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Review Article
Chitosan Nanoparticles based Drug Delivery: an Update

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Abstract

Nanotechnology is the "creation of functional devices in the nanometer range and the exploitation of the unique properties of these devices in various fields". Nanoparticles are one of the building blocks in nanotechnology. In recent years, nanotechnology and polymers have captivated a tremendous interest in many areas such as the pharmaceutical industry and therapeutic innovation among others. Chitosan is a versatile natural polymer which offers a valuable tool to novel drug delivery system in present scenario due to its inherent biological properties. They are potentially good drug delivery carriers due to their reduced size, better stability, low toxicity, least expensive, simple and mild preparation methods and their versatile routes of administration. Chitosan also have immense structural possibilities for chemical and mechanical modification due to the presence of reactive functional hydroxide and amine groups. Chemically modified chitosan have increased attention for their wide pharmaceutical applications. Therefore, considerable research efforts have been directed towards developing safe and efficient chitosan nanoparticulate based drug delivery systems. The present review outlines the major new findings on the pharmaceutical applications of chitosan nanoparticulate based drug delivery systems published over the past decade. This review is an insight into exploitation of various properties of chitosan to encapsulate drug. Various techniques used for their preparation, drug loading, drug release characteristics, applications and usefulness of these systems in delivering the bioactive molecules will also been discussed. It has been realized that research activities on chitosan nanoparticulate drug delivery systems have increased at the rapid rate.

Introduction

The discovery and development of drug has always been highly challenging, laborious and expensive process. Most of the drugs fail to achieve favorable clinical outcomes in the clinical phase as they do not reach the target site of action. New drug delivery technologies are revolutionizing the drug discovery; development and creating research and development (R&D) focused pharmaceutical industries to increase the momentum of global advancements[1]. Drugs are generally administered through oral, parental and topical routes. The oral route is considered the most convenient, safe and economical method for drug administration, because of its non-invasive nature. In parental route, the drug is introduced directly across the body's barrier defenses into the systemic circulation. However, these administrations are irreversible and may cause pain, fear, local tissue damage, and infections.

Topical application is used when a local effect of the drug is desired. Topical application may be either trans-dermal or mucosal. In trans-dermal, the drug is directly applied to the skin. The rate of absorption of drug can vary markedly, depending on the physical characteristics of the skin at the site of application as well as the lipid solubility of the drug. In mucosal, drug is administered to any mucosal membrane like nose, ear, eye, colon etc. These surfaces are used to deliver the drug for a prolonged period of time at a controlled rate by use of mucoadhesive agent. Therefore, to develop a drug delivery system that optimizes the pharmaceutical action of a drug while reducing its toxic side effects *in vivo* is a challenging task. In this regard, novel drug delivery systems (NDDS) have many benefits, which include improved efficacy, reduced toxicity, improved patient compliance and

convenience. One approach is the use of colloidal drug carriers that can provide specific or targeted drug delivery combined with optimal drug release profiles. The idea of using colloidal drug carriers for drug targeting comes after hundred years of drug-loaded magic bullets by Paul Ehrlich. Among colloidal drug carrier liposomes and nanoparticles act as drug carriers that can provide specific or targeted drug delivery combined with optimal drug release profiles. Liposomes present some technological limitations including poor reproducibility and stability, low drug entrapment efficiency. Therefore, nanoparticles are extensively investigated as a drug delivery carrier.

Nanoparticles

Nanoparticles are the solid colloidal particles with diameters ranging from 1-1000 nm. The word 'Nano' has been derived from Latin word, which means dwarf. Nano size refers to one thousand millionth of a meter. The word 'nanotechnology' for the first time was used by Prof. Taniguchi in 1974. In 1986 Eric Drexler's popularized the innovative possibilities of nanotechnology and specifically mentioned the "Grey Goo" phenomena. Then in 2000 National Nanotechnology Initiative (NNI) was established by US President Bill Clinton. Nanoparticles are all around us right now and have been all around us throughout human history. The ideas and concepts behind nanoscience and nanotechnology started with a talk entitled "There's Plenty of Room at the Bottom" by physicist Richard Feynman in 1959. In this talk, he explored the possibility of manipulating material at the nano scale by imagining the whole of the Encyclopedia Britannica written on the head of a pin and also described a process through which scientists would able to manipulate the individual atom or molecule as a more powerful tool of scientific chemistry than those used at that time. The artisans were known to be the first nanotechnologists they develop Medieval Stained by trapping gold nanoparticles in the 'glass matrix' in order to generate the ruby red color and silver nanoparticles. Deruta Ceramicists were developed by Italians; they produced iridescent or metallic glazes by using silver and copper particles. The Lycurgus Cup was made by the Romans with gold and silver nanoparticles. It appears green in reflected light and red in transmitted light. But now a day's nanoparticles act as a drug carrier in which the active ingredient is dissolved, dispersed, entrapped, encapsulated, adsorbed or chemically attached. Over the period various applications have been developed using nanoparticles. Today nanoparticles act as a drug carrier in which the active ingredient may be dispersed, dissolved, encapsulated, entrapped, adsorbed or chemically attached. Nanoparticles include nanospheres, nanocapsules, liposomes, dendrimers, polymeric nanoparticles and solid lipid nanoparticles. Among these polymeric nanoparticles may offer a new advancement in drug discovery. They can be prepared from either synthetic polymer or natural polymer. Among natural polymers chitosan are widely explored for biomedical and pharmaceutical applications.

Chitosan

Chitosan (CS) is a natural, linear carbohydrate polymer composed of randomly distributed β-(1-4)-linked D-glucosamine (deacetylated unit) and N-acetyl-D-glucosamine (acetylated units). It was first described by Rouget in 1859 and formally named by Hoppe-Seyler in 1894. This cationic polysaccharide is easily available, biocompatible, biodegradable, non toxic, low-immunogenic in nature and has inherent pharmacological properties. Thus it has drawn increasing attention for pharmaceutical and biomedical applications. Chitosan is a deacetylated form of chitin which is a polysaccharide found in exoskeleton of shellfish such as shrimp, lobster or crabs and cell wall of fungi etc. The extraction of polymer involves preparation of the samples from collected crude skeleton followed by deproteinization with 4M NaOH and crushed into pieces. The ground exoskeleton is then demineralised using 1% HCl and followed by deacetylation with 50% NaOH to obtain chitosan and finally extracted by dissolving chitosan in acetic acid and reprecipitated with pH change. Commercially available CS has an average molecular weight ranging between 3800 and 20,000 daltons and is 66 to 95% deacetylated. CS has a p*K*a of approximately 6.5 on the amine groups. It is insoluble at neutral pH, but at acidic pH it is soluble as the amino group of chitosan gets protonated and become positively charged. The solubility of chitosan in neutral and basic pH can be increased by quaternization. The physicochemical and biological properties of chitosan are greatly influenced by its molecular weight and degree of deacetylation. The degree of deacetylation is affected by the number of protonatable amine groups and it determines the polymer properties including solubility, hydrophobicity, and the ability to interact electrostatically with polyanions. The molecular weight has also fundamental importance. Generally chitosan having lower molecular weights and lower degrees of deacetylation exhibits greater solubility and faster degradation than their high molecular weight counterparts. Detailed characteristics of chitosan for biomedical applications are well described in a comprehensive review[2]. Due to the presence of reactive functional group CS has been modified in various forms like thiolated, carboxyalkyl, bile acid-modified, quaternized (N, N, N-trimethyl chitosan; TMC), sugar-bearing and cyclodextrin-linked chitosan. For example, thiolation of chitosan remarkably improves its mucoadhesive properties because of the formation of disulfide bonds with cysteine-rich sub domains of mucus glycoproteins[3]. Different types of CS based formulation already used in drug delivery systems have been summarized in **Table 1**. The present review addresses the recent trends in the area of chitosan nanoparticulate based drug delivery systems.

Table: 1 Chitosan based formulations prepared by various methods for different kind of drugs

Types of system	Method of preparation	Drug
Tablets	Matrix coating	Mesalamine[4], Glipizide[5]
Capsules	Capsule shell	5-ASA [6]
Microsphere/ Microparticles	Emulsion cross-linking	Clarithromicin[7]
	Coacervation/precipitation	Interleukin-2[8]
	Spray-drying	Vitamin D-2[9]
	Ionic gelation	Felodipine[10]
	Sieving method	Clozapine[11]
Nanoparticles	Emulsion-droplet coalescence	Gadopentetic acid[12]
	Coacervation/ precipitation	Ovalbumin[13-14]
	Ionic gelation	Ascorbic acid[15], Ampicillin [16], Insulin[17], Rivastigmine[18], Doxorubicin hydrochloride[19-20], Gemcitabine [21], 5-fluorouracil[22-23], Doxorubicin[24-55], Curcumin[26], Chloroquine[27], Thiocolchicoside[28], Docetaxel[29], Rizatriptan benzoate[30], Temoxifen citrate[31]
	Reverse micellar method	Doxorubicin[32]
Beads	Coacervation/ precipitation	Insulin[33]
Films	Solution casting	Ofloxacin[34]
Gel	Cross-linking	Theophylline[35]

Properties of Chitosan Nanoparticles

CS has been explored as a material of choice to form nanoparticles for the last decade. The properties of chitosan have been enhanced by making their nanoparticles. The unique character of NP for their small size and quantum size effect could make CSNP exhibit superior activities They are simple and inexpensive to manufacture and scale-up and have unique size and large surface-to-volume ratio. They are mucoadhesive and hydrophyllic in nature due to which they provide good protection to encapsulated drug, increase its clearance time and stability in the body. Thus they are applicable to a broad category of drugs; small molecules, proteins and polynucleotides. The benefits of encapsulating active agents in a polymer matrix include their protection from the surrounding medium or processing conditions and their controlled release.

Toxicity of chitosan nanoparticles

CS is approved as a non-toxic, biocompatible polymer by US Food and Drug Administration (FDA) for wound dressing. The LD_{50} of CS in mice after oral administration is 16 g/kg body weight, which is almost equal to household sugar or salt. No side effects were reported in human up to 4.5 g/day oral administration of CS. However, when taken regularly for 12 weeks, it showed mild nausea and constipation in humans[36.] Although CS alone is considered to be safe for oral administration, its properties may change completely upon chemical modification[37]. Moreover, it is well-known that the pharmacokinetic properties of a drug or excipient change considerably when included in a nanoparticulate system[37.] Thus there in vivo fate is decided by the size, charge and surface modifications of the NPs. This in turn can alter the toxicity profile of the NP itself, as these properties influence the way the NPs interacts with different types of cells, thus modifying their cellular uptake, absorption through the GIT, tissue distribution and excretion[37]. This is the reason that the generally recognized as safe (GRAS) status of CS does not apply for nanoparticulate formulations and primarily depends upon the conditions of intended use[37]. In addition, molecular weight and degree of deacetylation of chitosan affects the

pharmacokinetic properties and toxicity. Thus, each and every derivative should be assessed individually both in the free form and nanoparticulate form. A recently published review by Kean and Thanou[37] has summarized all the current findings related to CS toxicity.

Preparation of chitosan nanoparticles

Different methods have been used to prepare chitosan nanoparticles (CSNPs). Selection of any of the methods depends upon factors such as particle size requirement, thermal and chemical stability of the active agent, reproducibility of the release kinetic profiles, stability of the final product and residual toxicity associated with the final product [38]. In this review different methods for preparation of chitosan nanoparticles are discussed. However, selection of any of these methods depends upon nature of the active molecule as well as the type of the delivery device.

Emulsion Cross-Linking

This method was for the first time employed in 1994 [39] for the preparation of chitosan nanoparticles containing 5-fluorouracil, using glutaraldehyde as cross linking agent. This method utilizes the reactive functional amine group of chitosan to cross-link with aldehyde groups of the cross-linking agent. In this method, water-in-oil (w/o) emulsion is prepared by emulsifying the chitosan aqueous solution in the oil phase. These aqueous droplets are stabilized by using a suitable surfactant. The stable emulsion is then cross-linked by using an appropriate cross-linking agent such as glutaraldehyde to harden the droplets. The nanospheres obtained are filtered and washed repeatedly with n-hexane followed by alcohol and then dried. In this method the size of aqueous droplets pays a vital role in controlling the particles size. The particle size of final product depends upon the extent of cross-linking agent used while hardening in addition to speed of stirring during the formation of emulsion. However, this emulsion cross-linking method has few drawbacks since it involves tedious procedures as well as use of harsh of organic solvents and cross linking agents which adversely affect the stability of proteins. Simultaneously, there is difficulty in complete removal of the unreacted cross linking agent involved in the process. Moreover, glutaraldehydes cross linked nanoparticles present negative effects on cell viability.

Coacervation/precipitation

This method is developed in 1986 [40]. It utilizes the physicochemical property of CS that it is insoluble in alkaline pH medium, but precipitates/coacervates when it comes in contact with alkaline solution. Particles are produced by blowing CS solution into an alkali solution like sodium hydroxide, NaOH-methanol or ethane diamine using a compressed air nozzle to form coacervate droplets (fig1). Separation and purification of particles is done by filtration/centrifugation followed by successive washing with hot and cold water. Varying compressed air pressure or spray-nozzle diameter controls the size of the particles and then using a cross linking agent to harden particles can control the drug release.

Another technique was developed in 1996 [41]. They have added sodium sulphate solution drop wise to an aqueous acidic solution of chitosan containing a surfactant under stirring and ultrasonication for 30 min. Nanospheres were purified by centrifugation and re-suspended in demineralised water. Particles were cross-linked with glutaraldehyde. Particles produced by this method have better acid stability than observed by other methods.

Spray-drying

In 2010[42] chitosan–iron oxide nanoparticles was prepared with varying ratios of chitosan and iron oxide by spray-drying. Spray-drying is a well-known technique to produce powders, granules or agglomerates from the mixture of drug and excipient solutions as well as suspensions. In this method, chitosan is first dissolved in aqueous acetic acid solution. To this solution drug is dissolved or dispersed in the solution and then, a suitable cross-linking agent is added. Then this solution or dispersion is then atomized in a stream of hot air. Atomization leads to the formation of small droplets, from which solvent evaporates instantaneously and free flowing particles are formed (fig 2). Particle size depends upon the size of nozzle, spray flow rate, atomization pressure, inlet air temperature and extent of cross linking and also high negative voltage to instantly evaporate the particles sprayed from the nozzle as elctrospray disposition technique (new technique in which instead of the pressure high negative voltage is used), which atomizes the solids particles.

Emulsion-droplet coalescence method

The method was developed in 1999[43] which utilize the principles of both emulsion cross-linking and precipitation. However, in this method, instead of cross-linking the stable droplets, precipitation is induced by allowing coalescence of chitosan droplets with NaOH droplets. First, a stable emulsion containing aqueous solution of chitosan along with drug is produced in liquid paraffin oil and then, another stable emulsion containing chitosan aqueous solution of NaOH is produced in the same manner. When both emulsions are mixed under high-speed stirring, droplets of each emulsion would collide at random and coalescence thereby precipitating chitosan droplets to give small size particles (fig 3). Then the separation of particles takes place by centrifugation and washing.

Ionic gelation method

This method was developed by Calvo and co worker in 1997 [44]. In this method polymer solutions and polyanion solutions are mixed to form nanoparticles. The CSNPs are formed due to the electrostatic interaction between positively charged amine group of chitosan and negatively charge group of polyanion such as tripolyphosphate. This technique offers a simple and mild preparation method in the aqueous environment. First, chitosan can be dissolved in acetic acid in the absence or presence of stabilizing agent, such as poloxamer, Tween80 which can be added in the chitosan solution before or after the addition of polyanion. Polyanion such as TPP (sodium tripolyphosphate) was then added and nanoparticles were spontaneously formed under mechanical stirring at room temperature (fig 4). The material undergoes transition from liquid to gel phase due to interaction. Using this method different proteins and peptides has been loaded.

Reverse Micellar method

In2001[45]for the first time use this technique to encapsulated doxorubicin- dextran conjugate in CSNPs. In this method surfactant is first dissolved in an organic solvent to produce reverse micelles. Reverse micelles are thermodynamically stable liquid mixtures of water, oil and surfactant. To this, an aqueous solution of chitosan and drug are added with constant vortexing to avoid any turbidity. The organic solvent is then evaporated to obtain the transparent dry mass. The material is dispersed in water, followed by the addition of a suitable salt ($CaCl_2$), which helps to precipitate the excess surfactant out. It is then centrifuged and the supernatant is decanted, which contains the drug-loaded nanoparticles (fig 5). The aqueous dispersion is immediately dialyzed through dialysis membrane. For 1 hr and the liquid is lyophilized to dry powder. This technique offers a narrow size distribution of less than 100 nm and the particle size can be controlled by varying the amount of glutaraldehyde that alters the degree of cross-linking. Nevertheless, some disadvantages exist such as the use of organic solvent, time-consuming preparation process, and complexity in the washing step.

Sieving method

In this method nanoparticles are prepared by cross-linking chitosan to obtain a non-sticky glassy hydrogel followed by passing through a sieve[38]. A suitable quantity of CS is dissolved in 4% acetic acid solution to form a thick jelly mass that is cross-linked by adding glutaraldehyde. The non-sticky cross-linked mass is passed through a sieve with a suitable mesh size to get nanoparticles. Nanoparticles are washed with 0.1 N NaOH solutions to remove the unreacted excess glutaraldehyde and dried overnight in an oven at 40^oC (fig 6).

Drug Loading into –Nanoparticles of Chitosan

Drug can be loaded in chitosan nanoparticles by active or passive loading. In active loading drug is loaded during the preparation of particles (incorporation). In this method drug is physically embedded into the matrix or adsorbed onto the surface. By this method maximum drug can be loaded but it may get affected by the process parameters such as method of preparation, presence of additives, etc. Both water soluble and water-insoluble drugs can be loaded into chitosan-based particulate systems. In passive loading drug is loaded after the formation of particles (incubation). In this method drug was incubated with the pre-formed nanospheres for 48 h for the sufficient entry of the drug. Drug loading depends on (1) drug solubility in core phase; (2) Surfactant can be added to stabilize dispersion, (3) Characterization of nanoparticles, (4) Size and morphology, (5) Surface charge and electrphoretic mobility, (6) Surface hydrophobicity, (7) Density.

Mechanism of drug release from particulate system

Drug release from chitosan nanoparticulate systems depends upon the extent of cross-linking, morphology, size and density of the particulate system, physicochemical properties of the drug as well as the presence of adjuvant. *In vitro* release also depends upon pH, polarity and presence of enzymes in the dissolution media. CSNPs releases the drug either by swelling of nanoparticles due to hydration followed by release of drug through diffusion or by an enzymatic reaction resulting in rupture or cleavage or degradation of polymer site of delivery or simply by dissociation of drug from the polymer and its de-adsorption release from swelled nanoparticles. In majority of cases, drug release follows more than one type of mechanism. More the drug loading greater the burst and faster the release rate. For example, PLA nanoparticles containing 16.7% savoxepine released 90% of their drug load in 24 h, as opposed to particles containing 7.1% savoxepine, which released their content over 3 weeks[46]. The initial burst release is thought to be caused by poorly entrapped drug, or drug adsorbed onto the outside of the particles. When using polymers, which interact with a drug, like PLGA with a free COOH group and proteins, the burst release is lower and in some cases absent, and drug release is prolonged[46].

To develop a successful nanoparticulate system, both drug release and polymer biodegradation are important consideration factors. In general, drug release rate depends on (1) solubility of drug, (2) desorption of the surface bound/ adsorbed drug, (3) drug diffusion through the nanoparticle matrix, (4) nanoparticle matrix erosion/degradation and (5) combination of erosion/diffusion process[47]. Thus solubility, diffusion and biodegradation of the matrix materials govern the release process. Furthermore release rate can also be affected by ionic interaction between the drug and addition of auxiliary ingredients. When the drug forms a less water soluble

complex by the interaction with the auxiliary ingredients, then the drug release can be very slow with almost no burst release effect. To develop a successful nanoparticulate system, both drug release and polymer biodegradation are important consideration factors. In general, drug release rate depends on (1) solubility of drug, (2) desorption of the surface bound/ adsorbed drug, (3) drug diffusion through the nanoparticle matrix, (4) nanoparticle matrix erosion/degradation and (5) combination of erosion/diffusion process [47]. Thus solubility, diffusion and biodegradation of the matrix materials govern the release process. Furthermore release rate can also be affected by ionic interaction between the drug and addition of auxiliary ingredients. When the drug forms a less water soluble complex by the interaction with the auxiliary ingredient, then the drug release can be very slow with almost no burst release effect.

Benefits of using nanoparticles drug delivery system over conventional method

There are some issues associated with conventional method that about an estimated 40% of new drug are hydrophobic and lipophylic nature. Due to which they possess low aqueous solubility and have poor distribution profiles. They also plague with unfavorable pharmokinetics and are toxic to some tissues which prevent full exploitation of their therapeutic potential. These problems can overcome by using nanoparticulate drug delivery system **(**DDS). Due to reduced size nanoparticles increase surface area which results in faster dissolution of active agents in aqueous environment such as human body. Faster dissolution equates with greater bioavailability, small drug, less toxicity which improves the therapeutic effect of drug**.**

Modification of chitosan nanoparticles for targeted delivery

In order to improve targeting and bioavailability of chitosan nanoparticles, an increasing number of studies are focusing on modification of chitosan. Modified chitosan nanoparticles can be characterized by pH sensitivity, thermo sensitivity, and targeting accuracy. Positive charges of chitosan have selective adsorption and neutralizing effects on the tumor cell surface. As a drug carrier, it has a targeting function to liver, spleen, lung, and colon [48]. In 2008 Xu *et al*[49] prepared In 2008 Xu *et al*[49] prepared Doxorubicin–chitosan polymeric micelles. These micelles had excellent drug-loading properties and were found suitable for targeting the liver and spleen, and considerably reduced drug toxicity to the heart and kidney.

Modification for pH Sensitivity

In a drug delivery system, the pH-sensitive nanocarrier increases drug release by changing carrier properties under certain acid-base environment *in vivo*, and targets the lesion tissue. Poly propyl acrylic acid (PPAA) is a polymer that is highly sensitive to pH. In 2004, Kiang *et al*[50]added PPAA to chitosan–DNA complex to improve gene transfection efficiency. The results showed that adding PPAA to chitosan– DNA complexes enhanced gene expression in both HEK293 and He La cells compared with chitosan nanoparticles alone. In 2008, Fan L *et al*[51] seen that Camptothecine-loaded N-isopropylacrylamide–chitosan nanoparticles were sensitive to pH. The nanoparticles were most sensitive to tumor pH when the charge ratio of N-isopropylacrylamide: chitosan was 4:1. At a pH of 6.8, cumulative release rate of camptothecine was highest and cell toxicity was significantly stronger. Cell toxicity was minimum at a pH of 7.4. Thus, N-isopropylacrylamide–chitosan nanoparticles have a good potential for use as a targeted anticancer drug carrier. Chitosan nanoparticles prepared with sodium tripolyphosphate and glycidoxy propyl trimethoxysilane have been found to be pH sensitive with antihuman IgG antibody as a model protein drug, in which case the release of antibody was increased from 5.6 to 50% when solution pH was adjusted from 7.4 to 6.0.

Modification for Thermo sensitivity

Drug release is regulated by structural change of thermo sensitive drug carriers at different temperatures. Poly (N-isopropylacrylamide) is a well-known thermosensitive polymer widely used in drug carriers[52]. Chitosan polyvinyl caprolactan graft copolymer nanoparticles were sensitive to temperature, with a critical solution temperature at 38^0C. Chung *et a*l uses 5-fluorouracil as a model drug and found that drug release mainly occurred above 38^0C with high toxicity to tumor cells but low toxicity to normal cells.

Modification of sustained release

Chitosan was modified with lactic acid for high drug loading and prolonged drug release. By grafting D, L-lactic acid onto amino groups of chitosan without using a catalyst increases the drug encapsulation and prolong drug release by increasing the chitosan solubility in solution of neutral pH. Unlike chitosan, which is generally soluble only in acid solution, the chitosan modified with lactic acid can be prepared from solutions of neutral pH, offering an additional advantage of allowing proteins or drugs to be uniformly incorporated in the matrix structure with minimal or no denaturization. Chitosan–alumino-silicate nanoparticles with significant sustained/controlled release effects exhibited that pH of the environment and chitosan: alumino silicate ratio also influence drug release [53].

Modification of targeting

Active targeting can be obtained CSNPs through chemical modification, so as to make the drug identify the target accurately. In 2006 Yao Q[54] prepared chitosan nanoparticles with resveratrol as a model drug and modified the

nanoparticles by using ligands of both avidin and biotin. The resulting delivery system passively targeted the liver and positively targeted hepatoma cells [55]. In 2005 Kim *et al* [56] used hydrophobic cholanic acid to modify glycol chitosan and prepared nanoparticles through self-assembly. The antitumor drug cisplatin could be encapsulated easily in a hydrophobic core of nanoparticles. It was proven that due to prolonged circulating time in vivo and strengthened cell permeability and drug effect, drug-loaded nanoparticles were concentrated in tumor tissues of mice successfully with better antitumor effect and lower toxicity. In 2008, Min *et al* [57] prepared hydrophobic chitosan nanoparticles by using dialysis method. He used chemical binding to bind hydrophobic 5β-cholanic acid with the skeleton of hydrophilic glycol chitosan (HGC and encapsulate camptothecine, with drug-loading of more than 80%). The drug Camptothecine (CPT) was effectively protected from hydrolyzation when loaded in HGC nanoparticles and showed a significant antitumor effect as well as they are high targeting to MDA-MB231 human breast cancer cells.

Applications of chitosan nanoparticles as delivery vehicles:

Oral drug delivery

The nanoparticles prevent the enzymatic degradation of labile drugs in the gastrointestinal tract (GIT). This leads to the development of nanoparticles as oral delivery systems for macromolecules, proteins and polynucleotides. Among polymeric nanoparticles, CSNPs has been widely used as an oral drug delivery vehicle. CSNPs decrease the trans-epithelial electric resistance (TEER) of cell monolayers and protect the drug from being degraded in the stomach. Chitosan solutions also increase Trans and *para*-cellular permeability in a reversible and dose-dependent manner which depends on the degree of deacetylation and molecular weight of the chitosan. Furthermore, *in vitro* studies in Caco-2 cells have shown that chitosan have been able to induce a transient opening of tight junctions. CS interact with tight junction protein like occluding and zona occludens (ZO-1) and slight destabilization of plasma membrane due to redistribution of F-actin and tight junction open which increases the membrane permeability particularly for polar drugs, including peptides and proteins. Recent studies have shown that only protonated soluble chitosan, in its uncoiled configuration, can trigger the opening of the tight junctions, thereby, facilitating the paracellular transport of hydrophilic compounds. This property implies that chitosan would be effective as an absorption enhancer only in a limited area of the intestinal lumen where the pH values are below or close to its pKa. CSNPs also promote the absorption of drug by increasing the residence time in gastrointestinal tract (GIT) through mucous adhesion or by increasing the cell or tissue entry payer's patches and M cell mediated uptake by endocytosis. Mechanism of CSNPs transport across GIT is most probably through adsorptive endocytosis. Electrostatic interaction between positively charged chitosan and negatively charged sialic acid of mucin causes association of CSNPs to the mucus layer and subsequently internalization via endocytosis. CSNPs internalization has been found to be higher in the jejunum and ileum than in duodenum. Lin *et al* [58] reported the preparation of nanoparticles composed of chitosan and poly-γ-glutamic acid (γ-PGA) for insulin delivery. The induced diabetic rats show the hypoglycemic effect for at least 10 hours after oral administration of chitosan nanoparticles.

Parental drug delivery

CSNPs can be administered intravenously because the diameter of smallest blood capillary is 4μm. The particles greater than 100 nm can be rapidly taken up by reticuloendothelial system (RES) in the liver, spleen, lungs and bone marrow, while smaller particles tend to have a prolonged circulation time. The larger CSNPs can be coated by nonionic surfactant like polyethylene glycol to improve their circulation time. The OH group present in glycol binds with the protonated amino group of chitosan, as a result of which opsonins (negatively charged serum proteins that bind to substrates leading to their being taken up by the RES become unable to recognize CSNPs and consequently it escapes from phagocytosis and thereby improves the therapeutic efficacy of loaded drug particles. CSNPs can be used to deliver anti-infective drugs such as antibacterial, antiviral, antifungal and antiparasitic drugs. The low therapeutic index of antifungal drugs, short half-life of antivirals and the limited ability of antibiotics to penetrate infected cells in intracellular compartments make them ideal candidates for nanoparticle delivery. Thus, it has been suggested that nanoparticles improves the therapeutic efficacy while decreasing the toxic side effects of these drugs. In theory, chitosan NP are very attractive carrier system due to their nanosize and hydrophilic surface. The most promising drugs that have been extensively studied for delivery by this route are anticancer agents. Following intravenous injection, CSNPs exhibit a marked tendency to accumulate in a number of tumors.

Cancer-targeted drug delivery using chitosan nanoparticles

Despite of lot of research in treatment of cancer, chemotherapy and radiotherapy are major treatment methods for cancer. These two methods are although effective treatment of cancer, but cause serious side effects such as loss of hair, irritation in stomach and low counts of blood cells[59]. The critical bottleneck of conventional cancer chemotherapeutics includes high toxicity of most anticancer drugs, due to indiscriminate distribution of drugs towards disease and healthy cells following systemic administration. In addition, anticancer drugs are poorly soluble in water and thus used with the organic solvents or detergents for clinical

applications, resulting in undesirable side effects such as venous irritation and respiratory distress. Therefore, nanotechnology particularly nanomedicine has emerged as hope for treatment of cancer. By designing a distinct carrier system that encapsulates a large quantity of drugs and specifically targets tumor cells is indispensable for successful cancer therapy. Carrier-mediated drug delivery has emerged as a powerful methodology for the treatment of various types of cancer. The therapeutic index of traditional and novel drugs is enhanced via the increase of specificity due to targeting of drugs to a specific tissue, cell or intracellular compartment, the control over release kinetics, the protection of the active agent or a combination of the above[60]. Chitosan is an ideal carrier for anticancer drug as it can target these drugs to the diseased site by joining various ligands such as folic acid. Till now CSNPs are used to deliver drug to tumor cells. In cancer cells CSNPs enhance the therapeutic efficacy of drug both by passive targeting and active targeting. In passive targeting CSNPs enhances permeability and retention effect in tumor cells and drug may be released in extracellular matrix (ECM) and then diffuse through the tissues. Active targeting can be achieved by functionalizing the surface of nanoparticles with ligands that promote cell specific recognition and binding. The CSNPs can release the drug in close proximity to the target cell or it may attach to the cell membrane and act as an extracellular sustain release drug depot or it may be internalized in to the cell through receptor mediated endocytosis (RCM). For example, doxorubicin-conjugated glycol chitosan (DOX–GC) with a cis-aconityl spacer was synthesized by attaching of N-cisaconityl DOX to GC by means of carbodiimide chemistry[61]. When the DOX–GC nanoparticles were systemically administrated into the mice, they preferentially accumulated into the tumor tissues ascribed to the EPR effect. Doxorubicin loaded CSNPs showed regression in tumor growth and enhance survival rate of tumor-implanted rats after IV administration.

Another possibility is there that CSNPs can be coated with polysorbate 80 (P80) which adsorb apolipoprotein and seems to mimic lipoprotein particles that are able to interact with members of LDL receptor family are taken by receptor mediated endocytosis. Bound drugs may be further transported by diffusion. The *in vivo* fate of NPs coated with different surfactants confirmed the effectiveness of P80 in enhancing brain drug uptake. CSNPs are coated with coated with P80 before being utilized for brain targeting. Chitosan has been utilized to improve the brain targeting efficiency by the direct nose to brain pathway especially for drugs for treatment of central nervous system disorders. Further it has been used to combine the active drug for targeting to the olfactory region with controlled release. Besides this, the estradiol chitosan nanoparticles have higher estradiol concentration in CSF at each sampling time following intranasal delivery. This proves a better utility of chitosan nanoparticles as a suitable formulation for estradiol delivery to the central nervous system[62]. The CSNPs allow the access of non- transportable drugs across the BBB by masking their physicochemical characteristics through their encapsulation in these systems. CSNPs are also investigated for targeted delivery of antiretroviral drugs to HIV infected cells and to achieve sustained release kinetics. Their encapsulation into such systems may provide improved efficacy, decreased drug resistance, the reduction in dosage, a decrease in systemic. In 2011 Kaur SP *et al*[63] prepared Rivastigmine loaded chitosan nanoparticles by ionic gelation method. Three batches of chitosan used nanoparticles formulated shown to have appreciable entrapment efficiency with small size distribution. In-vitro release pattern showed sustained and controlled release for the short half-life drugs such as rivastigmine.

Transdermal drug delivery:

The human skin is a readily accessible surface for drug delivery. Transdermal drug delivery can be used as an alternative route of administration to accommodate patients who cannot tolerate oral dosage forms. It is also of great advantage in patients who are nauseated or unconscious. Drugs that cause gastrointestinal upset can be good candidates for transdermal delivery because this method avoids direct effects on internal organs such as the stomach and intestine. In addition, drugs that are degraded by the enzymes and acids in the gastrointestinal system may also be good targets. Chitosan has irreplaceable film forming ability, penetration enhancing competence without causing much stress to skin, skin compatibility and good adhesive properties[64]. The electrostatic interaction takes place between positively charged amino group of between chitosan and negatively charged glycoprotein residue on the cell surface which smoothes the progress of passive diffusion and result in successful absorption of drug in underlying epithelium. As penetration enhances chitosan disrupts the epithelial tight junctions on the skin and facilitates the drug permeation. This epithelial disruption takes place for very short period and is reversible. In 2006 Varhosaz J *et al*[65] prepared the gel containing lidocaine (LC) as a local anesthetic agent with three different molecular weights (MW) and concentrations of chitosan for prolonging anesthetic effect of this drug for transdermal delivery.

Ocular drug delivery

Ocular drug delivery has been a major challenge to pharmacologists and drug delivery researchers due to the eye's unique anatomy and physiology. The most common route of administration for the treatment of various ocular diseases is the topical application of drugs to the eye. Because of drainage of the excess fluid via the nasolacrimal duct and elimination by tear turnover, the intra ocular bioavailability of topically administered drugs is poor. Among mucoadhesive polymers, chitosan has attracted a great deal of attention as an ocular drug delivery carrier

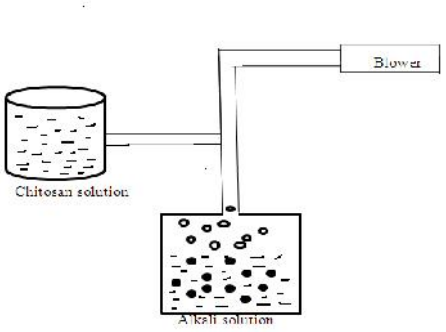

Figure 1: Coacervation phase separation method

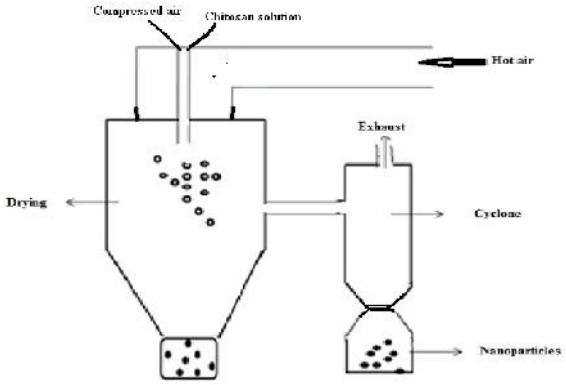

Figure 2: Spray drying method

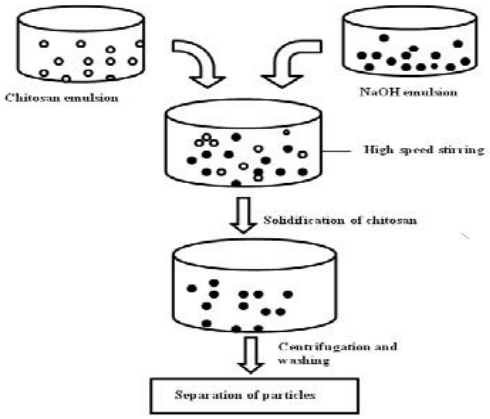

Figure 3: Emulsion-droplet coalescence method

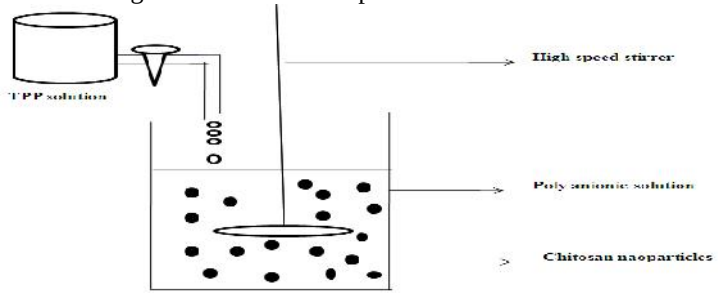

Figure 4: Ionic gelation method

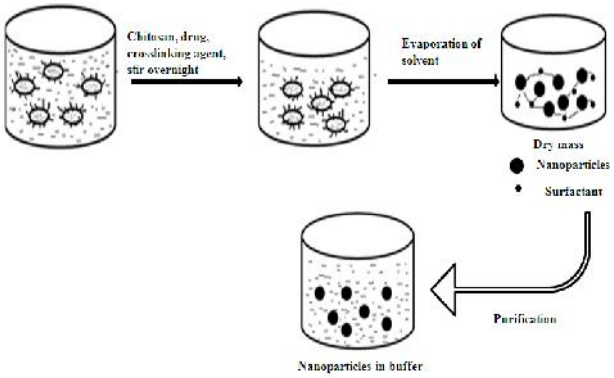

Figure 5: Reverse Miscellar method

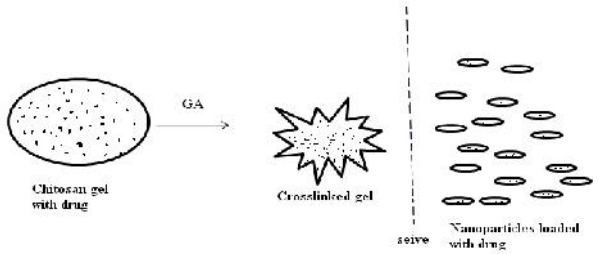

Figure 6: Sieving method

because of its absorption promoting effect. It not only enhances corneal contact time through its mucoadhesion mediated by electrostatic interaction between its positively charged chitosan and negatively charged mucin. CSNPs are able to open tight junction and improve drug bioavailability and prolonged the cornea resident time of antibiotic in rabbits. The same effects were also observed by De Campos[66], they noticed that CSNPs remained attached to the rabbits' cornea and conjunctiva for at least 24 hrs. Chitosan is a low toxic material; ophthalmic formulation based on chitosan exhibited an excellent tolerance after applying chitosan onto the rabbit's corneal surface[66]. Besides employing CSNPs to improve drug transport via ocular, chitosan-coated nanoparticles can also be utilized as it exhibited ability to enhance the corneal penetration. It is also found that after ocular administration of CSNPs in rabbits, most of drug was found in extraocular tissue, cornea and conjunctiva, while negligible drug were found in intraocular tissues, iris/ciliary body and aqueous humor [66].

Nasal drug delivery

The nasal mucosa is an attractive route for the delivery of vaccines due to its large absorptive surface and having low proteolytic activity. Importantly, nasally administered vaccines can induce both local and systemic immune responses. However, most proteins are not well absorbed from the nasal cavity when administered as simple solutions. The major factors which limits the absorption of nasally administered proteins are the poor ability to cross the nasal epithelia, and the mucociliary clearance, which rapidly removes protein solutions from the absorption site. CSNPs have received much attention to overcome these obstacles and deliver protein antigens via the nasal route, the cations present in chitosan bind to negatively charged sialic acid and tenders excellent mucoadhesive properties and increases the nasal residence time of the drug. CSNPs also have reversible and momentary action on tight junction and steps up the drug paracellulary. Thus, it increases the bioavailability of drug from mucosa to submucosa layer of nose. In 2008, Zhang *et al*[67] used polyethylene glycol-grafted chitosan nanoparticles to improve the systemic absorption of insulin, following nasal administration. *In vitro* release studies showed an initial burst, followed by a slow release of insulin. The nasal delivery of insulin using chitosan-acetyl-L-cysteine nanoparticles was proposed by In 2009 Wang *et al*[68] observed that intranasal administration of modified chitosan-based nanoparticles in rats enhanced the absorption of insulin by the nasal mucosa, as compared with unmodified chitosan nanoparticles as well as control (free insulin solution).

Future prospects

From this review, it is concluded that chitosan nanoparticles are the prospective promising drug delivery carriers that are suitable for a broad category of drugs including macromolecules and labile drugs. As drug delivery system, chitosan nanoparticles have attracted increasing attention because of their good biocompatibility, degradability, nontoxicity, mucoadhesivity and hydrophyllicity. Their nano-size facilitates the drug uptake through the cell membrane. Together, the absorption enhancing effect and smaller size exhibits ability to improve drug bioavailability. Modified Chitosan nanoparticles have great utility in controlled release of bioactive molecules and targeting. While great progress has been achieved in the application of chitosan nanoparticles as drug carriers, some problems remain to be resolved urgently. Majority of studies carried out so far are only in i*n vitro* conditions. More *in vivo* studies need to be carried out. Biggest challenge is approval of drug based on chitosan nanoparticles from food and drug administration (FDA). More research is needed in this area. Chemical modifications of chitosan are important to get the desired physicochemical properties such as solubility, hydrophilicity, etc. For example, chitosan has poor solubility and unmodified chitosan nanoparticles can encapsulate only some hydrophilic drugs. However, studies toward optimization of process parameters and scale up from the laboratory to pilot plant and then, to production level are yet to be undertaken. Although chitosan can be modified easily to encapsulate hydrophobic drugs, further investigations are required on the biocompatibility of modified chitosan and its derivatives. It can be concluded that, chitosan and its derivatives as drug carriers have potential for a wider application. The published literature

indicates that in the near future, chitosan nanoparticulate systems will have more commercial status in the market than in the past.

References

1.Rajan, M., and Raj, V. 2013. Potential Drug Delivery Applications of Chitosan Based Nanomaterials``. International Review of Chemical Engineering (I.RE.CH.E.). 5:145-155.

2. Patel, M.P., Patel, R.R., and Patel, J.K. 2010. Chitosan Mediated Targeted Drug Delivery System: A Review. J. Pharm. Pharma. Sci., 13:536 – 557.

3. Jie, J., Wu, W.Z., Zhong, Z.R., Guang, X.T.X., Shu, L.Z., and Wang, L. 2011 Recent Advances of Chitosan Nanoparticles. Int. J. Nanomedicine. 6:765–774.

4. Patel, N.V. 2009. Formulation and in-Vitro evaluation of mesalamine matrix tablets using chitosan for colonic drug delivery. J. Pharmacy Res., 2:1319-1323.

5. Reddy, T.R., Dhachinamoorthi, D., and Chandrasekhar, K.B. 2010.Independent release behavior of Glipizide matrix release tablets containing Chitosan and xanthan gum. Int. J. Pharmaceutical and Biomedical Res**.,** 1: 64-70.

6. Tozaki, H., Odoriba,T., Okada, N., Fujita,T., http://www.sciencedirect.com/science/article/pii/S0168365902000846Terabe, A., Suzuki, T., Okabe, S., Muranishi, S., and Yamamoto, A. 2002. Chitosan capsules for colon-specific drug delivery: enhanced localization of 5-aminosalicylic acid in the large intestine accelerates healing of TNBS-induced colitis in rats. J. Controlled Release. 82: 51–61.

7. Kotadiya, R., Patel, V., Patel, H., and Koradiya, H. 2009. Effect of cross-linking on physicochemical properties of chitosan mucoadhesive microspheres: A factorial approach. Int. J. Green Pharm., 3: 58-62.

8. Ozbas-Turan, S., Akbuga, J., and Aral C. 2002. Controlled release ofinterleukin-2 from chitosan microspheres. J. Pharm. Sci., 91:124–125.

9. Shi, X.Y., and Tan, T.W. 2002. Preparation of chitosan/ethylcellulosecomplex microcapsule and its application in controlled release of vitamin D-2. Biomaterials.23:4469–4473.

10. Ko, J.A., Park, H.J., Hwang, S.J., Park, J.B., and Lee, J.S. 2002. Preparation and characterization of chitosan microparticles intended for controlled drug delivery. In.t J. Pharm., 249:165-74.

11. Agnihotri, S.A., and Aminabhavi, T.M. 2004. Controlled release of clozapine through chitosan microparticles prepared by a novel method. J. Controlled Release. 96:245-259.

12. Hiroyuki, T., Hideki, I., and Yoshinobu, F. 1999. Chitosan-Gadopentetic Acid Complex Nanoparticlesfor Gadolinium Neutron-Capture Therapy of Cancer: Preparation by Novel Emulsion-Droplet CoalescenceTechnique and Characterization. Pharm. Res., 1 6:1830-1835.

13. Van Der Lubben, I.M., Konings ,F.A.J., Borchard, G., Verhoef, J.C., and Junginger, H.E. 2001. In vivo uptake of chitosan microparticles by murine Peyer's patches: visualization studies using confocal laser scanning microscopy and immuno-histochemistry. J. Drug Target. 9: 39-47.

14. Van Der Lubben, I.M., Kersten, G., Fretz, M.M., Beuvery, C., Verhoef, J.C., and Junginger, H.E. 2003.Chitosan microparticles for mucosal vaccination against diphtheria: oral and nasal efficacy studies in mice.Vaccine. 21**:** 1400-1408.

15. Jang, K., and Lee, H.G.2008. Stability of Chitosan Nanoparticles for l-Ascorbic Acid during Heat Treatment in Aqueous Solution. J. Agric Food Chem., 56: 1936-1941.

16. Saha, P., Goyal, A.K., and Rath, G. 2010.Formulation and Evaluation of Chitosan-Based Ampicillin Trihydrate Nanoparticles. Trop. J. Pharm. Res., 9:483-488.

17. Avadi, M.R., Sadeghi, A.M., Mohammadpour, N., Abedin, S., Atyabi, F., Dinarvand, R.,and Rafiee-Tehrani, http://www.ncbi.nlm.nih.gov/pubmed?term=Rafiee-Tehrani%20M%5bAuthor%5d&cauthor=true&cauthor_uid=19447202M. 2010. Preparation and characterization of insulin nanoparticles using chitosan and Arabic gum with ionic gelation method. Nanomedicine. 6:58-63.

18. Fazil, M., Md, S., Haque, S., Kumar, M., Baboota, *S*., Sahni J.K.,and Ali, J.. 2012. Development and evaluation of rivastigmine loaded chitosan nanoparticles for brain targeting. Eur. J. Pharm. Sci., 47:6-15.

19. Feng, C., Wang, Z., Jiang, C., Kong, M., Zhou, X., Li, Y., Cheng, X., and Chen X. 2013.Chitosan/o-carboxymethyl chitosan nanoparticles for efficient and safe oral anticancer drug delivery: *In vitro* and *in vivo* evaluation. In.t J. Pharma., 457:158–167.

20. Song, H., Su, C., Cui, W., Zhu, B., Liu, L., Chen, Z., and Zhao, L. 2013. Folic acid-chitosan conjugated nanoparticles for improving tumor-targeted drug delivery. Bio Med. Res. Int. Article ID, 723158

21. Hosseinzadeh, H., Atyabi, F., Dinarvand, R., and Ostad, S.N. 2012. Chitosan–pluronic nanoparticles as oral delivery of anticancer gemcitabine: preparation and *in vitro* study. *Int. J.* Nanomedicine. **7**: 1851-1863.

22. Anitha, A., Chennazhi, K.P., Nair, S.V., and Jayakumar, R. 2012. 5-Flourouracil loaded N, O-carboxymethyl chitosan nanoparticles as an anticancer nanomedicine for breast cancer. *J* Biomed Nanotechnol.,8: 29-42.

23. Saravanabhavan, S.S., Bose, R., Skylab, S., and Dharmalingam, S. 2013. Fabrication of chitosan/TPP nano--particles as a carrier towards the treatment of cancer. Int. J. Drug Deliv., 5: 35-42.

24. Naruphontjirakul, P., and Viravaidya-Pasuwat, K. 2011.International Conference on Biomedical EngineeringTechnology. IPCBEE.

25. Jadhav, S.S., and Bhalerao, A.V. 2013.Formulation and characterization of chitosan nanoparticles loaded with rizatriptan benzoate. Scholars Research Library Der. Pharmacia Lettre**.**5: 218-223.

26. Le, T.M.P., Pham, V.P., Dang, T.M.L., La, T.H., Le, T.H., and Le, Q. 2013. Preparation of curcumin-loaded pluronic F127/chitosan nanoparticles for cancer therapy. Adv. Nat. Sci. Nanosci. Nanotechnol.**,** 4: 025001.

27. Anbarasan, B., Vennya, V. M., Niranjana, V. A., and Ramaprabhu, S. 2013. Optimization of the formulation and *in-vitro* evaluation of chloroquine loaded chitosan nanoparticles using ionic gelation method. J. Chem. and Pharma. Sci. 6:106-112.

28. Nanda, R.K., Patil, S.S., and Navathar, D.A. 2012. Chitosan Nanoparticles Loaded with Thiocolchicoside. Der Pharma. Chemica., 4:1619-1625.

29. Henglun, L., Jian, T., Jing, L., Fuwei, Z., and Baoqin, R. 2011. Preparation and evaluation of docetaxel-loaded chitosan nanoparticles *in vitro*. Chongqing Med. J., 40: 3154-3156.

30. Jadhav, S.S., and Bhalerao, A.V. 2013. Formulation and characterization of chitosan nanoparticles loaded with rizatriptan benzoate. Der Pharmacia. Lettre. 5:218-223

31. Patel, P.N., Patel, L.J., and Patel, J.K. 2011**.**Development and testing of novel temoxifen citrate loaded chitosan nanoparticles using ionic gelation method. Pelagia Research Library Der Pharmacia Sinica.2:17-25.

32. Chenguang, L., Yulong,T., Chengsheng,L., Xiguang, C.,and LejunY. 2007. Preparations, characterizations and applications of chitosan-basednanoparticles. J. Ocean University of China. 6: 237-243

33. Kang, W., and Zhimin, H. 2002 Alginate-konjac glucomannan-chitosan beads as controlled release matrix. Int. J. Pharmceutics**.** 244:117-126.

34. Bhardwaj,V., Shukla,V. , Goyal,N. , Malviya, R., and Sharma PK. 2010. Formulation and Evaluation of Different Concentration Chitosan based Periodontal Film of Ofloxacin. *J.Pharmacy Res.*,3: 528-532.

35. Zhang, W.F., Chen, X.G., Li, P.W., He, Q.Z., and Zhou, H.Y. 2008**.** Preparation and characterization of theophylline loaded chitosan/beta-cyclodextrin microspheres. J. Mater. Sci. Mater. Med., 1:305-10.

36. Baldrick, P. 2010.The safety of chitosan as a pharmaceutical excipient. Regul. Toxicol. Pharmacol., 56:290–299.

37. Kean, T., and Thanou M. 2010. Biodegradation, biodistribution and toxicity of chitosan. Adv. Drug Deliv. Rev., 62: 3–11

38. Agnihotri, S.A., Mallikarjuna, N.N., and Aminabhavi, T.M. 2004. Recent advances on chitosan-based micro- and nanoparticles in drug delivery. J. Control Release. 100: 5–28.

39. Ohya, Y., Shiratani, M., Kobayashi, H., and Ouchi, T. 1994. Release behavior of 5-fluorouracil from Chitosan-gel Nanospheres Immobilizing5-fluorouracil coated with polysaccharides and their cell specific cytotoxicity. J. Macromol.Sci. Part *A*; 31:629–642.

40. Nishimura, K., Nishimura, S., Seo, H., Nishi, N., Tokura, S., and Azuma I. 1986.Macrophage activation with multiporous beads prepared from partially deacetylated chitin. J. Biomed Mater.Res., 20:1359– 1372.

41. Berthod, A., and Kreuter, J. 1996. Chitosan microspheres– improved acid stability and change in physicochemical properties bycross-linking. Proc In.t Symp.Control Release Bioact. Mate*r*; 23:69– 370.

42. Huang, H.Y., Shieh, Y.T., Shih, C.M., and Twu, Y.K. 2010.Magnetic chitosan/iron (II, III) oxide nanoparticles prepared by spray-drying. Carbohydr. Polym., 81: 906–910.

43.Tokumitsu, H., Ichikawa, H.,and Fukumori, Y. 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.

44. Calvo ,P., Remunan-Lopez, C., Vila-Jata, J.L., and Alonso, M.J. 1997. Chitosan and chitosan: ethylene oxide-propylene oxide block copolymer nanoparticles as novel carriers for protein and vaccines. *Pharm. Res.,* 14: 1431–143.

45. Mitra, S., Gaur, U., Ghosh, P.C., and Maitra, A.N. 2001. Tumor targeted delivery of encapsulated dextran–doxorubicin conjugate using chitosan nanoparticles as carrier. *J.* Control Release. 74:317– 323.

46. Leroux,J.C., Allemann,E., Jaeghere,F.D., Doelker,E ., and Gurny,R. 2010. Biodegradable nanoparticles —from sustained release formulations to improved Park JH., Saravanakumar G., Kim K., Kwon IC. Targeted delivery of low molecular drugs using chitosan and its derivatives. *J Adv. Drug Deliv.Rev.,* 62:28–41.

47. Mohanraj, V.J., and Chen, Y. 2006. Nanoparticles– A Review.Tropical J.Pharmaceutical Research. 5: 561-57.

48. Park, J.H., Saravanakumar, G., Kim, K.,and Kwon, I.C. 2010. Targeted delivery of low molecular drugs using chitosan and its derivatives. J. Adv. Drug Deliv. Rev., 62:28–41.

49. Xu, X.Y., Zhou, J.P., Li, L. 2008**.** Preparation of doxorubicin-loaded chitosan polymeric micelle and study on its tissue biodistribution in mice. Acta. Pharm. Sin.,43: 743–748.

50. Kiang, T., Bright, C., Cheung, C., Stayton, P., Hoffman, A., and Leong K. 2004.Formulation of Chitosan-DNA Nanoparticles with poly (propyl acrylic acid) Enhances Gene Expression. Biomater. Sci. Polym., 15:1405–1421.

51. Fan, L., Wu, H., Zhang, H., Li, F., Yang, T.H., Gu, C.H., and *Yang*,Q. 2008. Novel super pH-sensitive nanoparticles responsive to tumor extracellular pH. Carbohydr. Polym., 73: 390–400.

52. Chung, J., Yokoyama, M., Aoyagi, T., Sakurai, Y.,and Okano T. 1998. Effect of molecular architecture of hydrophobically modified poly (N-isopropylacrylamide) on the formation of thermoresponsive core-shell micellar drug carriers. J. Control Release. 53**:**119 -130.

53. Yuan, Q., Shah, J., Hein, S.,and Misra, R.D.K. 2010. Controlled and extended drug release behavior of chitosan-based nanoparticle carrier. *Acta. Biomater.*, 6: 1140–1148.

54. Yao, Q. 2006. Study on the two-ligand modified chitosan nanoparticles actively targeting to malignant liver cells. Doctoral Paper of Sichuan University**.** 6: 765–774.

55. Huang, Y., Lin, A.H., and Zhang, X. 2008. Targeting Binding of chitosan Nanoparticles with Glycyrrhizin Surface Modification to Hepatic Parenchymal Cells *Invitro*. Tradit. Chin. Drug *Res.* Pharmacol., 19**:**495–498.

56. Kim, K., Kwon, S., Park, J.H., Chung, H., Jeong, S.Y., Kwon, I.C., and Kim, I.S. 2005. Physicochemical Characterizations of Self-assembled Nanoparticles of Glycol Chitosan–Deoxycholic acid conjugates. Biomacromolecules. 6: 1154–1158.

57. Min, K., Park, K., Kim, Y.s., Bae, S.M., Lee, S., Jo, H.G., *Park* Jeong, S.Y., *Kim, K.*, Kwon, I.C. 2008. Hydrophobically modified glycol chitosan nanoparticles-encapsulated camptothecin enhance the drug stability and tumor targeting in cancer therapy. J. Control Release.127**:** 208–218.

58. Lin, Y.H., Mi, F.L., Chen, C.T., Chang, W.C., Peng, S.F., Liang, H.F., and Sung, H.W. 2007. Preparation and characterization of nanoparticles shelled with chitosan for oral insulin delivery. Biomacromolecules. *8*:146–152.

59. Kumar, N., Patel, A.K., Kumari, N.,and Kumar, A. 2014. A review on chitosan nanoparticles for cancer treatment. Int. J. Nanomaterials and Biostructures. 63-65.

60. Sharma, H., Sahu, G., and Sahu, S. 2014. Recent approaches of drug delivery for skin cancer. World J. pharmacy and pharmaceutical sciences 3: 300-315.

61. Kumar, N., Patel, A,K., Kumari, N., and Kumar A. 2014. A review on chitosan nanoparticles for cancer treatment. Int. J. Nanomaterials and Biostructures. 4:63-65.

62. Wang, X., Chi, N.,and Tang X. 2008. Preparation of estradiol chitosan nanoparticles forimproving nasal absorption and brain targeting. Eur. J. Pharm. Biopharm**.,** 70, 735-740

63. Kaur ,S.P., Rao, R., Hussain, A.,and Khatkar, S. 2011. Preparation and Characterization of Rivastigmine Loaded Chitosan Nanoparticles. J. Pharm. Sci. Res., 3: 1227-1232.

64. He, P., Davis, S.S., and Illum, L. 1998 In vitro evaluation of the mucoadhesive properties of chitosan microspheres. Int .J. Pharm.,166: 75-78.

65. Varshosaz, J., Jaffari, F., and Karimzadeh, S. 2006. Development of bioadhesive chitosan gels for topicaldelivery of lidocaine. Scientia Pharmaceutica Sci. Pharm., 74: 209-223

66. Gaudana, R., Ananthula, H., Parenky, A., and Mitra, A. 2010. Ocular drug delivery. AAP*S***.** *12*:348–360.

67. De Campos, A.M., Sánchez, A., and Alonso, M.J. 2001.Chitosan nanoparticles: a new vehicle for the improvement of the delivery of drugs to the ocular surface. Application to cyclosporin A. Int. J. Pharm., 224:159-168.

68. Wang, X., Chi, N.,and Tang ,X. 2008.Preparation of estradiol chitosan nanoparticles forimproving nasal absorption and brain targeting. Eur. J. Pharm. Biopharm.70:735-740.