PharmTech
International Journal of PharmTech Research
CODEN (USA): IJPRIF ISSN : 0974-4304
Vol.2, No.4, pp 2271-2282, Oct-Dec 2010

Chitosan: A Platform for Targeted Drug Delivery

Kushwaha Swatantra K.S.[1]*, Rai Awani K.[1], Singh Satyawan[2]

[1]Pranveer Singh Institute of Technology, Kanpur, INDIA

[2]Saroj Institute of Technology & Management, Lucknow, INDIA

*Corres.author: swatantrakushwaha@yahoo.co.in

Abstract: Chitosan has prompted the continuous impetus for the development of safe and effective drug delivery systems because of its unique physicochemical and biological characteristics. The primary hydroxyl and amine groups located on the backbone of chitosan allow for chemical modification to control its physical properties. When the hydrophobic moiety is conjugated to a chitosan molecule, the resulting amphiphile may form self-assembled nanoparticles that can encapsulate a quantity of drugs and deliver them to a specific site of action. Chemical attachment of the drug to the chitosan throughout the functional linker may produce useful prodrugs, exhibiting the appropriate biological activity at the target site. The main objective of this review is to provide an insight into various target-specific carriers, based on chitosan and its derivatives, towards low molecular weight drug delivery. The pace of development of delivery systems that could target drugs to specific body sites and control the release of drugs for prolonged periods of time have been steady though slow. Till a decade ago, mostly microspheres or nanoparticles were widely studied and applied in cancer treatment. However, due to shortcomings of these systems, there has been a surge in interest for in situ hydrogels.
Key Words: *Chitosan, Glycerophosphate, Hydrogel, Drug delivery, Cancer.*

INTRODUCTION:

Chitosan is a natural mucopolysaccharide of marine origin having structural characteristics similar to glycosaminoglycans that is present in the exoskeleton of crustacean [1], arthropod and fungi. It consists of a linear (1-4)-linked 2-amino-2-deoxy-_-D glucan and can be chemically prepared from naturally occurring chitin i.e. its N-acetyl product by treatment with alkali at elevated temperature [2]. The structure of chitosan is shown below in Fig. 1.

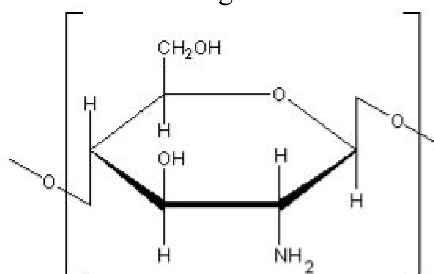

Fig. 1: Structure of Chitosan

Chitosan has been used for a wide variety of biomedical applications, such as in the drug delivery system [3], charcoal encapsulated chitosan beads for toxin removal [4], dental and orthopedic materials. This is primarily due to its biodegradable, nontoxic and biocompatible features. Other applications in biomedical field includes fibers for fat blocker, digestible sutures, liposome stabilization, anti bacterial, anti viral and anti tumor agents, haemostatic and hypocholestertemic and hypolipidemic agent.

Chitosan (CS) and its derivatives are examples of value-added materials. They are produced from chitin, which is a natural carbohydrate polymer found in the skeleton of crustaceans, such as crab, shrimp and lobster, as well as in the exoskeleton of marine zooplankton spp., including coral and jellyfishes. Insects, such as butterflies and ladybugs, also have chitin in their wings and the cell walls of yeast, mushrooms and other fungi also contain this substance. Industrial-scale CS production involves

four steps: demineralization (DM), deproteinization (DP), decoloration (DC) and deacetylation (DA). Despite the widespread occurrence of chitin in nature, presently crab and shrimp shells remain the primary commercial sources.

Chitin is the second most abundant natural polymer in the world after cellulose. Upon deacetylation, it yields the novel biomaterial Chitosan, which upon further hydrolysis yields an extremely low molecular weight oligosaccharide. Chitosan possesses a wide range of useful properties. Specifically, it is a biocompatible, antibacterial and environmentally friendly polyelectrolyte, thus lending itself to a variety of applications including water treatment, chromatography, additives for cosmetics, textile treatment for antimicrobial activity, novel fibers for textiles, photographic papers, biodegradable films, biomedical devices, and microcapsule implants for controlled release in drug delivery.

CHEMISTRY OF CHITIN:

Chitin is a polysaccharide. A polysaccharide is a polymer - a giant molecule consisting of smaller molecules of sugar strung together. Chitin can be described as a biopolymer composed of N-acetyl-D-glucosamine; a chemical structure very close to cellulose except that the hydroxyl group in C (2) of cellulose is being replaced by an acetamido group in chitin. One can associate this chemical similarity between cellulose and chitin as serving similar structural and defensive functions.

How to extract chitin from the crustacean (hard) shells? While there exists many extraction methods of the chitin from the crustacean shells, the principles of chitin extraction are relatively simple. The proteins are removed by a treatment in a dilute solution of sodium hydroxide (1-10%) at high temperature (85-100°C). Shells are then demineralized to remove calcium carbonate. This is done by treating in a dilute solution of hydrochloric acid (1-10%) at room temperature. Depending on the severity of these treatments such as temperature, duration, concentration of the chemicals, concentration and size of the crushed shells, the physico-chemical characteristics of the extracted chitin will vary. For instance, the three most important characteristics of the chitin i.e., degree of polymerization, acetylation and purity, will be affected. Shell also contains lipids and pigments. Therefore, a decolorizing step is sometimes needed to obtain a white chitin. This is done by soaking in organic solvents or in a very dilute solution of sodium hypochlorite. Again, these treatments will influence the characteristics of the chitin molecule.

Chitosan - another important derivative of chitin Chemists love to play with molecules. They did not spare chitin, the polymer either and made chitosan. The term chitosan is used when chitin could be dissolved in weak acid. When chitin is heated in a strong solution of sodium hydroxide (>40%) at high temperature (90-120°C), chitosan is formed. This harsh treatment removes acetylic grouping on the amine radicals to a product (chitosan) that could be dissolved. It is said that at least 65% of the acetylic groups should be removed on each monomeric chitin to obtain the ability of being put in solution. The degree of deacetylation will vary according to the duration, the temperature and the concentration of the sodium hydroxide. Furthermore, many chemical characteristics of the chitosan (molecular weight, its polydispersity, the purity) are greatly dependant on the method, the equipment used and also of the source of the shells. It is therefore, crucial to control precisely methods of production of the chitosan to obtain the exact characteristics needed for end use application of the product.

Finally, the purity of the product is vital particularly for high-value product (biomedical or cosmetic area). This purity is quantified as the remaining ashes, proteins, insolubles, and also in the bio-burden (microbes, yeasts and moulds, endotoxins). Even in the lower value chitosan such as that used for the wastewaters treatment, the purity is a factor because the remaining ashes or proteins tend to block active sites, the amine grouping. Being not available to bind, a greater amount of chitosan is needed to be effective. Here comes the multitude of applications & hellip; Chitosan is a biological product with cationic (positive electrical charge) properties. It is of great interest, all the more so because most polysaccharides of the same types are neutral or negatively charged. By controlling the molecular weight, the degree of deacetylation and purity, it is possible to produce a broad range of chitosans and derivatives that can be used for industrial, dietary, cosmetic and biomedical purposes. Together these properties have led to the development of hundreds of applications so far. There is plethora of literature, books and conference proceedings that documented the multiple uses of the Chitosan [5]. It is out of the scope of this article to describe extensively every applications of chitosan. We will concentrate on the major uses of chitosan and the most promising future applications. Applications of chitosan can be classified mainly in 3 categories according to the requirement on the purity of the chitosan:

- Technical grade for agriculture and water treatment
- Pure grade for the food and cosmetics industries
- Ultra-pure grade for biopharmaceutical uses in agriculture: Chitosan offers a natural alternative to the use of chemical products that are sometimes harmful to humans and their environment. Chitosan triggers the defensive mechanisms in

plants (acting much like a vaccine in humans), stimulates growth and induces certain enzymes (synthesis of phytoalexins, chitinases, pectinases, glucanases, and lignin). This new organic control approach offers promise as a biocontrol tool. In addition to the growth-stimulation properties and fungi, chitosans are used for:

- Natural and biodegradable
- A powerful competitor for synthetic chemical products

CHITOSAN-BASED DELIVERY SYSTEMS:

Chitosan and chitosan derivatives because of their excellent mucoadhesive and absorption-enhancing properties (further elaborated in the following sections) have been extensively studied for delivery of therapeutic proteins and antigens particularly via mucosal routes. Chitosan (derivatives) can interact with mucus and epithelial cells and induced a redistribution of cytoskeletal F-actin and the tight junction protein ZO-1 resulting in opening of cellular tight junctions and increasing the paracellular permeability of the epithelium [6-8]. Besides their charge, other structural elements of these polymers likely contribute to their penetration-enhancing activity, since cationic polysaccharides such as quaternized diethyl aminoethyl (DEAE)-dextrans were ineffective as an enhancer [9]. Inmany studies, it has been demonstrated that chitosan-based formulations were superior in enhancing absorption of therapeutic proteins as well as induction of antibodies after mucosal vaccination [10].

CHITOSAN (DERIVATIVES):

Chitosan [α (1–4) 2-amino 2-deoxy β-D glucan], a copolymer of glucosamine and N-acetylglucosamine, is obtained by deacetylation of chitin, a naturally abundantly available polymer (e.g. in crustaceans). Because of its favorable properties, as discussed in the present article as well as other articles in this issue, chitosan has been studied as a biomaterial and as a pharmaceutical excipient in drug formulations [11-13]. The primary amine groups introduce special properties that render chitosan very useful for pharmaceutical applications. As an example, the interaction of its protonated amine groups with cell membranes results in a reversible structural reorganization of protein associated tight junctions which is followed by their opening. Because of this activity, chitosan has been used for the preparation of mucoadhesive formulations [14, 15], for drug targeting systems [16], and for formulations that enhance the absorption of macromolecular therapeutics (peptides protein therapeutics and antigens as well as plasmid DNAs) [17, 18]. Chitosan is soluble, mucoadhesive and active as an absorption enhancer in its protonated form [19]. Because the pKa of the amine groups of chitosan is 6.2, chitosan at neutral pH hardly carries a charge, has a low solubility and is therefore essentially inactive. Because of the presence of functional groups (amine and hydroxyl) various chemical chitosan derivatives have been synthesized and studied for different applications. Thiolated chitosans, obtained by modification of the primary amine groups with cysteine, thioglycolic acid and 2-iminothiolane, are a class of derivatives that showed improved mucoadhesive properties and have been applied in mucoadhesive oral and nasal drug delivery systems. These thiolated chitosans have shown in situ gelling properties due to the formation of inter- and intramolecular disulfide bonds at physiological pH [20-22]. The strong mucoadhesive properties of the thiolated chitosans make them particularly suitable carriers for prolonged protein delivery at the mucosal sites.

Quaternary chitosan derivatives are, because of their permanent cationic charge, soluble over a wide pH range. Importantly, these derivatives have mucoadhesive and penetration-enhancing properties also at neutral pH. The first quaternized chitosan was synthesized by alkylation of the primary amine groups of chitosan with various aldehydes using sodium borohydride as reducing agent. These N-alkylated chitosan derivativeswere used as antibacterial and antifungal materials. N-trimethyl chitosan (TMC) is a partially quaternized and well water-soluble derivative of chitosan, which had been extensively studied for its mucoadhesive and absorption-enhancing effects for hydrophilic macromolecules in particular. The penetrationenhancing activity of TMCaswell as other properties, amongwhich is its biocompatibility, is discussed in more detail in the next section. Other quaternary chitosan derivatives have been synthesized by attaching a quaternary ammonium moiety to the amine groups of chitosan e.g. by reaction of N-chloroacyl-6-O-triphenylmethyl with chitosan. Xu et al. synthesized a quaternary derivative of chitosan, N-(2-hydroxyl) propyl-3-trimethyl ammoniumchitosan chloride (HTCC), by reaction of chitosan with glycidyl trimethyl ammonium. This chitosan derivative was used to prepare albumin-loaded nanoparticles by ionic gelation with sodium tripolyphosphate (TPP). These nanoparticles had a size between 110–180 nm and their encapsulation efficiency for albumin was up to 90%. In vitro release studies showed a burst effect followed by a slow release. Addition of poly (ethylene glycol) (PEG) significantly decreased both the burst release and the encapsulation efficiency, whereas the addition of alginate reduced the burst releasewhile protein loading remained high [23].

N-trimethyl chitosan (TMC):

In recent years much research has been done on cationic watersoluble chitosan derivatives in particularly N-trimethyl Chitosan (TMC). This polymer is obtained by methylation of amine groups of chitosan with methyl iodide. During this reaction some chain scission occurs which is likely due to the harsh reaction conditions (4 M NaOH, a temperature of 60 °C and relatively long reaction time (2–3 h)) [24].

By varying the degree of methylation, the water-solubility of TMC can be tailored [25]. Soluble TMC has both mucoadhesive properties and excellent absorption-enhancing effects (the latter depending on its degree of quaternization (DQ)) even at neutral pH. It has been shown in many studies that TMCs, depending on their degree of quaternization (DQ), enhance the permeation of hydrophilic macromolecules across the mucosal epithelia by opening the tight junctions. At physiological pH, only TMC with a degree of quaternization above 36% increased the absorption of hydrophilic model compounds such as mannitol and poly (ethylene glycol) 4000 across intestinal epithelia and nasal mucosa. The permeation-enhancing effect of TMC increases with an increasing degree of quaternization. It has been shown that TMC can also enhance the permeation of hydrophilic high molecular weight compounds (dextran Mw 4400 Da) across stratified epithelia such as buccal mucosa which lack tight junctions [26].

TECHNIQUES FOR THE PREPARATION OF CHITOSAN-BASED MICRO/NANOPARTICLE FORMULATIONS:

Chitosan-based particles loaded with proteins can be prepared by both chemical and physical methods. However major drawbacks are associated with the use of chemical crosslinking methods. Firstly, organic solvents used to make w/o emulsions may adversely affect the stability of proteins and, more importantly, the applied crosslinking agents can chemically modify proteins [27]. Secondly, complete removal of the unreacted and often toxic crosslinker is difficult to achieve. Consequently, methods by which chitosan and its derivatives are crosslinked by physical methods to yield particles are preferred. Spray drying is a relatively protein friendly technique that has been applied for the preparation of protein-loaded chitosan microparticles and nanoparticles suitable e.g. for pulmonary delivery [28]. Also other techniques, such as ionic crosslinking methods and drying processes have been used for the preparation of protein-loaded chitosanbased particles. These techniques are discussed in more detail in the following sections.

(a) Chemical crosslinking methods:

Chitosan-based particles can be formed by chemical processes, e.g. by reacting the primary amine groups of chitosan with a di-aldehyde (mostly glutaraldehyde) crosslinker. Here, first water-in-oil (w/o) emulsion of chitosan with the drug in a water-immiscible solvent (e.g. liquid paraffin) is formed, after which glutaraldehyde is added to crosslink chitosan to yield drug-loaded microspheres [29]. In another study, insulin-loaded chitosan microspheres were prepared by dissolving the protein and the polymer in an acetic acid solution. This solution was subsequently emulsified in mineral oil and chitosan was chemically crosslinked with ascorbyl palmitate or dehydroascorbyl palmitate. This preparation method yielded microparticles characterized by high loading levels of insulin, and they completely released the drug in an active form in about 80 h at an almost constant release rate. In this study, mineral oil was used as oil phase to make a w/o emulsion. Ascorbyl or dehydroascorbyl, palmitate, as interfacial crosslinkers, resides at the water/oil interface of the emulsion and might protect the protein from the high interfacial surface tension. Moreover, as compared to glutaraldehyde, ascorbyl or dehydroascorbyl, palmitate has substantially lower toxicity. It should be mentioned that the authors did not discuss a possible chemical modification of the proteins by the crosslinking agents [30]. Wang et al. prepared uniform-sized protein-loaded chitosan microspheres by a step-wise crosslinking [31]. First, a w/o emulsion of chitosan/insulin in paraffin/petroleum ether mixture was prepared and extruded through a membrane with uniform pores. Then, tripolyphosphate (TPP) was added as an ionic crosslinker followed by the addition glutaraldehyde to stabilize the microspheres (Fig.2).

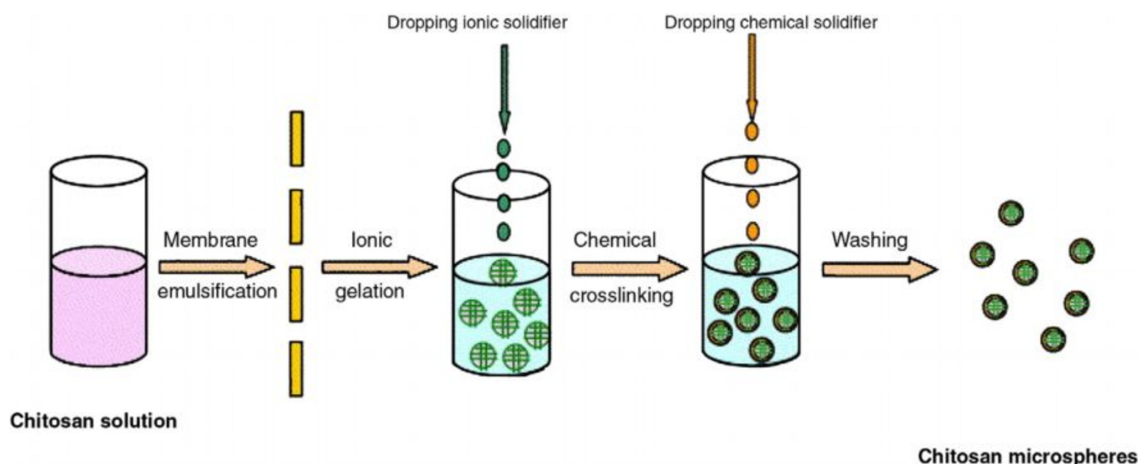

Fig.2. Preparation process of chitosan microspheres by double crosslinking method

(b) Ionic crosslinking methods:
The complexation between chitosan-based polymers and oppositely charged macromolecules can be exploited to prepare micro/nanoparticles suitable for drug delivery. The particles are prepared by ionic crosslinking through self-assembly of chitosan/chitosan derivatives and oppositely charged macromolecules or by addition of a low molecular weight anionic crosslinker, such as tripolyphophate (TPP), sodium sulfate or cyclodextrin (CD) derivatives to chitosan solutions. The ionic crosslinking methods mentioned above have received much attention in recent years for the preparation of protein formulations because the used processes are simple and mild to proteins, as they do not involve the use of chemical crosslinkers and avoid the use of organic solvents and high temperatures [32]. Coacervation/precipitation has been used to prepare a great variety of proteinloaded chitosan microparticles. In these methods, a coacervate, e.g. sodium sulfate, is added dropwise to an acidic solution of Chitosan under stirring and sonication to prepare ionically crosslinked particles. This method has been used to prepare chitosan-based microparticles loaded with interleukin-2 (IL-2) [33]. Although the coacervation/ precipitation method is more protein-friendly than the chemical crosslinking preparation technologies, sonication may harm the protein structure.
Self-assembled polyelectrolyte complexes (PECs) have been recently investigated for protein delivery. Oppositely charged polyelectrolytes can form stable intermolecular complexes. These PEC nanoparticles are either positively or negatively charged, and they showa pH-dependent destabilization. Schatz et al. synthesized a partially N-sulfated chitosan.Upon acidification of an aqueous solution of this amphoteric chitosan; nanoparticles were formed by electrostatic interactions between the non-sulfated protonated amine groups of chitosan and the negatively charged N-sulfated chitosan amines. These polyelectrolyte complexes can be used for encapsulation of macromolecules but loading and releases studies have not been reported [34].

(c) Drying techniques used for the preparation of protein-loaded chitosan-based particles:
There is a need for particle-formation processes which are simple and protein friendly can easily be scaled, offer the possibility to produce formulations with different particle sizes and yield particles with a good life. Different drying processes have been recently used for preparation of chitosan–protein powder formulations [35]. Spray drying is the most commonly used drying method for preparation of protein microparticles. It is efficient process and particles are formed in a one-step process. Protein-loaded chitosan microparticles were prepared by spraying of an aqueous solution of chitosan and a protein into a drying chamber, yielding protein–chitosan microparticles (size 4–7 μm) [36]. In another study, mannitol microspheres containing protein-loaded chitosan nanoparticles suitable for pulmonary delivery were prepared by spray drying of a chitosan–FITC-BSA nanoparticles suspension in an aqueous mannitol solution. Mannitol stabilizes the protein structure and improves aerosolization of protein drugs into lungs. FITC-BSA loaded chitosan nanoparticles (300–400 nm) were homogenously encapsulated in mannitol microparticles particles with a mean aerodynamic diameter of 2.7 μm, adequate for pulmonary delivery. However, the structural integrity of the encapsulated protein of these particles was not reported [37].
Supercritical fluid (SCF) drying has been recently investigated as an alternative process for producing powder formulations. SCF drying is a fast and mild process, is cost effective and offers the possibility to produce small microparticles suitable for inhalation. Above the critical points (temperature and pressure), a SCF has liquid-like viscosity and density, and gas-like diffusivity properties, and can therefore easily

penetrate into substances like a gas and dissolve materials like a liquid [38].

The most widely used SCF for pharmaceutical applications is carbon dioxide (CO_2) because it has a low critical temperature (31.2 °C) and pressure (75.8 bar), and it is non-flammable, non-toxic and inexpensive. Because proteins have a very low solubility in supercritical CO_2 (SC-CO_2), this fluid has been used as an antisolvent to precipitate proteins from their aqueous solutions. It is however possible to modify the solvent power of SC-CO_2 by adding volatile co-solvents such as ethanol [39].

CHITOSAN-BASED NANOPARTICLES AND MICROPARTICLES FOR PROTEIN DELIVERY:

The effects of molecular weight and degree of deacetylation (DD) of chitosan on cellular uptake of nanoparticles prepared from these polymers were studied using A549 cells. The uptake of nanoparticles was a saturable event and importantly, cell-associated chitosan nanoparticles were internalized, but not the cell-associated Chitosan polymers. It was shown that nanoparticles prepared at pH 6.2 using chitosan with a low DD contain more primary amines available for protonation had a higher positive charged and were more extensively taken up by the A459 cells than particles prepared with chitosan with a higher DD [40].

Chitosan particles for nasal delivery of peptides/proteins:

Several studies of chitosan particles or polyelectrolytes complexes for nasal delivery of therapeutic proteins have been done. It has been shown that insulin-loaded chitosan nanoparticles enhanced nasal absorption of proteins to a greater extent than chitosan solutions. Wang et al. showed that insulin-loaded thiolated chitosan nanoparticles substantially improved absorption of insulin across nasal mucosa as compared to non-thiolated Chitosan nanoparticles as well as soluble chitosan. Likely, thiolated Chitosan nanoparticles have higher mucoadhesion properties and thus a longer residence time in nasal cavity. Moreover, thiolated chitosan nanoparticles showed a faster swelling and release as compared to Chitosan nanoparticles, which might facilitate diffusion of the encapsulated drug. In vivo evaluations showed that after intranasal administration of the insulin-loaded thiolated nanoparticles to rats, the blood glucose levels of the animals rapidly decreased. The glucose levels of these animals were similar to those that received insulin subcutaneously [41].

In another study nanoparticles consisting of chitosan and negatively charged cyclodextrin (sulfobutylether-β-CD (SBE-β-CD) or carboxymethyl-β-CD (CM-β-CD) derivatives were prepared and characterized. It was demonstrated that chitosan–SBE-β-CD– TPP nanoparticles induced lower TEER values of Calu-3 cells than chitosan–CM-β-CD–TPP nanoparticles. However, both insulin-loaded nanoparticles showed similar effects on reduction of rats' plasma glucose levels upon intranasal administrations. It should be noted that, the plasma insulin concentrations of the treated animals which may give better indications in absorption enhancement properties of the formulations, were not determined [42].

Chitosan particles for pulmonary delivery of peptides/proteins:

Powder formulations of protein-loaded chitosan nanoparticles suitable for pulmonary delivery were prepared by spray drying. Insulin-loaded nanoparticles were obtained by ionic gelation of a chitosan solution with a TPP solution also containing insulin. The nanoparticles were suspended in a solution of mannitol and lactose. Spray drying yielded microparticle powders with a suitable aerodynamic diameter (1–3 μm) for alveolar deposition. The insulin-loaded chitosan nanoparticles had a good loading capacity (65–80%) and were fully recovered from the powder formulations after contact with an aqueous medium, and showed a fast release of insulin. However, no in vivo pulmonary delivery data with these powder formulations have been published so far. In another study by the same authors, the biocompatibility and penetration-enhancing effects of their Chitosan powder formulations were examined in vitro using A549 and Calu-3 cells as models for alveolar and respiratory epithelial cells, respectively. The formulations exhibited a very low cytotoxicity in both cell lines, but no effects on opening of tight junctions of the cells were reported. Further, CLSM studies did not reveal internalization of nanoparticles which contrasts previously reported studies. The authors speculated that the total amount of chitosan used in their study was lower than that used in other publications. Moreover, Lim et al. used a different chitosan salt (glutamate) than 'normal' chitosan, which probably did not lose its charge after dispersing the particles in buffer [43].

INJECTABLE CHITOSAN HYDROGELS FOR CANCER THERAPY:

Methods of preparation of chitosan gels:

(a) Crosslinking by ultraviolet irradiation

This method was firstly reported by Ono et al. in 2000 [44]. In this method, lactose moieties were introduced into chitosan to obtain much better water-soluble chitosan at neutral pH, and photoreactive azide groups were added to provide the ability to form a gel through crosslinking azide groups with amino groups. This photocrosslinkable chitosan (Az-CH-LA) was then

exposed to ultraviolet light (UV) irradiation to form an insoluble and adhesive hydrogel within 60 s. Az-CH-LA hydrogel has the consistency of transparent and soft rubber [45].
Azide and lactose moieties were introduced to Chitosan molecules through a two-step condensation reaction. Firstly, the lactose moiety was introduced to chitosan chains using lactobionic acid with the presence of EDC (1-ethyl-3-(3- dimethylaminopropyl carbodiimide). The reaction took place in solution of TEMED (N, N, N', N'-tetramethylethylenediamine) containing concentrated HCl. Subsequently, the azide moiety was introduced to the lactose-linked chitosan (CH-LA) using 4- azidobenzoic acid. Again, the reaction was completed in the presence of EDC in TEMED as in the first condensation.It has been estimated that between 2% and 2.5% of amino groups in the chitosan molecule were replaced by lactobionic acid and by azidobenzoic acid, respectively using this method. CH-LA exhibited a good aqueous solubility at neutral pH and lower. The introduction of azidobenzoic acid by 2.5% into CHLA showed no additional change in water solubility.

(b) Crosslinking by high temperature:
This method was invented by Chenite and colleagues, then developed and named as BST-Gel platform technology at Biosyntech Inc. (Laval, QC, Canada) [46]. It is based on the neutralization of a chitosan solution with a polyol counterionic dibase salt such as β-glycerophosphate. Chitosan/glycerophosphate (C/GP) is a thermosensitive solution which is liquid at room temperature and solidifies into a white hydrogel at body temperature. According to this report, chitosan solution was prepared in 0.1 M acetic acid. Glycerophosphate (GP) solution was prepared and chilled along with the chitosan solution in an ice bath for 15 min. Then, the cold GP solution was added dropwise to the cold chitosan solution with stirring to produce C/GP aqueous formulation with a pH value around neutral. Stability and viscosity of C/GP solutions was dependent on the deacetylation degree of chitosan. Solutions made of less deacetylated chitosan are more stable and their viscosity remains unchanged for a longer time.
The gelation rate was dependent on chitosan deacetylation degree, concentration of β-GP, temperature and pH of final solution as shown by another study [47]. More highly deacetylated chitosan turns to gel quicker when incorporated with GP, which can be explained by the higher cross-linking density between phosphate groups of GP and the ammonium groups of chitosan. The sol-gel transition starts as soon as the polyol salt is added, however, low temperature and low pH slow down the gelation process. The C/GP gel system having pH values between 6.9 and 7.2 is partially thermoreversible upon cooling to 5 °C because of the existence of remaining associations [48]. However, C/GP solutions having lower pH between 6.5 and 6.9 show complete thermoreversibility [49].
It was suggested that in C/GP systems, there are three types of interactions involved in the gelation process. They are (1) electrostatic attraction between the ammonium group of the chitosan and the phosphate group of the glycerophosphate; (2) hydrogen bonding between the chitosan chains as a consequence of reduced electrostatic repulsion after neutralization of the chitosan solution with GP; and (3) chitosan–Chitosan hydrophobic interactions. The observation that C/GP solutions gel with increasing temperature implies that some repulsive forces between the chitosan chains are stabilized at low temperature and weakened at high temperature. The polyol part of GP has been considered as the element which prevents or slows down gelation at low temperature. It was hypothesized that polyols reinforced the initial structure of chitosan, thus, more energy was needed to break it. Mi et al. reported the occurrence of ionic interactions between positively charged chitosan molecules and negatively charged tri-polyphosphates using FTIR spectra analysis [50, 51]. However, Cho et al. reported that hydrophobic interactions and reduced solubility are the main driving force for chitosan gelation at high temperature in the presence of β-GP.
Recently, Buschmann et al. have demonstrated that the solgel transition occurs via two steps [52]. Firstly, polyelectrolyte chitosan is partially neutralized and brought close to precipitation by the addition of a weak base such as dibasic sodium phosphate or GP. After the neutralization step, the solution is then heated and induces chitosan to release its proton. Phosphate salt acts as a proton sink. If the amount of phosphate is enough to accept these protons, the transfer of protons is sufficient to bring the chitosan to precipitation and induce the sol-gel transition. Therefore, there is no ionic crosslink between the phosphate or polyphosphate and chitosan chain, the gelation is a block precipitation of the chitosan resulting from the homogeneous neutralization of the polyelectrolyte induced by heating. The phosphate salt is then free to diffuse out of the gel.

(c) Crosslinking by high pH:
This method employs the pH-sensitive property of Chitosan solutions at low pH. Once injected into the body, these polymer solutions face different environmental pH conditions and form gels. *insitu* gel system which consisted of 3% (w/v) chitosan and 3% (w/v) GMO in 0.33 M Ganguly et al. developed a novel mucoadhesive pH-sensitive chitosan/glyceryl monooleate (C/GMO) in citric acid. Chitosan is normally insoluble in neutral or alkaline pH. However,

in dilute acids (pH≤5.0), it becomes soluble due to the protonation of free amino groups on the chitosan chains (RNH_3+). The solubility of chitosan in acidic medium also depends on its molecular weight. Acidic solutions of chitosan when exposed to alkaline pH or body biological pH lose this charge and form viscous gels [53].

CYTOTOXICITY OF CHITOSAN HYDROGELS:

Chitosan is a natural product which is safe to the human body and contains basic groups. Cells generally have a predominantly negative charge on their surfaces, thus they are known to adhere much more strongly to substrates with basic groups such as chitosan. However, the strongly positive charge causes metamorphosis of cells and inhibits cell growth. Prior studies have reported that epithelial cells grew on a film of chitosan, and fibroblast cells could grow on collagen-chitosan blended films [54]. The addition of chitosan to collagen increased cell attachment but decreased cell growth. It has been found to be difficult for cells to adhere to and grow on chitosan hydrogels having high water content.

In vitro cytotoxicity testing of Az-CH-LA gel showed that human skin fibroblasts, coronary smooth muscle cells, and endothelial cells do not adhere and grow on immobilized chitosan gels prepared by UV irradiation. However, they grow normally beside the hydrogels. Az-CH-LA and its gel did not influence both the adhesion and proliferation of these cells. The test confirmed that Az-CH-LA aqueous solutions and their gels are not toxic to the above cells. In vivo toxicity testing showed that mice with subcutaneously injected Az-CH-LA gels were alive for at least 1 month. The implanted chitosan hydrogel was partially biodegraded in vivo in about 10–14 days when implanted subcutaneously into the mouse back. After 1 month, at the site of administration, no chitosan gel could be visibly detected. In addition, toxicity tests for mutagenicity and cytotoxicity have shown the safety of both Ax-CH-LA and its hydrogel. These results show the safety of Az-CH-LA gels in medical use.

Several C/GP formulations with degree of deacetylation ranging from 40 to 95% were tested in vitro with several cell lines and in vivo with rats to investigate their cytocompatibility. The absence of any toxic elements was demonstrated by the ability of these C/GP preparations to maintain more than 80% cell viability over several weeks. The safety of these systems was also confirmed by histological analysis. Degree of deacetylation was found to be the key factor governing both the rate of degradation and the inflammatory response. While a lower degree of deacetylation resulted in a short residence time (few days) and inflammatory cell induction, a higher one showed longer residence time (several weeks) and no detectable inflammation. The cytotoxicity of chitosan/GMO systems have not been investigated yet. However, their low pH property imposes a large gap in pH between such delivery solutions and body biological environment, which facilitates gel forming but may lead to significant side effects.

DRUG AGENTS FORMULATED IN CHITOSAN HYDROGELS FOR CANCER TREATMENT:

Paclitaxel is in a class of drugs as taxanesa, originally extracted from the bark of the Pacific yew (Taxus brevifolia), known as a potent inhibitor of angiogenesis, cell migration, and collagenase production, and exhibits inhibitory action on tumor cell proliferation [55]. Paclitaxel did not stop cell growth but considerably diminished its rate. It exerts its main anti-tumoral activity by binding to and promoting the assembly of microtubules. This causes the microtubules to become resistant to depolymerization into tubulin. This means that paclitaxel blocks a cell's ability to break down the mitotic spindle during mitosis (cell division). With the spindle still in place the cell cannot divide into daughter cells. Consequently, the cells are arrested at the G2 and M phases of the cell cycle [56–58]. It also has been demonstrated that paclitaxel has significant anti-tumor activity against various solid tumors, including breast and colon cancer, ovarian carcinoma, lung cancer, head and neck carcinoma, malignant melanoma, esophageal adenocarcinoma and acute leukemia. Paclitaxel also induces apoptosis and could sensitize even multi-drug resistant tumor cells to radiation.

Traditionally, paclitaxel was administered by intravenous (IV) infusion, and cremophor EL was use as a solvent to enhance paclitaxel solubility. However, this solvent caused severe hypersensitive reactions, cytotoxicity, and was incompatible with polyvinyl chloride (PVC) which was commonly used in IV dosage form. Paclitaxel cannot differentiate between cancer and normal cells, resulting in major toxicity to normal cells. To minimize the cytotoxicity and side effects, localized and targeted delivery of paclitaxel need to be developed. For regional delivery, paclitaxel has been formulated in biodegradable polymeric microspheres, hydrogels, surgical pastes, and implants [59].

Camptothecin is an inhibitor of the DNA-replicating enzyme topoisomerase I, leading to the production of a double-strand DNA break during replication and resulting in cell death if the break is not repaired. Camptothecin is believed to break the topoisomerase I-induced single strand in the phosphodiester backbone of DNA, thus preventing replication. In preclinical studies, camptothecin was effective against colon, lung, breast, ovarian, and melanoma cancers.

However, camptothecin was not administered in any clinical trials systemically because of its low solubility in water, unexpected toxicity and low antineoplastic activity. In addition, the lactone ring of camptothecin and its analogs is unstable in the presence of human serum albumin which results in the conversion of the active drug to the inactive carboxylate form bound to albumin [60-62].

Alternatively, local delivery of camptothecin has attracted some attention. Camptothecin has been formulated in biodegradable polymeric implant devices, microspheres and hydrogels. Camptothecin was loaded into a controlled-release polymer (ethylene-vinyl acetate co-polymer; EVAc) for brain tumor treatment [63]. It was shown that local controlled delivery by this polymer system significantly extended survival: 59% of the treated animals were long-term survivors (N120 days) compared to 0% of controls. Biodegradable polyanhydride polymer devices and biodegradable poly (lactide-coglycolide) (PLGA) microspheres [64] were also studied to locally deliver camptothecin and showed promises. However, polymer devices require insertion by surgical intervention and microspheres do not form a continuous film or solid implant and may be poorly retained because of their small size, discontinuous nature and lack of adhesiveness. Recently, camptothecin was formulated in an injectable thermosensitive chitosan/glycerophosphate system for controlled release [65]. This system shows an effective sustained intratumoral delivery of camptothecin.

DISCUSSION AND CONCLUSIONS:

The Chitosan - based systems for delivery of therapeutic proteins/peptides and antigens, particularly after administration particles via mucosal (nasal and pulmonary) and parenteral routes. In many studies, it has been shown that bio/mucoadhesive chitosan (derivatives) can prolong the residence time of formulations at the mucosal sites, fairly protect the peptide/protein of interest from degradation and enhance its absorption across epithelial barriers. However, systematic studies on the bioavailability of proteins formulated with chitosan are lacking. It might be argued that the bioavailabilities of therapeutic proteins formulated with chitosans are low because of their poor transport to the sub-mucosal sites, and degradation of the protein either loaded into particles or in its soluble form. Further, it has been shown that chitosan-based nanoparticles are efficiently taken up by the epithelial cells, but not necessarily transported across the cells. Finally, most of the chitosan-based nanoparticles under investigation have limited colloidal stability particularly in biological fluids. As a consequence, they dissociate and/or aggregate at mucosal lumens before reaching the absorption site (the mucosal epithelial cells). Therefore, strategies to stabilize Chitosan particles need further attention.

Another aspect, hardly addressed in drug delivery literature so far is the potential immunogenicity of particulate drug delivery systems such as chitosan-based carriers. As described in this review, chitosan polymers have adjuvant properties, particularly in particulate form. After parenteral or mucosal administration of proteinloaded chitosan particles, they can be taken up and subsequently processed by APCs, which may initiate an immune response against the therapeutic protein. It is important to note that most of therapeutic proteins have to be repeatedly administered to patients that may increase the potential risk of antibody formation against the formulated therapeutic proteins and pose a safety concern.

Although several chitosan hydrogels have been investigated, only a few of them have in situ gelling properties. Therefore, most chitosan hydrogels were developed under the forms of microspheres or nanoparticles for cancer treatment. Some injectable chitosan hydrogels were invented and presented potential but have not tested in vivo or in preclinical trial for cancer application. They are thermosensitive poly (ethylene glycol)-grafted Chitosan system, temperature-responsive hydroxybutyl Chitosan, poly (vinyl alcohol)/chitosan-blended hydrogels; chitosan/ bifunctional aldehyde hydrogel; and a system composed of N-[(2-hydroxy-3-trimethylammonium) propyl] chitosan chloride/glycerophosphate.

Most in situ chitosan hydrogel systems have been tested to deliver cytotoxic drugs in vivo for treatment of cancer. However, the effect of toxic agents released from these hydrogel systems on other organs or tissues such as heart and skin have not been studied. Moreover, whether an in situ forming hydrogel can treat metastases has not been investigated yet. It has been widely known that metastasis (the spread of cancer in the body) is the main cause of death for patients with cancers. Malignant cancers can spread preferentially from one organ to another. Cancer cells can break away, leak, or spill from a primary tumor, enter the blood vessels, circulate through the bloodstream, and settle down to grow within normal tissues elsewhere in the body .

REFERENCES:

1. Chandy, T. and C.P. Sharma, 1995. Resorbable chitosan matrix-a promising biomaterials for the future. Biomed. Engg. Conf., Proc. of the Fourteenth Southern, pp: 282-285. New York: IEEE.
2. Hirano, S., N.Y. Yasuharu, J. Kinugawa, H. Higashijima and T. Hayashi, 1987. Chitin and Chitosan for use as a Novel Biomedical Material. In: Advances in Biomedical Polymers (Ed. C.G. Gebelain) pp: 285-297. Plenum Press, New York.
3. Shahidi, F.; Abuzaytoun, R. Chitin, chitosan, and co-products: chemistry, production, application, and health effects. *Adv. Food Nutr. Res*. **2005**, *49*, 93-135.
4. Tharanathan, R.N.; Kittur, F.S. Chitin -the undisputed biomolecule of great potential. *Crit. Rev. Food. Sci. Nutr*. **2003**, *43*, 61-87.
5. Gossen, M.F.A. (1997) Applications of Chitin and Chitosan. Technomic Publishing Company Book, Lancaster, 1997.
6. P. Artursson, T. Lindmark, S.S. Davis, L. Illum, Effect of chitosan on the permeability of monolayers of intestinal epithelial cells (Caco-2), Pharm. Res. 11 (1994) 1358–1361.
7. G. Borchard, H.L. Luessen, A.G. de Boer, J.C. Verhoef, C.M. Lehr, H.E. Junginger, Effects of chitosan–glutamate and carbomer on epithelial tight junctions in vitro, J. Control. Release 39 (1996) 131–138.
8. N.G. Schipper, S. Olsson, J.A. Hoogstraate, A.G. de Boer, K.M. Varum, P. Artursson, Chitosans as absorption enhancers for poorly absorbable drugs 2: mechanism of absorption enhancement, Pharm. Res. 14 (1997) 923–929.
9. G. Di Colo, S. Burgalassi, Y. Zambito, D. Monti, P. Chetoni, Effects of different Ntrimethyl chitosans on in vitro/in vivo ofloxacin transcorneal permeation, J. Pharm. Sci. 93 (2004) 2851–2862.
10. M. Singh, M. Briones, D.T. O'Hagan, A novel bioadhesive intranasal delivery system for inactivated influenza vaccines, J. Control. Release 70 (2001) 267-276.
11. O. Felt, P. Buri, R. Gurny, Chitosan: a unique polysaccharide for drug delivery, Drug Dev. Ind. Pharm. 24 (1998) 979–993.
12. L. Illum, Chitosan and its use as a pharmaceutical excipient, Pharm. Res. 15 (1998) 1326–1331.
13. H.E. Junginger, J.C. Verhoef, Macromolecules as safe penetration enhancers for hydrophilic drugs — a fiction? Pharm. Sci. Technol. Today 1 (1998) 370–376.
14. C.M. Lehr, J.A. Bouwstra, E.H. Schacht, H.E. Junginger, Invitro evaluation of mucoadhesive properties of chitosan and some other natural polymers, Int. J. Pharm. 78 (1992) 43–48.
15. H.L. Luessen, J.C. Verhoef, G. Borchard, C.M. Lehr, A.G. de Boer, H.E. Junginger, Mucoadhesive polymers in peroral peptide drug delivery. II. Carbomer and polycarbophil are potent inhibitors of the intestinal proteolytic enzyme trypsin, Pharm. Res. 12 (1995) 1293–1298.
16. E.E. Hassan, R.C. Parish, J.M. Gallo, Optimized formulation of magnetic Chitosan microspheres containing the anticancer agent, oxantrazole, Pharm. Res. 9 (1992) 390–397.
17. M. Bivas-Benita, K.E. van Meijgaarden, K.L. Franken, H.E. Junginger, G. Borchard, T.H. Ottenhoff, A. Geluk, Pulmonary delivery of chitosan–DNA nanoparticles enhances the immunogenicity of a DNA vaccine encoding HLA-A*0201-restricted T-cell epitopes of Mycobacterium tuberculosis, Vaccine 22 (2004) 1609–1615.
18. H.Q. Mao, K. Roy, V.L. Troung-Le, K.A. Janes, K.Y. Lin, Y. Wang, J.T. August, K.W. Leong, Chitosan–DNA nanoparticles as gene carriers: synthesis, characterization and transfection efficiency, J. Control. Release 70 (2001) 399–421.
19. A.F. Kotze, M.M. Thanou, H.L. Luebetaen, A.G. de Boer, J.C. Verhoef, H.E. Junginger, Enhancement of paracellular drug transport with highly quaternized N-trimethyl chitosan chloride in neutral environments: in vitro evaluation in intestinal epithelial cells (Caco-2), J. Pharm. Sci. 88 (1999) 253–257.
20. A. Bernkop-Schnurch, M. Hornof, T. Zoidl, Thiolated polymers–thiomers: synthesis and in vitro evaluation of chitosan-2-iminothiolane conjugates, Int. J. Pharm. 260 (2003) 229–237.
21. M.D. Hornof, C.E. Kast, A. Bernkop-Schnurch, In vitro evaluation of the viscoelastic properties of chitosan-thioglycolic acid conjugates, Eur. J. Pharm. Biopharm. 55 (2003) 185–190.
22. M. Roldo, M. Hornof, P. Caliceti, A. Bernkop-Schnurch, Mucoadhesive thiolated chitosans as platforms for oral controlled drug delivery: synthesis and in vitro evaluation, Eur. J. Pharm. Biopharm. 57 (2004) 115–121.
23. Y. Xu, Y. Du, R. Huang, L. Gao, Preparation and modification of N-(2-hydroxyl) propyl-3-trimethyl ammonium chitosan chloride nanoparticle as a protein carrier, Biomaterials 24 (2003) 5015–5022.

24. D. Snyman, T. Govender, A.F. Kotze, Low molecular weight quaternised Chitosan (I): synthesis and characterisation, Pharmazie 58 (2003) 705–708.
25. R.J. Verheul, M. Amidi, S. van der Wal, E. van Riet, W. Jiskoot, W.E. Hennink, Synthesis, characterization and in vitro biological properties of O-methyl free N, N, N-trimethylated chitosan, Biomaterials 29 (2008) 3642–3649.
26. G. Sandri, S. Rossi, M.C. Bonferoni, F. Ferrari, Y. Zambito, G. Di Colo, C. Caramella, Buccal penetration enhancement properties of N-trimethyl chitosan: influence of quaternization degree on absorption of a high molecular weight molecule, Int. J. Pharm. 297 (2005) 146–155.
27. W.E. Hennink, C.F. van Nostrum, Novel crosslinking methods to design hydrogels, Adv. Drug Deliv. Rev. 54 (2002) 13–36.
28. M. Yang, S. Velaga, H. Yamamoto, H. Takeuchi, Y. Kawashima, L. Hovgaard, M. van de Weert, S. Frokjaer, Characterisation of salmon calcitonin in spray-dried powder for inhalation. Effect of chitosan, Int. J. Pharm. 331 (2007) 176–181.
29. S.R. Jameela, T.V. Kumary, A.V. Lal, A. Jayakrishnan, Progesterone-loaded Chitosan microspheres: a long acting biodegradable controlled delivery system, J. Control. Release 52 (1998) 17–24.
30. F. Bugamelli, M.A. Raggi, I. Orienti, V. Zecchi, Controlled insulin release from chitosan microparticles, Arch. Pharm. (Weinh.) 331 (1998) 133–138.
31. L.Y. Wang, Y.H. Gu, Q.Z. Zhou, G.H. Ma, Y.H. Wan, Z.G. Su, Preparation and characterization of uniform-sized chitosan microspheres containing insulin by membrane emulsification and a two-step solidification process, Colloids Surf., B Biointerfaces 50 (2006) 126–135.
32. P. Calvo, C. RemunanLopez, J.L. VilaJato, M.J. Alonso, Novel hydrophilic chitosan–polyethylene oxide nanoparticles as protein carriers, J. Appl. Polym. Sci. 63 (1997) 125–132.
33. S. Ozbas-Turan, J. Akbuga, C. Aral, Controlled release of interleukin-2 from chitosan microspheres, J. Pharm. Sci. 91 (2002) 1245–1251.
34. C. Schatz, A. Bionaz, J.M. Lucas, C. Pichot, C. Viton, A. Domard, T. Delair, Formation of polyelectrolyte complex particles from self-complexation of N-sulfated chitosan, Biomacromolecules 6 (2005) 1642–1647.
35. M. Amidi, H.C. Pellikaan, H. Hirschberg, A.H. de Boer, D.J. Crommelin, W.E. Hennink, G. Kersten, W. Jiskoot, Diphtheria toxoid-containing microparticulate powder formulations for pulmonary vaccination: preparation, characterization and evaluation in guinea pigs, Vaccine 25 (2007) 6818–6829.
36. C. Kusonwiriyawong, W. Pichayakorn, V. Lipipun, G.C. Ritthidej, Retained integrity of protein encapsulated in spray-dried chitosan microparticles, J.Microencapsul. 26 (2009) 111–121.
37. A. Grenha, B. Seijo, C. Serra, C. Remunan-Lopez, Chitosan nanoparticle-loaded mannitol microspheres: structure and surface characterization, Biomacromolecules 8 (2007) 2072–2079.
38. E. Reverchon, J. Daghero, C. Marrone, M. Mattea, M. Poletto, Supercritical fractional extraction of fennel seed oil and essential oil: experiments and mathematical modeling, Ind. Eng. Chem. Res. 38 (1999) 3069–3075.
39. P.M. Gallagher, M.P. Coffey, V.J. Krukonis, N. Klasutis, Gas antisolvent recrystallization-new process to recrystallize compounds insoluble in supercritical fluids, Acs Symp. Ser. 406 (1989) 334–354.
40. M. Huang, E. Khor, L.Y. Lim, Uptake and cytotoxicity of chitosan molecules and nanoparticles: effects of molecular weight and degree of deacetylation, Pharm. Res. 21 (2004) 344–353.
41. X. Wang, C. Zheng, Z.M. Wu, D.G. Teng, X. Zhang, Z. Wang, C.X. Li, Chitosan- AC nanoparticles as a vehicle for nasal absorption enhancement of insulin, J. Biomed. Mater. Res. B, Appl. Biomater. 88B (2009) 150–161.
42. D. Teijeiro-Osorio, C. Remunan-Lopez, M.J. Alonso, New generation of hybrid poly/oligosaccharide nanoparticles as carriers for the nasal delivery of macromolecules, Biomacromolecules 10 (2009) 243–249.
43. A. Grenha, C.I. Grainger, L.A. Dailey, B. Seijo, G.P. Martin, C. Remunan-Lopez, B. Forbes, Chitosan nanoparticles are compatible with respiratory epithelial cells in vitro, Eur. J. Pharm. Sci. 31 (2007) 73–84.
44. K. Ono, Y. Saito, H. Yura, K. Ishikawa, Photocrosslinkable chitosan as a biological adhesive, J. Biomed. Mater Res., Part A 49 (2000) 289–295.
45. M. Ishihara,K.Obara, S.Nakamura, M. Fujita,K.Masuoka,Y.Kanatani, B. Takase, H. Hattori, Y. Morimoto, M. Ishihara, T. Maehara, M. Kikuchi, Chitosan hydrogel as a

drug delivery carrier to control angiogenesis, J.Artif. Organs 9 (2006) 8–16.
46. E. Ruel-Gariepy, M. Shive, A. Bichara, M. Berrada, D.L. Garrec, A. Chenite, J.-C. Leroux, A thermosensitive chitosan-based hydrogel for the local delivery of paclitaxel, Eur. J. Pharm. Biopharm. 57 (2004) 53–63.
47. A. Chenite, M. Buschmann, D. Wang, C. Chaput, N. Kandani, Rheological characterisation of thermogelling chitosan/glycerol-phosphate solutions, Carbohydr. Polym. 46 (2001) 39–47.
48. J.Cho, M.-C. Heuzey, A. Begin, P.J. Carreau, Physical gelation of chitosan in the presence of b-glycerophosphate: the effect of temperature, Biomacromolecules 6 (2005) 3267–3275.
49. A. Chenite, C. Chaput, D. Wang, C. Combes, M.D. Buschmann, C.D. Hoemann, J.C. Leroux, B.L. Atkinson, F. Binette, A. Selmani, Novel injectable neutral solutions of chitosan form biodegradable gels in situ, Biomaterials 21 (2000) 2155–2161.
50. F.-L. Mi, S.-S. Shyu, C.-Y. Kuan, S.-T. Lee, K.-T. Lu, S.-F. Jang, Chitosan-polyelectrolyte complexation for the preparation of gel beads and controlled release of anticancer drug. I. Effect of phosphorous polyelectrolyte complex and enzymatic hydrolysis of polymer, J. Appl. Polymer Sci. 74 (1999) 1868–1879.
51. F.-L. Mi, S.-S. Shyu, S.-T. Lee, T.-B. Wong, Kinetic study of chitosantripolyphosphate complex reaction and acid-resistive properties of the chitosan-tripolyphosphate gel beads prepared by in-liquid curing method, J. Polymer Sci. B Polymer. Phys 37 (1999) 1551–1564.
52. M. Buschmann, D. Filion, M. Lavertu, Gel formation of polyelectrolyte aqueous solutions by thermally induced changes in ionization state, Canada Patent WO 2007/0513112007.
53. S. Ganguly, A.K. Dash, A novel in situ gel for sustained drug delivery and targeting, Int. J. Pharm. 276 (2004) 83–92.
54. T. Koyano, N. Minoura, M. Nagura, K. Kobayashi, Attachment and growth of cultured fibroblast cells on PVA/chitosan-blended hydrogels, J. Biomed. Mater. Res. 39 (1998) 486–490.
55. O. Nativ, M. Aronson, O. Medalia, T. MolDavsky, E. Sabo, I. Ringel, V. Kravtsov, Anti-neoplastic activity of Paclitaxel on experimental superficial bladder cancer: in vitro and in vivo studies, Int. J. Cancer 70 (1997) 297–301.
56. J.J. Manfredi, J. Parness, S.B.Horwitz, Taxol binds to cellularmicrotubules, J. Cell Biol. 94 (1982) 688–696.
57. J. Parness, S.B. Horwitz, Taxol binds to polymerized tubulin in vitro, J. Cell Biol. 91 (1981) 479–487.
58. P.B. Schiff, J. Fant, S.B. Horwitz, Promotion of microtubule assembly in vitro by taxol, Nature 277 (1979) 665–667.
59. S. Nsereko, M. Amili, Localized delivery of paclitaxel in solid tumors from biodegradable chitin micropartical formulations, Biomaterials 23 (2002) 2723–2731.
60. J. Fassberg, V.J. Stella, A kinetic and mechanistic study of the hydrolysis of camptothecin and some analogues, J. Pharm. Sci. 81 (1992) 676–684.
61. R.P. Hertberg, M.J. Caranfa, K.G. Holden, D.R. Jakas, G. Gallagher, M.R. Mattern, S.M. Mong, J.O. Bartus, R.K. Johnson, W.D. Kingsbury, Modification of the hydroxy lactone ring of camptothecin: inhibition of mammalian topoisomerase I and biological activity, J.Med. Chem. 32 (1989) 715–720.
62. T.G. Burke, Z. Mi, The structural basis of camptothecin interactions with human serum albumin: impact on drug stability, J. Med. Chem. 37 (1994) 40–46.
63. J.D. Weingart, R.C. Thompson, B. Tyler, O.M. Colvin, H. Brem, Local delivery of the topoisomerase I inhibitor camptothecin sodium prolongs survival in the intracranial 9 L aliosarcoma model, Int. J. Cancer 62 (1995) 605–609.
64. R.A. Jain, The manufacturing techniques of various drug loaded biodegradable poly (lactide-co-glycolide) (PLGA) devices, Biomaterials 21 (2000) 2475–2490.
65. M. Berrada, A. Serreqi, F. Dabbarh, A. Owusu, A. Gupta, S. Lehnert, A novel non-toxic camptothecin formulation for cancer chemotherapy, Biomaterials 26 (2005) 2115–120.

*****