Recent Advances in Chitosan-Based Systems for Delivery of Anticancer Drugs

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Abstract

Problems in transporting drug molecules to tumor sites in required dose or constitution lead to low efficacy and significant side effects. Shielding the drug molecules in micelles, liposomes, or nanoparticles is a major line of investigation to improve chemotherapeutic treatment. Though compatibility for proper envelopment of the drug and timely release at the tumor site are required of such a carrier, protecting its own physicochemical and morphological integrity during transport is another precondition.

Because of its superior polymerization capability, biocompatibility, pH dependence, and charging characteristics, chitosan has been in the forefront of potential drug carriers. Numerous synthesis routes for chitosan-based nanocarriers have been suggested to the extent that a search of the literature published since 2000 with the keywords "novel + nano + chitosan" *in the title* results in 527 articles, indicating the bewildering quality and quantity of the new information.

This review was carried out not only to peruse this large amount of work on chitosan-based anticancer drug delivery but also to extract manageable patterns from numerous synthesis routes. The main conclusion is that the synthesis methods suggested in literature can be combined into two main routes, and the degree of hydrophobicity of the drug determines which route should be followed.

Keywords

Anticancer · Drug carrier(s) · Chitosan · Nanoparticle(s)

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7.1 Introduction

7.1.1 Cancer and Anticancer Drugs

7.1.1.1 Cancer

Cancer is the uncontrolled breakdown of the systematic mechanisms of cell growth and division to form new cells as required by the body to replace the old or damaged cells. Though it is required for the upkeep of the body, in the long run, this process leads to the development of cells with defective DNA. These cells which divide and grow without control may form growths called tumors. Though many a time the tumors are benign and remain dormant in the body, some are malignant masses of tissue and have the potential to spread into, or invade, nearby tissues. In addition, they can also detach and travel in the body through the blood or the lymph system and form new tumors in other organs removed from the original tumor.

Cancer is the second leading cause of death globally and has been responsible for an estimated 9.6 million deaths in 2018. This translates into the fact that about one in six deaths globally is due to cancer. Approximately 70% of deaths from cancer occur in low- and middle-income countries. Late-stage presentation and inaccessible diagnosis and treatment are common. In 2017, only 26% of low-income countries reported having pathology services generally available in the public sector. More than 90% of high-income countries reported treatment services are available compared to less than 30% of low-income countries. The economic impact of cancer is significant and is increasing. The total annual economic cost of cancer in 2010 was estimated at approximately US$ 1.16 trillion (WHO 2018).

The treatment of cancer is complicated and may require combination of various parallel or successive treatments, such as surgery with chemotherapy and/or radiation therapy depending on the nature and degree of cancer and, of course, on the patient. Though it is mainly treated using chemotherapy, radiation therapy, and surgery, there are several treatment methods available (https://www.cancer.gov):

Chemotherapy: It is the treatment of cancer by the use of cytotoxic and other drugs. It is considered a systemic therapy and affects the entire body. It also suffers from treatment-related side effects, off-target effects, and drug resistance limits.

Radiation Therapy: It is a type of cancer treatment which uses high-energy particles or waves, such as X-rays, gamma rays, electron beams, or protons, to destroy or damage cancer cells. Unlike chemotherapy, which usually exposes the whole body to cancer-fighting drugs, radiation therapy is usually a local treatment.

Surgery: It is a procedure where the tumor cells and nearby tissue are removed from the body during an operation. It can be curative, preventive, diagnostic, staging, debulking, palliative, supportive, or restorative surgery.

Immunotherapy: It helps immune system fight cancer by enhancing the body's antitumor immune functions. An immunotherapy approach includes monoclonal antibodies, immune checkpoint blockers, cancer vaccines, and cell-based therapies.

Targeted Therapy: It is a cancer treatment which uses drugs as chemotherapy with the difference that it works by targeting the cancer's specific genes, proteins, or the tissue environment that contributes to cancer growth and survival.

Hormone Therapy: It is usually used for the treatment of breast and prostate cancers which depend on hormones for growth. Hormone therapy acts by disrupting the mechanism of the hormone action and by keeping the hormone away from the hormone receptor cancer cells.

Stem Cell Transplant: Stem cell transplants are procedures that restore blood-forming stem cells in cancer patients who have had theirs destroyed by very high doses of chemotherapy or radiation therapy. Stem cells can function as novel delivery platforms by homing to and targeting both primary and metastatic tumors, secretion of bioactive factors, and immunosuppression.

Precision Medicine: It is an evolving approach to cancer treatment which aims to leverage the pathogenesis of cancer to more precisely target therapy. Precision medicine helps doctors select treatments that are most likely to help patients based on a genetic understanding of their disease.

7.1.1.2 Some Common Anticancer Drugs

Chemotherapy, or the forms of it, is considered the most effective treatment method by targeting cancer cells for termination, thereby stopping the spread or slowing the cancer cell from growing. There are numerous compounds which are commercially available as chemotherapeutic anticancer drugs. A summary of the major classes of these compounds is discussed below.

Alkylating Agents: Alkylating agents are compounds that work by adding an alkyl group to the guanine base of the DNA molecule, preventing the strands of the double helix from linking as they should. This causes breakage of the DNA strands, affecting the ability of the cancer cell to multiply. Eventually, the cancer cell dies. The five traditional categories of alkylating agents are nitrogen mustards (e.g., bendamustine, chlorambucil, cyclophosphamide, ifosfamide, mechlorethamine, melphalan), nitrosoureas (e.g., carmustine, lomustine, streptozocin), alkyl sulfonates (e.g., busulfan), triazines (e.g., dacarbazine, temozolomide), and ethylenimines (e.g., altretamine, thiotepa).

Antibiotics/Antineoplastics: It is an antibiotic compound which inhibits the growth of bacteria (bacteriostatic effect) or destroys them (bactericidal effect). The antibiotic effect can be obtained by different mechanisms which damage the microbial DNA.

Antimetabolites: Antimetabolites are drugs that interfere with one or more enzymes or their reactions that are necessary for DNA synthesis. They affect DNA synthesis by acting as a substitute to the actual metabolites that would be used in the normal metabolism (e.g., antifolates interfere with the use of folic acid). Folic acid antagonist: methotrexate. Pyrimidine antagonist: 5-fluorouracil, floxuridine, cytarabine, capecitabine, and gemcitabine. Purine antagonist: 6-mercaptopurine and 6-thioguanine. Adenosine deaminase inhibitor: cladribine, fludarabine, nelarabine, and pentostatin.

Hormones/Antineoplastics: The activated hormone receptor complexes, binding to specific receptors of chromatin, react with the role of various components of the nucleus, which causes DNA replication and cell division by a series of enzymatic reactions, thus affecting the physiological function of cells. These antineoplastic drugs include aromatase inhibitors, aromatase inactivators, estrogens, antiestrogens, progestins, androgens, anti-androgens, luteinizing hormone agonists, glucocorticoids hormones, adrenal blockers, and others.

Platinum Compounds: Strategies for improving platinum-based anticancer drugs usually involve changes in the neutral spectator ligands, in the nature of the anions (halides vs various carboxylates), and in the oxidation states of the metal (PtII vs PtIV).

Vinca Alkaloids: Vinca alkaloids are obtained from the Madagascar periwinkle plant. There are four major vinca alkaloids in clinical use: vinblastine, vinorelbine, vincristine, and vindesine. These are sometimes called monoterpenoid indole alkaloids in the scientific literature. All vinca alkaloids are administered intravenously. They are eventually metabolized by the liver and excreted. The vinca alkaloids are cytotoxics – they halt the division of cells and cause cell death. The main mechanisms of vinca alkaloid cytotoxicity are due to their interactions with tubulin and disruption of microtubule function, particularly of microtubules comprising the mitotic spindle apparatus, directly causing metaphase arrest. Nevertheless, the vinca alkaloids also have an effect on both nonmalignant and malignant cells in the nonmitotic cell cycle, because microtubules are involved in many nonmitotic functions (Moudi et al. 2013).

Protein Tyrosine Kinase Inhibitors: A protein kinase inhibitor is a type of enzyme inhibitor that can block the action of protein kinases. Protein kinases add a phosphate group to a protein in a process called phosphorylation, which can turn a protein on or off, therefore affecting its level of activity and function.

Antineoplastic Interferons: Antineoplastic interferons are interferons (alpha) that are manufactured using recombinant DNA technology and used therapeutically to treat certain types of cancers and viral infections.

The use of alkylating agent mechlorethamine, a nitrogen mustard, to treat lymphomas in the 1940s and antimetabolite methotrexate to cure a solid tumor in the 1956 was the first in cancer treatment. In 1957, 5-fluorouracil to cure tumor was first of pyrimidine analogs. Since then many anticancer drugs have been developed and used with some success. Numerous studies have focused on plant-derived compounds with curative potential and have been used widely in medicines (Verma et al. 2008; Shi et al. 2006). A review by Nahata (2017) compiles the most promising anticancer agents and lists their major cancer curative potentials. Some of the specific agents discussed by Nahata (2017) are summarized below:

Paclitaxel: Paclitaxel (Taxol by Bristol-Myers Squibb) (Wani et al. 1971; Schiff et al. 1979; Honore et al. 2004) blocks a cell's ability to break down the mitotic spindle during mitosis (cell division). It is given intravenously. It irritates the skin and mucous membranes on contact and is most effective against ovarian

carcinomas and advanced breast carcinomas. *Taxus baccata* and *Taxus brevifolia* are members of the yew family (Taxaceae). It is not water soluble.

Docetaxel: Rhone-Poulenc Rorer has trademarked Docetaxel as Taxotere. Like paclitaxel, it prevents the mitotic spindle from being broken down by stabilizing the microtubule bundles, but clinical trials indicate that it is about twice as effective as paclitaxel in doing so. Docetaxel, which is also given intravenously, is being tested on carcinomas of the bladder, cervix, lung, and ovaries, on malign antimelanoma and on non-Hodgkin's lymphoma. It's water solubility is threefold higher than paclitaxel.

Beta-lapachone and Lapachol: It is a quinone derived from lapachol (a naphthoquinone), which can be isolated from the lapacho tree (*Tabebuia avellanedae*), a member of the catalpa family (Bignoniaceae). ß-Lapachone inhibits DNA topoisomerase I. Beta-lapachone keeps the chromosomes wound tight, and so the cell can't make proteins. As a result, the cell stops growing. Because cancer cells grow and reproduce at a much faster rate than normal cells, they are more vulnerable to topoisomerase inhibition than are normal cells. Beta-lapachone is effective against several types of cancer, including lung, breast, colon, and prostate cancers and malignant melanoma. The use of beta-lapachone in humans has been limited due to its toxicity.

Colchicine: It is a water-soluble alkaloid found in the autumn crocus that blocks or suppresses cell division by inhibiting mitosis. Specifically, it inhibits the development of spindles as the nuclei are dividing. Because cancer cells divide much more rapidly than normal cells, cancers are more susceptible to being poisoned by mitotic inhibitors such as colchicine, paclitaxel, and the vinca alkaloids, vincristine, and vinblastine.

Natural Anticancer Agents: Besides curing cancer, the synthetic drugs also harm the normal cells of the body and are producing severe side effects that are not only long living but may pose threat to human's life and are more toxic to body. Therefore there has been numerous work to test the anticlastogenic, antimutagenic, and anticarcinogenic activity of natural plants and herbs which have been traditionally known to have anti-inflammatory, antifungal, antiallergenic, anthelminthic, and other biological curing properties. Though the list can be extended much further, some of these natural plants and herbs standing out in cancer research have been summarized below:

Ganoderma lucidum (reishi mushroom): Ganoderma lucidum is a natural medicine that is widely used and recommended by Asian physicians and naturopaths for its supporting effects on the immune system. Laboratory research and a handful of preclinical trials have suggested that *Ganoderma lucidum* carries promising anticancer and immunomodulatory properties. However, there is no systematic review that has been conducted to evaluate the actual benefits of *Ganoderma lucidum* in cancer treatment (Gao et al. 2004; Nahata 2017, Nahata et al. 2011, 2012a, b, 2013; Chi et al. 2013).

Sphaeranthus indicus (Compositae): Also known as East Indian globe thistle, this herb is found mostly in southern India. It has been demonstrated to have remarkable anti-allergic effects in vitro in preventing mast cell degranulation.

In vitro studies with cancer cell lines demonstrated 80–100% anti-proliferative effect, which competed with many reference drugs used in cancer therapy. However, all of these studies are highly preliminary (Nahata et al. 2011, 2013).

Radix sophorae: This herb is the root of *Sophora flavescens* Ait. and has been used to treat abscess, edema, dysentery, eczema, ulcers, skin burns, skin itch, and atopic dermatitis in traditional medicine for thousands of years. It has also been shown to have antitumor effects (Cheung et al. 2007).

Punica granatum: This is a pomegranate extract rich in polyphenols and has demonstrated antiproliferative, antimetastatic, and anti-invasive effects on various cancer cells line in vitro as well as in vivo animal model or human clinical trial (Lanksy et al. 2007).

Betulinic acid: Betulinic acid is a pentacyclic triterpenoid of plant origin that is widely distributed in the plant kingdom throughout the world. For example, considerable amounts of betulinic acid are available in the outer bark of a variety of tree species, e.g., white-barked birch trees. It has been shown to have a range of biological effects including potent antitumor activity (Pisha et al. 1995).

Turmeric: This herb is a spice grown in many Asian countries and is also known as Indian saffron, jiang huang, haridra, and haldi. It belongs to the ginger family and is a main ingredient of curry powder. The main active ingredient in turmeric is curcumin or diferuloyl methane. Laboratory studies have shown curcumin has anticancer effects on cancer cells (Yasmin et al. 1998; Gupta et al. 2010; Andriani et al. 2015)

Glinus lotoides: This is used as a dietary vegetable and medicinal plant in Asia and Africa. The seed of *Glinus lotoides* has been shown to have antitumor, antifungal, and anthelminthic activity which has been attributed to its saponin and flavonoid content (Kavimani et al. 1999).

Andrographis paniculata: Andrographis paniculata belongs to the family Acanthaceae or Kalmegh and is commonly known as "king of bitters." It is extensively used as home remedy for various diseases in Indian traditional system as well as in tribal system in India for multiple clinical applications. It has been tested to have anticlastogenic, antimutagenic, and anticarcinogenic properties (Kumar et al. 2002).

β-Hydroxyisovaleryl-shikonin: This compound which is isolated from the roots of the plant *Lithospermum radix* has been shown to have inhibitory ability on the proliferation of various human cancer cells, notably the lung and cervical cancers (Masuda et al. 2004).

Saussurea lappa: This plant which has been long used in certain systems of alternative medicine, including Ayurveda and traditional Chinese medicine, is also known as snow lotus. It has been shown to have an effect on asthma, inflammatory diseases, ulcers, and stomach problems in Korea, China, and Japan. Several studies have suggested that it also has anticancer effects in neuroblastoma, lung cancer, hepatocellular carcinoma, gastric cancer, and prostate cancer (Ming et al. 2003; Tian et al. 2017).

Litchi fruit pericarp extract: Litchi is a nonclimacteric subtropical fruit that, once harvested, loses its red pericarp color because of browning reactions probably

involving polyphenols. Litchi fruit pericarp (LFP) extract contains significant amounts of polyphenolic compounds and exhibits powerful antioxidant activity against fat oxidation in vitro and has been shown to have anticancer activity (Wang et al. 2006).

Lignans from stem wood of Cedrus deodara: The lignan mixture of this plant comprising lignans from stem wood of *Cedrus deodara* consisting of wikstromal, matairesinol, and dibenzylbutyrolactone has been shown to demonstrate in vitro cytotoxicity against human cancer cell lines (Singh et al. 2007).

Pine needles: Pine needles (*Pinus densiflora* Siebold et Zuccarini) have long been used as a traditional health-promoting medicinal food. It has been shown that pine needle oil could induce DNA damage in a dose-dependent manner and has potential anticancer effects and antioxidant, antimutagenic, and antitumor activities (Kwak et al. 2006).

Polyalthia longifolia: Polyalthia longifolia is a lofty evergreen tree found in India and Sri Lanka. It has been shown that the methanolic extract from the leaves of *Polyalthia longifolia* has significant anticancer potential (Verma et al. 2008).

Ashwagandha: This plant is a popular Ayurvedic herb used in Indian traditional home medicine and has been shown to have anti-inflammatory effects in addition to relaxing the central nervous system in animals. Other research suggest that Ashwagandha extract and its purified component withanone are selective in killing of cancer cells (Widodo et al. 2007).

7.1.2 Problems Associated in Using Anticancer Drugs

Use of anticancer drugs depends on several factors: (1) the type and location of the cancer, (2) its severity, (3) the type of therapy (surgery/radiation), and (4) the side effects associated with the drug. Though some can be taken orally or can be injected intramuscularly or intrathecally (within the spinal cord), most anticancer drugs are administered intravenously.

The chemotherapeutic treatment is complicated in that the anticancer drugs are generally toxic and they cannot differentiate normal cells from the cancer cells. This leads to harming of the normal cells causing serious side effects, some of which are life-threatening. Some commonly encountered side effects include low blood counts, tiredness, mouth soreness, nausea, vomiting, loss of appetite, constipation or diarrhea, hair loss, skin changes or reactions, pain or nerve changes, and changes in fertility and sexuality. In rare instances prolonged use of anticancer drugs can also lead to the development of secondary cancers. One way of encountering this problem and reducing the side effects of the anticancer drugs is application of multidrug therapy on the patient. This method is based on the nanoscale drug co-delivery systems, which loads at least two anticancer drugs with different physicochemical and pharmacological properties into a combined delivery system. Different types of anticancer drugs exert their effects in a certain part of the cell cycle (e.g., cell growth phase, cell division phase, resting phase). Thus, while one drug

may be used to stop the growth of cancer cells in a certain phase, another agent may work at a different phase. Nano-drug co-delivery systems are said to synergistically inhibit the growth of the tumor compared with the free drugs. Qi et al. (2017) highlighted the current state of co-delivery nanoparticles and the most commonly used nanomaterial. They discussed challenges and strategies and prospect future development.

However, the very wide spectrum of the observed side effects clearly indicates that the real problem in drug-based cancer treatment lies in the targeting stage of the drugs, in other words successful transport and delivery of the drugs in the body. It seems that they do not reach the targeted cancer cell and they do not arrive there in one piece. The result is the spread of a cytotoxic agent into the body in free form, hence the side effects. This is the main reason for the fact that though there have been numerous compounds which have been demonstrated to be very successful in destroying or killing the cancer cells in laboratories, they have been short of showing progressive improvement when applied in the human bodies. Hence, the targeted drug delivery and controlled drug release stand out as one of the most important and promising avenues in increasing the efficiency of the anticancer drugs, which in laboratory show considerable success, while reducing the side effects significantly. To summarize, there are two main impediments on the way of cancer treatment agent no matter how exceptional it is in reaching the tumor area with maximum efficacy and destroying the cancer cells: the first is the lack of success in preventing the agent from interacting the healthy non-cancer cells, especially in free form (side effect prevention), and the second is the problems in proper arrival (to the right address in desired form) of the drug into tumor site. Therefore, drug carriers (vehicles) have become the essential tools by which one can deliver drugs into tumor cells in the desired form and concentration with minimum drug leakage into normal cells. This will be the subject of the following paragraphs.

7.1.3 Common Drug Carrier Systems for Anticancer Drugs

As explained in the above paragraphs, the major problems encountered in conventional chemotherapy are poor bioavailability, high-dose requirements, hostile side effects, and low therapeutic records.

Most chemotherapeutic drugs have low solubility in water, hence in body fluids. The poor water solubility of hydrophobic anticancer drugs creates difficulties in loading and delivery and limits their overall therapeutic efficacy, hence their clinical use, unless the solubility is increased by some modification or the drug is enveloped in a soluble environment. The remaining hydrophilic anticancer agents which are freely soluble in bloodstream also face complexities in treatment because of their interactions with the blood components such as proteins.

Therefore, enveloping the anticancer agents whether they are hydrophobic or hydrophilic in nature has become one of the most sought-after preparation methods for increasing the effectiveness of chemotherapeutic treatment. To start with these anticancer drug carriers must offer nontoxicity, good biodegradation, or

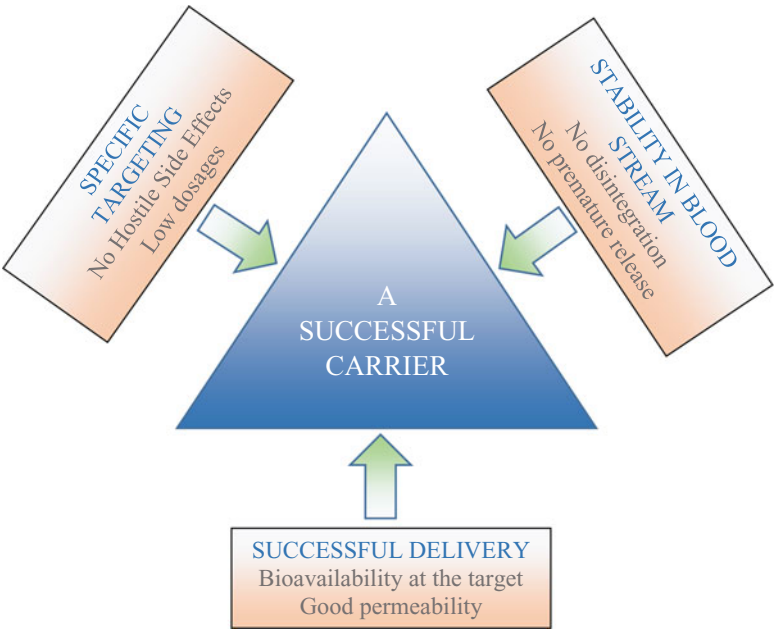

Fig. 7.1 A schematic of the general requirements expected from a successful drug carrier

bioavailability characteristics. In addition, the carrier must also fulfill the following requirements:

- The correct chemical properties to be able to dissolve and envelop the drug in its structure
- Sufficient stability in the circulation system to protect the drug during transport
- Proper attributes to deliver the drug once it reaches the target cells

The problems associated with a carrier if it does not satisfy the above requirements are summarized schematically in Fig. 7.1. Therefore, numerous research has been carried out in the literature for addressing these problems and developing successful drug delivery vehicles for anticancer drugs to the desired sites of therapeutic action with minimal adverse side effects. Many types of materials with different structural characteristics as popular delivery vehicles for chemotherapeutic agents-anti-cancer drugs that have emerged from these studies are summarized below:

Polymers: Polymeric nanoparticles are solid, biocompatible, and biodegradable systems. They have easy structural modification and allow wanted properties to be built into the nanoparticle. Polymeric nanoparticles can be prepared from synthetic polymers, e.g., poly(lactic acid) (PLA), poly(ε-caprolactone) (PCL), poly(lactic-co-glycolic acid), *N*-(2-hydroxypropyl)-methacrylamide copolymer

(HPMA), and poly(styrene-maleic anhydride) copolymer, or from natural polymers, such as gelatin, dextran, guar gum, chitosan, and collagen (Hartwell et al. 1971; Cragg et al. 1993; Kumar et al. 2000; Newman et al. 2003; Shi et al. 2006; Park et al. 2008; Parveen and Sahoo 2008).

Lipids: Liposomes are small, spherical, self-closed structures with at least one concentric lipid bilayer and an encapsulated aqueous phase in the center. They have biocompatible-biodegradable nature and unique ability to encapsulate hydrophilic agents (hydrophilic drugs, DNA, RNA, etc.) in their inner aqueous core and hydrophobic drugs within the lamellae, which makes them versatile therapeutic carriers (Hande et al. 1998; Chabner and Lango 2001; Mo et al. 2014; Dong et al. 2014).

Inorganic Carriers: Inorganic nanocarriers have great advantages, such as large surface area, good drug loading capacity, bioavailability, low toxic side effects, controlled drug release, and their tolerance toward organic solvents (most of them). Quantum dots, carbon nanotubes, layered double hydroxides, mesoporous silica, and magnetic nanoparticles are commonly used in cancer treatment in various ways (Wani et al. 1971; Bianco et al. 2008; Zrazhevskiy, et al. 2010; Kairdolf et al. 2013; Li et al. 2015).

Polymeric Hydrogels: Hydrogels are three-dimensional polymeric and hydrophilic networks that can absorb large amounts of water. The key success of hydrogel development is in situ gelation. The gelation process is time and concentration dependent and can be triggered by an external stimulus, such as pH, temperature, or light (Schiff et al. 1979; Peppas et al. 2000; Lin and Metters 2006; Tomme et al. 2008; Qi et al. 2015; Ghosh et al. 2015).

Micelles: Micelles are spherical and amphiphilic colloids formed by self-assembly of amphiphilic block copolymers in an aqueous solution, resulting in a hydrophobic core and a hydrophilic shell. They can be formed spontaneously under certain concentrations (critical micelle concentration (CMC)) and temperatures. The hydrophobic core serves as a reservoir for hydrophobic drugs, whereas the hydrophilic shell stabilizes the hydrophobic core and renders both polymer and hydrophobic drugs water soluble (Park et al. 2008; Deshayes et al. 2013; Shi et al. 2014; Jin et al. 2016; Gilbreth et al. 2016; Kumari et al. 2017).

Protein-Based Nanocarriers: Albumin-based nanocarriers have high binding capacity for various drugs and they are nontoxic, non-immunogenic, biocompatible, and biodegradable, and have a long half-life in circulating plasma. Albumin has functional groups as amino and carboxylic groups to easily bind targeting ligands and other surface modifications (Dreis et al. 2007; Hawkins et al. 2008; Zhao et al. 2010; Elzoghby et al. 2012).

Note that the reference list is much longer and should be taken only as an example for the specific vehicle since they have been more exhaustively summarized in excellent review papers recently (Senapati et al. 2018; Zhu and Liao 2015; Dong et al. 2019).

As response to the characteristics required of a drug carrier summarized in Fig. 7.1, the research has focused on developing nanoscale alternative delivery systems such as micelles, polymeric nanoparticles, and liposomes.

Compared with the direct administration of bare chemo-drugs, drug encapsulation in a carrier offers a number of advantages, such as protection from degradation in the bloodstream, better drug solubility, enhanced drug stability, targeted drug delivery, decreased toxic side effects, and improved pharmacokinetic and pharmacodynamics drug properties. To date, an impressive library of various drug delivery vehicles has been developed with varying sizes, architectures, and surface physicochemical properties with targeting strategies. There are excellent review papers (Senapati et al. 2018; Qi et al. 2017; Zhu and Liao 2015) that summarize some examples of drug delivery systems that have either been approved or are in clinical or preclinical development stages. These structures are expected to encapsulate the hydrophobic or hydrophilic anticancer agents for minimizing the side effects due to disintegration during intravenous delivery and improving the therapeutic efficacy through the enhanced availability, permeability, and retention at the target.

Amphiphilic block copolymers which contain chemically tethered hydrophilic and hydrophobic segments have been used extensively for the purpose of delivering therapeutic compounds with low water solubility in body fluids. In aqueous solutions, these polymers associate into nano-sized core/shell structures called micelles above a critical concentration (the critical micelle concentration or CMC). The hydrophobic core section of a micelle serves as a reservoir for the hydrophobic drug molecules, whereas the hydrophilic shell (corona) provides water solubility. Once stabilized inside a micelle's core, the probability of the drug molecules to avoid premature degradation and ingestion before reaching the target tissues is expected to increase significantly (Gaucher et al. 2005; Sachs-Barrable et al. 2007; Plapied et al. 2011; Hunter et al. 2012; Ensign et al. 2012; Maeda et al. 2013; Xu et al. 2013; Talelli et al. 2008). Nevertheless, efficiency of the micelles as drug carriers is lower than desired. Interaction of the micelles with the native blood plasma components is one obvious obstacle. If present, such interactions may alter the conformation, size, and surface properties of the carrier and negatively influence both the drug holding capacity and activity at the target site. The most potent binding partners in blood are albumin, immunoglobulins, fibrinogen, apolipoproteins, and complement cascade proteins. Therefore, there have been lots of studies on the stability of polymeric micelles in aqueous solutions (including body fluid), and these studies have been summarized by some excellent reviews addressing these issues (Owen et al. 2012; Shi et al. 2017; Zhou et al. 2016).

In recent years, there have been several studies on the fixation of anticancer drugs in the core of polymeric micelles to increase their intravenous stability (Kabanov et al. 2002; Batrakova and Kabanov 2008). Very recent findings by the authors of this paper demonstrate that dilution of the micellar nanocarriers must also be taken into account in devising drug carriers from polymeric surfactant aggregates (Polat et al. 2019). The most obvious solution to this would be encapsulation of the micelles by a third phase as suggested by the same authors in their previous work (Cihan et al. 2017). In that study, we designed and developed spherical chitosan

nanoshells which enveloped the micelles of a polymeric surfactant whose cores provided the necessary solvation characteristics and safeguarded delivery of strongly lipophilic drug.

In summary, nanoparticles have immense potential as drug-delivery carriers due to their unique physicochemical properties whether they directly accommodate the drug molecules or envelop other nanoscale structures which contain the drug molecules (such as micelles; see Cihan et al. 2017). These particles have the potential to improve the pharmacological and therapeutic properties of the anticancer drugs by controlling release rates and targeted delivery process, which eliminate the limitations of conventional anticancer treatment methods. A wide variety of inorganic or organic materials such as silica and polystyrene have been employed to synthesize the nanoparticle-based drug carriers. However, among these materials, natural polymers stand out as the most promising agents due to their biodegradability, biocompatibility, and favorable physicochemical responses. The following section gives a summary account of such materials as nanoparticle feedstocks and will describe why chitosan emerges as one of the most promising natural polymers as an anticancer drug carrier.

7.2 Chitosan as an Anticancer Drug Carrier

7.2.1 Characteristic Properties of Chitosan

Natural polymers such as cellulose, starch, chitosan, carrageenan, alginates, etc. are among the preferred materials for drug delivery applications due to their chemically inert, nontoxic, biocompatible, and biodegradable structures and availability. There are several studies on the use of these materials for enveloping the anticancer drugs for developing efficient chemotherapeutic treatment systems (Toti and Aminabhavi 2004; Kaur et al. 2013; Gao et al. 2013; Mazumder et al. 2018; George et al. 2019; Singh and Singh 2019). Among these materials, chitosan has been receiving special attention due to its superior characteristics such as (i) high drug-carrying capacity, (ii) multifunctionality, (iii) prolonged circulation ability, and (iv) favorable targeting and penetrability of the cell membranes because of the primary amine groups in its structure. Moreover, molecular weight and molecular fraction of glucosamine units in the chitosan structure influence the solubility and antimicrobial and biological activity (Tikhonov et al. 2006). Because of these properties, chitosan has received an immense attention in the literature. A search of the papers in Web of Science with the word "chitosan" *in the title alone* results in 33,502 published journal articles between the years 2000 and 2019 (Fig. 7.2a). It can be seen that the interest in chitosan has grown drastically in the last 20 years.

Chitosan is natural polysaccharide containing β-(1-4)-D-glucosamine and N-acetyl-β-(1-4)-D-glucosamine units. It is produced by deacetylation of chitin. Deacetylation process includes treatment of chitin with aqueous NaOH at 110–115 °C for several hours without oxygen. When the deacetylation degree is over 50% the product is termed "chitosan" (Chang et al. 1997). Dissimilar to

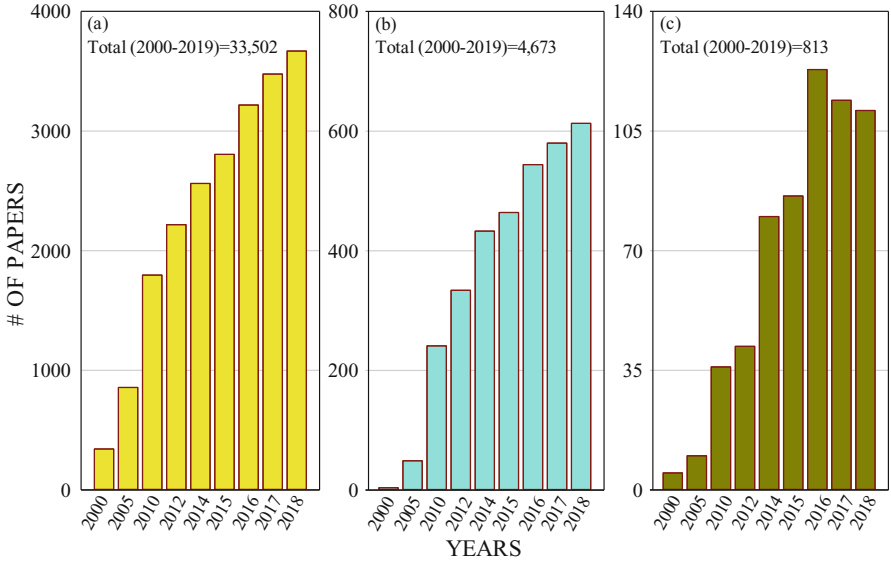

Fig. 7.2 The trend in the number of articles published in respected journals with keywords (**a**) *chitosan∗*, (**b**) *chitosan + (nanoparticle∗ or nano-particle∗ or nano-particle∗)*, and (**c**) *chitosan + (tumor∗ or cancer∗) in the title* in Web of Science between 2000 and 2019

cellulose, chitosan contains hydroxyl groups, acetylamine, or free amino groups that provide many unique properties. Also these amino groups and hydroxyl groups present in its structure provide flexible sites for managing the type and degree of modification for the purposes of the requirements of the end user. It is found that mucoadhesion of chitosan increases with an increasing deacetylation degree and decreases with an increase in the cross-linking (George et al. 2006). These groups are responsible for its outstanding properties such as its cationic nature, pH sensitivity, in situ gelation ability, antimicrobial activity, and permeability which have important implications for targeted drug delivery and controlled drug release. All these features make chitosan nanocomposites ideal candidates for applications such as biomedical scaffolds but also for the delivery of macromolecular therapeutics, like protein and peptides.

Chitosan is soluble in weakly acidic solutions (formic acid, acetic acid, hydrochloric acid, etc.) depending on the number of its amino groups but insoluble in water and alkaline solutions (Krajewska 2004). Because of this it can be used as a pH-dependent material and must be regarded if any system consists of chitosan. It has been observed that protonation of chitosan in different acidic environments depends on pH and pK value of the acid. Though most of the polysaccharides have been observed to have neutral or negatively charged surface in acidic media, chitosan molecules are charged positively when it is dissolved in acidic environment since the amino groups ($-NH_2$) of the glucosamine become protonated ($-NH_3^+$).

Chitosan is widely used in oral delivery relying on its mucoadhesive property. Because of the negative charge of mucosal surfaces, strong mucoadhesive force

occurs between such surfaces and chitosan. It is found that mucoadhesion of chitosan increases with an increasing deacetylation degree and decreases with an increase in the cross-linking (George et al. 2006). In addition, thiolation and trimethylation increase mucoadhesion of chitosan. These properties of chitosan make it a preferred material for controlled release of orally delivered drugs.

Because of all these reasons outlined in above paragraphs, various forms of chitosan materials such as beads, films, microspheres, nanoparticles, nanofibers, hydrogels, and nanocomposites have been developed and tested as drug delivery devices and applications. The recent review papers by Prabaharan (2015), Elgadir (2015), Ali et al. (2018), Pella et al. (2018), and Naskar et al. (2019) report the vast literature available on chitosan-based materials in drug delivery applications. Some representative pictures of chitosan beads, foams, and sheets synthesized in the authors' laboratories are presented in Fig 7.3.

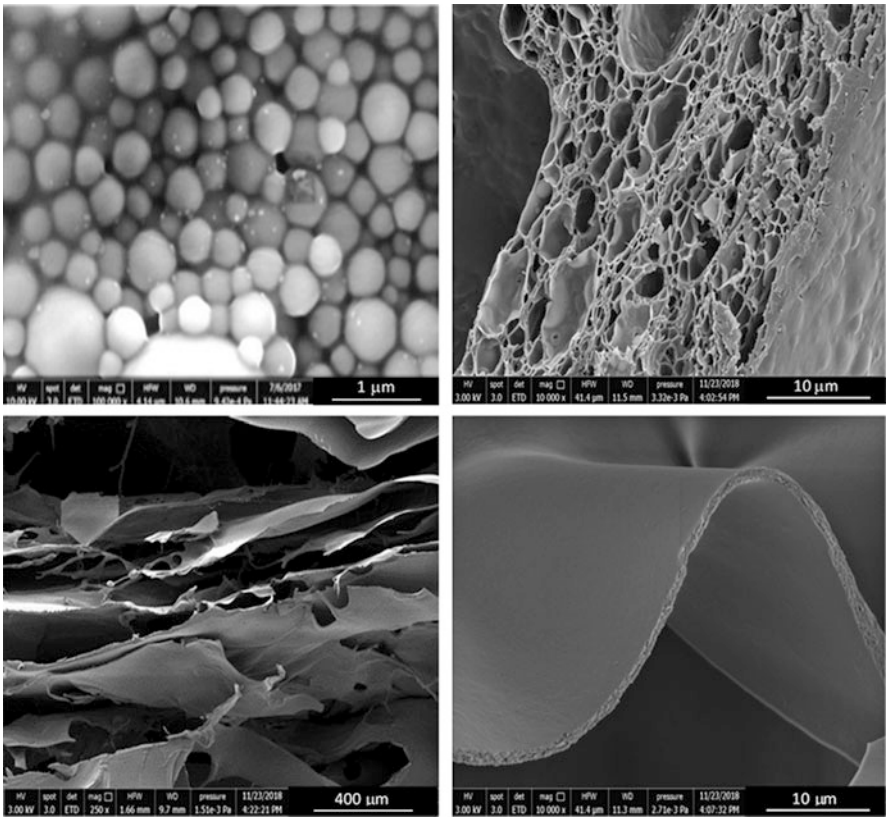

Fig. 7.3 Different forms of chitosan nanostructures synthesized in the authors' laboratories: *nanoparticles (top left), nanofoam (top right), microsheets (bottom left), porous microfilms (bottom right)*

7.2.2 Preparation of Drug-Loaded Chitosan Nanoparticles

Complications always exist in the synthesis of the chitosan nanoparticles even in the absence of any drug in the structure due to the difficulties in controlling the size, morphology, and integrity of the synthesized particles which makes the design of chitosan carriers a state of art. Despite these difficulties, and most probably because of them, the literature on the synthesis of chitosan nanoparticles is immense. Searching the papers in Web of Science with the word "chitosan" and "nanoparticle" *in the title alone* results in 4,473 published journal articles between the years 2000 and 2019 (Fig. 7.2b). It can been seen that there is a similar growth trend in using chitosan to synthesize nanoparticles in the last 20 years to that observed with the articles dealing with chitosan alone.

Various synthesis methods of chitosan nanoparticles are summarized by Grenha (2012), Vyas et al. (2016), Naskar et al. (2019) and Shanmuganathan et al. (2019). The grouping of the methods suggested by these authors is presented below without change in the terminology in order to make comparison with the literature easier. However, it should be stressed that many of the methods proposed are actually variation of one of the few main routes to creating chitosan nanoparticles. Therefore, we have added our specific comments where more need to be said or where certain misunderstandings to be corrected. Therefore, a reordering and generalizing of the grouping of these methods will also be presented at the end paragraphs of this section in Fig. 7.4 based on our comments in this sections.

Ionic Gelation/Polyelectrolyte Complexation: This is the most straightforward of the methods employed in the literature. It is based on contacting the cationic chitosan molecules dissolved in an aqueous phase with a negatively charged cross-linking agent slowly to allow complex formation, polymerization, and precipitation of chitosan by electrostatic forces (Kawashima et al. 1985a, b; Fernandez-Urrusuno et al. 1999; Pan et al. 2002; Ahmad et al. 2012; Aydin et al. 2012; Rampino et al. 2013; Motwani et al. 2008; Nanjwade et al. 2010; Meng et al. 2011; Alam et al. 2012; de Campos et al. 2001; Wu et al. 2005; Bhattarai et al. 2006; Pawar et al. 2013; Xue et al. 2015a; Gao et al. 2016; Andriani et al. 2015).

Nevertheless, in our experience, control of this system to manufacture particles of desired size and morphology is somewhat difficult, and if applied directly, the method usually results in precipitation of a chitosan phase in an uncontrolled manner. This is the main reason for introducing separate components or phases (such as creating some form of an emulsion system as mentioned in the following paragraphs) for controlling polymerization reactions to certain sizes and shapes.

However, as we have seen in our studies, introduction of the micelles into the system to present nano-sized nucleation sites for the chitosan polymerizing may allow creation of particles of desired size and morphology. Such approach also lets a more proper use of the micelles (see the reverse micellization method below for the misleading use of the micelle term).

Modified Ionic Gelation with Radical Polymerization: In this approach, a polymeric acid such as polyacrylic acid or polymethacrylic acid is added to the aqueous chitosan solution. Polymerization takes place due to the interaction between the

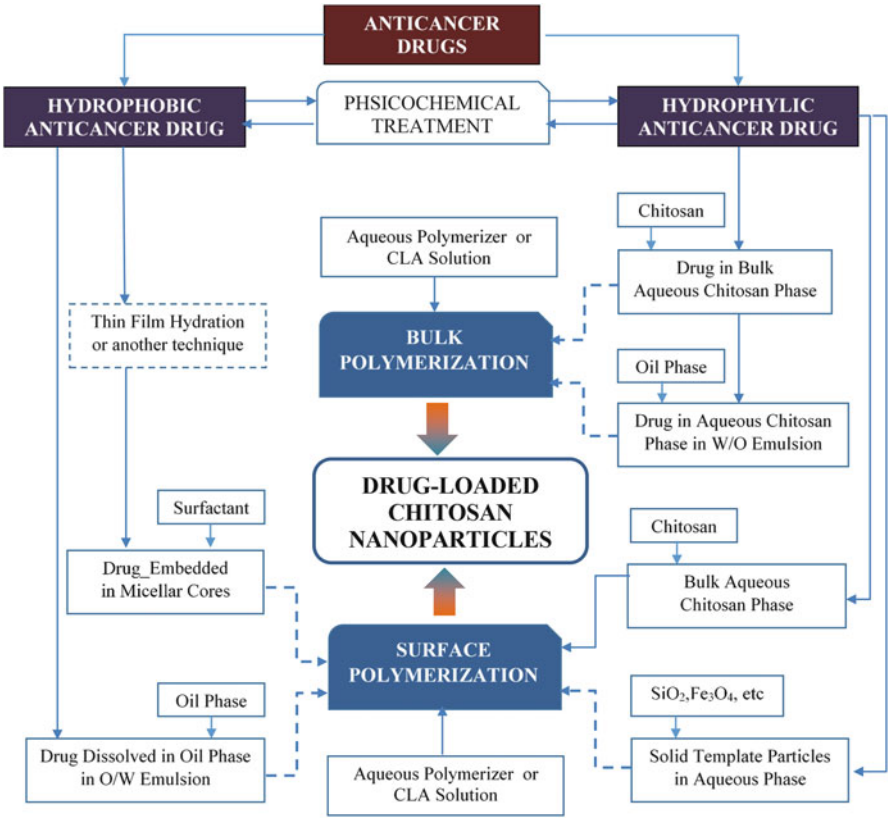

Fig. 7.4 A combination of various synthesis methods for drug-loaded chitosan nanoparticles. *Only one of the inputs shown by the broken lines is added to the system depending on the synthesis procedure*

cation of chitosan and the anionic polymeric acid. In some cases, the acid added in monomeric form is polymerized by use of a polymerization agent such as potassium persulfate to initiate polymerization of the acid in chitosan solution leading to precipitation of chitosan nanoparticles (Hu et al. 2002; Sajeesh and Sharma 2005, 2006). Nevertheless, as the name implies, this is a variation of the ionic gelation method with the difference that instead of a more traditional cross-linking agent such as TPP, a monomeric or polymeric acid is employed.

Desolvation: This method is also a straightforward approach to chitosan polymerization in that it simply is based on decreasing the solubility of chitosan in an aqueous solution by addition of a precipitation agent rather than a cross-linking agent. The precipitating agents could be electrolytes such as sodium sulfate or solvents such as acetone in the presence of some stabilizing agents if necessary. In some cases, a polymerizing agent can also be added to create a more compact and

sturdy chitosan phase (Jiang et al. 2018). However, since precipitation takes place due to desolubilization of chitosan, controlling the size, morphology, and stability of the synthesized particles could be a challenge as was the case with the ionic gelation/polymerization method discussed in the previous paragraph.

Emulsification Cross-Linking: This is one of the main methods in creating chitosan nanoparticles. In this method, a W/O emulsion is created by introducing aqueous chitosan solution into an oil phase to create dispersed chitosan solution droplets which act as micro- or nano-reactors. Then, a polymerization agent such as glutaraldehyde is added into the emulsion and is expected to diffuse into the dispersed aqueous chitosan droplets initiating the polymerization within the confined volume of the droplets by cross-linking through the aldehyde groups of the polymerizer and the amino groups of the chitosan molecules to form nanoparticles. A suitable stabilizing surfactant is usually employed to keep the aqueous phase dispersed in oil (Ohya et al. 1994; Grenha 2012; Yuan et al. 2010). In this case, the drug should be dissolved in the chitosan solution previously by use of a suitable method if the drug molecules is to be incorporated into the chitosan nanoparticles.

W/O Emulsion Droplet Coalescence: This is a variation of the emulsification cross-linking method above with the difference that instead of a single W/O emulsion into which the polymerizer is added in aqueous phase directly, two W/O emulsions are prepared separately in hydrophobic organic phases. Of the two W/O emulsions, one includes an aqueous solution of chitosan with or without the dissolved drug molecules, and the other may contain a strongly aqueous solution of a base such as sodium hydroxide or a cross-linking agent for initiating chitosan precipitation. When the two systems are mixed under high shear conditions, the droplets from the two W/O emulsions collide randomly and coalesce, creating droplet-size reactors where drug-loaded chitosan solution is precipitated by the base, or the polymerizer, in the form of nanoparticles. The use of a stabilizer to ensure emulsification through surface tension and viscosity reduction followed by steric or electrostatic stabilization and high-speed homogenization conditions may also be necessary (Reddy et al. 2013; Tokumitsu et al. 1999).

Emulsion Solvent Diffusion: In this method, a hydrophilic drug is initially dissolved in an organic phase with the help of a solvent. The drug-containing organic phase is then dispersed in chitosan solution with the help of a stabilizer. Under high-pressure evaporation conditions, the organic phase evaporates, while the solvent diffuses into the aqueous phase. While the diffusion of the solvent into aqueous solution brings about polymerization of chitosan, evaporation of the organic phase precipitates the drug leading to drug-loaded chitosan nanoparticles (El-Shabouri et al. 2002; Niwa et al. 1993; Anto et al. 2011). The same procedure can be used to load hydrophobic drugs into the organic hydrophobic phase directly without the use of any solvent. However, precipitation of chitosan may be achieved by addition of a separate cross-linking agent slowly into the emulsion phase if this is the case.

Reverse Micellization: A surfactant is dissolved in an organic phase or a combination of organic phases initially. Then, aqueous chitosan solution is slowly added to the organic phase creating a W/O emulsion. During this phase, the surfactant molecules transfer to the oil-water interface and stabilize the droplets. Introduction

of the polymerization agent to the emulsion system allows the cross-linking agent to slowly diffuse into the emulsion droplet which acts as micro reactors to precipitate chitosan (Banerjee et al. 2002; Mitra et al. 2001; Kafshgari et al. 2012).

It should be noted that the use of the reverse micelle term in the literature is erroneous and misleading in this case since the emulsion droplets stabilized this way by the surfactant (polar heads looking into the droplet while the hydrocarbon chains extending into continuous organic phase) are not true micelles. They are simply aqueous droplets stabilized by the surfactant molecules in an organic hydrophobic phase. Introduction of the drug into this system could be made by dissolving the drug in the aqueous phase by employing proper solvation routes which would lead to drug-loaded chitosan particles after polymerization.

Nanoprecipitation: This is another variation of the emulsification methods with the difference that chitosan is dissolved in a suitable organic solvent instead of an aqueous phase. The organic phase is then gradually added into another phase which is not miscible with it and in which chitosan is insoluble (such as ethanol). The diffusion of the chitosan molecules from the organic phase into the dispersed phase creates the precipitated nanoparticles. The formed particles can be kept dispersed by adding a stabilizing surfactant into the dispersing phase (Fessi et al. 1989; Bilati et al. 2005; Luque-Alcaraz et al. 2016).

Spray-Drying: This is a rather classical method of particle formation where aqueous chitosan solution is atomized using a spray dryer. Then small droplets obtained via atomizer are mixed with a drying gas to evaporate the liquid phase to obtain chitosan nanoparticles (Ngan et al. 2014; Mehrotra et al. 2010; Liu et al. 2018)

It can be observed from the description of the methods in the above paragraphs that all the methods described can actually be combined into two main categories (Fig. 7.4) which can be best expressed as:

- Methods where chitosan polymerization is achieved in bulk solution
- Methods where chitosan polymerization is achieved on the surface of some template material such as micelles, oil droplets, or solid particles

It can be seen that either bulk polymerization or surface polymerization can be employed to create drug-loaded chitosan nanoparticles depending on the degree of hydrophobicity of the drug.

Bulk Polymerization: This method is employed for hydrophilic drug molecules unless drug molecules are intentionally imparted hydrophobicity by a suitable physicochemical treatment such as grafting by a specific end group or by a surfactant. In bulk polymerization, the hydrophilic drug is dissolved in an aqueous chitosan solution initially. Polymerization can be carried out by direct addition of the polymerization agent into this solution. In this case, the polymerization agent and chitosan diffuse into each other in bulk and precipitation takes place. But, controlling the size and morphology of the precipitated particles is a challenge.

To achieve better control, the chitosan solution containing the dissolved drug is first used in creating a W/O emulsion such that the drug is within the dispersed

micron or nano-sized droplets of the aqueous chitosan phase such that each droplet acts as a micron or nano-sized reactor for the polymerization reactions. Introduction of the aqueous polymerizer solution to initiate chitosan precipitation can be made by direct addition, which creates a W/O emulsion of the polymerizer solution in bulk, or by dispersing the polymerizer solution in an oil phase before creating the W/O emulsion of the polymerizer separately. In either case, the precipitation reactions occur when the droplets from both W/O emulsions, one containing the polymerizer and the other chitosan and the drug, collide and coalesce, leading to a slower and a better controlled synthesis step.

Surface Polymerization: This method is employed (when chitosan polymerization is achieved on the surface of micelles and oil droplets not solid particles) for hydrophobic drug molecules unless drug molecules are intentionally made hydrophilic by grafting by a specific end group or by a surfactant. In surface polymerization, the hydrophobic drug is dissolved either in the micellar core of a polymeric or directly in an oil phase. In either case, the micellar solution or the oil phase containing the drug molecules is dispersed in an aqueous chitosan solution. The micelles and the drug-containing oil droplet act both as nucleation sites and soft templates on the surface of which polymerization of chitosan takes place upon introduction of the polymerizer. In some cases, chitosan is precipitated on the surface of solid particles (such as silica, calcium ferric, iron oxide, etc.) together with the drug in order to create particles with magnetic properties or with a better strength.

Figure 7.5 presents some recent results from our laboratory where we have prepared micelles of the polymeric surfactant Pluronic P-123 to dissolve a strongly hydrophobic drug probucol which were then camouflaged with chitosan using surface polymerization. Dissolving the drug in the micelle structure has been carried out by thin-film hydration in this case (Cihan et al. 2017). In some cases, however, a physicochemical treatment can be applied to modify the drug to impart a hydrophilic nature instead of using micelles as stated above.

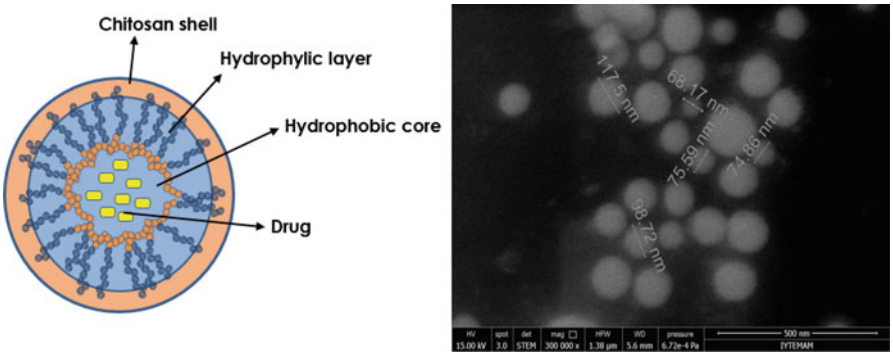

Fig. 7.5 A schematic view (left) of the chitosan nanoshells which camouflage Pluronic P-123 micelles in whose cores a strongly hydrophobic drug probucol is dissolved and the SEM pictures of the nanoshells (right)

In addition, a variation of the bulk polymerization method, which is named as self-assembly in literature, has been suggested by various researches (Yang et al. 2014; Petrov et al. 2008; Mu et al. 2019a, b). The main aspect of this method is that the dissolved chitosan molecules in acidic aqueous phase are made hydrophobic by attaching selected monomers (e.g., acid-anhydrides of benzoic or valeric acids) onto the amine groups. This is similar to decreasing the degree of solvation of the chitosan molecules, in this case brought about by making the molecules hydrophobic in an aqueous solution, leading to creation of chitosan aggregates (or chitosan micelles) into which hydrophobic drug molecules are incorporated.

7.2.3 Characterization of Chitosan Nanoparticles

Nanoscale materials may present different properties than from their bulk counterparts, as their high surface-to-volume ratio results in an exponential increase of the reactivity at the molecular level. In this section, we describe various surface and structure characterization methods which are widely employed to characterize nanostructures such as the chitosan nanoparticles we deal in this paper. While these techniques can be employed exclusive for the study of a particular property, they can be used in combination in most cases to describe a better picture of the system under analysis. The techniques which are employed most commonly will be discussed in detail in the following paragraphs. An excellent summary of the techniques has been carried out by Mourdikoudis et al. (2018). A schematic view of the characterization techniques is presented in Fig. 7.6 below.

Scanning Electron Microscopy: SEM is a type of electron microscope that produces images of a sample by scanning the surface with a focused beam of electrons. The electrons interact with atoms in the sample, producing various signals that contain information about the surface topography and composition of the sample. The electron beam is scanned in a raster scan pattern, and the position of the beam is combined with the detected signal to produce an image. SEM can achieve resolution better than 1 nanometer. SEM may be used to determine the surface morphologies and structural integrity of micro- and/or nano-sized particles. A representative SEM photograph of nano-sized silica particles manufactured in our laboratories using Stöber synthesis is presented in the top left section of Fig. 7.7.

Scanning Transmisson Electron Microscopy: STEM is a type of transmission electron microscope (TEM). In STEM, the electron beam is focused to a fine spot (0.05–0.2 nm) unlike the conventional transmission electron microscope and then scanned over the sample in a raster illumination system constructed in a way that at each point sample is illuminated with the beam parallel to the optical axis. Scanning transmission electron microscopy (STEM) analysis can be used for the investigation of surface and morphology of chitosan and micelle-embedded chitosan nanoparticles. Again a representative STEM photograph of the drug-loaded Pluronic P-123 micelles prepared in our laboratories using thin-film hydration method is presented in the bottom left section of Fig. 7.7.

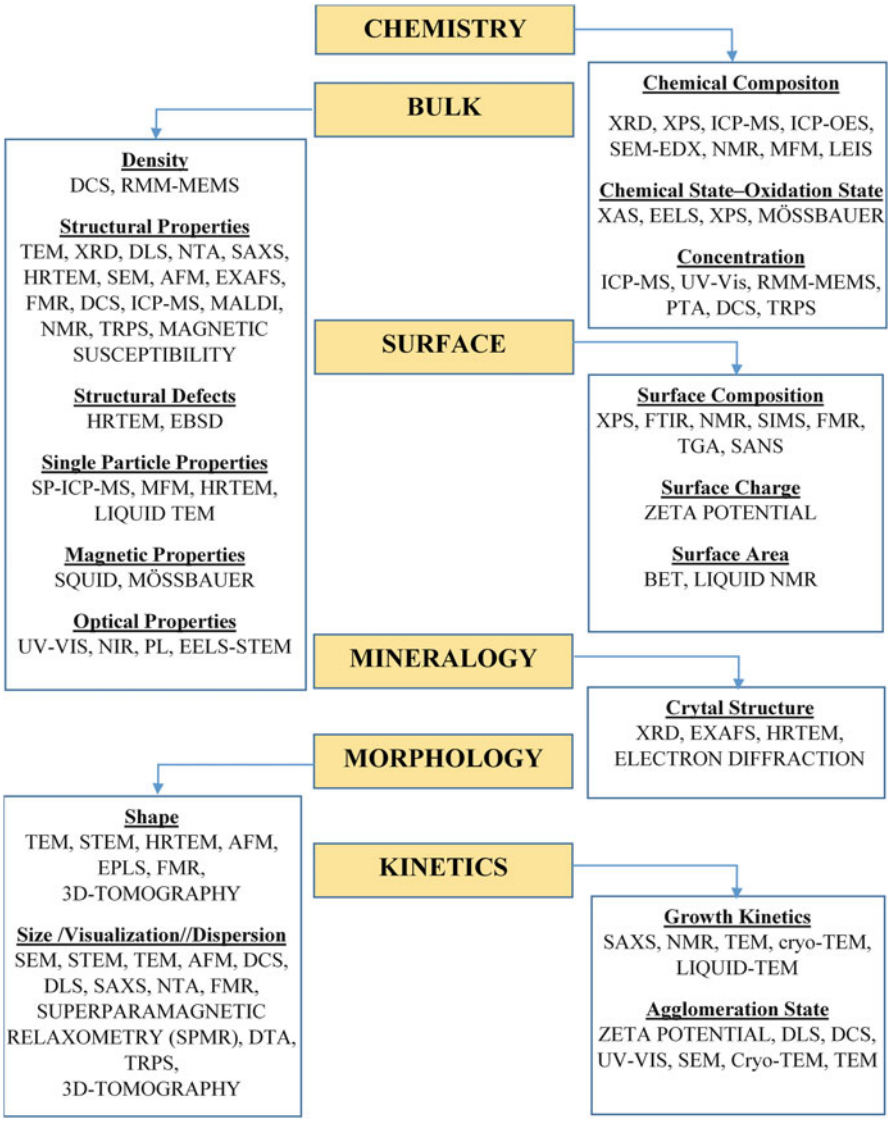

Fig. 7.6 Nanoparticle characterization methods

Transmission Electron Microscopy: TEM is a very powerful tool for material science. A high-energy beam of electrons is shone through a very thin sample, and the interactions between the electrons and the atoms can be used to observe features such as the crystal structure and features in the structure like dislocations and grain boundaries. TEM can reveal the finest details of internal structure – in some cases as small as individual atoms. TEM can be used for the investigation of surface and

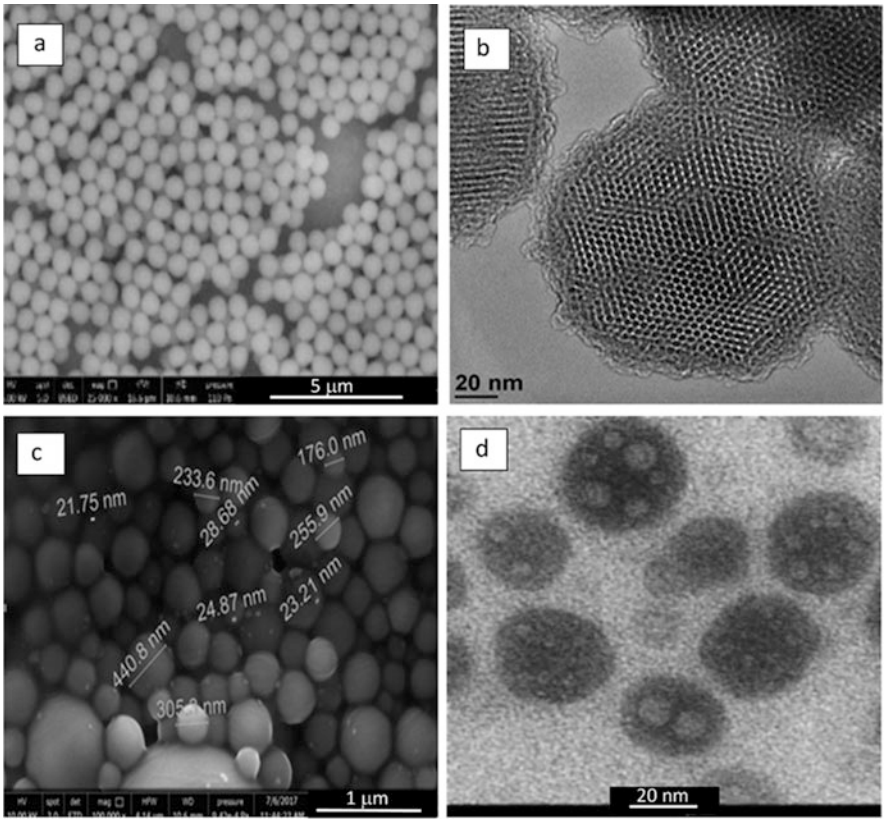

Fig. 7.7 Some examples of the SEM, STEM, and TEM pictures of the nanoparticles synthesized in the authors' laboratories
Mono-sized mesoporous silica particles; photographs obtained with SEM (a) and TEM (b)
Drug-loaded Pluronic P-123 micelles; photographs obtained with STEM (c) and TEM (d)

structure of chitosan and drug-embedded chitosan nanoparticles. TEM has priority on imaging samples with a great magnification rather than SEM and other electron microscope techniques, so it makes TEM the best way to determine morphological properties of nanoparticles and drug-loaded micelles in samples. Two representative TEM photographs of the silica particles and drug-loaded Pluronic micelles are presented in the top and bottom right sections of Fig. 7.7.

Dynamic Light Scattering: Size measurements of chitosan nanoparticles can also be obtained using DLS methods. Size of particles which is measured by the method is inversely proportional to angle seen after the particles scatter light. These particles pass through a focused laser beam during the laser diffraction measurement. A series of photosensitive detectors are used to get the angular intensity of scattered light. Particle size is calculated by using the map of scattering intensity versus angle. Particles are moving because of Brownian motion which is due to random collision

with the molecules of the liquid that surrounds the particle. Stokes-Einstein equation defines the relationship between size of particle and its speed due to Brownian motion.

Fourier Transform Infrared Spectroscopy: FTIR is used in the qualitative analysis of drug-loaded chitosan nanoparticles. The presence or absence of functional groups is investigated. FTIR spectroscopy is based on vibrational energy as a consequence of radiation absorption on atoms. Vibrational energy leads to determine functional groups and bonds in compounds.

Small-Angle X-Ray Scattering: SAXS is a nondestructive method for investigating nanostructures in liquids and solids. In a SAXS experiment, an X-ray beam is aimed at a nanostructured sample (for example proteins, macromolecules, or nanoparticle dispersions). The beam is scattered following its interaction with the electrons of the sample and collected by a detector. The detected scattering pattern is characteristic for the nanostructures of the sample and can be used to determine important structural parameters such as particle size, shape, internal structure, porosity, and arrangement (orientation). Small-angle X-ray scattering (SAXS) ideally complements microscopic methods (AFM, TEM) since it provides representative structural information about a large sample area.

Nuclear Magnetic Resonance Spectroscopy: NMR or nuclear magnetic resonance spectroscopy is a technique used to determine a compound's unique structure. It identifies the carbon-hydrogen framework of an organic compound. Using this method and other instrumental methods including infrared and mass spectrometry, scientists are able to determine the entire structure of a molecule. Even though there are many other spectrometers including C-NMR and N-NMR, hydrogen (H-NMR) was the first and is the most common atom used in nuclear magnetic resonance spectroscopy. The atomic nucleus is a spinning charged particle, and it generates a magnetic field. Without an external applied magnetic field, the nuclear spins are random and spin in random directions. But, when an external magnetic field is present, the nuclei align themselves either with or against the field of the external magnet.

7.3 Modification of Chitosan Nanoparticles for Anticancer Therapies

Multitudinous work exists in the literature on the use of chitosan-based nanoparticles as anticancer drug carriers, and the number has been increasing at a greater rate in the last 20 years. Searching the papers in the Web of Science with the word "chitosan + cancer or tumor" *in the title* results in 813 published journal articles between the years 2000 and 2019 (see Fig. 7.2c). It can been seen that there is a growth trend in the last 20 years in employing chitosan in anticancer research in one form or another. Majority of this work is about synthesizing chitosan nanoparticles for use in anticancer therapy by one of the routes described in Sect. 7.2.2 and summarized in Fig. 7.4. The literature reviewed in the following paragraphs deal specifically with modifying the chitosan nanoparticles for more efficient tumor-targeted anticancer

drug delivery applications. The following paragraphs contain a concise classification of these latter studies.

Chemical Modification: Chitosan is insoluble in water except acidic media and in other organic solvents due to the presence of amine groups in its structure. Various chemically modified chitosan derivatives have been produced through modification of the amine or hydroxyl functional groups to increase its solubility, which is extremely important in controlling the interaction of the polymer with the drugs (Casettari et al. 2012; Kurita et al. 2006; Ahsan et al. 2018; Shukla et al. 2013; Gorochovceva et al. 2004; Luckachan et al. 2006; Hua et al. 2008; Tang et al. 2007; Radhakumary et al. 2007; Sutirman et al. 2018; Shariatinia et al. 2019).

Rahimi et al. (2019) prepared chitosan-quinoline nanoparticles as hydrophobic anticancer drug nanocarriers using 2-chloro-3-formylquinoline and 3-formylquinolin-2(1H)-one as nontoxic modifying agents via Q/W nanoemulsion technique. Their characterization using FTIR, UV-vis spectrophotometry, XRD, SEM, AFM, and DLS techniques demonstrated that the drug-loaded chitosan-quinoline nanoparticles have a regular nanorod shape and monolithic structure with a particle size range of between 141 and 1745 nm and a zeta potential range between −2.4 and −14.1 mV. They found that the loading capacity and encapsulation efficiency with the hydrophobic anticancer drug quercetin were between 4.8–9.6% and 65.8–77%, respectively.

Zhang et al. (2019) studied the utilization of the marker GX1 to fabricate a multifunctional vascular targeting of docetaxel-loaded nanoparticles with N-deoxycholic acid glycol chitosan as the carrier and GX1-PEG-deoxycholic acid conjugate as the targeting ligand for gastric cancer therapy. They found that in vitro drug release tests showed sustained and pH-dependent release. In vivo delivery of marker/carrier/drug composite nanoparticles inhibited tumor growth in mice at a rate of 67.05%.

Razmi et al. (2013) studied platinum-based anticancer drugs that have limited applications due to their severe toxicity. They described a drug delivery system comprising a platinum complex (bipyridine morpholine dithiocarbamate Pt (II) nitrate) within nanoparticles composed of casein and chitosan. They studied the effect of pH using UV-vis spectrometry, dynamic light scattering (DLS), and scanning electron microscopy (SEM). They observed that the optimum pH for complex formation is between the pI of casein (5.3) and the pKa of chitosan (6.5), and there is an enhancement in the cytotoxicity and cellular uptake of platinum by its entrapment in casein-chitosan nanovehicles. Their findings suggest that this drug delivery system enables drugs to be thermodynamically stable in aqueous solutions and is potentially useful for targeted oral delivery applications.

Sutar et al. (2018) introduced a chitosan-polylactic acid-drug conjugate, and its iron transport protein (transferring) receptor targeted polyelectrolyte complex nanoparticles, encapsulating free drug to increase its potency and specificity. The model drug was strongly hydrophobic curcumin and incorporated in the system in both conjugated and encapsulated form. Chitosan-polylactic acid-curcumin copolymer was characterized by 1 H NMR, FTIR, UV-visible spectrometry, differential scanning calorimetry, and zeta potential measurements. The nanoparticles

demonstrated high curcumin loading over 92% with extended periods for release (60% and 85% at pH 7.4 and 5, respectively, even after 8 days). It was concluded that curcumin-loaded transferrin-chitosan-polylactic acid-curcumin nanoparticles may provide an efficient and targeted delivery for cancer treatment.

In some other studies, chitosan was modified by grafting polymer chains onto the surface of magnetic chitosan (Cesano et al. 2015; Kloster et al. 2015; Liu et al. 2009; Suzariana Samuri et al. 2016; Roveimiab et al. 2012; Jiang et al. 2012; Bagheri et al. 2015; Niu et al. 2014). In these studies, three types of grafting methods "grafting from," "grafting through," and "grafting onto" were employed. The "grafting onto" method is based on using pre-synthesized polymer chains. Among these three methods, "grafting from" (Hua et al. 2008; Jiang et al. 2012) can be performed using controlled radical polymerization techniques (Hojjati et al. 2008). Atom transfer radical polymerization (Renggli et al. 2017; Lanzalaco et al. 2017), reversible addition-fragmentation chain transfer (Hua et al. 2008; Cao et al. 2015; Yamamoto et al. 2014; Hosseinzadeh et al. 2018), and living free radical nitroxide-mediated polymerization (Hua et al. 2008; Ballard et al. 2017; Nicolás et al. 2013) are among the controlled radical polymerization techniques (Yamamoto et al. 2014). Hosseinzadeh et al. (2019) prepared a unique stimuli-responsive hydrogel nanocomposite via surface reversible addition fragmentation chain transfer copolymerization of acrylic acid and N-isopropyl acrylamide onto chitosan and subsequent in situ synthesis of magnetic Fe_3O_4 nanoparticles for anticancer drug doxorubicin delivery. The maximum of doxorubicin loading efficiency of nanocomposite was 89%, and 82% of total doxorubicin was released from the hydrogel within 2 days. In this study, temperature and pH responsiveness of the nanocomposite were demonstrated, and they suggested that the chitosan-based nanocomposite may be utilized as a promising drug carrier for controlled and sustained release of anticancer drugs.

Another chemical modification technique is the use of carboxymethyl moieties. The water solubility of carboxymethyl chitosan at various pH environments is governed by the carboxymethylation degree. Shariatinia (2018) has reviewed the literature on carboxymethyl chitosan and its properties and biomedical applications. The most recent applications of carboxymethyl chitosan derivatives with antimicrobial, anticancer, antitumor, antioxidant, and antifungal biological activities in various areas like wound healing, tissue engineering, drug/enzyme delivery, bio-imaging, and cosmetics have been discussed in their review.

Tana et al. (2013) also synthesized a glycol chitosan-carboxymethyl-cyclodextrins (G-chitosan-CM-dextrins) for delivering different hydrophobic anticancer drugs. They showed that the three anticancer drugs (5-fluorouracil, doxorubicin, and vinblastine) could be successfully loaded into the cavities of the covalently linked CM-dextrins. pH-sensitive release of doxorubicin has been observed and suggested that different drugs should be released in different ways.

Coating on Nanoparticles: Parsian et al. (2016) studied the targeted delivery of the hydrophilic gemcitabine to increase its cellular uptake and efficacy. For this purpose, chitosan-coated iron oxide nanoparticles have been synthesized by coprecipitation that encapsulates gemcitabine as described in Fig. 7.4. They

optimized the loading of gemcitabine as 30 μM with the highest drug release as 65% at pH 4.2, while it was 8% at pH 7.2. This is desired since pH of tumor tissue and endosomers is acidic. They tested the cellular uptake and targetability of these nanoparticles on MCF-7 breast cancer cell lines and indicated the increased efficacy of gemcitabine when loaded onto nanoparticles. Kamaraj et al. (2018) have developed curcumin-loaded hybrid nanoparticles of vanillin-chitosan coated with paramagnetic calcium ferrite nanoparticles using ionic gelation method. The vanillin-chitosan nanoparticles were functionally modified by the Schiff base reaction to enhance the hydrophobic drug encapsulation efficiency. Calcium ferrite nano particles were added to the system to improve the biocompatibility. The maximum encapsulation efficiency obtained was 98.3% under the conditions of 0.1, 0.75, and 1.0 for the drug to chitosan-vanillin, CFNP to chitosan-vanillin, and TPP to chitosan-vanillin ratios, respectively. They executed the curcumin release at various pH, initial drug loading concentrations, and magnetic fields and predicted the drug release mechanism by fitting the experimental kinetic data with various drug release models. The cytotoxicity test of nanocarriers is performed against MCF-7 breast cancer cell line to check the anticancer property of the hybrid nanocarrier with the curcumin drug. Chen et al. (2019) created composite structures by cross-linking chitosan onto drug-loaded mesoporous silica nanoparticles through disulfide bonds. This created a thin film of chitosan which led to site-specific and timely drug delivery. The system was also able to trigger drug release by the changes in such factors which are common to cancer cells. They suggested that this surface chemical modification strategy promises a powerful approach constructing smart drug delivery systems for efficient and safe chemotherapy.

Sasirekha et al. (2019) investigated the use of a mesoporous, biodegradable nanomaterial obtained from the natural silica found in the diatom species Amphora subtropica for drug delivery applications. The cultures of this material were cleaned and chemically treated to obtain Amphora frustules (exoskeleton) followed by surface functionalization with chitosan. Results of their experiments demonstrated high drug loading, strong luminescence, and biodegradable and biocompatible nature of the doxorubicin tethered diatom.

Rao et al. (2018) proposed in situ preparation method of Au NPs (hexagonal and rod-shaped structures) in the lumen as well as the surface cage of biocompatible halloysite nanotubes using curcumin as anticancer drug and subsequently coating with bioadhesive chitosan. The anticancer potential of halloysite nanotube hybrid nanoparticles on MCF-7 cancer cells was studied and showed efficient anticancer activity under intracellular tumor cell environment (pH 5.5) than extracellular conditions (pH 7.4). They suggested that the developed halloysite nanotube hybrid nanoparticles consisting of Au nanoparticles (NIR-responsive property) and pH-responsive curcumin release could make it suitable for cancer cell-targeted drug delivery.

Self-Assembled Amphiphilic Chitosan Nanoparticles: A review on the recent progresses in the design and fabrication of chitosan-based self-assembled nanomaterials and their applications in the delivery of different therapeutic agents was done by Yang et al. (2014). In a recent study, Petrov et al. (2008) produced a

drug delivery system by encapsulating quercetin into pH-sensitive self-assembled amphiphilic chitosan nanoparticles. Up to 83% of quercetin was entrapped by the nanoparticles. They found that the payload release is larger at an acidic pH of 5.0 than at the physiological pH of 7.4. They further revealed that quercetin maintains its metabolism inhibition against MCF-7 cells after encapsulation and that nanoparticles accumulate on the cell surface.

Mu et al. (2019a, b) synthesized quercetin-chitosan conjugate for oral delivery of doxorubicin to improve its oral bioavailability by increasing its water solubility, opening tight junction, and bypassing the P glycoprotein. The prepared quercetin-chitosan self-assembled into micelles which could encapsulate doxorubicin with high encapsulation rate, small particle size (137 nm), and strong zeta potential (+16.2 mV). Quercetin-chitosan-doxorubicin micelles displayed sustained-release profile in gastrointestinal simulation fluid (pH 1.2/pH 7.4). Quercetin-chitosan micelles could promote cellular uptake of doxorubicin, which was 2.2-fold higher than that of free doxorubicin. They showed that quercetin-chitosan micelles are promising vehicles for the oral delivery of insoluble anticancer drugs.

Chitosan-Mediated Co-delivery: Different anticancer drugs affect different parts of the cell and should be used together in chemotherapy. Nanoparticle systems which allow the simultaneous use of multiple drugs are called the co-delivery systems and synergistically inhibit the tumor growth. Qi et al. (2017) highlighted the current state of co-delivery nanoparticles and the most commonly employed nanomaterials. They discussed challenges and strategies and prospect future development. Afkham et al. (2018) designed chitosan-mediated co-delivery nanoparticles for the efficient encapsulation of the anticancer drugs SN38 (7-Ethyl-10-hydroxy-camptothecin) and snail-specific small interfering RNAs (siRNA). They found that chitosan nanoparticles encapsulating SN38 and snail-specific siRNA may represent huge potential as an effective anticancer drug delivery system for the treatment of prostate cancer.

There are also studies which employ chitosan nanoparticles for immunotherapy of cancers. The use of chitosan nanoparticles in this field is summarized in a review paper by Naskar et al., (2019) where various types of chitosan nanoparticles have been listed. The paper discusses the attempts made to form new therapeutic approaches that can restore immune competence in cancer patients (Li et al. 2011; Zhao et al. 2011a, b; Xue et al. 2015b; Garg et al. 2016; Jesus et al. 2017; Yaguchi et al. 2011).

7.4 Drug Release Studies with Chitosan Nanoparticles

The most important feature of chitosan-based nanoparticles/nanoshells is their pH-dependent release behavior. Chitosan structures degrade at the acidic pH environment of the cancer cells and release the contained drug. The pH-dependent release of drug with a minimal release at the physiological pH of 7.4 makes the chitosan carriers as one of the most promising candidates for chemotherapeutic cancer treatment with reduced side effects.

Qiu et al. (2013) studied the release profiles of doxorubicin from self-assembled chitosan nanoparticles. The self-assembled phytosterol-fructose-chitosan nanoparticles have been synthesized from water-soluble fructose-chitosan solutions. The procedure consisted of forming fructose-chitosan polymeric material by adding fructose to chitosan-acetic solution and polymerization by sodium borohydride as a first step. Then, phytosterol hemisuccinate was coupled to fructose-chitosan for self-assembly by succinyl linkages with phytosterols as hydrophobic moieties. In the final step doxorubicin was physically entrapped inside the self-assembled nanoparticles by the dialysis method. A slow sustained release of doxorubicin over a 48 h period was observed, and the release rate in phosphate buffered saline solution at pH 7.4 was much slower than that in pH 5.5 and pH 6.5.

Wang et al. (2015) prepared chitosan-modified polylactic acid nanoparticles as carriers for encapsulation of docetaxel by anti-solvent precipitation method. They characterized the polylactic acid/chitosan nanoparticles by SEM, DLS, FTIR, and XPS. The particles were 250 nm in size and had a zeta potential value of +53.9 mV. They showed that docetaxel releases from the prepared polylactic acid/chitosan nanoparticles with 40% initial burst release in 5 h and 70% cumulative release within 24 h. The release of docetaxel from the polylactic acid nanoparticles, on the other hand, was 65% in 5 h. The study suggested that polylactic acid/chitosan nanoparticles prolong drug release while decreasing the initial burst release.

Sun et al. (2017) studied the sustained-release properties of the biodegradable nano-drug delivery systems to improve the residence time of the chemotherapeutic agent in the body. The 5-fluorouracil-loaded chitosan nanoparticles have been prepared in their study. They found that when the mass ratio of 5-fluorouracil and chitosan was 1:1, the maximum drug loading of nanoparticles was around 20% with an encapsulation efficiency of around 44%. The mean size of the particles was 284 nm and the measured zeta potential was 45.3 mV. The prepared nanoparticles had both burst-release and sustained-release phases in vitro release studies.

Karimi et al. (2018) developed κ-carrageenan-cross-linked magnetic chitosan with different molecular weights as pH-responsive carriers for controlled release of the hydrophobic anticancer drug sunitinib. They found that drug encapsulation efficiency and release performance were influenced by the size of magnetic nanoparticles. Encapsulation efficiencies of sunitinib by low, medium, and high molecular weights of magnetic chitosan carriers were found to be 62.4, 69.6, and 78.4%, respectively. The in vitro sunitinib release from magnetic chitosan/κ-carrageenan carriers has shown to be pH-dependent and followed a Fickian release mechanism. They showed that sunitinib was efficiently released from magnetic carriers into the environment under acidic pH and the release rate was size- and molecular weight-dependent.

These sample drug release studies demonstrate that the use of chitosan nanoparticles as drug carriers presents a decisively positive contribution. In many cases, however, the research tends to employ chitosan together with other materials to create a synergy for delivery purposes. However, it is important to note that one should be careful about not trading away the outstanding properties of this

biopolymer (such as its pH-dependent degradation and positive charge) when it is employed with other materials to design a composite drug carrier.

References

Afkham A, Aghebati-Maleki L, Siahmansouric H, Sadreddini S, Ahmadia M, Dolati S, Jadidi-Niaragh F, Yousefi M, Siahmansouri H, Afkham NM, Akbarzadeh P, Yousefib M et al (2018) Chitosan (CMD)-mediated co-delivery of SN38 and Snail-specific siRNA as a useful anticancer approach against prostate cancer. Pharmacol Rep 70:418–425

Ahmad FJ, Islam F, Alam S, Mustafa G, Bhatnagar A, Khan ZI et al (2012) Development and evaluation of thymoquinone encapsulated chitosan nanoparticles for nose-to-brain targeting: a pharmaco scintigraphic study. Int J Nanomed 7:5705–5718

Ahsan SM, Thomas M, Reddy K, Sooraparaju SG, Asthana A, Bhatnagar I et al (2018) Chitosan as biomaterial in drug delivery and tissue engineering. Int J Boil Macromol 110:97–109

Alam S, Khan ZI, Mustafa G, Kumar M et al (2012) Development and evaluation of thymoquinone-encapsulated chitosan nanoparticles for nose-to-brain targeting: a pharmacoscintigraphic study. Int J Nanomedicine 7:5705–5718

Ali A, Ahmed S et al (2018) A review on chitosan and its nanocomposites in drug delivery. Int J Biol Macromol 109:273–286

Andriani Y, Grasianto C, Siswanta D, Mudasir M (2015) Retracted-glutaraldehyde-crosslinked chitosan-pectin nanoparticles as a potential carrier for curcumin delivery and its in vitro release study. Int J Drug Deliv 7:167–173

Anto SM, Kannan C, Kumar KS, Kumar SV, Suganeshwari M et al (2011) Formulation of 5-fluorouracil loaded chitosan nanoparticles by emulsion droplet coalescence method for cancer therapy. Int J Pharm Biol Arch 2:926–931

Aydin RST, Pulat M et al (2012) 5-Fluorouracil encapsulated chitosan nanoparticles for pH-stimulated drug delivery: evaluation of controlled release kinetics. J Nanomater 2012:10

Bagheri M, Younesi H, Hajati S et al (2015) Application of chitosan-citric acid nanoparticles for removal of chromium (VI). Int J Biol Macromol 80:431–444

Ballard N, Aguirre M, Simula A, Leiza JR, Van Es S, Asua JM et al (2017) Nitroxide mediated suspension polymerization of methacrylic monomers. Chem Eng J 316:655–662

Banerjee T, Mitra S, Singh AK et al (2002) Preparation, characterization and biodistribution of ultrafine chitosan nanoparticles. Int J Pharm 243:93–105

Batrakova EV, Kabanov AV (2008) Pluronic block copolymers evolution of drug delivery concept from inert nanocarriers to biological response modifiers. J Control Release 130(2):98–106. https://doi.org/10.1016/j.jconrel.2008.04.013

Bhattarai N, Ramay HR, Chou SH et al (2006) Chitosan and lactic acid-grafted chitosan nanoparticles as carriers for prolonged drug delivery. Int J Nanomed 1:181–187

Bianco A, Kostarelos K, Prato M (2008) Opportunities and challenges of carbon-based nanomaterials for cancer therapy. Expert Opin Drug Deliv 5:331–342

Bilati U, Allemann E, Doelker E (2005) Development of a nanoprecipitation method intended for the entrapment of hydrophilic drugs into nanoparticles. Eur J Pharm Sci 24:67–75

Cao Y, Xu Y, Zhang J, Yang D, Liu J et al (2015) Well-controlled atom transfer radical polymerizations of acrylates using recyclable niobium complex nanoparticle as photocatalyst under visible light irradiation. Polymer 61:198–203

Casettari L, Castagnino E, Vllasaliu D, Stolnik S, Howdle S, Illum L et al (2012) PEGylated chitosan derivatives: synthesis, characterizations and pharmaceutical applications. Prog Polym Sci 37(5):659–685

Cesano F, Fenoglio G, Nisticò R, Carlos L et al (2015) One-step synthesis of magnetic chitosan polymer composite films. Appl Surf Sci 345:175–181

Chabner BA, Lango DL (2001) Topoisomerase II inhibitors: the epipo- dophyllotoxins, m–AMSA and the ellipticine derivatives. In: Cancer chemo-theraphy and biotherapy: principles and practice. Lippoincott Williams and Wilkins, Philadelphia

Chang K, Liang B et al (1997) Heterogeneous N-deacetylation of chitin in alkaline solution. Carbohydr Res 303(3):327–332

Chen J, Ma YD, Wang LC, Han WY et al (2019) Preparation of chitosan/SiO2-loaded graphene composite beads for efficient removal of bilirubin. Carbon 143:352–361

Cheung CSF, Chung KKW, Lui JC, Lau CP, Hon PM et al (2007) Leachi- anone A as a potential anticancer drug by induction of apoptosis in human hepatoma HepG2 cells. Cancer Lett 253 (2):224–235

Chi HJK, Amalini CJ, Karen SB, Marcus PG, Lynnette RF et al (2013) Anticancer activities of Ganoderma lucidum: active ingredients and pathways. Funct Food Health Dis 3(2):48–65

Cihan E, Polat M, Polat H et al (2017) Designing of spherical chitosan nano-shells with micellar cores for solvation and safeguarded delivery of strongly lipophilic drugs. Colloids Surf A Physicochem Eng Asp 529:815–823

Cragg GM, Schepartz SA, Suffness M, Grever MG et al (1993) The taxol supply crisis. new NCI policies for handling the large scale production of novel nat-ural product anticancer and anti-HIV agents. J Nat Prod 56(10):1657–1668

De Campos AM, Sanchez A, Alonso MJ et al (2001) Chitosan nanoparticles: a new vehicle for the improvement of the delivery of drugs to the ocular surface. Application to cylosporin A. Int J Pharm 224:159–168

Deshayes S et al (2013) Phenylboronic acid-installed polymeric micelles for targeting sialylated epitopes in solid tumors. J Am Chem Soc 135:15501–15507

Dong Y et al (2014) Lipid-like nanomaterials for simultaneous gene expression and silencing in vivo. Adv Healthc Mater 3:1392–1397

Dong P, Rakesh KP, Manukumar HM, Mohammed YHE, Karthik CS, Sumathi S, Mallud P, Qin HL (2019) Innovativenano-carriersinanticancerdrugdelivery-acomprehensive review. Bioorg Chem 85:325–336

Dreis S et al (2007) Preparation, characterisation and maintenance of drug efficacy of doxorubicin-loaded human serum albumin (HSA) nanoparticles. Int J Pharm 341:207–214

Elgadir MA, Adam A, Uddin MS, Sarker MZI, Ferdosh S, Chowdhury AJK et al (2015) Impact of chitosan composites and chitosan nanoparticle composites on various drug delivery systems: a review. J Food Drug Anal 23(4):619–629

El-Shabouri MH et al (2002) Positively charged nanoparticles for improving the oral bioavailability of cyclosporin-A. Int J Pharm 249:101–108

Elzoghby AO, Samy WM, Elgindy NA (2012) Albumin-based nanoparticles as potential controlled release drug delivery systems. J Control Release 157:168–182

Ensign LM, Cone R, Hanes J et al (2012) Oral drug delivery with polymeric nanoparticles: the gastrointestinal mucus barriers. Adv Drug Deliv Rev 64(6):557–557

Fernandez-Urrusuno R, Cavlo P, Remunan LC et al (1999) Enhancement of nasal absorption of insulin using chitosan nanoparticles. Pharm Res 16:1576–1581

Fessi H, Puisieux F, Devissaguet JP, Ammoury N, Benita S (1989) Nanocapsule formation by interfacial polymer deposition following solvent displacement. Int J Pharm 55:R1–R4

Gao JJ, Nakamura N, Min BS, Hirakawa A, Zuo F et al (2004) Quantitative determination of bitter principles in specimens of Ganoderma lucidum using high-performance liquid chromatography and its application to the evaluation of Ganoderma products. Chem Pharm Bull 52(6):688–695

Gao X, He C, Xiao C, Zhuang X, Chen X (2013) Biodegradable pH-responsive polyacrylic acid derivative hydrogels with tunable swelling behavior for oral delivery of insulin. Polymer 54:1786–1793

Gao P, Xia G, Bao Z, Feng C, Cheng X, Kong M, Liu Y, Chen X et al (2016) Chitosan based nanoparticles as protein carriers for efficient oral antigen delivery. Int J Biol Macromol 91:716–723

Garg T, Rath G, Goyal AK et al (2016) Inhalable chitosan nanoparticles as antitubercular drug carriers for an effective treatment of tuberculosis. Artif Cells Nanomed Biotechnol 44:997–1001
Gaucher G, Dufresne MH, Sant VP, Kang N, Leroux JC, Maysinger D et al (2005) Block copolymer micelles: preparation, characterization and application in drug delivery. J Control Release 109:169–188
George M, Abraham TE et al (2006) Polyionic hydrocolloids for the intestinal delivery of protein drugs: alginate and chitosan-a review. J Control Release 114(1):1–14
George A, Shah PA, Shrivastav PS (2019) Guar gum: versatile natural polymer for drug delivery applications. Eur Polym J 112:722–735
Ghosh R, Goswami U, Ghosh SS, Paul A, Chattopadhyay A (2015) Synergistic anticancer activity of fluorescent copper nanoclusters and cisplatin delivered through a hydrogel nanocarrier. ACS Appl Mater Interfaces 7:209–222
Gilbreth RN et al (2016) Lipid- and polyion complex-based micelles as agonist platforms for TNFR superfamily receptors. J Control Release 234:104–114
Gorochovceva N, Makuska R et al (2004) Synthesis and study of water-soluble chitosan-0-poly (ethylene glycol) graft copolymers. Eur Polym J 40:685–691
Grenha A (2012) Chitosan nanoparticles: a survey of preparation methods. J Drug Target 20 (4):291–300
Gupta NK, Nahata A, Dixit VK et al (2010) Development of spectrofluorimetric method for the determination of curcumin. Asian J Trad Med 5(1):12–18. https://www.cancer.gov
Hande KR et al (1998) Etopside: four decades of development of a topoisomerase II inhibitor. Eur J Cancer 34(10):1514–1521
Hartwell JL et al (1971) Plants used against cancer – a survey. Lloydia 34(4):204–255
Hawkins MJ, Soon-Shiong P, Desai N (2008) Protein nanoparticles as drug carriers in clinical medicine. Adv Drug Deliv Rev 60:76–885
Hojjati B, Charpentier PA et al (2008) Synthesis and kinetics of graft polymerization of methyl methacrylate from the RAFT coordinated surface of nano-TiO2. J Polym Sci A 46 (12):3926–3937
Honore S, Kamath K, Braguer D, Horwitz SB et al (2004) Synergistic suppression of microtubule dynamics by discodermolide and paclitaxel in non-small cell lung carcinoma cells. Cancer Res 64:4957–4964
Hosseinzadeh H, Pashaei S, Ramin S, Hosseinzadeh S, Khodaparast Z, Saadat Y et al (2018) Preparation of novel multi-walled carbon nanotubes nanocomposite adsorbent via RAFT technique for the adsorption of toxic copper ions. Sci Total Environ 640:303–314
Hosseinzadeh H, Pashaei S, Ramin S, Hosseinzadeh S, Khodaparast Z, Saadat Y et al (2019) Synthesis of stimuli-responsive chitosan nanocomposites via RAFT copolymerization for doxorubicin delivery. Int J Biol Macromol 121:677–685
Hu Y, Jiang X, Ding Y, Ge H, Yuan Y, Yang C (2002) Synthesis and characterization of chitosan poly (acrylic acid) nanoparticles. Biomaterials 23:3193–3201
Hua D, Tang J, Cheng J, Deng W, Zhu X et al (2008) A novel method of grafting modification of chitosan via REFT polymerization agent. Carbonhydr Polym 73:98–104
Hunter AC, Moghimi SM, Elsom J, Wibroe PP et al (2012) Polymeric particulate technologies for oral drug delivery and targeting: a pathophysiological perspective. Nanomed Nanotechnol 8:5–20
Jesus S, Soares E, Borchard G, Borges O et al (2017) Poly-e-caprolactone/chitosan nanoparticles provide strong adjuvant effect for hepatitis B antigen. Nanomedicine 12:2335–2348
Jiang J, Pan X, Cao J, Jiang J, Hua D, Zhu X et al (2012) Synthesis and property of chitosan graft copolymer by RAFT polymerization with tosylic acid–chitosan complex. Int J Biol Macromol 50(3):586–590
Jiang L, Duan H, Ji X et al (2018) Application of a simple desolvation method to increase the formation yield, physical stability and hydrophobic drug encapsulation capacity of chitosan-based nanoparticles. Int J Pharm 545:117–127

Jin X et al (2016) Efficient intravesical therapy of bladder cancer with cationic doxorubicin nanoassemblies. Int J Nanomed 11:4535–4544
Kabanov AV, Batrakova EV, Alakhov VY et al (2002) Pluronic block copolymers as novel polymer therapeutics for drug and gene delivery. J Control Release 82:189–212
Kafshgari MH, Khorram M, Mansouri M et al (2012) Preparation of alginate and chitosan nanoparticles using a new reverse micellar system. Iran Polym J 21:99–107
Kairdolf BA et al (2013) Semiconductor quantum dots for bioimaging and biodiagnostic applications. Annu Rev Anal Chem 6:143–162
Kamaraj S, Palanisamy UM, Mohamed MSBK, Gangasalam A, Maria GA, Kandasamy R et al (2018) Curcumin drug delivery by vanillin-chitosan coated with calcium ferrite hybrid nanoparticles as carrier. Eur J Pharm Sci 116:48–60
Karimi MH, Mahdaviniab GR, Massoumia B et al (2018) pH-controlled sunitinib anticancer release from magnetic chitosan nanoparticles crosslinked with κ-carrageenan. Mater Sci Eng C 91:705–714
Kaur G, Mahajan M, Bassi P (2013) Derivatized polysaccharides: preparation, characterization, and application as bioadhesive polymer for drug delivery. Int J Polym Mater Polym Biomater 62:475–481
Kavimani S, Manisenthilkumar KT, Ilango R, Krishnamoorthy G et al (1999) Effect of the methanolic extract of Glinus lotoides on Dalton's ascitic lym- phoma. Biol Pharm Bull 22 (11):1251–1252
Kawashima Y, Handa T, Takenaka H, Lin SY, Ando Y et al (1985a) Novel method for the preparation of controlled-release theophylline granules coated with a polyelectrolyte complex of sodium polyphosphate-chitosan. J Pharmacol Sci 74:264–268
Kawashima Y, Handa T, Takenaka H, Lin SY, Ando Y et al (1985b) The effect of thickness and hardness of the coating film on the drug release of theophylline granules. Chem Pharm Bull 33:2469–2474
Kloster GA, Meiorin C, Marcovich NE, Mosiewicki MA, Muraca DR, Pirota K et al (2015) Magnetic characterization of chitosan-magnetite nanocomposite films. Eur Polym J 72:202–211
Krajewska B (2004) Application of chitin- and chitosan-based materials for enzyme immobilizations: a review. Enzym Microb Technol 35(2–3):126–139
Kumar MN, Kumar N, Domb AJ (2000) Pharmaceutical polymeric controlled drug delivery systems. Adv Polymer Sci 160:45–117
Kumar A, Sridevi K, Kumar NV, Nanduri S, Rajagopal S et al (2002) Hypericin in cancer treatment: more light on the way. Int J Biochem Cell Biol 34(3):221–241
Kumari P et al (2017) Cholesterol-conjugated poly (D, L-lactide)-based micelles as a nanocarrier system for effective delivery of curcumin in cancer therapy. Drug Deliv 24:209–223
Kurita K et al (2006) Chitin and chitosan: functional biopolymers from marine crustaceans. Mar Biotechnol 8:203–226
Kwak CS, Moon SC, Lee MS et al (2006) Antioxidant, antimutagenic, and anti-tumor effects of pine needles (Pinus densiflora). Nutr Cancer 56(2):162–171
Lanksy EP, Robert AN et al (2007) Punica granatum (pomegranate) and its potential for prevention and treatment of inflammation and cancer. J Ethnopharmacol 109(2):177–206
Lanzalaco S, Scialdone O, Galia A, Fantin M, Matyjaszewski K, Isse AA, Gennaro A et al (2017) Atom transfer radical polymerization with different halides (F, Cl, Br, and I): is the process "living" in the presence of fluorinated initiators? Macromolecules 50(2):192–202
Li P, Wang Y, Zeng F et al (2011) Synthesis and characterization of folate conjugated chitosan and cellular uptake of its nanoparticles in HT-29 cells. Carbohydr Res 346:801–806
Li Z et al (2015) Quantum dots loaded nanogels for low cytotoxicity, pH-sensitive fluorescence, cell imaging and drug delivery. Carbohydr Polym 121:477–485
Lin CC, Metters AT (2006) Hydrogels in controlled release formulations: network design and mathematical modeling. Adv Drug Deliv Rev 58:1379–1408
Liu X, Fang Z, Zhang X, Zhang B, Hu Q et al (2009) Magnetic chitosan nanocomposites: a useful recyclable tool for heavy metal ion removal. Langmuir 25(1):3–8

Liu SS, Yang SL, Paul CH et al (2018) Intranasal administration of carbamazepine-loaded carboxymethyl chitosan nanoparticles for drug delivery to the brain. Asian J Pharm Sci 13:72–81

Luckachan GE, Pillai CKS et al (2006) Chitosan/oligo L-lactide graft copolymers: effect of hydrophobic side chains on the physico-chemical properties and biodegradability. Carbohydr Polym 64:254–266

Luque-Alcaraz AG, Lizardi-Mendoza J, Goycoolea FM et al (2016) Preparation of chitosan nanoparticles by nanoprecipitation and their ability as a drug nanocarrier. RSC Adv 6:59250–59256

Maeda H, Nakamura H, Fang J et al (2013) The EPR effect for macromolecular drug delivery to solid tumors: improvement of tumor uptake, lowering of systemic toxicity, and distinct tumor imaging in vivo. Adv Drug Deliv Rev 65:71–79

Masuda Y, Shima G, Aiuchi T, Horie M, Hori K et al (2004) Involvement of tumor necrosis factor receptor-associated protein 1 (TRAP1) in apoptosis induced by beta-hydroxyisovalerylshikonin. J Biol Chem 279(41):42503–42515

Mazumder A, Cerella C, Diederich M (2018) Natural scaffolds in anticancer therapy and precision medicine. Biotechnol Adv 36:1563–1585

Mehrotra A, Nagarwal RC, Pandit JK et al (2010) Fabrication of lomustine loaded chitosan nanoparticles by spray drying andin vitrocytostatic activity on human lung cancer cell line L132. J Nanomed Nanotechnol 1:1–7

Meng J, Sturgis TF, Youan BC et al (2011) Engineering tenofovir loaded chitosan nanoparticles to maximize microbicide mucoadhesion. Eur J Pharm Sci 44:57–67

Ming SC, Wan S Jr, Jaw DM, Jin LJ, Hee LG et al (2003) Cytotoxic sesquiter-pene lactones the root of Saussurea lappa. J Nat Prod 66(9):1175–1180

Mitra S, Gaur U, Ghosh PC et al (2001) Tumour targeted delivery of encapsulated dextran-doxorubicin conjugate using chitosan nanoparticles as carrier. J Control Release 74:317–323

Mo R, Jiang T, Gu Z (2014) Recent progress in multidrug delivery to cancer cells by liposomes. Nanomedicine 9:1117–1120

Motwani SK, Chopra S, Talegaonkar S et al (2008) Chitosan-sodium alginate nanoparticles as submicroscopic reservoirs for ocular delivery: formulation, optimization and in vitro characterization. Eur J Pharm Biopharm 68:513–525

Moudi M, Go R, Yien CSK, Nazre M et al (2013) Vinca alkaloids. Int J Prev Med 4(11):1231–1235

Mourdikoudis S, Pallares RM, Thanh TNK et al (2018) Characterization techniques for nanoparticles: comparison and complementarity upon studying nanoparticle properties. Nanoscale 10:12871–12934

Mu Y, Fu Y, Li J, Yu X, Li Y, Wang Y, Wu X, Zhang K, Kong M, Feng C, Chen X et al (2019a) Multifunctional quercetin conjugated chitosan nano-micelles with P-gp inhibition and permeation enhancement of anticancer drug. Carbohyd Polym 203:10–18

Mu Y, Li J, Yu X, Li Y, Wang Y, Wu X, Zhang K, Kong M, Feng C, Chen X, Fu Y et al (2019b) Multifunctional quercetin conjugated chitosan nano-micelles with P-gp inhibition and permeation enhancement of anticancer drug. Carbohydr Polym 203:10–18

Nahata A, Dixit VK et al (2011) Sphaeranthus indicus attenuates testosterone induced prostatic hypertrophy in albino rats. Phytother Res 25(12):1839–1848

Nahata A, Dixit VK et al (2012a) Ganoderma lucidum is an inhibitor of testosterone induced prostatic hyperplasia in rats. Andrologia 44(1):160–174

Nahata A, Dixit VK et al (2012b) Ameliorative effects of stinging nettle (Urtica dioica) on testosterone-induced prostatic hyperplasia in rats. Andrologia 44(1):396–409

Nahata A et al (2013) Ganoderma lucidum: a potent medicinal mushroom with numerous health benefits. Pharm Anal Acta 4:e159

Nahata A (2017) Anticancer agents: a review of relevant information on important herbal drugs. Int J Clin Pharmacol Toxicol 6(2):250–255

Nanjwade BK, Singh J, Parikh KA et al (2010) Preparation and evaluation of carboplatin biodegradable polymeric nanoparticles. Int J Pharm 385:176–180

Naskar S et al (2019) Chitosan-based nanoparticles: an overview of biomedical applications and its preparation. J Drug Deliv Sci Technol 49:66–81
Newman DJ, Cragg GM, Snader KM et al (2003) Natural products as sources of new drugs over the period 1981–2002. J Nat Prod 66(7):1022–1037
Ngan LTK, Wang SL, Hiep ĐM et al (2014) Preparation of chitosan nanoparticles by spray drying, and their antibacterial activity. Res Chem Intermed 40:2165–2175
Nicolás P, Ferreira ML, Lassalle V, Saleta M, Troiani H, Zysler R et al (2013) Preparation of iron oxide nanoparticles stabilized with biomolecules: experimental and mechanistic issues. Acta Biomater 9(1):4754–4762
Niu XF, Tian F, Wang LZ, Li XM, Zhou G, Fana YB et al (2014) Synthesis and characterization of chitosan-graft-poly(lactic acid) copolymer. Chin J Polym Sci 32(1):43–50
Niwa T, Takeuchi H, Hino T, Kunou T, Kawashima Y et al (1993) Preparations of biodegradable nanospheres of water-soluble and insoluble drugs with D,L-lactide/glycolide copolymer by a novel spontaneous emulsification solvent diffusion method and the drug release behavior. J Contr Release 25:89–98
Ohya Y, Shiratani M, Kobayashi H, Ouchi T et al (1994) Release behaviour of 5-fluorouracil from chitosan-gel nanospheres immobilizing 5-fluorouracil coated with polysaccharides and their cell specific cytotoxicity. Pure Appl Chem A 31:629–642
Owen SC, Dianna CPY, Shoichet MS et al (2012) Polymeric micelle stability. Nano Today 7:53–65
Pan Y, Li Y, Zhao H et al (2002) Bioadhesive polysaccharide in protein delivery system: chitosan nanoparticles improve the intestinal absorption of insulin in vivo. Int J Pharm 249:139–147
Park JH et al (2008) Polymeric nanomedicine for cancer therapy. Prog Polym Sci 33:113–137
Parsian M, Unsoy G, Mutlu P, Yalcin S, Tezcaner A, Gunduz U et al (2016) Loading of Gemcitabine on chitosan magnetic nanoparticles increases the anticancer efficacy of the drug. Eur J Pharmacol 784:121–128
Parveen S, Sahoo SK (2008) Polymeric nanoparticles for cancer therapy. J Drug Target 16:108–123
Pawar D, Mangal S, Goswami R, Jaganathan KS et al (2013) Development and characterization of surface modified PLGA nanoparticles for nasal vaccine delivery: effect of mucoadhesive coating on antigen uptake and immune adjuvant activity. Eur J Pharm Biopharm 85:550–559
Pella MCG, Lima-Tenório MK, Tenório-Neto ET, Guilherme MR, Muniz EC, Rubira AF et al (2018) Chitosan-based hydrogels: from preparation to biomedical applications. Carbohydr Polym 196:233–245
Peppas N, Bures P, Leobandung W, Ichikawa H (2000) Hydrogels in pharmaceutical formulations. Eur J Pharm Biopharm 50:27–46
Petrov P, Yuan J, Yoncheva K, Müller AHE, Tsvetanov CB et al (2008) Wormlike morphology formation and stabilization of pluronic P123 micelles by solubilization of pentaerythritol tetraacrylate. J Phys Chem B 112:8879–8883
Pisha E, Chai H, Lee IS, Chagwedera TE, Farnsworth NR et al (1995) Discovery of betulinic acid as a selective inhibitor of human melanoma that functions by induction of apoptosis. Nat Med 1 (10):1046–1051
Plapied L, Duhem N, des Rieux A, Preat V et al (2011) Fate of polymeric nanocarriers for oral drug delivery. Curr Opin Colloid Interface Sci 16:228–237
Polat H, Kutluay G, Polat M (2019) Dilution induced disintegration of micellar drug carriers in the presence of extra and intra micellar species, Submitted to macromolecules
Prabaharan M et al (2015) Chitosan-based nanoparticles for tumor-targeted drug delivery. Int J Biol Macromol 72:1313–1322
Qi X et al (2015) Fabrication and characterization of a novel anticancer drug delivery system: salecan/poly (methacrylic acid) semi-interpenetrating polymer network hydrogel. ACS Biomater Sci Eng 1:1287–1299
Qi SS, Sun JH, Yu HH, Yu SQ (2017) Co-delivery nanoparticles of anti-cancer drugs for improving chemotherapy efficacy. Drug Deliv 24:1909–1926
Qiu Y, Zhu J, Wang J, Gong R, Zheng M, Huang F et al (2013) Self-assembled phytosterol-fructose-chitosan nanoparticles as a carrier of anticancer drug. Nanosci Nanotechnol 13:1–7

Radhakumary C, Nair PD, Mathew S, Reghunadhan Nair CP et al (2007) Synthesis, characterization, and properties of poly(vinyl acetate)- and poly(vinyl alcohol)-grafted chitosan. J Appl Polym Sci 104(3):1852–1859

Rahimi S, Khoee S, Ghandi M (2019) Preparation and characterization of rod-like chitosan-quinoline nanoparticles as pH-responsive nanocarriers for quercetin delivery. Int J Biol Macromol 128:279–289

Rampino A, Borgogna M, Blasi P, Bellich B, Cesàro A et al (2013) Chitosan nanoparticles: preparation, size evolution and stability. Int J Pharm 455:219–228

Rao KM, Kumar A, Suneetha M, Han SS et al (2018) pH and near-infrared active; chitosan-coated halloysite nanotubes loaded with curcumin-Au hybrid nanoparticles for cancer drug delivery. Int J Biol Macromol 112:119–125

Razmi M, Divsalar A, Saboury AA, Izadi Z, Haertlé T, Mansuri-Torshizi H et al (2013) Beta-casein and its complexes with chitosan as nanovehicles for delivery of a platinum anticancer drug. Colloids Surf B Biointerfaces 112:362–367

Reddy YD, Dhachinamoorthi D, Chandra SKB et al (2013) Formulation and in vitro evaluation of antineoplastic drug loaded nanoparticles as drug delivery system. Afr J Pharm Pharmacol 7:1592–1604

Renggli K, Nussbaumer MG, Urbani R, Pfohl T, Bruns N et al (2017) Biocatalytic atom transfer radical polymerization in a protein cage nanoreactor. Polym Chem 8:2133–2136

Roveimiab Z, Mahdavian AR, Biazar E, Saeed Heidari K et al (2012) Preparation of magnetic chitosan nanocomposite particles and their susceptibility for cellular separation applications. J Colloid Sci Biotechnol 1:82–88

Sachs-Barrable K, Lee SD, Wasan EK, Thomton SJ, Wasan KM et al (2007) Enhancing drug absorption using lipids: a case study presenting the development and pharmacological evaluation of a novel lipid-based oral amphotericin B formulation for the treatment of systemic fungal infections. Adv Drug Deliv Rev 60:692–701

Sajeesh S, Sharma CP (2005) Novel pH responsive polymethacrylic acid-chitosanpolyethylene glycol nanoparticles for oral peptide delivery. J Biomed Mater Res B Appl Biomater 76B:298–305

Sajeesh S, Sharma CP (2006) Cyclodextrin-insulin complex encapsulated polymethacrylic acid based nanoparticles for oral insulin delivery. Int J Pharm 325:147–154

Sasirekha R, Sheenab TS, Deepikac MS, Santhanama P, Townleye HE, Jeganathanb K, Kumara SD, Premkumard K et al (2019) Surface engineered Amphora subtropica frustules using chitosan as a drug delivery platform for anticancer therapy. Mat Sci Eng C-Mater 94:56–64

Schiff PB, Fant J, Horwitz SB et al (1979) Promotion of microtubule assembly in vitro by taxol. Nature 277(5698):665–667

Senapati S, Mahanta AK, Kumar S, Maiti P et al (2018) Controlled drug delivery vehicles for cancer treatment and their performance. Signal Transduct Target Ther 3:7

Shanmuganathan R, Edison TNJI, Lewis Oscar F, Ponnuchamy K, Shanmugame S, Pugazhendhi A et al (2019) Chitosan nanopolymers: an overview of drug delivery against cancer. Int J Biol Macromol 130:727–736

Shariatinia Z et al (2018) Carboxymethyl chitosan: properties and biomedical applications. Int J Biol Macromol 120:1406–1419

Shariatinia Z, Mazloom-Jalali A et al (2019) Chitosan nanocomposite drug delivery systems designed for theifosfamide anticancer drug using molecular dynamics simulations. J Mol Liq 273:346–367

Shi M, Cai Q, Yao L, Mao Y, Ming Y et al (2006) Antiproliferation and apoptosis induced by curcumin in human ovarian cancer cells. Cell Biol Int 30(3):221–226

Shi C et al (2014) Actively targeted delivery of anticancer drug to tumor cells by redox-responsive star-shaped micelles. Biomaterials 35:8711–8722

Shi Y, Lammers T, Storm G, Hennink WE et al (2017) Physico-Chemical stability and drug retention of polymeric micelles for tumor-targeted drug delivery. Macromol Biosci 7(1):11

Shukla SK, Mishra AK, Arotiba OA, Mamba BB et al (2013) Chitosan-based nanomaterials: a state-of-the-art review. Int J Biol Macromol 59:46–58

Singh B, Singh B (2019) Developing a drug delivery carrier from natural polysaccharide exudate gum by graft-copolymerization reaction using high energy radiations. Int J Biol Macromol 127:450–459

Singh SK, Shanmugavel M, Kampasi H, Singh R, Mondhe DM et al (2007) Chemically standardized isolates from cedrus deodara stem wood having anticancer activity. Planta Med 73(6):519–526

Sun L, Chen Y, Zhou Y, Guo D, Fan Y, Guo F, Zheng Y, Chen W (2017) Preparation of 5-fluorouracil-loaded chitosan nanoparticles and study of the sustained release in vitro and in vivo. Asian J Pharm Sci 12:418–423

Sutar YB, Telvekar YN et al (2018) Chitosan based copolymer-drug conjugate and its protein targeted polyelectrolyte complex nanoparticles to enhance the efficiency and specificity of low potency anticancer agent. Mat Sci Eng C-Mater 92:393–406

Sutirman ZA, Sanagi MM, Abd Karim J, Abu Naim A, Wan Ibrahim WA et al (2018) New crosslinked-chitosan graft poly(N-vinyl-2-pyrrolidone) for the removal of Cu(II) ions from aqueous solutions. Int J Biol Macromol 107(Part A):891–897

Suzariana Samuri N, Hussein-Al-Ali SH, Kura A, Zobir Hussein M, Fakurazi S, Halim Shaari A et al (2016) Synthesis and characterization of magnetic chitosan-L-dopa nanocomposite. Curr Nanosci 12:487–492

Talelli P, Waddingham W, Ewas A, Rothwell JC, Ward NS et al (2008) The effect of age on task-related modulation of interhemispheric balance. Exp Brain Res 186:59–66

Tana H, Qina F, Chenb D, Hanb S, Luc W, Yaoa X et al (2013) Study of glycol chitosan-carboxymethyl -cyclodextrins as anticancer drugs carrier. Carbohydr Polym 93:679–685

Tang ZX, Qian JQ, Xi LE et al (2007) Preparation of chitosan nanoparticles as carrier for immobilized enzyme. Biotechnol 136:77–96

Tian X, Song HS, Cho YM, Park B, Song YJ, Jang S, Kang SC (2017) Anticancer effect of Saussurea lappa extract via dual control of apoptosis and autophagy in prostate cancer cells. Medicine (Baltimore) 96:7606

Tikhonov VE, Stepnova EA, Babak VG, Yamskov IA, Palma-Guerrero J, Jansson HB, Lopez-Llorca LV, Salinas J, Gerasimenko DV, Avdienko ID, Varlamov VP et al (2006) Bactericidal and antifungal activities of a low molecular weight chitosan and its N-/2(3)-(dodec-2-enyl) succinoyl/-derivatives. Carbohydr Polym 64(1):66–72. https://doi.org/10.1016/j.carbpol.2005.10.021

Tokumitsu H, Ichikawa H, Fukumori Y et al (1999) Chitosan-gadopentetic acid complex nanoparticles for gadolinium neutron capture therapy of cancer: preparation by novel emulsion droplet coalescence technique and characterization. Pharm Res 16:1830–1835

Tomme SRV, Storm G, Hennink WE (2008) In situ gelling hydrogels for pharmaceutical and biomedical applications. Int J Pharm 355:1–18

Toti US, Aminabhavi TM (2004) Modified guar gum matrix tablet for controlled release of diltiazem hydrochloride. J Control Release 95:567–577

Verma M, Singh SK, Bhushan S, Sharma VK, Datt P et al (2008) In vitro cytotoxic potential of Polyalthia longifolia on human cancer cell lines and induction of apoptosis through mitochondrial-dependent pathway in HL-60 cells. Chem Biol Interact 171(1):45–56

Vyas R, Gupta N, Nimesh S (2016) Nanobiomaterials in cancer therapy. In: Chitosan nanoparticles for efficient and targeted delivery of anticancer drugs, 7th edn, Bucharest, pp 281–306

Wang X, Yuan S, Wang J, Lin P, Liu G et al (2006) Anticancer activity of litchi fruit pericarp extract against human breast cancer in vitro and in vivo. Toxicol Appl Pharmacol 215 (2):168–178

Wang LS, Wang CY, Yang CH, Hsieh CL, Huang KS et al (2015) Synthesis and anti-fungal effect of silver nanoparticles–chitosan composite particles. Int J Nanomed 10:2685–2696

Wani MC, Taylor WHC, Coggon ME, Phail MC et al (1971) Plant antitumor agents. The isolation and structure of taxol, a novel antileukemic and antitumor agent from Taxus brevifolia. J Am Chem Soc 93(9):2325–2327

WHO (2018). https://www.who.int/news-room/fact-sheets/detail/cancer

Widodo N, Kaur K, Shrestha BG, Takagi Y, Ishii T et al (2007) Selective killing of cancer cells by leaf extract of Ashwagandha: identification of a tumor-inhibitory factor and the first molecular insights to its effect. Clin Cancer Res 13(7):2298–2306

Wu Y, Yang W, Wang C, Hu J, Fu S et al (2005) Chitosan nanoparticles as a novel delivery system for ammonium glycyrrhizinate. Int J Pharm 295:235–245

Xu Z, Graham K, Foote M, Liang F, Rizkallah R, Hurt M, Wang Y, Wu Y, Zhou Y et al (2013) 14-3-3 protein targets misfolded chaperone-associated proteins to aggresomes. J Cell Sci 126:4173–4186

Xue MY, Steven HU, Lu YF, Zhang Y, Jiang XN, An S et al (2015a) Development of chitosan nanoparticles as drug delivery system for a prototype capsid inhibitor. Int J Pharm 495:771–782

Xue B et al (2015b) Intracellular degradable hydrogel cubes and spheres for anti-cancer drug delivery. ACS Appl Mater Interfaces 7:13633–13644

Yaguchi T, Sumimoto T, Kudo-Saito C, Tsukamoto N, Ueda R, Iwata Kajihara T, Nishio H, Kawamura N, Kawakami Y et al (2011) The mechanisms of cancer immune escape and development of overcoming strategies. Int J Hematol 93:294–300

Yamamoto K, Yoshida S, Kadokawa JI et al (2014) Surface-initiated atom transfer radical polymerization from chitin nanofiber macroinitiator film. Carbohydr Polym 112:119–124

Yang Y, Wang S, Wang Y et al (2014) Advances in self-assembled chitosan nanomaterials for drug delivery. Biotechnol Adv 32:1301–1316

Yasmin H, Mazumdar T, Mittal P, Chakravarty AK et al (1998) Turmeric mediated activation of lymphocytes and induction of apoptosis of tumor cells. J Immunol Cell Biol 16(5):221–232

Yuan Q, Shah J, Hein S, Misra RDK et al (2010) Controlled and extended drug release behavior of chitosan-based nanoparticle carrier. Acta Biomater 6:1140–1148

Zhang E, Xing R, Liu S, Li K, Qin Y, Yu H, Li P et al (2019) Vascular targeted chitosan-derived nanoparticles as docetaxel carriers for gastric cancer therapy. Int J Biol Macromol 126:662–672

Zhao D et al (2010) Preparation, characterization, and in vitro targeted delivery of folate-decorated paclitaxel-loaded bovine serum albumin nanoparticles. Int J Nanomed 5:669–677

Zhao LM, shi LE, Zhang ZL, Chen JM, Shi DD, Yang J, Tang ZX et al (2011a) Preparation and application of chitosan nanoparticles and nanofibers. Braz J Chem Eng 28:353–362

Zhao K, Shi XM, Zhao Y, Wei HX, Sun QS, Huang TT et al (2011b) Preparation and immunological effectiveness of a swine influenza DNA vaccine encapsulated in chitosan nanoparticles. Vaccine 29:8549–8556

Zhou W, Li C, Wang Z, Zhang W, Liu J (2016) Factors affecting the stability of drug-loaded polymeric micelles and strategies for improvement. J Nanopart Res 18:275–293

Zhu Y, Liao L (2015) Applications of nanoparticles for anticancer drug delivery: a review. J Nanosci Nanotechnol 15:4753–4773

Zrazhevskiy P, Sena M, Gao X (2010) Designing multifunctional quantum dots for bioimaging, detection, and drug delivery. Chem Soc Rev 39:4326–4354

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