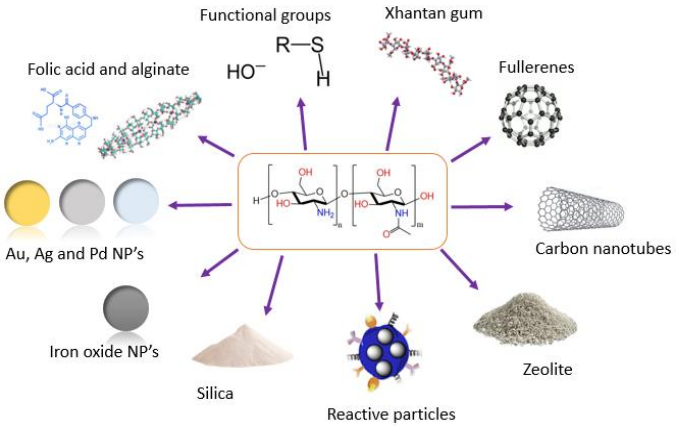

Figure 6. Chitosan-related and compatible materials for use in chemo drug delivery systems.

Table 1. Some studies conducted for the development of carriers of chemotherapy drugs that include chitosan and other materials.

General system	Components	Drug	Compatibility and interactions with chitosan	Interaction with the biological environment and the tumor microenvironment	Ref.
Chitosan + bioactive molecules or crosslinking agent	Chitosan + TPP, bis trimethyl acetamide + 4-bromomethyl -3-benzoic	5- fluorouracil	-Through the components, it was possible to covalently conjugate the drug 5-fluorouracil with the low molecular weight chitosan, by means of a photo permeable linker capable of being separated in a controlled manner to effectively release the drug.	- Drug release tests were not performed in solutions at different pHs simulating the physiologic environment and the tumor microenvironment. - Instead, it could be measured after radiation. The photocleavable crosslinker containing an ortho-nitrobenzyl derivative decomposes upon irradiation with λ=365nm UV-A light (which is safe for human cells) to form o-nitrobenzaldehyde.	[53]
	Chitosan + O-methyl-O′-succinyl polyethylene glycol + oleic acid	Camptothecin	- A two-step carbodiimide coupling of the components was formed with the chitosan leaving hydrophilic and hydrophobic spots. -This allowed the formation of polymeric micelles and at the same time the encapsulation of the hydrophobic drug with an EE of 78%.	- The resulting particles with an average size of 140 nm, intended for oral administration, were evaluated in gastrointestinal fluids (in vitro) for drug release. These showed low release in the gastric environment and controlled release in intestinal fluids.	[54]
	Chitosan + glycol carboxymethyl + β-cyclodextrins	5- fluorouracil, Doxorubicin and Vinblastine	- A complex was formed that made it possible to anchor the cyclodextrins to the polymeric matrix and within them encapsulate the drug molecules with hydrophobic characteristics by means of covalent bonds.	- The release behaviors with changes in pH of the three drugs were analyzed, For 5-Flu and VBL, the carrier was able to release the drugs at pH 5.0 (simulating the pH of the tumor microenvironment) but not at pH 7.0 (simulating the pH of the environment. physiologic). Instead, DOX was found to be pH sensitive in its release.	[55]
	Chitosan + stearic acid-O-carboxymethyl	Paclitaxel	- The assembled conjugate of chitosan with the component was obtained by an amide bond (chemical coupling). Then the drug was encapsulated.	- Faced with acidic pH stimuli simulating tumor micro-environment pH (5.0), unpacking and rapid drug release was caused with about 85% in 48 hours. On the other hand, simulating the pH of the physiologic environment (7.4), about 65% was released in the same time.	[56]
	Lauryl Chitosan + polyethyleneimine	p53 gene and doxorubicin	- Chitosan was initially modified with the addition of lauryl chloride and then mixed with polyethyleneimine. - The gene and drug were then loaded into different systems respectively.	- The release profile that was carried out for doxorubicin at pH 5.0 and 7.4 showed that delivery was faster at pH 5.0 with around 59% in the first 24 hours, with respect to the release of 19% of the drug at pH 7.4.	[57]
	Chitosan + Pluronic F-127 +TPP	Gemcitabine	- The chitosan solution was first prepared in acid medium and then various concentrations of TPP were added. These solutions were then mixed with different concentrations of Pluronic F-127.	- The release profile test was done under conditions of physiological medium pH (7.4) at 37 ° C, indicating that at 72 hours the chitosan system delivered 85.86% of the drug. -The inclusion of Pluronic in different concentrations in the rest of the	[58]

			- In addition, solutions with different concentrations of the drug and TPP were made to be loaded onto the nanoparticle systems by ionic gelling.	solutions influenced to decrease the percentage of drug release in this medium with 79.58%, 71.23%, and 57.74 for chitosan-PF (10%, 15%, and 20%) respectively.	
Modified chitosan + other materials	Thiolated Chitosan + thiolated halloysite NT's	Doxorubicin	- The thiol group was included in the components of the system to cross-link them and to be able to obtain hydrogels with electrostatic interactions. - The DOX was initially loaded onto the halloysite NT's and then a cross-linked polymeric matrix was formed with the modified chitosan.	- The route of administration of this system is visualized as a local injection into the tumor. - The gel was pH-sensitive to release the drug. At pH of the physiologic medium (7.4,) the gel released about 64% of DOX in 120h and at tumor microenvironment pH (5.5) it released 100% of DOX in 120h.	[59]
	Carboxymethyl chitosan + alginate + folic acid + mesoporous silica	5- Fluorouracil and Bis-demethoxy curcumin	- First, the synthesis of amino-functionalized mesoporous silica NPs was carried out. Then separately the folic acid-conjugated o-carboxy methyl chitosan was synthesized. - Finally, when these materials were mixed, the synthesis of nanoparticles of aminated mesoporous silica - alginate / folic acid conjugated with O-carboxy methyl chitosan-gelatin was obtained. In the end, the drugs were loaded into the system.	- The drug delivery profile was evaluated in solutions of pH 1.2 and 7.4 simulating gastrointestinal conditions, resulting in that the highest drug release occurring at pH 7.4 and is lower at pH 1.2 due to the nature of the material. - Likewise, hemocompatibility studies were performed with PBS as a negative control and Na_2CO_3 as a positive control. The results indicated a hemolysis percentage > 3%, complying with the regulations.	[60]
Chitosan + other materials	Chitosan + magnetic nanoparticles	Telmisartan	- The chitosan covered the magnetic nanoparticle through the interactions between the OH- groups and the protonated amino groups of the chitosan. - Telmisartan was then loaded into the system through the amide bond between the remaining free protonated amino groups and the carboxylic groups of the drug.	- The drug is not soluble in a biological medium and although it is fully exposed on the chitosan coat, the objective is to improve the bioavailability of the chemotherapy agent and to be able to guide the magnetic nanoparticles externally. - The acidic tumor microenvironment favors the hydrolysis of the amide bond and thus the release of the drug.	[61]
	Chitosan + magnetic nanoparticles + TPP	Gemcitabine	- A co-precipitation of Fe salts in the presence of chitosan and TPP was performed, expecting the TPP to cross-link the chitosan chains around the magnetic cores. Finally, gemcitabine was loaded into the system.	- The drug release profile was performed in acetate buffer solutions (pH 4.2 and 5.2) and in phosphate buffer solution (pH 7.2) simulating pHs of the tumor microenvironment and physiological environment. - In 24 hours in pH 4.2 solution, the system released 65.4% of the drug, in pH 5.2 solution it released 33%, and in pH 7.2 solution it released 8% of Gemcitabine.	[62]
	Chitosan + montmorillonite	Doxorubicin	- In both cases, carriers of montmorillonite and chitosan + montmorillonite, the positively charged chemotherapeutic drug bound strongly to the negatively charged aluminosilicate. With the inclusion of cationic chitosan, it is expected to neutralize this strong bond.	- The reduced solubility of the aluminosilicate/chitosan nanocomposite at gastric pH (1.2) offers advantages for oral administration. Furthermore, the mucoadhesive property of chitosan can improve bioavailability in the gastrointestinal tract. - At pH of the physiologic medium (7.4), in 60 hours the systems release up to 26% of the drug, and at tumor microenvironment pH (5.3) up to 29%, indicating controlled release.	[63]
	Chitosan + vanillin + calcium ferrite + TPP	Curcumin	- Vanillin was covalently linked from its aldehyde functional group to the nitrogen of the free amino group of the molecular weight chitosan (40-60 KDa) to enhance the hydrophobic interaction with Curcumin. The TPP helped cross-link the chitosan molecules. - The cover of the calcium ferrite nanoparticles was easily incorporated into the system.	- In the simulation with pH of the physiologic medium (7.4), Curcumin carrier nanosystem, released about 59.4% in 24 hours. Simulating gastric pH (1.2) for a possible oral route of administration, the system released at least 78.3% of the curcumin, at the same time. -In the tests carried out to see the release with the magnetic effect, the system released 86.2% and 79.5% in 4.5 hours with the application of magnetic fields of 10mT and 5mT, respectively.	[64]
	Chitosan + magnetic nanoparticles (Fe_3O_4) + Pluronic F-68	Gemcitabine	- The Fe_3O_4 nanoparticles were obtained from $FeCl_2$ and $FeCl_3$ chlorides. Then a solution of chitosan in acetic acid with Pluronic was prepared to which the MNPs were added. Finally, by the coacervation method, a sodium sulfate solution was precipitated on the solution with the main components. - Gemcitabine loading was performed by entrapment (included in the same initial aqueous solution) and adsorption method.	- Drug release assays were performed in solutions of pH 7.4 (simulating physiological environment) and pH 5.0 (simulating tumor microenvironment) for the two loading methods. - The fastest delivery of GB was found to be for the adsorption loading method in both pH 7.4 and pH 5.0 solutions. Adsorption method: in 2 hours it has already released 100% of the drug at both pHs. Entrapment method: more controlled release, at pH 7.4 in 125 hours it released 100% of the drug and at pH 5.0 in 48 hours it released the same percentage of the drug.	[65]
	Chitosan + magnetic nanoparticles (Fe_3O_4) + TPP	Doxorubicin	- During the in situ co-precipitation of Fe salts, together with chitosan and TPP, the polymer tends to envelop the anionic centers of Fe_3O_4 while TPP is crosslinking the chitosan molecules around these particles. - Finally, the Doxorubicin was loaded into the chitosan layers.	- Drug delivery was performed at two different pHs (4.2 and 5.0) simulating endosomal conditions (external and internal tumor microenvironment). - A burst release was observed in the initial 30 min stage, of 20% - 30% at pH 4.2 and 15% - 20% at pH 5.0. Then in 15 hours, the sample (S_1) released 61% DOX at pH 4.2 and 35% DOX at pH 5.0.	[66]

4. HYPOTHESIZED CHEMO PHARMACEUTICAL DELIVERY MECHANISMS

Whether from systems where chitosan is the protagonist in the form of modified and/or cross-linked structure or in composite systems with other organic and inorganic materials, it is essential to visualize the mechanisms in chemo pharmaceutical delivery from the synthesis and characterization stages.

According to Singh et al [67], the main mechanisms involved in drug release are diffusion, which involves the penetration of the dissolution fluid through the coating to the interior, where it dissolves the contents and is expelled through the pores; dissolution, which as the name implies, refers to the dissolution of the coating material where it will expose the internal contents. In the osmosis mechanism, the coating material acts as a semi-permeable membrane that will then create a pressure difference to expel the dissolved contents. Finally, erosion comprises the susceptibility of the coating material to the pH of the medium and/or enzymatic hydrolysis, thus causing the release of the contents.

For their part, Xie L et al [68], point out that there are release strategies to direct drugs to the target site. Guided drug delivery can be passive, active, or physicochemical. Passive delivery comprises the natural form of delivery where the increased permeability-retention effect (EPR) of the tumor site is exploited. Active delivery refers to the addition of biomarkers and active molecules on the surface of the particle so that they can then interact with specific receptors guiding drug delivery. Physicochemical delivery involves the release of the drug by the application of physical or chemical methods, including the application of a magnetic field and/or light to guide and release the drug. Combinations of these strategies can also be considered in the design and development of new chemo pharmaceutical delivery systems.

Precisely, there is research where the responses to stimuli in chitosan-based chemo pharmaceutical delivery systems are studied. One example is the work carried out by Rajalakshmi et al [69], who, with the aim of avoiding premature drug release through response to dual stimuli such as pH and light, synthesized a WO_3-Fe-Au@C nanocomposite to transport Doxorubicin. This system showed that the functionalization of Au-Np's improved the photoactive property of the material, biocompatibility in fibroblast cells, and a doxorubicin loading efficiency of about 70.2%. The obtained particle sizes ranging from 18 - 26 nm would allow a passive release strategy. The release assay showed that it is pH sensitive, being higher at acidic pH (5), and under visible light irradiation the drug is also efficiently released at the same pH. With these results, it is evident that the nanocomposite was also designed thinking of a directed physicochemical strategy and release by erosion or dissolution mechanisms.

3D printed systems for colon-specific delivery of camptothecin loaded in chitosan micelles were synthesized by Almeida et al [70], in order to improve the efficacy of therapy by oral administration. The characterization results of these systems show particle sizes ranging from 130 to 262 nm after incorporation of all the materials and drug loading. In addition, the micelles present a highly positive surface charge which indicates that interaction with epithelial cells can be favored. The micelles were sealed with an enteric coating to protect the nanosystems from the harsh gastrointestinal environment (GIT). Simulated digestion tests after oral administration and intestinal permeability were performed. The systems remained intact at gastric pH and only released the micelles at colonic pH, effectively delivering the drug directly to the target site.

Thanks to the carboxylated chitosan included in a nanocomposite based on aptamer-mesoporous silica nanoparticles for the treatment of breast cancer, the efficacy of the doxorubicin delivery system is improved. The promising strategy accompanying this study is the nanoparticle targeting of overexpressed cell surface receptors belonging to an active delivery form. These bioconjugates synthesized by Lohiya and Katti [71], proved to be highly reproducible and monodisperse, spherical in shape, and with an average particle size of < 100nm. The chitosan coating provided the systems with high pH sensitivity (higher delivery at pH 5.5 in 21 days compared to pH 7.4; 28% and 3.9%, respectively) and endo/lysosomal escape capacity, increasing drug delivery into the tumor cell. Partial carboxylation of the polymeric shell allowed a higher drug delivery in a short time. The aptamer-mesoporous silica included in the system played a very important role as a calcination agent, which in this case, in synergy with the rest of the materials and structures produced an improvement in the release efficiency and in the active and physicochemical targeting to the breast cancer cells.

As mentioned above, knowledge of biology and biochemical expressions of cells in the different organs makes it possible to study cases of active direction with overexpression of factors or genes. This is the case of the epidermal growth factor receptor (EGFR) associated with lung cancer. The work of Maya et al [72], consisted of evaluating the effect of chitosan nanoparticles cross-linked with PGA, loaded with docetaxel, and targeted to the EGF receptor to act on non-small cell lung cancer cells. Obtaining particles with sizes ranging from 20 to 200 nm, confirming the results of EGFR specificity assays of nanoparticles with increased uptake rate and cytotoxicity studies by MTT / LDH and flow cytometry, this nanocomposite becomes an effective alternative to conventional non-specific chemotherapy. Of course, it alludes to an active drug delivery strategy.

The administration of two or more chemotherapeutic agents in a single therapy has visibly improved the efficacy of treatment in shrinking tumors and extending the life expectancy of patients. In the development of new delivery systems based on chitosan and other materials, it is possible to think about the inclusion of several drugs and the synergy of the materials, to improve the therapy and to continue avoiding the unpleasant side effects. Thus, the work of Khoshtabiat et al [73], was based on developing a therapy based on a Fenton system for magnetic hydroxyapatite and chitosan, loaded with a double drug against colon cancer (cisplatin and methotrexate). In vitro and in vivo experiments showed higher toxicity of the final system compared to free drugs. Drug delivery assays (in solutions at pH 7.4 and 4.5, with and without SMF), indicated that the nanocomposite is sensitive to pH and magnetic field, releasing the highest % of drug in conditions of pH 4.5 and pH 4.5 with SMF, The most effective antitumor activity occurred when cells treated with the new system were exposed to SMF 0.9 T and no damage to healthy cells and tissues was observed. What was observed was the following: Fe ions released inside the cancer cells triggered the Fenton reaction, while other factors such as the release of chemo pharmaceuticals, the reduction of intracellular glutathione, and the application of SMF aggravated the Fenton reaction. This consequently led to the generation of reactive oxygen species (ROS) and the induction of apoptosis. Active targeting by methotrexate and magnetic targeting by hydroxyapatite under a magnetic field increased tumor selectivity and enhanced the accumulation and cellular uptake of the nanocomposite in the tumor, also being considered a nano platform responsive to redox and pH stimuli.

Another example of dual inclusion and transport of chemo pharmaceuticals is shown in the study by Tao et al [74]. They fabricated alginate-coated hollow chitosan nanospheres by the hard template method. Inside them they loaded paclitaxel by adsorption and on the surface through electrostatic interactions they added doxorubicin, to evaluate the effect on A549 non-small cell lung cancer cells. The approximate size of the nanosystems was 130 nm, these demonstrated in cellular uptake experiments that it effectively accumulated in the cytoplasm. Also, the alginate/chitosan structure alone showed no toxicity unlike the system loaded with the two chemotherapeutic agents. The biodegradability of the complex in acetate buffer solution pH 4.5 was evidenced. Drug release delivery studies showed that the first drug to be released was DOX followed by PTX. In a medium at pH 6.8 and at 37°C, at 24 hours approximately 77% and 55% were released, respectively.

The intrinsic loading and transport restrictions of some drugs call for the innovative design of delivery systems. In this case, chitosan as a biocompatible, biodegradable biopolymer, with all its crosslinking, modification, and mixing capabilities with other materials, can provide unique features to the system to improve therapy. In Nazli and Açıkel's study [75], resveratrol loading was performed in pH-sensitive alginate/chitosan smart hydrogels for controlled release. Resveratrol is a drug used for the treatment of breast, skin, brain, and prostate cancer. It has characteristics that restrict its administration such as low stability against oxidation, high photosensitivity, insolubility in water or low solubility, and insufficient systemic transport. To solve these challenges, beads (hydrogels) were synthesized as polyelectrolyte complexes with alginate and chitosan with an approximate size of 2.286 mm. Understanding that their administration can be by oral route, the delivery profile and kinetic study were performed in solutions of pH 7.4; 6.8; 5.5, and 1.2 representing different parts of the gastrointestinal tract (GIT) and blood. The results of resveratrol delivery kinetics may finally suggest that drug release occurs mainly by diffusion phenomena in gastric medium and in the ascending and descending columns of the large intestine. It further considers that diffusion and swelling mechanisms predominantly control the transport of resveratrol at pH 6.8 representing the medium of the small intestine and at pH 7.4 representing the blood.

The characteristics of the amphiphilic materials that can be formed with the use of chitosan in chemo pharmaceutical delivery systems allow overcoming the challenge of not being able to deliver drugs with hydrophobic characteristics to the tumor site. The work of Qu et al [76], suggests the synthesis of paclitaxel micelles based on pH-responsive amphiphilic chitosan and functionalized with Anisamide, for sigma-1 receptor-targeted therapy in prostate cancer. With an approximate size of 162.8, nm these micelles were able to encapsulate the drug paclitaxel with hydrophobic characteristics. The maleic and phosphoryl groups cooperatively contributed to the pH-responsive drug release due to an internal conversion in the endo/lysosomal acidic microenvironment from hydrophilic to hydrophobic. The delivery profile in pH 7.4 and 4.5 solutions indicated paclitaxel delivery of approximately 21.5 and 76%, respectively, within 48 hours. The strategy of targeting the system with the inclusion of anisamide and its affinity for the sigma-1 receptor overexpression in prostate tumors, allowed for enhanced internalization and rapid release of the drug intracellularly, increasing cytotoxicity. In addition, *in vivo* studies verified that the carrier system could accumulate to a great extent at the tumor site after 24 hours of administration, which evidenced tumor inhibition and a prolonged survival period.

The combination of drug delivery strategies and different release methods allows the design of new chemo pharmaceutical delivery systems to improve their efficacy by being able to target cancer cells without affecting healthy cells. In the study by Fathi et al [77], multifunctional magnetic nanoparticles based on chitosan and conjugated with methotrexate were designed for the targeted delivery of Erlotinib in the targeted therapy of ovarian cancer. Modification of chitosan with sodium dodecyl sulfate and maleic anhydride yielded a polymerizable organic soluble precursor. Then, thermo- and pH-sensitive monomers were grafted onto the chitosan by free radical polymerization. With the chitosan copolymer, a ferrofluid of magnetic nanoparticles was then prepared by an inclusion complex between the carboxylic groups of the Cs and the MNP's. Methotrexate was conjugated to the amino groups of Cs (based on structural similarity to folic acid) and acted as a targeting ligand. These particles with an approximate size of 112 nm were able to encapsulate up to 86% of Erlotinib and release it in a heat and pH-dependent behavior. A higher drug delivery occurred in solution pH 5.5 at 37°C and was lower in solution pH 7.4 at 37°C. The cellular uptake of the nanosystem was validated by high internalization (thanks to methotrexate targeting) and high toxicity on OVCAR-3 cancer cells. Rightly, the array presents itself as a great option for targeted therapy of various solid tumors by considering active and physicochemical strategies in chemo pharmaceutical delivery.

Kinase inhibitor drugs act by blocking the abnormal protein that sends multiplication signals to cancer cells. Very useful in preventing the spread of cancer cells, they are being included and studied in chitosan-based drug delivery nanosystems.

This is the case of the work of Zhao et al [78], who synthesized chitosan/sulfo butylether-B-cyclodextrin nanoparticles loaded with the hydrophobic kinase inhibitor, Ibrutinib. Nanoparticles with average sizes of 277.9 nm, demonstrated mucoadhesive properties (perfect for considering oral drug delivery). In vitro release assays showed that the ibrutinib-loaded nanoparticles occurred in two phases in the gastric environment, starting with a burst release (2 hours) and then becoming more delayed with a slower rate (totaling approximately 30% in 12 hours). On the other hand, the release profile performed in simulated intestinal conditions showed a profile similar to the gastric one, demonstrating that ibrutinib-loaded nanoparticles present pH-independent release. In this case, a dose reformulation of the chemo pharmaceutical is required. Basically, in this design, a chemo pharmaceutical with hydrophobic characteristics was incorporated, with rapid pH-independent absorption, taking advantage of the highly adhesive properties of the chitosan biopolymer for oral administration.

Various pathways to reach the same tumor (in a specific organ) can be evaluated in the design of these new chemo pharmaceutical delivery systems. This is the case for CT26 cancer cells of colorectal carcinoma, where an intravenous targeted therapy can be estimated. Bhattacharya's study [79] shows the fabrication and characterization of chitosan-based polymeric nanoparticles carrying Imatinib for application in colorectal cancer. With particles of approximately 208 nm in size free to circulate in the blood medium, hemolysis studies show only 0.46%, indicating that the nanocomposite is safe for I.V administration. In addition, histopathological studies showed no damage to healthy cells. The drug release profile was performed in buffer pH 6.8 at 37°C where the nanosystem released 86.45% of imatinib after 84 hours. The release occurred in two phases, up to 2 hours it was a fast delivery and then it had a slower and controlled behavior, the latter due to the activation of the polymeric matrix diffusion.

5. CHITOSAN-BASED ANTICANCER DRUG DELIVERY SYSTEMS IN CLINICAL TRIALS

The U.S. Food and Drug Administration (FDA) is the agency in charge of controlling the drug approval process and is responsible for reviewing and evaluating new medical devices and drugs before companies can sell them. The center as such does not test the drugs themselves but carries out in-depth research in the areas of quality control, safety, and efficacy of the drugs. Before reaching approval by this agency and complying with all the requirements, there are the clinical trials that comprise one of the big and final steps to reach the consumers who need them.

Although many initial studies have been carried out on the design of new chitosan-based chemo pharmaceutical delivery systems, few are being tested in clinical phases. The National Library of Medicine [80] provides detailed information on the systems in clinical phases, Table 2.

Table 2. Current clinical phase studies of chitosan-based delivery systems for chemotherapeutic agents

NCT	Study title	Condition disease	Drug or device	Study type	Phase	Subjects Number
02967146	Anti-adhesive Effect and Safety of a Mixed Solid of Poloxamer, Gelatin and Chitosan(Mediclore®) After Axillary Dissection for Breast Cancer	Breast cancer Surgery: Axillary Dissection	Device: adhesive barrier	Interventional	Phase 3	170
03712371	Study of Chitosan for Pharmacologic Manipulation of AGE (Advanced Glycation Endproducts) Levels in Prostate Cancer Patients	Prostate Cancer	Drug: Chitosan	Interventional	Phase 1 Phase 2	45
03202446	Randomized Clinical Trial Evaluating the Use of the Laser-Assisted Immunotherapy (LIT/inCVAX) in Advanced Breast Cancer	Breast Cancer Stage IIIA Breast Cancer, Stage IIIB Breast Cancer Stage IV	Drug: 1% Glycated Chitosan Device: Photothermal Laser Drug: Placebo	Interventional	Phase 3	18
03993678	Intratumoral Injection of IP-001 Following Thermal Ablation in Patients With Advanced Solid Tumors.	Advanced Solid Tumors	Drug: IP-001 Device: Thermal Ablation	Interventional	Phase 1 Phase 2	39

Of the studies found, two are in recruitment status (03712371 and 03993678). Completed is 03202446 and lastly with unknown status without any reference is 02967146.

Many of the reasons why clinical studies fail are related to failures in the simulation of original laboratory conditions, not scalable; complex experimental design that affects the mass production of the drug or device; the drug does not meet quality control standards and some inconclusive results obtained from animal tests to trials in a few patients. Definitely, the technology and development of computational models together with increased testing in animals and humans under conditions could come to provide more information and conclusive results in these stages of approval of new delivery systems.

CONCLUSIONS

Many if not all types of tumor cancers have differences in biological behavior and affinity to organs for metastasis. In addition to this, other characteristics such as the stage of tumor progression, immune system status, and other diseases that the patient has influenced the form of treatment of cancer he/she suffers from. A series of pharmaceutical forms and different possible routes of administration are then derived to improve the efficacy of chemotherapy treatment.

Opportunities for treatment enhancement based on chemotherapeutic agents are booming, thanks to a better understanding of the biology and biochemistry of the tumor environment.

Biocompatible and biodegradable, non-toxic biopolymers of the biocompatible and biodegradable type have gained momentum in the design of chemo pharmaceutical delivery systems, thanks to the characteristics they can bring to the process. Chitosan has been extensively studied for some years for use in biomedicine in the area of drug delivery systems. Its affinity, modification capacity, and compatibility with other materials make it a versatile material to succeed in systems based on it, allowing the improvement of the therapy.

REFERENCES

1. OMS, "Cáncer," *Organización Mundial de la Salud*, 2018. [Online]. Available: https://www.who.int/es/news-room/fact-sheets/detail/cancer. [Accessed: 10-Oct-2019].
2. International Agency for Research on Cancer, "Cancer Tomorrow," *Global Cancer Observatory*, 2018. [Online]. Available: https://gco.iarc.fr/tomorrow/graphic-isotype?type=0&population=900&mode=population&sex=0&cancer=39&age_group=value&apc_male=0&apc_female=0. [Accessed: 10-Oct-2019].
3. N. C. Institute, "A to Z List of Cancer Types - National Cancer Institute." [Online]. Available: https://www.cancer.gov/types. [Accessed: 13-Sep-2021].
4. E. S. dos Santos, V. P. Wagner, J. Cabral Ramos, D. W. Lambert, R. M. Castilho, and A. F. Paes Leme, "Epigenetic modulation of the tumor microenvironment in head and neck cancer: Challenges and opportunities," *Crit. Rev. Oncol. Hematol.*, vol. 164, no. May, p. 103397, 2021.
5. D. Ribatti, T. Annese, and R. Tamma, "Controversial role of mast cells in breast cancer tumor progression and angiogenesis," *Clin. Breast Cancer*, 2021.
6. Y. Deng, S. Y. Liu, S. L. Chua, and B. L. Khoo, "The effects of biofilms on tumor progression in a 3D cancer-biofilm microfluidic model," *Biosens. Bioelectron.*, vol. 180, no. February, p. 113113, 2021.
7. S. Mukhopadhyay, K. K. Mahapatra, P. P. Praharaj, S. Patil, and S. K. Bhutia, "Recent progress of autophagy signaling in the tumor microenvironment and its targeting for possible cancer therapeutics," *Semin. Cancer Biol.*, 2021.
8. Q. Hu and Y. Luo, "Recent advances of polysaccharide-based nanoparticles for oral insulin delivery," *Int. J. Biol. Macromol.*, vol. 120, pp. 775–782, 2018.
9. Cancer.Net, "Understanding Chemotherapy | Cancer.Net," *2019*. [Online]. Available: https://www.cancer.net/navigating-cancer-care/how-cancer-treated/chemotherapy/understanding-chemotherapy. [Accessed: 14-Sep-2021].
10. Z. Mármol, G. Páez, M. Rincón, K. Araujo, and C. Aiello, "Quitina y Quitosano polímeros amigables. Una revisión de sus aplicaciones Chitin and Chitosan friendly polymer A review of their applications," *Rev. Tecnocientifica URU*, no. August 2016, pp. 53–58, 2011.
11. M. T. Taghizadeh, H. Ashassi-Sorkhabi, R. Afkari, and A. Kazempour, "Cross-linked chitosan in nano and bead scales as drug carriers for betamethasone and tetracycline," *Int. J. Biol. Macromol.*, vol. 131, pp. 581–588, 2019.
12. S. Naskar, S. Sharma, and K. Kuotsu, "Chitosan-based nanoparticles: An overview of biomedical applications and its preparation," *J. Drug Deliv. Sci. Technol.*, vol. 49, no. June 2018, pp. 66–81, 2019.
13. S. Rodrigues, M. Dionísio, C. R. López, and A. Grenha, "Biocompatibility of Chitosan Carriers with Application in Drug Delivery," *J. Funct. Biomater.*, vol. 3, no. 3, pp. 615–641, 2012.
14. E. M. Costa, S. Silva, M. Veiga, P. Baptista, F. K. Tavaria, and M. E. Pintado, "Textile dyes loaded chitosan nanoparticles: Characterization, biocompatibility and staining capacity," *Carbohydr. Polym.*, vol. 251, no. August 2020, p. 117120, 2021.
15. B. Lima Santos Klienchen Dalari, C. Lisboa Giroletti, L. Dalri-Cecato, D. Gonzaga Domingos, and M. E. Nagel Hassemer, "Application of heterogeneous photo-Fenton process using chitosan beads for textile wastewater treatment," *J. Environ. Chem. Eng.*, vol. 8, no. 4, p. 103893, 2020.
16. S. Sady, A. Błaszczyk, W. Kozak, P. Boryło, and M. Szindler, "Quality assessment of innovative chitosan-based biopolymers for edible food packaging applications," *Food Packaging Shelf Life*, vol. 30, no. September 2021.
17. Z. Wu, S. Tang, W. Deng, J. Luo, and X. Wang, "Antibacterial chitosan composite films with food-inspired carbon spheres immobilized AgNPs," *Food Chem.*, vol. 363, no. June, p. 130342, 2021.
18. H. Park *et al.*, "Flexible and disposable paper-based gas sensor using reduced graphene oxide/chitosan composite," *J. Mater. Sci. Technol.*, vol. 101, no. 2, pp. 165–172, 2022.
19. T. Ahmed *et al.*, "Bioengineered chitosan-magnesium nanocomposite: A novel agricultural antimicrobial agent against Acidovorax oryzae and Rhizoctonia solani for sustainable rice production," *Int. J. Biol. Macromol.*, vol. 168, pp. 834–845, 2021.
20. P. L. Kashyap, X. Xiang, and P. Heiden, "Chitosan nanoparticle-based delivery systems for sustainable agriculture," *Int. J. Biol. Macromol.*, vol. 77, pp. 36–51, 2015.
21. S. M. Ahsan, M. Thomas, K. K. Reddy, S. G. Sooraparaju, A. Asthana, and I. Bhatnagar, "Chitosan as a biomaterial in drug delivery and tissue engineering," *Int. J. Biol. Macromol.*, vol. 110, pp. 97–109, 2018.
22. C. Vauthier, C. Zandanel, and A. L. Ramon, "Chitosan-based nanoparticles for in vivo delivery of interfering agents including siRNA," *Curr. Opin. Colloid Interface Sci.*, vol. 18, no. 5, pp. 406–418, 2013.
23. N. Lal, J. Dubey, P. Gaur, N. Verma, and A. Verma, "Chitosan-based in situ forming polyelectrolyte complexes: A potential sustained drug delivery polymeric carrier for high dose drugs," *Mater. Sci. Eng. C*, vol. 79, pp. 491–498, 2017.
24. M. Prabaharan, "Chitosan-based nanoparticles for tumor-targeted drug delivery," *Int. J. Biol. Macromol.*, vol. 72, pp. 1313–1322, 2015.
25. X. Zhang, X. Yang, J. Ji, A. Liu, and G. Zhai, "Tumor targeting strategies for chitosan-based nanoparticles," *Colloids Surfaces B Biointerfaces*, vol. 148, pp. 460–473, 2016.
26. S. Wei, Y. C. Ching, and C. H. Chuah, "Synthesis of chitosan aerogels as promising carriers for drug delivery: A review," *Carbohydr. Polym.*, vol. 231, no. November 2019, p. 115744, 2020.
27. A. M. Olaru, L. Marin, S. Morariu, G. Pricope, M. Pinteala, and L. Tartau-Mititelu, "Biocompatible chitosan-based hydrogels for potential application in local tumor therapy," *Carbohydr. Polym.*, vol. 179, no. August 2017, pp. 59–70, 2018.
28. R. Sedghi, M. Gholami, A. Shaabani, M. Saber, and H. Niknejad, "Preparation of novel chitosan derivative nanofibers for prevention of breast cancer recurrence," *Eur. Polym. J.*, vol. 123, p. 109421, 2020.
29. A. Ali and S. Ahmed, "A review on chitosan and its nanocomposites in drug delivery," *Int. J. Biol. Macromol.*, vol. 109, pp. 273–286, 2018.
30. R. Shanmuganathan, T. N. J. I. Edison, F. LewisOscar, P. Kumar, S. Shanmugam, and A. Pugazhendhi, "Chitosan nanopolymers: An overview of drug delivery against cancer," *Int. J. Biol. Macromol.*, vol. 130, pp. 727–736, 2019.
31. C. Galo, S. Monsalve-Rozas, and L. Vergara-González, "Microencapsulation of Erlotinib and Nanomagnetite Supported in Chitosan as Potential Oncologic Carrier," *Polymers (Basel).*, vol. 13, no. 1244, p. 22, 2021.
32. Z. Shariatinia, "Pharmaceutical applications of chitosan," *Adv. Colloid Interface Sci.*, vol. 263, pp. 131–194, 2019.
33. N. Islam, I. Damour, and M. O. Taha, "Degradability of chitosan micro/nanoparticles for pulmonary drug delivery," *Heliyon*, vol. 5, no. 5, p. e01684, 2019.
34. M. A. Mohammed, J. T. M. Syeda, K. M. Wasan, and E. K. Wasan, "An overview of chitosan nanoparticles and its application in non-parenteral drug delivery," *Pharmaceutics*, vol. 9, no. 4, 2017.
35. Y. Xiao and D. Yu, "Tumor microenvironment as a therapeutic target in cancer," *Pharmacol. Ther.*, vol. 221, p. 107753, 2021.
36. J. Shim, J. Kang, and S. Il Yun, "Chitosan–dipeptide hydrogels as potential anticancer drug delivery systems," *Int. J. Biol. Macromol.*, vol. 187, no. July, pp. 399–408, 2021.

37. S. Inphonlek, P. Sunintaboon, M. Léonard, and A. Durand, "Chitosan/carboxy methylcellulose-stabilized poly(lactide-co-glycolide) particles as bio-based drug delivery carriers," *Carbohydr. Polym.*, vol. 242, no. January, p. 116417, 2020.
38. M. A. Khapre, S. Pandey, and R. M. Jugade, "Glutaraldehyde-cross-linked chitosan–alginate composite for organic dyes removal from aqueous solutions," *Int. J. Biol. Macromol.*, vol. 190, no. July, pp. 862–875, 2021.
39. Y. Ma, K. J. Thurecht, and A. G. A. Coombes, "Development of enteric-coated, biphasic chitosan/HPMC microcapsules for colon-targeted delivery of anticancer drug-loaded nanoparticles," *Int. J. Pharm.*, vol. 607, no. June, p. 121026, 2021.
40. F. Khatami, M. M. Martin, N. M. Danesh, A. R. Bahrami, K. Abnous, and S. M. Taghdisi, "Targeted delivery system using silica nanoparticles coated with chitosan and AS1411 for combination therapy of doxorubicin and antimiR-21," *Carbohydr. Polym.*, vol. 266, no. April, p. 118111, 2021.
41. X. Chen *et al.*, "Magnetic and self-healing chitosan-alginate hydrogel encapsulated gelatin microspheres via covalent cross-linking for drug delivery," *Mater. Sci. Eng. C*, vol. 101, no. April, pp. 619–629, 2019.
42. Z. Khorsandi, M. Borjian-Boroujeni, R. Yekani, and R. S. Varma, *Carbon nanomaterials with chitosan: A winning combination for drug delivery systems*. Elsevier B.V., 2021.
43. S. A. Agnihotri, N. N. Mallikarjuna, and T. M. Aminabhavi, "Recent advances on chitosan-based micro-and nanoparticles in drug delivery," *J. Control. Release*, vol. 100, no. 1, pp. 5–28, 2004.
44. G. Cárdenas-Triviño, M. Burgos, and C. Von Plessing, "Microencapsulation of oxolinic acid with chitosan beads," *J. Chil. Chem. Soc.*, vol. 63, no. 4, pp. 4229–4238, 2018.
45. S. Salalah and I. W. Lenggoro, "Nanoparticles carrying biological molecules: Recent advances and applications," *KONA Powder Part. J.*, vol. 2018, no. 35, pp. 89–111, 2018.
46. S. Yu, X. Xu, J. Feng, M. Liu, and K. Hu, "Chitosan and chitosan coating nanoparticles for the treatment of brain disease," *Int. J. Pharm.*, vol. 560, no. February, pp. 282–293, 2019.
47. E. Zhang, R. Xing, S. Liu, Y. Qin, K. Li, and P. Li, "Advances in chitosan-based nanoparticles for oncotherapy," *Carbohydr. Polym.*, vol. 222, no. June, p. 115004, 2019.
48. G. P. Barbosa, H. S. Debone, P. Severino, E. B. Souto, and C. F. Da Silva, "Design and characterization of chitosan/zeolite composite films - Effect of zeolite type and zeolite dose on the film properties," *Mater. Sci. Eng. C*, vol. 60, pp. 246–254, 2016.
49. A. Khani Oushani, M. Soltani, N. Sheikhzadeh, M. Shamsaie Mehrgan, and H. Rajabi Islami, "Effects of dietary chitosan and nano-chitosan loaded clinoptilolite on growth and immune responses of rainbow trout (Oncorhynchus mykiss)," *Fish Shellfish Immunol.*, vol. 98, no. July 2019, pp. 210–217, 2020.
50. M. V. Dinu, A. I. Cocarta, and E. S. Dragan, "Synthesis, characterization and drug release properties of 3D chitosan/clinoptilolite biocomposite cryogels," *Carbohydr. Polym.*, vol. 153, pp. 203–211, 2016.
51. X. Liu, X. Li, R. Zhang, L. Wang, and Q. Feng, "A novel dual microsphere-based on water-soluble thiolated chitosan/mesoporous calcium carbonate for controlled dual drug delivery," *Mater. Lett.*, vol. 285, p. 129142, 2021.
52. L. Guo *et al.*, "Combinatorial photothermal and Immuno cancer therapy using chitosan-coated hollow copper sulfide nanoparticles," *ACS Nano*, vol. 8, no. 6, pp. 5670–5681, 2014.
53. H. Horo, S. Das, B. Mandal, and L. M. Kundu, "Development of a photoresponsive chitosan conjugated prodrug nano-carrier for controlled delivery of antitumor drug 5-fluorouracil," *Int. J. Biol. Macromol.*, vol. 121, pp. 1070–1076, 2019.
54. A. Almeida *et al.*, "Novel amphiphilic chitosan micelles as carriers for hydrophobic anticancer drugs," *Mater. Sci. Eng. C*, vol. 112, no. November 2019, p. 110920, 2020.
55. H. Tan, F. Qin, D. Chen, S. Han, W. Lu, and X. Yao, "Study of glycol chitosan-carboxymethyl β-cyclodextrins as anticancer drugs carrier," *Carbohydr. Polym.*, vol. 93, no. 2, pp. 679–685, 2013.
56. F. Luo *et al.*, "pH-responsive stearic acid-O-carboxymethyl chitosan assemblies as carriers delivering small molecular drug for chemotherapy," *Mater. Sci. Eng. C*, vol. 105, no. August, p. 110107, 2019.
57. R. Rajesh, M. R. Rekha, and C. P. Sharma, "Evaluation of lauryl chitosan graft polyethyleneimine as a potential carrier of genes and anticancer drugs," *Process Biochem.*, vol. 47, no. 7, pp. 1079–1088, 2012.
58. H. Hosseinzadeh, F. Atyabi, R. Dinarvand, and S. N. Ostad, "Chitosan-Pluronic nanoparticles as oral delivery of anticancer gemcitabine: Preparation and in vitro study," *Int. J. Nanomedicine*, vol. 7, pp. 1851–1863, 2012.
59. R. Li *et al.*, "Injectable halloysite-g-chitosan hydrogels as drug carriers to inhibit breast cancer recurrence," *Compos. Part B Eng.*, vol. 221, no. May, p. 109031, 2021.
60. T. S. Anirudhan, C. Sekhar V., and S. S. Nair, "Polyelectrolyte complexes of carboxymethyl chitosan/alginate-based drug carrier for targeted and controlled release of a dual drug," *J. Drug Deliv. Sci. Technol.*, vol. 51, no. December 2018, pp. 569–582, 2019.
61. R. P. Dhavale *et al.*, "Chitosan coated magnetic nanoparticles as carriers of anticancer drug Telmisartan: pH-responsive controlled drug release and cytotoxicity studies," *J. Phys. Chem. Solids*, vol. 148, no. July 2020, p. 109749, 2021.
62. M. Parsian, G. Unsoy, P. Mutlu, S. Yalcin, A. Tezcaner, and U. Gunduz, "Loading of Gemcitabine on chitosan magnetic nanoparticles increase the anti-cancer efficacy of the drug," *Eur. J. Pharmacol.*, vol. 784, no. May, pp. 121–128, 2016.
63. Q. Yuan, J. Shah, S. Hein, and R. D. K. Misra, "Controlled and extended drug release behavior of chitosan-based nanoparticle carrier," *Acta Biomater.*, vol. 6, no. 3, pp. 1140–1148, 2010.
64. S. Kamaraj, U. M. Palanisamy, M. S. B. Kadhar Mohamed, A. Gangasalam, G. A. Maria, and R. Kandasamy, "Curcumin drug delivery by vanillin-chitosan coated with calcium ferrite hybrid nanoparticles as a carrier," *Eur. J. Pharm. Sci.*, vol. 116, no. April 2017, pp. 48–60, 2018.
65. J. L. Arias, L. H. Reddy, and P. Couvreur, "Fe 3O 4/chitosan nanocomposite for magnetic drug targeting to cancer," *J. Mater. Chem.*, vol. 22, no. 15, pp. 7622–7632, 2012.
66. G. Unsoy, R. Khodadust, S. Yalcin, P. Mutlu, and U. Gunduz, "Synthesis of Doxorubicin loaded magnetic chitosan nanoparticles for pH-responsive targeted drug delivery," *Eur. J. Pharm. Sci.*, vol. 62, pp. 243–250, 2014.
67. N. Singh, G. J. S. Jenkins, R. Asadi, and S. H. Doak, "Potential toxicity of superparamagnetic iron oxide nanoparticles (SPION)," *Nano Rev.*, vol. 1, no. 1, p. 5358, 2010.
68. L. Xie, W. Jin, H. Chen, and Q. Zhang, "Superparamagnetic iron oxide nanoparticles for cancer diagnosis and therapy," *J. Biomed. Nanotechnol.*, vol. 15, no. 2, pp. 215–235, 2019.
69. R. Rajalakshmi, S. Sivaselvam, and N. Ponpandian, "Chitosan grafted Fe-doped WO3 decorated with gold nanoparticles for stimuli-responsive drug delivery systems," *Mater. Lett.*, vol. 304, no. March, p. 130664, 2021.
70. A. Almeida, V. Linares, G. Mora-Castaño, M. Casas, I. Caraballo, and B. Sarmento, "3D printed systems for colon-specific delivery of camptothecin-loaded chitosan micelles," *Eur. J. Pharm. Biopharm.*, vol. 167, no. June, pp. 48–56, 2021.
71. G. Lohiya and D. S. Katti, "Carboxylated chitosan mediated improved efficacy of mesoporous silica nanoparticle-based targeted drug delivery system for breast cancer therapy," *Carbohydr. Polym.*, p. 118822, 2021.
72. S. Maya, B. Sarmento, V. K. Lakshmanan, D. Menon, V. Seabra, and R. Jayakumar, "Chitosan cross-linked docetaxel loaded EGF receptor-targeted nanoparticles for lung cancer cells," *Int. J. Biol. Macromol.*, vol. 69, pp. 532–541, 2014.
73. L. Khoshtabiat, A. Meshkini, and M. M. Martin, "Fenton-magnetic based therapy by dual-chemo drug-loaded magnetic hydroxyapatite against colon cancer," *Mater. Sci. Eng. C*, vol. 127, no. February, p. 112238, 2021.
74. L. Tao, J. Jiang, Y. Gao, C. Wu, and Y. Liu, "Biodegradable Alginate-Chitosan Hollow Nanospheres for Codelivery of Doxorubicin and Paclitaxel for the Effect of Human Lung Cancer A549 Cells," *Biomed Res. Int.*, vol. 2018, 2018.
75. A. B. Nazlı and Y. S. Açıkel, "Loading of cancer drug resveratrol to pH-Sensitive, smart, alginate-chitosan hydrogels and investigation of controlled release kinetics," *J. Drug Deliv. Sci. Technol.*, vol. 53, no. April, p. 101199, 2019.
76. D. Qu *et al.*, "Anisamide-functionalized pH-responsive amphiphilic chitosan-based paclitaxel micelles for sigma-1 receptor-targeted prostate cancer treatment," *Carbohydr. Polym.*, vol. 229, p. 115498, 2020.
77. M. Fathi, J. Barar, H. Erfan-Niya, and Y. Omidi, "Methotrexate-conjugated chitosan-grafted pH- and thermo-responsive magnetic nanoparticles for targeted therapy of ovarian cancer," *Int. J. Biol. Macromol.*, vol. 154, pp. 1175–1184, 2020.
78. L. Zhao *et al.*, "Chitosan/Sulfobutylether-β-Cyclodextrin Nanoparticles for Ibrutinib Delivery: A Potential Nanoformulation of Novel Kinase Inhibitor," *J. Pharm. Sci.*, vol. 109, no. 2, pp. 1136–1144, 2020.
79. S. Bhattacharya, "Fabrication and characterization of chitosan-based polymeric nanoparticles of Imatinib for colorectal cancer targeting application," *Int. J. Biol. Macromol.*, vol. 151, pp. 104–115, 2020.
80. "Home - ClinicalTrials.gov." [Online]. Available: https://www.clinicaltrials.gov/. [Accessed: 14-Nov-2021].