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CHITOSAN-BASED PARTICULATE SYSTEM FOR ORAL VACCINE DELIVERY: A REVIEW

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ABSTRACT

Mucosal vaccination is a promising alternative to classical parental vaccination, as it is non-invasive and, in principle, capable of eliciting strong systemic and local immune responses. Chitosan has already gained considerable attentions as vehicles for protein drug delivery due to its excellent biocompatibility, biodegradability and non-toxicity. However, its poor aqueous solubility and loss of penetration-enhancing above pH 6 are major drawbacks for its use as oral vaccine carrier. In recent years, the area of focus has shifted from chitosan to chitosan derivatized polymers for the preparation of nanoparticles due to their vastly improved properties, such as acid resistivity, better drug retention capability, improved permeation, enhanced mucoadhesion and sustained release of therapeutic antigens. Additionally, interaction with counter ions such as sulfates or polyphosphates facilitates its usefulness in the coating or entrapment of antigenic molecules as a vaccine candidate. The current review focuses on the recent advancements in the field of oral vaccine delivery via chitosan-based particulates, describes the various methods used to design and synthesis and discusses about their *in vitro* and *in vivo* implications.

Keywords: Adjuvant, intestinal epithelial cells, M-cell targeting, mucosal immunity, vaccination

INTRODUCTION

Vaccination is regarded as the most effective therapeutics for protection against debilitating infectious diseases and has contributed significantly to an increase in life expectancy, especially in children, in many parts of the world.[1-4] Most of the vaccines are almost exclusively administered by parenteral injections or infusions.[5, 6] Compared to traditional routes of administration, oral vaccine delivery offers several attractive advantages such as lower costs, ease of administration, higher patient compliance, reducing the need for trained personnel and averting vaccine-related infections correlated to the disposal and reuse of needles in systemic delivery as well as higher capacity of much immunizations.[7-10] Importantly, orally administered antigens can induce both local and systemic immune responses, providing a complete immune response.[11] A local immune response can be induced by efficient delivery of an antigen to the gut-associated lymphoid tissue (GALT) as well as antigen-presenting cell (APC) lining between intestinal epithelial cells.

Many efforts have been made to develop an ideal oral antigen delivery vehicle that can satisfy the requirement of stability, targetibility, and antigenicity.[12, 13] There are two major hurdles in the development of successful vaccine delivery.[14, 15] First, protection of the antigen of interest from low gastric pH and digestive enzymes, and second, delivery of antigens to the professional antigen presenting cells. To overcome these barriers, antigens should be formulated with proper excipients that maintains the antigen in a stable form, protects from enzymatic degradation and harsh condition in the stomach and intestine, adhere to mucosal surface, ensure the antigen remains in the gastro intestinal tract region long enough for the antigen to interact

with the lymphatic system and efficiently stimulates innate responses and evoke adaptive immune responses that are appropriate for the target pathogen.[16-20] Because the different parts of gastrointestinal tract have their own specific barriers in terms of accessibility, epithelial cell types and gastric environment, the properties of delivery systems for therapeutic antigens have to be tailored according to the route of administration.[21]

Most of the advance particulate drug carriers have been developed by utilising either synthetic or natural polymers or by their combination with their specialized properties.[22] In recent years, soluble and particulate carriers based on CS and its derivatives have received particular interest for the oral delivery of protein/DNA.[23-27] CS-based polymers are less toxic, mucoadhesive, capable of opening the tight junctions between epithelial cells and are able to control the release of therapeutic agents.[28-34] CS has free amino and hydroxyl functional groups which allow chemical modification and enhance cross-linking capability to make it an ideal candidate for fabricating oral particulate drug delivery system.

The objective of this review is to give an overview of the recent advancements in the field of CS-based particulate systems which have been utilized for oral vaccine delivery and its *in vivo* and *in vitro* implications. This review also describes the various methods used to design and synthesize CS-based nanoparticle/microparticle formulation uses as oral vaccine carrier.

CS-BASED DELIVERY SYSTEMS

The efficacy of oral vaccine is currently limited by the poor immunogenic properties of the vaccine antigens and a very inefficient delivery of these antigens to the intestinal surfaces, which can be mainly attributed to gastrointestinal degradation and poor uptake by intestinal epithelial cells and antigen presenting cells (APC). A wide variety of CS derivatives have been synthesized for wide spectrum of solubility at different pH, better mucoadhesiveness, enhanced protection against degradation and/or its immunostimulatory properties to develop effective oral vaccine delivery. CS derivatives can interact with mucus and epithelial cells, induce a redistribution of cytoskeletal F-actin and the tight junction protein ZO-1 resulting in opening of cellular tight junctions, and thus, enhancing the paracellular permeability of the epithelium.[35-36] The pH-dependent CS solubility is important from the perspective of its application for oral vaccine delivery. In practice, this means that CS administered orally as an aqueous solution is likely to precipitate upon reaching the intestinal region due to the increase in pH (around 6.5-7.5). Alternatively CS administered orally as a powder is believed to dissolve in the acid gastric pH and then precipitate at the intestinal pH. The insolubility of CS at physiological pH could be thought to interfere with its application in drug delivery. In many studies, it has been demonstrated that CS-based formulations were superior in enhancing absorption of therapeutic proteins as well as induction of antibodies after mucosal vaccination.[37-39]

Unmodified chitosan: Due to its natural origin, CS cannot be defined as unique chemical structures but as a family of polymers that present a high variability in their chemical and physical properties. This often variability is related not only to the origin of the samples but also to their method of preparation. Hence, the polymeric chain is generally described as a copolymeric structure comprising D-glucosamine with variable amounts of N-acetyl residues. Indeed, CS defines a family of linear polysaccharides consisting of varying levels of β-(1, 4)-linked residues of 2-amino-2-deoxy-D-glucose and N-acetyl-2-amino-2-deoxy-D-glucose, forming a long chain linear polymer (Fig. 1).[40-42]

From a biopharmaceutical standpoint, CS has the special feature of adhering to mucosal surfaces, favouring the interaction of the drug with the mucous layer covering different epithelial surfaces.[43-44] This fact makes CS very useful for oral drug delivery. Indeed, due to the protonated amine groups, CS is able to interact with the negatively charged mucus components, results in a reversible structural reorganization of protein associated tight junctions which is followed by their opening.[45-46] Mucus affects free drug permeability and particle uptake by forming both a physical barrier to diffusion and favouring electrostatic interaction with cationic molecules.[47] Incubation of Caco-2 cells with 50 µg/ml solutions of CS having various molecular weights and degrees of deacetylation (31 kDa, 99% DA and 170 kDa, 65%DA) increased permeation of the drug across cells.[48]

Nanoparticle can be easily obtained from CS and are very efficient as well as nontoxic absorption enhancer for oral administration of vaccines, proteins and peptides.[49, 50] The ovalbumin was incorporated into CS microparticles and the uptake of ovalbumin associated with CS microparticles in murine Peyer's patches was demonstrated using confocal laser scanning microscopy.[51] In another study, the ability of CS microparticles was investigated the ability of CS microparticles to enhance both systemic and local immune responses against diphtheria toxoid (DT) vaccine after the oral administration in mice.

Systemic and local IgG and IgA immune responses against DT associated to CS microparticles were strongly enhanced after the oral delivery in mice.[52] Oral administration to mice of CS nanoparticles complexed with DNA coding for a peanut allergen Arah2 elicited elevated secretary IgA and serum IgG2a titres, as well as a reduced increase in IgE.[53] This immune response was not observed for mice given naked plasmid DNA. Delivery of the CS-DNA nanoparticles also mitigated the anaphylactic response peanut challenge, possibly through redirection of the immune response away from an allergic, IgE-based response to a more T_H1-dominated response. CS-DNA nanparticles have also been successfully used to generate an immune response against native dust mite allergen Der p 1.[54] Oral feeding of DNA-loaded CS nanoparticles containing 50 µg Der p 1 DNA to mice was followed by an intramuscular boost with 50 µg Der p 1 DNA in saline and electroporation. While intramuscular injection alone was unable to generate immune response to Der p 1, oral priming led to detectable levels of IgG2a and low level of IgA. Nanoparticles might also facilitate mucoadhesion and DNA uptake by the host cells, leading to enhanced transfection efficiency. Oral delivery of CS DNA nanoparticles was also evaluated as an oral vaccine strategy against intracellular parasite *Toxoplasma gondii*.[55] In this study, both CS nanoparticles loaded with parasite protein GRA_1 encoding DNA plasmid (pDNA) and CS microparticles loaded with recombinant GRA_1 protein were compared for their ability to elicit GRA-1-specific immune responses after intragastric administration using different prime boost regimen. Boosting with GRA_1 DNA vaccine resulted in high anti- GRA_1 antibody levels, characterized by a mixed IgG2a/IgG1 ratio.[55]

Though all these studies with unmodified CS used almost similar preparatory conditions and formulations parameters, the results indicate that the encapsulation efficiency and release properties of the particles were more depended on the nature of the drug molecule itself rather than the inherent property of the CS- system. Thus, to effectively control the release of its content, it was required to develop some advance CS particulate systems which could precisely anticipate the drug release on oral administration, yet enhance the bioavailability of the drug. Hence at different stages, CS derivatives were utilised or CS was combined with other compounds to prepare the particulate oral vaccine delivery for mucosal immunization.

MODIFIED CHITOSAN

Various CS derivatives have been synthesized and studied for oral vaccine delivery formulation.

Quaternized derivatives of CS: Quaternary derivatives of CS (Figure 2), obtained by introducing various alkyl groups of CS molecule structure, were extensively studied for oral protein and peptide delivery.[56] These derivatives are drastically more soluble in neutral and alkaline environments and have mucoadhesive and penetration-enhancing properties over a wide pH range. The first quaternized CS was synthesized by alkylation of the primary amine groups of CS with various aldehydes using $NaBH_4$ as reducing agent and were evaluated as antibacterial and antifungal materials.[57]

A quaternary derivative of CS, N-(2-hydroxyl) propyl-3-trimethyl ammonium chitosan chloride (HTCC), was synthesized by reaction of CS with glycidyl trimethyl ammonium.[58] This CS 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 release while protein loading remained high.[59] In another study, quaternary CS derivative was synthesized by reaction of N-chloroacyl-6-O-triphenylmethyl with CS.[58]

Recently, CS nanoparticles were prepared from newly synthesised di ethyl methyl CS (CS DEM) by ionotropic gelation method or by polyelectrolyte complexation method.[60] The nanoparticle was prepared from newly synthesised tri-ethyl CS (CS TE) and di methyl ethyl CS (CS DME).[61, 62] In both these studies, drug encapsulation efficiency was reported to high due to electrostatic interactions between the negatively charged acidic groups of proteins with the positively charged amino groups of CS derivatives.[63] These quaternary derivatives of CS (CS TM, CS DEM, CS TE, CS DME) as free soluble polymers can improve the paracellular transport of protein across the Caco-2 cell monolayer much more than the corresponding nanoparticle.[64] This was because the positive charge available on the surface of the NPs decreased, and hence, the particles were unable to open the tight junctions of the Caco-2 cell monolayer.

***N,N,N*-tri methyl CS:** *N,N,N*-tri methyl CS (CS TM) (Figure 3) derived through amine functionalization of CS was found to have enhanced solubility, strength, porosity, absorption efficiency, chemical resistant, and non-antigenic properties. CS TM was synthesized based on the alkylation of primary amines of CS in strong alkaline condition with iodomethane using N-methyl 2-pyrrolidone (NMP) as solvent.[65] The reaction condition leads to dimethylated polymer with 10-15% of quaternization and polymer chain scission due to relative vigorous reaction conditions. The process was modified with respect to the solvent/reagent addition sequence leads to partial and uncontrolled methylation of the C-3 and C-6 hydroxyl group of CS.[66] The reaction was carried out in multiple steps using various solvent systems like NaOH and dimethylaminopyridine along with NMP as bases.[67-69] A combination of two bases and increasing number of reaction steps limited to 12.5-34.4% DQ accompanied by O-methylation. An alternative sequence for the synthesis of CS TM was reported using dimethyl sulphate as the reactive agent wherein CS in solution of NaOH and NaCl is mixed and refluxed with methylated agent at room temperature or at 70 ^{o}C.[70] The undesirable O-methylation and chain scission were also observed to take place for the reaction. In another study, CS TM was synthesized using iodemethane and DMF/H_2O mixture (1:1) as solvent system without the aid of a catalyst sodium iodide.[71] The reaction significantly reduced O-methylation and DQ varied from 0-74% depending on the reaction conditions. Quaternized N-alkyl CS derivatives containing alkyl substituent of different chain length was also synthesized in two steps.[72] In the first step, CS reacted with aldehyde and the resulting Schiff bases were reduced with $NaBH_4$. In the last step, N-alkyl CS derivatives were quaternized with iodomethane in presence of sodium hydroxide and NMP. Several adjustments to the method were presented introducing various reaction conditions and altering solvent system.[73-75] Recently CS-TM was synthesized introducing formic acid-formaldehyde instead of $NaBH_4$ as the reducing agent to synthesize *N,N*-dimethylated chitosan (CS DM).[76] Quaternization of CS DM was performed using iodomethane in NMP without the assistance of a catalyst for the last step.

The water-solubility of CS TM can be tailored by varying the degree of methylation.[76] Soluble CS TM has both mucoadhesive properties and excellent absorption-enhancing effects even at neutral pH.[78-80] The permeation of hydrophilic macromolecules across the mucosal epithelia by opening the tight junctions can be modified depending on DQ of CS TM.[68, 81-84] CS TM with a DQ above 36% increased the absorption of hydrophilic model compounds such as mannitol and poly (ethylene glycol) 4000 across intestinal epithelia and nasal mucosa at physiological pH.[85] In several studies, it has been shown that CS TM with a DQ of 40–60% showed the best absorption-enhancing properties for small proteins and peptides and a further increase in DQ of CS TM did not considerably improve its absorption properties but increased its toxicity. The O-methyl free CS TM had stronger membrane permeability activity as demonstrated by a larger decrease of the TEER of Caco-2 cells as compared to O-methylated CS TM synthesized by the conventional method.[76] Nanoparticles were formulated of three different Mw of CS-TM intended for vaccine delivery and reported cytotoxicity is independent of change of Mw.[86] The concomitant use of limiting doses of the fully nontoxic LTK63 mutant as a mucosal adjuvant and CS-TM as a delivery system allowed the reduction of each of the component for the induction of antibody and responses similar to or higher than those induced by parenteral administration.[87]

Thiolated CS: Thiolated polymers have gained considerable interest, especially for vaccine delivery, because they are one of the most promising polymers with multifunctional properties including strong mucoadhesivity, enhanced permeation effects, protection ability, stability, and enhanced bioavailability of drugs (Figure 4).[88-92] Among various thiomer-based carriers, thiolated CS are highly popular because of their strong mucoadhesiveness and ability to control and extend drug release profiles with improved permeation ability.[29, 93] The primary amino groups of CS were coupled with the thiol-bearing functional groups to form thiolated CS demonstrated 6–100-fold augmented mucoadhesive property and enhanced paracellular permeation when compared to unmodified CS. Thiolated CS nanoparticles made from thioglycolic acid and CS were first prepared to deliver DNA to the cells via the oral route.[94-97] This nanoparticle showed significant improvement in transfection rate when compared to DNA encapsulated with unmodified CS. Though the results were promising for accomplishing successful non-viral gene delivery, further investigation is required before clinical application. Since the strong mucoadhesive property of thiolated CS is primarily due to disulphide bond formation between the thiolated polymer and cysteine-rich mucus layer, the addition of polyanionic excipients during ionotropic gelation of nanoparticles could actually lead to a reduced mucoadhesive and permeation-enhancing properties of CS due to loss of its inherent positive charge.

Several approaches were studied to elaborate the application of thiolated CS by preserving its mucoadhesive property and permeation-enhancing ability. One of them was the emulsion polymerization method to prepare thiolated CS nanoparticles coated with poly-isobutylcyanoacrylate.[98-101] Their results indicated improved penetration through the mucus layer due to enhanced bioadhesion when CS of high Mw was utilised for the preparation of nanoparticles. This was because more thiol groups were present to form more covalent bonds with the cysteine-rich residues of the mucus glycoproteins.[102] In another approach, a thiolated CS nanoparticle was developed by emulsion polymerization method utilising different molecular weights of CS with polyhydroxyl ethyl methacrylate (pHEMA) as coating material. This formulation also revealed the importance of formulation parameters on the permeability and mucoadhesive properties of the nanoparticles.[32]

TECHNIQUES FOR THE PREPARATION OF CS-BASED MICRO / NANOPARTICLE FORMULATIONS

CS-based particles loaded with antigens/DNA for oral vaccine delivery can be prepared by both chemical and physical methods. Selection of the method depends upon physicochemical factors and 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.

Ionotropic gelation: The use of complexation between oppositely charged macromolecules to prepare CS microspheres has attracted much attention because the process is very simple and mild.[103, 104] In this process, an aqueous solution of counterion is added dropwise to an aqueous acidic solution of CS at ambient temperature under stirring. CS nanoparticles are formed due to complexation of the oppositely charged components.[105] Depending on the conditions, particles with different properties can be obtained including differences in size, zeta potential, and stability at different pH values or loading characteristics. The molecular interactions of the ionic cross-linking of CS with TPP have been investigated and have been reported regarding the linking types and corresponding interaction energies.[106]

CS nanoparticles loaded with tetanus toxoid have been prepared using this method and investigated as vaccine delivery vehicles.[107] In this study, tetanus toxoid (TT)-loaded CS nanoparticles, with an average size about 350 nm and a positive surface charge, showed a high loading efficiency (around 50–60%). *In vitro* release studies showed an initial burst followed by a sustained release of antigenically active toxoid for 16 days. CS microparticle was encapsulated with an atrophic rhinitis vaccine by ionotropic gelation, and after administration, enhanced cytokine and nitric oxide were produced.[108]

Emulsification and ionotropic gelation: An aqueous solution of CS is added to a nonaqueous continuous phase (isooctane and emulsifier) to form a water-in-oil emulsion. Sodium hydroxide solution is then added at different intervals, leading to ionotropic gelation.[109] CS 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 ethanediamine using a compressed air nozzle to form coacervate droplets. Gelatin is also used along with CS forming CS/gelatine emulsion under coagulation conditions at a low temperature.[110] Sodium sulphate or sodium citrate can be used for surface medication leads to smooth surface of nanoaprticles. Increase of stirring speed produces decrease in diameter and a narrower size distribution. The emulsion and ionotropic gelation method was adopted for microencapsulation of diphtheria toxoid (DT).[111] This study showed that the loading efficiency of CS microspheres depends on the Mw and the type of cross-linker used. Microspheres prepared by high MW CS and glutaraldehyde (cross-linking agent) had the highest DT loading level. Size distribution studies showed that the particle size of microspheres prepared by low and medium Mw CS solutions with a concentration of 1 % w/v was below 10 mm.

Complex coacervation: Sodium alginate, sodium carboxymethyl cellulose, κ-carrageenan, and sodium polyacrylic acid can be used for complex coacervation with CS derivatives to form microspheres/nanoparticles after the inter-ionic interaction between oppositely charged polymers.[112] Potassium chloride and calcium chloride were used to formulate the coacervate capsules of CS–alginate and CS–κ-carrageenan, respectively, and the obtained capsules were hardened in the counterion solution before washing and drying. Plasmid-DNA loaded CS nanoparticles for expression of Interleukin-12 were prepared using complex coacervation process at different N/P ratios. Strong attachment of the DNA to CS was observed after polyplex formation. The transfection efficiency of the prepared complexes were higher than these of naked DNA when N/P ratio was inbetween 16 and 60.[113]

Emulsion crosslinking method: This method utilizes the reactive functional amine group of CS to cross-link with aldehyde groups of the cross-linking agent. Water insoluble reagents can be simply dispersed in CS solution and entrapped by the emulsion crosslinking process. Glutaraldehyde, formaldehyde, and genipin have been widely used as crosslinking agents for the preparation of CS nanoparticle/microparticle.[114, 115] Size of the particles can be controlled by controlling the size of aqueous droplets, extent of cross-linking agent, speed of stirring during the formation of emulsion. The emulsion cross-linking method has few drawbacks since it involves tedious procedures as well as use of harsh cross-linking agents, which might possibly induce chemical reactions with the DNA/protein antigens and complete removal of the un-reacted cross-linking agent may be difficult in this process.

Coacervation/precipitation: Since CS is insoluble in alkaline pH medium, 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 ethanediamine using a compressed air nozzle to form coacervate droplets.[116] Varying compressed air pressure or spray-nozzle diameter controlled the size of the particles and then using a cross-linking agent to harden particles can control the drug release. CS microparticles loaded with recombinant human interleukin-2 (rIL-2) have been prepared by dropping of rIL-2 with sodium sulfate solution in acidic CS solution.[117] The rIL-2 was released from microspheres in a sustained manner for up to 3 months. Efficacy of the systems developed was studied by using two model cells viz., HeLa and L-strain cell lines. CS DNA nanoparticles have been prepared using the complex coacervation technique.[118] Important parameters such as concentrations of DNA, CS, sodium sulfate, temperature, pH of the buffer and molecular weights of CS and DNA have been investigated.

CS-BASED PARTICULATE SYSTEM: BIODISTRIBUTION AND TOXICITY

Systemic absorption and distribution of CS nanoparticles through oral delivery largely depend on the Mw of CS and its modification. It is very likely that oligomers (3.8 kDa) could show better absorption and higher plasma concentration than that of high Mw (230 kDa).[119] GFP Expression study revealed CS TM oligomers/DNA nanoparticles were taken up in the gastric and duodenal mucosa and to some extent in the jejunum mucosa, ileal mucosa and large intestinal mucosal cells.[120] The biodegradation of CS occurring predominantly in gut was found to be species dependent. In the same study, the digestion of N-stearoyl CS was negligible, indicating that the enzymatic degradation is dependent on NH_2 availability of CS[121] Generally, CS is relatively non-toxic, biocompatible material and approved by Food and Drug Administration (USA) for wound dressing.[122] Although, CS alone is considered to be safe for oral administration, modifications made to CS could make it more or less toxic. Moreover, the pharmacokinetic properties of a drug or excipient change considerably when included in a nanoparticulate system.[123] Thus, each and every derivative should be assessed individually both in the free form and nanoparticulate form. Systematic study on biodistribution, *in vivo* and *in vitro* toxicity using various CS (Mw and DD) and derivatives would provide data that could help correlate CS structure and safety profile.[124-129]

CONCLUSION AND FUTURE PERSPECTIVES

CS holds enormous promise as an idle oral vaccine delivery vehicle for among various investigated vaccine carriers. This review has discussed and evaluated various methods for preparation of CS derivatives and CS-based particulate system which could help to design more and better functionalized oral vaccine carrier systems. This study demonstrated that vaccine-loaded CS micro/nano particulate system could be prepared with suitable and appropriate particle sizes, which is a very important factor in the delivery of the vaccine to the induction site of mucosa-associated lymphoid tissue for proper immune stimulation. Additionally, both systemic and local immune responses can be induced in a dose- and time-dependent manner through vaccine-loaded particulate systems. The many advantages of CS, including safety, biodegradability, ease of modification, ease of DNA or protein complex formation, widespread availability, and low cost justify the continuing development of this promising drug and gene delivery system. Furthermore, modified CS like quaternized CS, tri-methylated CS, thiolated CS, and carboxy-methylated CS etc with various degree of substitution improve various properties such as increased mucoadhesivity, membrane permeability, stability, and controlled/extended release of the encapsulated vaccine and makes its strengths as a promising candidate for a potent vaccine carrier system. Considering these factors, carefully designed and better functionalized CS-based particulate system could be prepared for fruitful future application. More research needs to be conducted on these topics for the rational design of the next generation of oral CS drug and gene delivery systems.

However, there are many challenges including low physical and mechanical stability, irregular particle size and distribution, and low target specificity that have hindered the efficacy, practical use, and commercialization of CS particulate systems. It will be important in the future for additional studies to better define the key or unique immune mechanisms invoked by encapsulated vaccines, mechanistic insight and information such as barriers in the macroscopic transport of these antigen bearing particles across the mucosal surface, biodistribution in different tissues, types of cells transfected, transgene expression kinetics, and extra- and intracellular release of the antigen and DNA from the particles. The human immune system has evolved sophisticated mechanisms for recognizing and responding to pathogens, making it logical for researchers to focus on incorporating biomimetic features into vaccine formulations. In conclusion, addition of biomimetic features to CS-base particulate vaccines presents excellent opportunities in the development of new oral vaccines as well as improvement in the effectiveness of existing ones.

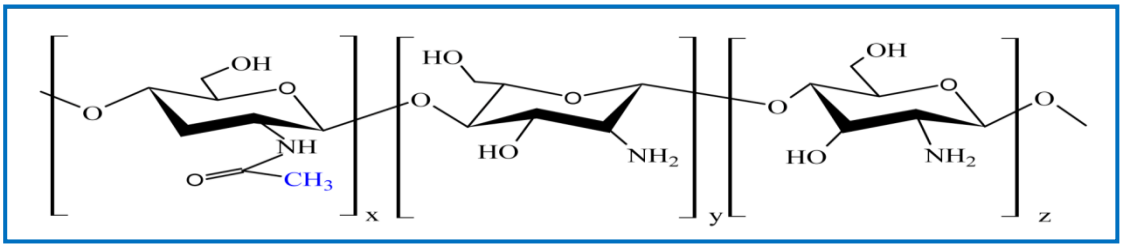

Figure 1 Chemical structure of chitosan. The degree of de-acetylation (x) is variable

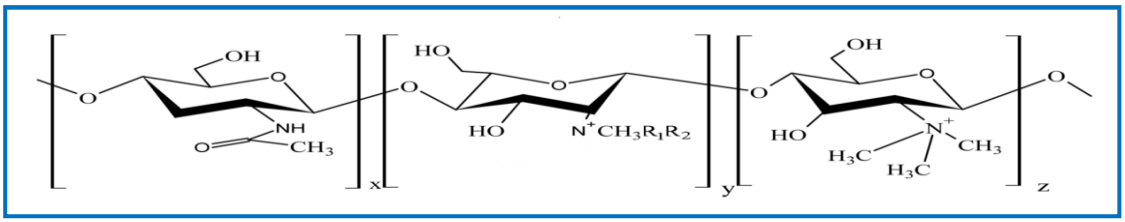

Figure 2 Quaternized derivatives of CS, alkylation of chitosan followed by quaternization (R_1=H, R_2=CH_3)

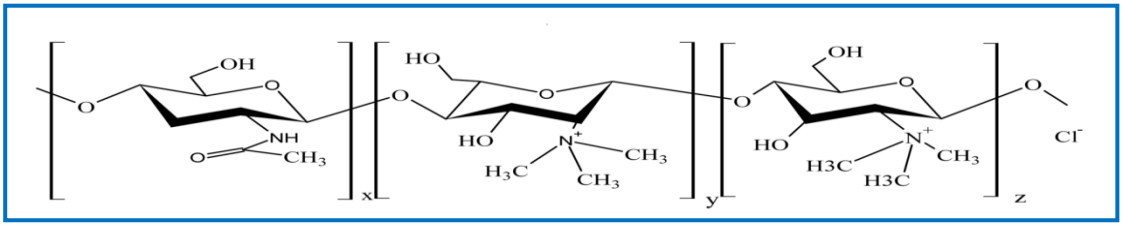

Figure 3 The chemical structure O-methylated free CS TM. CS TM can vary in extent of di- and tri-methylated groups and residual acetyl groups.

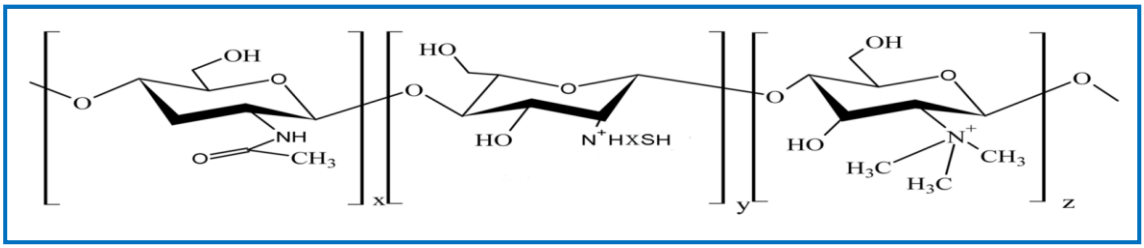

Figure 4 General structure of thiolated CS as modified by an –SH, X: linker

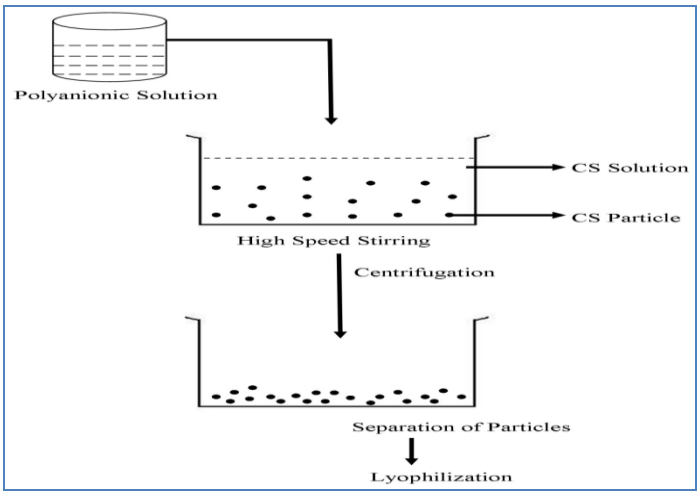

Figure 5 Schematic representations of chitosan particulate formulation by ionotropic gelation method

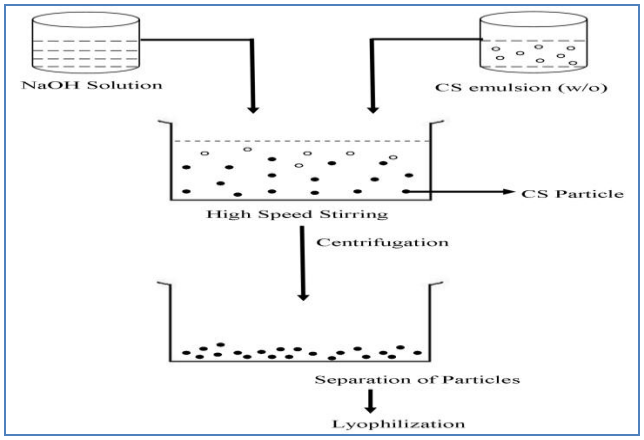

Figure 6 Schematic representations of chitosan particulate formulation by emulsification and ionotrpic gelation method.

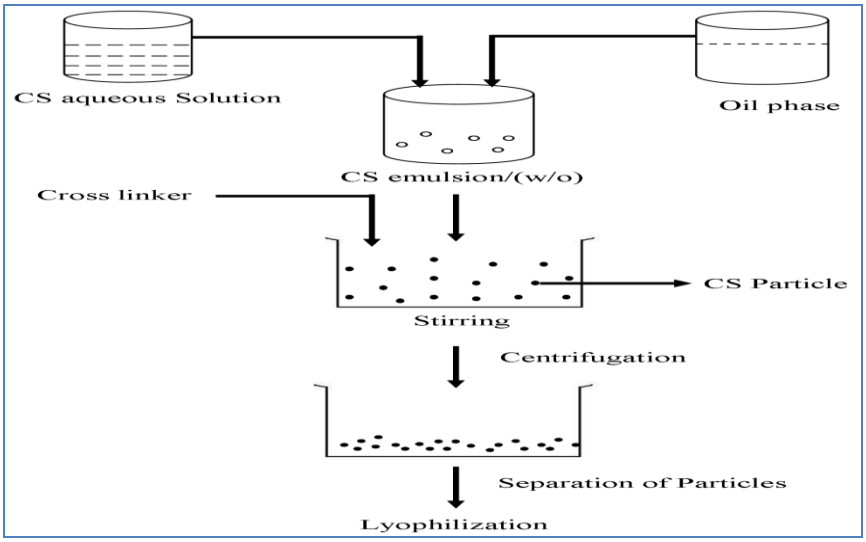

Figure 7 Schematic representations of chitosan particulate formulation by emulsion cross-linking method.

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