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REVIEW ARTICLE

Novel Techniques of Preparations of Chitosan Based Nanoparticles and it's Applications: A Critical Review

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ABSTARCT
Nanotechnology has been used as one of the methods in the development of novel drug delivery system through the incorporation of drugs into the nanoparticulate system. Chitosan (CHT) is a polysaccharide similar in structure like cellulose. CHT and cellulose are prepared by linear h-(1Y4)-linked monosaccharides. CHT's primary amine groups have unique characteristics that make it ideal for medicinal purposes. CHT possesses a positive charge when compared to other natural polymers. Applications of CHT NPs in various therapies like cancer, vaccine delivery, vaginal delivery, mucosal drug delivery, nasal drug delivery and buccal drug delivery is well recognized. The present review focus on the importance of chitosan in different treatment approaches via nano drug delivery.
Keywords*: Novel drug delivery system, Chitosan (CHT), Nanoparticles, Applications of CHT NPs.*

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INTRODUCTION

Drug delivery is a conventional terminology that refers to the formulation as well as administration of a pharmacologically active compound to provide a sufficient drug plasma concentration and bring the drug to the specific site of action. To overcome some drug stability problems in the gastrointestinal tract (GIT), manage drug release, improve transmucosal absorption, and get the drug into its precise site of action, numerous techniques have been used. Microfabrication is a technique for fabricating materials on a micrometer scale that has been shown to improve diagnosis and biomedical applications. The alteration of material shape, surface character, and release kinetics are the consequence of this micronization [1]. Nanotechnology has been used as one of the methods in the development of novel drug delivery system (NDDS) through the incorporation of drugs into the nanoparticulate system [2]. The nanoparticles (NPs) possess the unique physical as well as chemical properties because of their high surface area and nano scale size [9]. The polymeric NPs are synthesized by natural as well as synthetic polymers. Polymeric NPs have some advantages such as being available from marine source like chitin and from agricultural source such as cellulose, starch, and pectin [3]. CHT is a polysaccharide similar in structure like cellulose. CHT and cellulose are prepared by linear h-(1Y4)-linked monosaccharides [4]. CHT's primary amine groups have unique characteristics that make it ideal for medicinal purposes. CHT possesses a positive charge when compared to other natural polymers [5]. CHT is the most important chitin derivative, and it is made by removing the acetate moiety from the chitin. CHT works as a penetration enhancer by opening epithelial tight junctions [6]. The present review aims to discuss the preparation techniques and essential applications of CHT based polymers importance of chitosan in different treatment approaches.

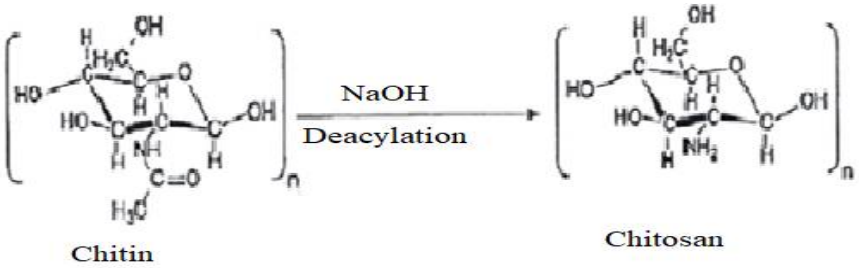

Fig 1: Deacylation of CHT [7]

METHODS OF PREPARATION OF CHT NPs

CHT is widely used as a perfect carrier for numerous drug delivery systems. CHT NPs can prepared by numerous ways.

- Spray drying
- Emulsion cross linking
- Reverse Micellization
- Nanoprecipitation
- Emulsion droplets coalescence
- Ionic gelation
- Desolvation

Spray drying

Spray drying is the most common method of formulation of CHT NPs. The spray drying technique is considered completely successful when atomized droplets get dried in the hot air creek. CHT solutions can be made by combining CHT powder with glacial acetic acid (GAA) solution, letting it dry naturally, and then atomizing the mixture to create tiny droplets. Once the liquid has dissipated from a tiny droplet, CHT NPs can instantly form [8-13].

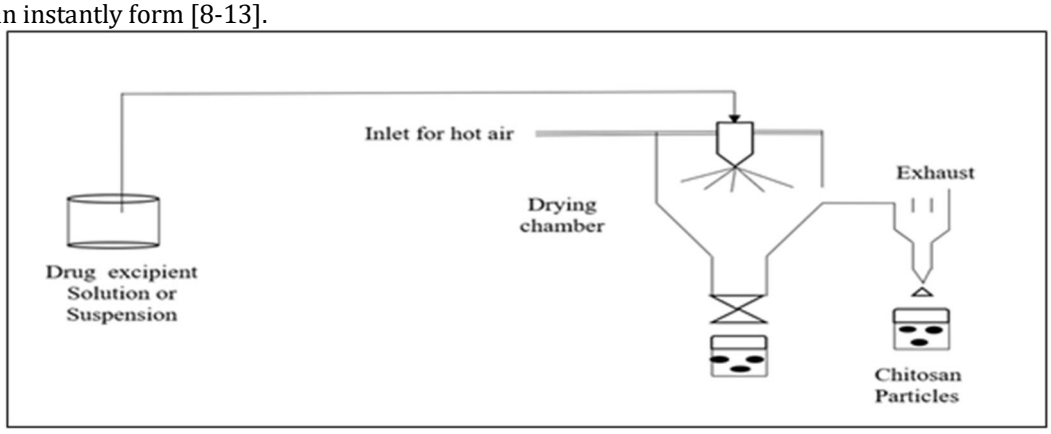

Fig 2: Schematics Illustration of Spray Drying Method [14]

Emulsion cross-linking

To create a water-in-oil emulsion, a hydrophilic CHT solution will be first mixed in the oil droplets. Secondly, hydrophilic drops are stabilised utilizing an appropriate surfactant (TWEEN 80). One of most adaptable cross-linking ingredients, such as fumaric acid and polyethylene glycol, is used after creating the homogeneous mixture. [15] Here, CHT amino sites and glutaraldehyde's carbonyl groups interact to form a cross-linking process that precipitated particles [8-17].

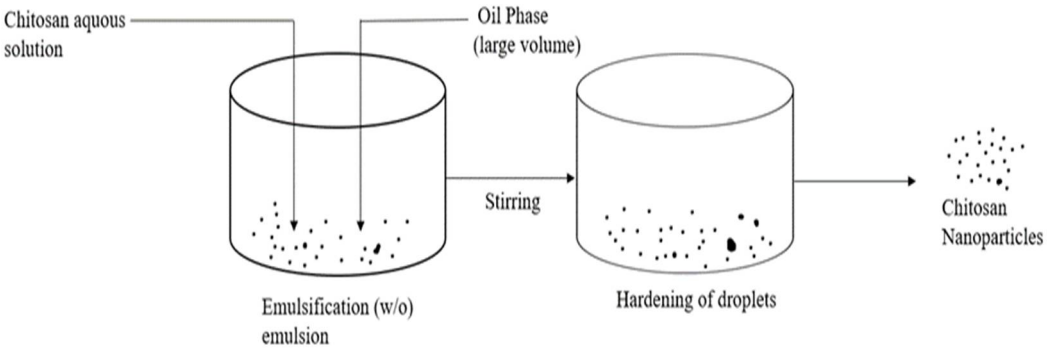

Fig 3: Schematics Illustration of Emulsion Cross linking Method [18]

Technique of reverse micellization

Reverse microspheres are the name given to water-in-oil spheres that have undergone the reversing micellar process [19, 20]. In order to create a water in oil phase emulsified mixture, a lipid soluble surfactant (like cetyl trimethyl ammonium bromide) must first be dissolved in an appropriate extraction liquid (such as n-hexane). Following that, to prevent turbulence, an aqueous CHT solution, medication, and glutaraldehyde are added to the mixture phase. NPs extraction is completed in the end [21, 23].

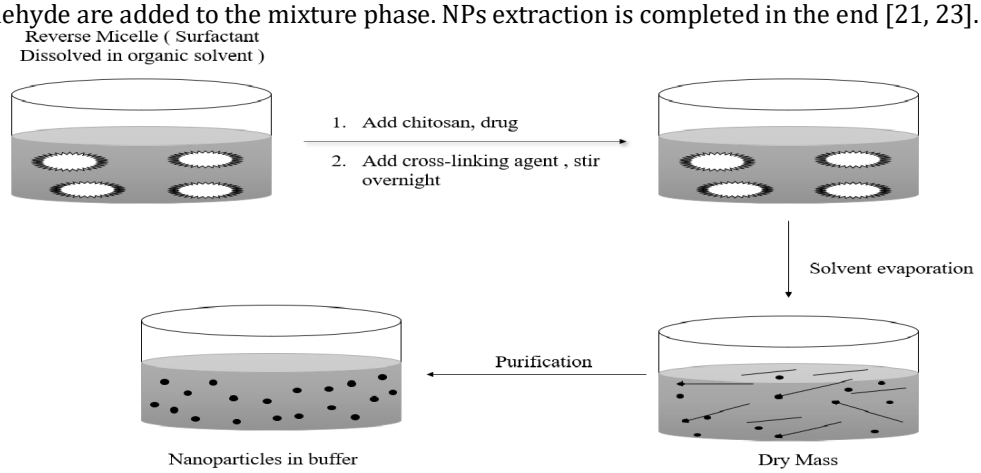

Fig 4: Schematic Illustration of Reverse micellization Technique [24].

Nanoprecipitation

The diffused nanoprecipitation is achieved by dissolving CHT in relevant solvent which is known as diffusion phase. Followed by magnetic stirring, methanol is added to the dispersion phase. The diffusing phase is then incorporated into dispersion phase with the help of peristaltic pump having a needle positioned 2 cm overhead the superficial edge at 0.86 mL min^{-1}. Then, irrespective of the non-solvent to solvent volume proportion, a very insignificant quantity of Tween -80 is, incorporated to the non-solvent phase to yield smaller NPs [8, 25].

Emulsion droplet coalescence

Emulsion coalescence approach is utilised to produce two stable emulsion droplets. The liquid paraffin oil holding medication is combined with a CHT aqueous solution in which the first stable emulsion is incorporated. Later on, the liquid paraffin oil is combined with a second stable emulsion incorporated with CHT aqueous solution of sodium hydroxide. In the end two emulsified mixture is mixed with the help of magnetic stirrer. While mixing of these two emulsified phases, the droplets from both the emulsion will collide at random and consolidate. Leading to generation of precipitated form CHT droplets to form tiny particles [26, 27].

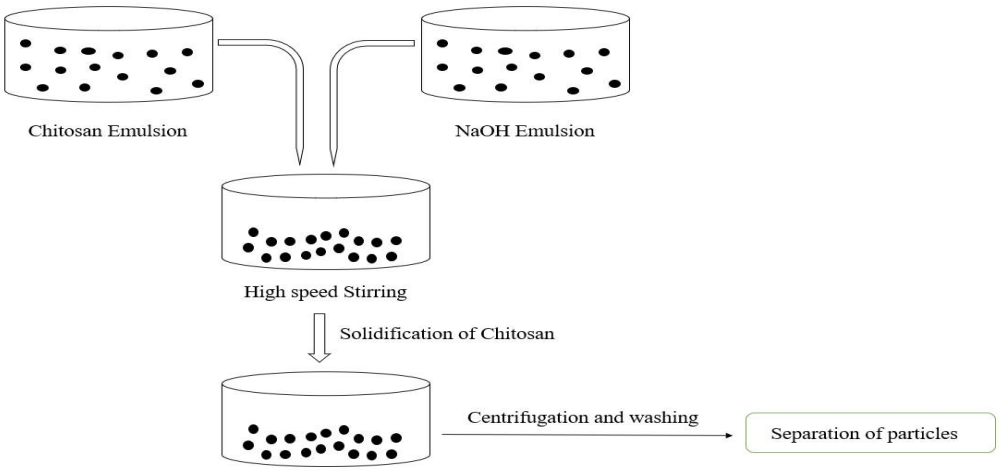

Fig 5: Schematic Depiction of Emulsion Droplet Coalesance.[28]

Ionic gelation

This approach is another better technique for formulation of CHT NPs [4]. This technique involve preparation of CHT solution by dissolving 0.1 to 1% CHT in 100ml of 1% v/v acetic acid, later on preparation of 0.1 % tripolyphosphate solution this can be achieved by dissolving 100mg tripolyphosphate in 100 ml of distilled water along with addition of 100mg of API to be added to the tripolyphosphate solution. Mix the solution at 1500 rpm for 30 minutes on an ultrasonicator and add the tripolyphosphate solution dropwise along with continuous stirring for 3 hours on homogenizer. The phenomenon of addition of tripolyphosphate solution to the CHT solution produces NPs. The CHT NPs may then be spun for 10 minutes at 15000rpm in high-speed in the centrifuge. The formation of CHT NPs will occur due to interaction between the negative charge of tripolyphosphate and positive charge of CHT amino groups.[4,29] as shown in [figure 6].

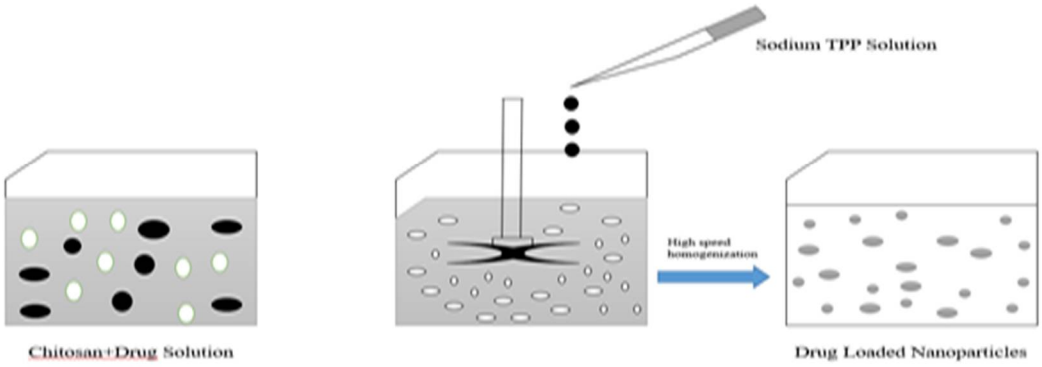

Fig 6: Depiction of Ionic Gelation Procedures.[30]

Desolvation

The desolvation procedure involve preparing CHT solution using relevant solvent, and a solvent that act as competing agent with great hydrophilicity. Example of such case is addition of sodium sulfate to CHT solution. Water gets evaporated when the salt combines with moist environment of CHT solution which lead to a stronger affinity of water in the salt.

This procedure solubilizes the polymers, which lead to precipition [31, 32]. Acetone and Sodium sulphate are widely used for such purposes.[33-38] To keep NPs suspension stable TWEEN 80 is best choice ingredient considered followed by that crosslinking is achieved by glutaraldehyde which harden the NPs 8,31 as shown in [figure 7]

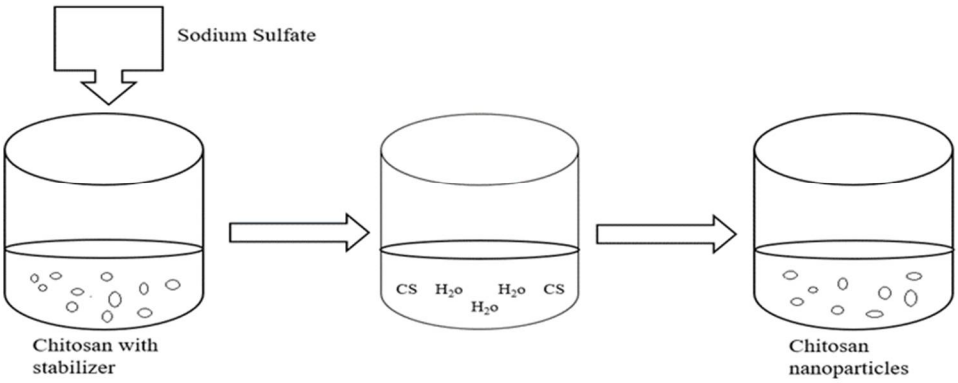

Fig 7: Illustration of Desolvation Approaches. [39]

CROSS LINKING AGENTS FOR CHT NPS

Tripolyphosphate

Sodium tripolyphosphate is another name for tripolyphosphate. Tripolyphosphate is a non-poisonous, colourless salt with a polyanion (penta-anion) crosslinker [40, 41]. The NH2 groups of chitosan can undergo reversible physical cross-linking with the negatively charged tripolyphosphate molecules via electrostatic contact, resulting in the creation of nanoparticles [42, 43].

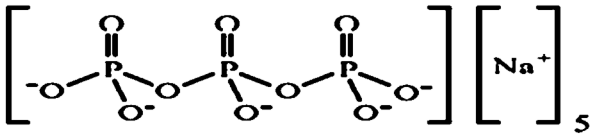

Fig 8: Structure of Sodium Tripolyphosphate.

Genipin

The iridoid glucoside geniposide found in the fruit of Gardenia jasminoides Ellis is used to make genipin. Genipin is a naturally occurring cross-linking agent that improves casein's mechanical and barrier properties. In NH2, Genipin is dyed a vivid blue colour [44, 45]. Genipin has a similar chemical structure to glutaraldehyde, but it has better heat stability [44].

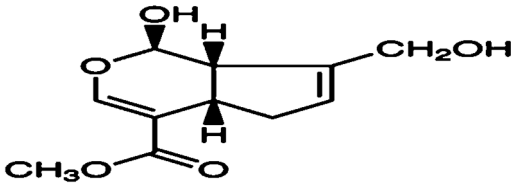

Fig 9: Structure of Genipin

Citric Acid

Thermal cross-linking is one of the physical crosslinking methods. It involves adding citric acid, a typical cross-linking agent, to an aqueous chitosan acidic solution in a fixed molar ratio of citric acid to chitosan solution.[46] This combination is cooled to 0°C before being stirred into an oily phase such as corn oil or sesame oil and cooled to 0°C again. At 120°C, the emulsion is thermally crosslinked, and the resulting microspheres are filtered, washed, and dried [46, 47].

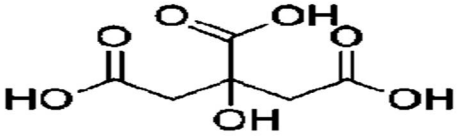

Fig 10: Structure of Citric acid

Glutaraldehyde

The glutaraldehyde is dialdehyde, its production is affordable and form a thick cationic emulsion, generally glutaraldehyde used in versatile crosslinker [48-50] The covalent crosslinking can be occur between aldehyde groups of glutaraldehyde and amino groups of chitosan, resulting in the formation of nanoparticle [51].

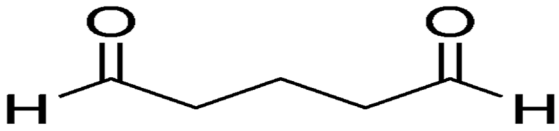

Fig 11: Structure of Glutaraldehyde.

APPLICATIONS OF CHT NPS

Cancer Drug Delivery

Medication given to patients during chemotherapy has crucial effects on unhealthy and healthy cells. Cancer chemotherapy are highly toxic, particularly anticancer medication which are meant for systemic administration. In many situations, anticancer medication shows poor water solubility or less hydrophilic nature. Low hydrophilic drugs demand use of the organic solvents and detergents in clinical applications with unwanted adverse effects like irritation in veins which may lead to respiratory distress. To achieve successful cancer therapy, a particular carrier system that holds huge number of medications specifically which targets tumour cell [52]. CHT is most preferred in such situation because of its biocompatible and biodegradable nature.

Ocular drug delivery

CHT is nontoxic, biodegradable and biocompatible and that's why it chosen as a candidate for ocular DDS. CHT also has mucoadhesive property which help in extended release of medication on the eye surface. CHT also shows gelling capabilities and thus it can be prepared in gel form in liquid state when required to apply on ocular surface. CHT NPs prolong the duration of action of medication and improvise the effectiveness of the drug [52-55]. Santhi, et al. created antifungal medication CHT NPs using an automatic emulsification technique and a cross-linking technique. The study uses the cup-plate technique with common eye drop to evaluate the antifungal activity of CHT NPs. CHT NPs were determined to have a particle size of 152 nm. Additionally, it was discovered that the medication sorption capacity of all CHT NPs loaded with drugs was well within an optimal range (50%). fluconazole CHT NPs had the highest overall loading levels, fungicidal activity, and extended-release action [55].

Transdermal medication administration

It is challenging to deliver any diagnostics material into the circulatory system through topical delivery due to the skin's enormous surface covering and role as a penetrating resistance. The strata primary function as a barricade and the epidermal' top layer, which is composed of compacted kerato fibers coated by a keratinized sheath and encircled by numerous membranous bilayers, are primarily responsible for this epithelial barrier. medicines used topically may offer a difficulty for medications used for local and systemic effects. Both macro and low-molecular-weight drugs can be designed as cutaneous or percutaneous DDS [56, 57]. Katas H *et al* synthesized hydrocortisone NPS by using tripolyphosphate to ionically crosslink high- and low-molecular-weight CHT and N-trimethyl CHT, Hydrocortisone (HC)-loaded CHT NPs. By using FT-IR, the HC loading into CHT NPs was verified. According to in-vitro penetration experiments, HC was effectively liberated from of the CHT NPs in Quality verification cream whereas CHT NPs in aqueous cream gave HC a prolonged release. As a result, it is expected that CHT NPs will be a successful means of delivering anti-inflammatory medications [58].

Colon drug delivery

Chitosan was used to deliver medications to the colon since it breaks down in colon easily. Hyaluronic acid-coupled CHT NPs containing 5-fluorouracil were synthesized using ionotropic crosslinking for controlled drug delivery to colonic tumours [59]. 5-Flurouracil CHT NPs boost cellular uptake by HT-29 carcinoma cells in contrast to uncoupled NPs [60]. CHT NPs with pH-sensitive properties and specific biodegradable nature can be synthesized for colon-targeted delivery of satranidazole [61].

Mucosal Drug delivery

CHT amplifies absorption of hydrophilic glimmers (Protein and peptides drugs) which add benefit in enhancement of mucosa targeted drug delivery. Mucus is comprised of mucin and glycoprotein. Mucin is a sialic acid residue with pKa 2.6 which makes it completely negatively charged at biological pH [62, 63]. CHT is penetration enhancer, mucoadhesive and offers ideal tool for mucosal drug delivery [64, 6]. The smaller size CHT NPs allows more penetration into mucosal layers. CHT offers paracellular drug transport particularly of macromolecular medicines by opening intracellular snug junctions. Positive charges of CHT NPs allows attachment to the cell membrane, it also lower the inter epithelial resistance of monolayers of cells and enhance permeability across the cells. The CHT solution allows intercellular as well as paracellular permeability which depend on molecular weight and degree of deacylation of CHT as well.[65] Because of their mucoadhesion or close interactions with the gut wall, CHT NPs have been shown to be able to sustain

and improve the body's absorption of salmon calcitonin [66].

Delivery of Vaccines

CHT is frequently found in vaccine carriers [67, 68]. To enhance mucosal immunity while also promoting absorption Systemic immunizations are administered using CHT as an additive. The activation of macrophages appears to be caused by CHT absorption [69, 70]. Deoxyribonucleic acid-containing mucosal vaccines have routinely employed CHT (DNA). In a study, Illum et al. developed a CHT-based DNA flu vaccine that produced significant antibody responses in mice after being administered intranasally [69]. After being administered orally to mice, the plasmids pCMVArah2 that encodes the peanuts allergenic gene may be fully integrated into CHT NPs with adequate antigen presentation and resistance [70, 72]. It has been demonstrated that the incorporation of vaccines into various microparticles like NPs enhances the antigenic ingestion by mucosa lymphoid cells, hence generating potent systemic as well as mucosa immunological response against the pathogens [73].

Delivering controlled substances

CHT NPs are suitable for regulated drug carriers. It is possible to disclose the processes of CHT NPs, such as chemically bridging, ionic crosslinking, and ionic complex formation. In order to manage the medication-controlled release, the potential replacement for CHT through chemical functionalization is helpful. Bioactive components can be attached to polymers in this way. CHT can therefore be utilized as a capping material for liposome formulation due to its high reactivity for cellular membrane [74].

Drug administration to kidney

CHT drug ligands can be used to direct medications to the kidneys in the type of drug moiety. Drug adducts frequently have issues with bioactivity, nephrotoxicity, and Cardiac adverse effects [75, 76]. Therefore, scientists have concentrated on creating extremely secure drug - delivery systems. 50% N-acetylated low - molecular - weight CHT can assemble in the kidneys of mice after intravenous injection, notably in the nephron tubes [77, 78]. To create a drug carrier for renal selectivity, the authors linked prednisolone with low molecular weight CHT (19 k Da) using a succinic acid spacer [78].

Liver drug administration

For liver delivery, NPS trapping by reticuloendothelial (REM) and proactive detection of CHT NPs, it is possible to take advantage of the connection between both the liver receptors and the ligand-containing aggregates [78, 79]. N-succinyl-CHT and lactose are capable of degrading aminated using sodium cyanoborohydride to create lactosaminated N-succinyl CHT (Lac-Suc), a medication carrier that is unique to the liver [78, 80., 81]. In a different experiment, metabolizes sugar (glucose can be attached to the interface of CHT NPs using an ionotropic gelation method [78].

Vaginal drug delivery

El-Kamel et al [82] compress the polymers largely cross-linked using fumaric acid and sodium alginate even without microcrystalline cellulose [MCC] to create metronidazole-holding CHT vaginal tablets. The pills have such a low infiltration index and excellent release characteristics at pH 4.8 and pH 7. The pills of metronidazole showed strong adherence. Acyclovir was used as a model drug by Sandri et al [83]. developed four different chitosan derivatives' mucoadhesive and permeation-enhancing properties via the vaginal and buccal mucosa. These derivatives included 5-methyl-pyrrolidinone chitosan, second low molecular mass chitosan, and a partially re-acetylated chitosan. The structures surrounding the vaginal and buccal cavities were strengthened thanks to the high mucoadhesive and penetration concentration of the chitosan made from methyl-pyrrolidinone. the ability to increase acyclovir's penetration or permeation

Nasal Drug Administration

Nasal obstructions frequently have quite poor bioavailability of intranasal delivered drugs due to low permeability, large amount of bloating in the nasal passage, and shorter recovery period. The employment of permeability enhancers with a clogged bioadhesion intranasal delivery device is by far the most efficient strategy to increase intranasal absorption of the drug, according to research. By delaying the mucus secretion of the formulations, they can maintain lengthy contact with pharmaceutical formulation and uptake and accumulation in the nasal cavity. As previously mentioned, such mucoadhesive systems can be liquid in situ gels or powders. CHT has been researched as a potential suspect in the nasal medication delivery mechanism, primarily because of its rated qualities and penetration [84].

Delivery of drugs intravenously

In order to determine if CHT and thiolated CHT NPs may delay the time required for drugs to permeate in the urinary tracts, Barthelmes et al. examined at their mucoadhesion in the intravesical mucous. Thiolated CHT NPs have been discovered to be an advantageous help in the iv delivery of medications, prolonging the half-life of the medication and assuring lengthy sustainable diffusion for a lengthy time [85].

Drug delivery via buccal route

Effective for conveying medications to the insertion point is the Buccal route. The Buccal path is perfect for

drug targeting to the intended site since it avoids hepatic first-pass processing and breakdown in the gastro intestinal tract, and it has an increased patient adherence. A reliable buccal method of delivery must show sustainable effect in the oral cavity for longer period. Polymers which offer mucous adhesion property lengthen the time the medicine remains in the mouth [86]. CHT is a potential polymer which will be employed for buccal distribution since it has bioadhesive and absorbing qualities. Sandri et al detail how trimethylated CHT is being used as a suitable additive in DDS that are intended to be applied to the buccal mucosa to improve the absorption of hydrodynamic molecules. In pigs effectiveness of thiolated CHT for peptide delivery methods via buccal mucosa was assessed [87].

CONCLUSION

Chitosan nanoparticles are very high efficacy in controlled release and targeting studies of almost all phases of bioactive molecules. The chemical modifications of chitosan are very essential for obtaining the desired physicochemical properties. Recently, chitosan was further tested in genetic delivery. Chitosan nanoparticles can effectively deliver drug at specific sites by retaining the drug locally to permit an extended time for drug absorption. The present review discussed importance of chitosan NPs in different treatment approaches.

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CONFLICT OF INTEREST

The author declares that there is no conflict of interest.

REFERENCES

1. Chirra HD, Desai TA. (2012). Emerging microtechnologies for the development of oral drug delivery devices. Adv drug deliv rev. 64(14):1569-1578.
2. Des Rieux A, Fievez V, Garinot M, Schneider YJ, Preat V. (2006). Nanoparticles as potential oral delivery systems of proteins and vaccines: a mechanistic approach. J Control Release.116(1):1-27.
3. K. Divya. M. S. Jisha. (2017). A review on Chitosan nanoparticles preparation and applications. Environmental chemistry Letters. 101-12.
4. Agnihotri SA, Mallikarjuna NN, Aminabhavi TM. (2004). Recent advances on chitosan-based micro-and nanoparticles in drug delivery. J Control Release. 100(1):5-28.
5. Berscht PC, Nies B, Liebendorfer A, Kreuter J. (1994). Incorporation of basic fibroblast growth factor into methylpyrrolidinone chitosan fleeces and determination of the in vitro release characteristics. Biomaterials. 15(8):593-600.
6. Mohammed MA, Syeda J, Wasan KM, Wasan EK. (2017). An overview of chitosan nanoparticles and its application in non-parenteral drug delivery. Pharm;9(4):53.
7. Okoya AA, Akinyele AB, Amuda OS, Ofoezie IE. (2016). CHT-grafted carbon for the sequestration of heavy metals in aqueous solution. American Chemical Science Journal. 11(3):1-4.
8. Naskar S, Das SK, Sharma S, Kuotsu K. (2021). A Review on Designing Poly (Lactic-co-glycolic Acid) Nanoparticles as Drug Delivery Systems. Pharma Nanotechnol. 9(1):36-50.
9. Ohya Y, Shiratani M, Kobayashi H, Ouchi T. (1994). Release behavior of 5-fluorouracil from chitosan-gel nanospheres immobilizing 5-fluorouracil coated with polysaccharides and their cell specific cytotoxicity. J. Micromol. Sci. - Pure Appl. Chem. 31(5):629-642.
10. Wang SL, Hiep ĐM, Luong PM, Vui NT, Dinh TM, Dzung NA. (2014). Preparation of chitosan nanoparticles by spray drying, and their antibacterial activity. Res. Chem. Intermed. 40(6):2165-75.
11. Sinsuebpol C, Chatchawalsaisin J, Kulvanich P. (2013). Preparation and in vivo absorption evaluation of spray dried powders containing salmon calcitonin loaded chitosan nanoparticles for pulmonary delivery.Drug Des. Devel. Ther. ; 7:861.
12. Li FQ, Ji RR, Chen X, You BM, Pan YH, Su JC. (2010). Cetirizine dihydrochloride loaded microparticles design using ionotropic cross-linked chitosan nanoparticles by spray-drying method. Arch. Pharm. Res.;33(12):1967-73.
13. Mehrotra A, Nagarwal RC, Pandit JK. (2010). Fabrication of lomustine loaded chitosan nanoparticles by spray drying and in vitro cytostatic activity on human lung cancer cell line L132. J Nanomed NanotechnoL. 1:103.
14. Ahmed TA, Aljaeid BM. (2016). Preparation, characterization, and potential application of chitosan, chitosan derivatives, and chitosan metal nanoparticles in pharmaceutical drug delivery. Drug design, development and therapy. 10:483.
15. Wang JJ, Zeng ZW, Xiao RZ, Xie T, Zhou GL, Zhan XR, Wang SL. (2011). Recent advances of chitosan nanoparticles as drug carriers. Int J Nanomed. 6:765.
16. Grenha A. (2012). Chitosan nanoparticles: a survey of preparation methods. J Drug Target. 20(4):291-300.

17. Songjiang Z, Lixiang W. (2009). Amyloid-beta associated with chitosan nano-carrier has favorable immunogenicity and permeates the BBB. AapsPharm Sci Tech. 10(3):900-5.
18. Naskar S, Kuotsu K, Sharma S. (2019). Chitosan-based nanoparticles as drug delivery systems: a review on two decades of research. Journal of drug targeting. 21;27(4):379-93.
19. Melo EP, Aires-Barros MR, Cabral JM. (2004). Reverse micelles and protein biotechnology.87-90
20. Pileni MP. (2006). Reverse micelles used as templates: a new understanding in nanocrystal growth. J Exp Nanosci. 1(1):13-27.
21. Banerjee T, Mitra S, Singh AK, Sharma RK, Maitra A. (2002). Preparation, characterization and biodistribution of ultrafine chitosan nanoparticles. Int J Pharma. 243(1-2):93-105.
22. Kafshgari MH, Khorram M, Mansouri M, Samimi A, Osfouri S. (2012). Preparation of alginate and chitosan nanoparticles using a new reverse micellar system. Iran. Polym. J. (2):99-107.
23. Mitra S, Gaur U, Ghosh PC, Maitra AN. Tumour targeted delivery of encapsulated dextran–doxorubicin conjugate using chitosan nanoparticles as carrier. J Control Release. 2001;74(1-3):317-23.
24. Wang Y, Li P, Truong-Dinh Tran T, Zhang J, Kong L. Manufacturing techniques and surface engineering of polymer based nanoparticles for targeted drug delivery to cancer. Nanomaterials. 2016 Feb 1;6(2):26.
25. Luque-Alcaraz AG, Lizardi-Mendoza J, Goycoolea FM, Higuera-Ciapara I, Argüelles-Monal W. Preparation of chitosan nanoparticles by nanoprecipitation and their ability as a drug nanocarrier. RSC advances. 2016;6(64):59250-6.
26. Tokumitsu H, Ichikawa H, Fukumori Y. Chitosan-gadopentetic acid complex nanoparticles for gadolinium neutron-capture therapy of cancer: preparation by novel emulsion-droplet coalescence technique and characterization. Pharm res. 1999 ;16(12):1830-5.
27. Reddy YD, Dhachinamoorthi D. Formulation and in vitro evaluation of antineoplastic drug loaded nanoparticles as drug delivery system. AJJP 2013 ;7(23):1592-604.
28. Agarwal M, Nagar DP, Srivastava N, Agarwal MK. Chitosan nanoparticles-based drug delivery: An update. Int. J. Adv. Multidiscip. Res. 2015;2(4):1-3.
29. Nagarajan E, Shanmugasundaram P, Ravichandiran V, Vijayalakshmi A, Senthilnathan B, Masilamani K. Development and evaluation of chitosan based polymeric nanoparticles of an antiulcer drug lansoprazole. J Appl Pharma Sci. 2015;5(4):20-5.
30. Tyagi S, Pandey VK. Research & Reviews: Journal of Pharmaceutics and Nanotechnology. JPN. 2016;4:2-12.
31. Alonso MJ. Nanoparticulate drug carrier technology. InMicroparticulate systems for the delivery of proteins and vaccines 2020: (pp. 203-242).
32. Poncelet D. Microencapsulation: fundamentals, methods and applications. In Surface chemistry in biomedical and environmental science 2006 (pp. 23-34). Springer, Dordrecht.
33. Mao HQ, Roy K, Troung-Le VL, Janes KA, Lin KY, Wang Y, August JT, Leong KW. Chitosan-DNA nanoparticles as gene carriers: synthesis, characterization and transfection efficiency. J Control Release. 2001;70(3):399-421.
34. Borges O, Borchard G, Verhoef JC, de Sousa A, Junginger HE. Preparation of coated nanoparticles for a new mucosal vaccine delivery system. Int J Pharm. 2005;299(1-2):155-66.
35. Atyabi F, Talaie F, Dinarvand R. Thiolated chitosan nanoparticles as an oral delivery system for amikacin: in vitro and ex vivo evaluations. J Nanosci Nanotechnol. 2009;9(8):4593-603.
36. Mao HQ, Roy K, Troung-Le VL, Janes KA, Lin KY, Wang Y, August JT, Leong KW. Chitosan-DNA nanoparticles as gene carriers: synthesis, characterization and transfection efficiency. J Control Release. 2001;70(3):399-421.
37. Borges O, Borchard G, Verhoef JC, de Sousa A, Junginger HE. Preparation of coated nanoparticles for a new mucosal vaccine delivery system. Int J Pharm. 2005 ;299(1-2):155-66.
38. Agnihotri SA, Aminabhavi TM.(2007). Chitosan nanoparticles for prolonged delivery of timolol maleate. Drug Develop Ind Pharma. ;33(11):1254-62.
39. Rajalakshmi R, Aruna U, Vinesha V, Rupangada V. (2014). Chitosan nanoparticles-An emerging trend in nanotechnology. International Journal of Drug Delivery. 25;6(3):204-29.
40. Corbridge DE. (1960). The crystal structure of sodium triphosphate, Na5P3O10, phase I. Acta Crystallographica. ;10;13(3):263-9.
41. Liu Z, Jiao Y, Wang Y, Zhou C, Zhang Z. (2008). Polysaccharides-based nanoparticles as drug delivery systems. Adv Drug Delivery Rev. 60(15):1650-62.
42. Konecsni K, Low NH, Nickerson MT. (2012). Chitosan–tripolyphosphate submicron particles as the carrier of entrapped rutin. Food chemistry. 134(4):1775-9.
43. Hu B, Pan C, Sun Y, Hou Z, Ye H, Hu B, Zeng X. (2008). Optimization of fabrication parameters to produce chitosan-tripolyphosphate nanoparticles for delivery of tea catechins. J Agri Food Chem. ;56(16):7451-8.
44. Tomasula PM. (2009). Using dairy ingredients to produce edible films and biodegradable packaging materials. In Dairy-Derived Ingredients pp. 589-624.
45. Bigi A, Cojazzi G, Panzavolta S, Roveri N, Rubini K. (2002). Stabilization of gelatin films by crosslinking with genipin. Biomaterials. 23(24):4827-32.
46. Orienti I, Aiedeh K, Gianasi E, Bertasi V, Zecchi V. (1996). Indomethacin loaded chitosan microspheres. Correlation between the erosion process and release kinetics. J Microencap. ;13(4):463-72.
47. Ahmed TA, Aljaeid BM. (2016). Preparation, characterization, and potential application of chitosan, chitosan derivatives, and chitosan metal nanoparticles in pharmaceutical drug delivery. Drug design, develop ther. 10:483.

48. Andriani Y, Grasianto C, Siswanta D, Mudasir M. (2015). Retracted-glutaraldehyde-crosslinked chitosan-pectin nanoparticles as a potential carrier for curcumin delivery and it's in vitro release study. Int J Drug Deliv. 7:167-73.
49. Zhao LM, Shi LE, Zhang ZL, Chen JM, Shi DD, Yang J, Tang ZX. (2011). Preparation and application of chitosan nanoparticles and nanofibers. Brazil J Chem Eng. ;28(3):353-62.
50. Pfafflin, James R.; Ziegler, Edward N. (2006). Encyclopedia of Environmental Science and Engineering: A-L. CRC Press. p. 235.
51. Banerjee T, Mitra S, Singh AK, Sharma RK, Maitra A. (2002). Preparation, characterization and biodistribution of ultrafine chitosan nanoparticles. Int J pharm. 28;243(1-2):93-105.
52. Torchilin VP. (2004). Targeted polymeric micelles for delivery of poorly soluble drugs. Cellular and Molecular Life Sciences CMLS.;61(19):2549-59.
53. Garg U, Chauhan S, Nagaich U, Jain N. (2019). Current advances in chitosan nanoparticles-based drug delivery and targeting. Adv pharm bull. 195.
54. Ahmed TA, Aljaeid BM. Preparation, characterization, and potential application of chitosan, chitosan derivatives, and chitosan metal nanoparticles in pharmaceutical drug delivery. Drug design, develop ther. 2016; 10:483.1
55. Paudel KS, Milewski M, Swadley CL, Brogden NK, Ghosh P, Stinchcomb AL. Challenges and opportunities in dermal/transdermal delivery. Therapeutic delivery. 2010 Jul;1(1):109-31.
56. Kim DG, Jeong YI, Choi C, Roh SH, Kang SK, Jang MK, Nah JW. (2006). Retinol-encapsulated low molecular water-soluble chitosan nanoparticles. IntJ Pharm. 319(1-2):130-8.
57. Katas H, Hussain Z, Ling TC. (2012). Chitosan nanoparticles as a percutaneous drug delivery system for hydrocortisone. Journal of Nanomaterials. 1;2012.
58. Park JH, Saravanakumar G, Kim K, Kwon IC. (2010). Targeted delivery of low molecular drugs using chitosan and its derivatives. Adv drug deliv rev. 62(1):28-41.
59. Jain SK, Jain A. (2008). Target-specific drug release to the colon. Expert opinion on drug delivery. 5(5):483-98.
60. Tan YL, Liu CG.(2009). Self-aggregated nanoparticles from linoleic acid modified carboxymethyl chitosan: Synthesis, characterization and application in vitro. Colloids and Surfaces B: Biointerfaces. 69(2):178-82.
61. Deacon MP, Mcgurk S, Roberts CJ, Williams PM, Tendler SJ, Davies MC, Davis SS, (2000). Harding SE. Atomic force microscopy of gastric mucin and chitosan mucoadhesive systems. Biochem J. 348(3):557-63.
62. George M, Abraham TE. (2006). Polyionic hydrocolloids for the intestinal delivery of protein drugs: alginate and chitosan-a review. J control release. 14(1):1-4.
63. Dodane V, Khan MA, Merwin JR. (1999). Effect of chitosan on epithelial permeability and structure. Int j pharm. ;182(1):21-32.
64. Prego C, Fabre M, Torres D, Alonso MJ. (2006). Efficacy and mechanism of action of chitosan nanocapsules for oral peptide delivery. Pharm res. 23(3):549-56.
65. Van der Lubben IM, Verhoef JC, Borchard G, Junginger HE. (2001). Chitosan for mucosal vaccination. Adv drug deliv rev. 52(2):139-44.
66. Murata Y, Toniwa S, Miyamoto E, Kawashima S. (1999). Preparation of alginate gel beads containing chitosan nicotinic acid salt and the functions. Eur J Pharm and Biopharm. 48(1):49-52.
67. Seferian PG, Martinez ML. (2000). Immune stimulating activity of two new chitosan containing adjuvant formulations. Vaccine. 19(6):661-8.
68. Tiyaboonchai W. (2013). Chitosan nanoparticles: a promising system for drug delivery.NUJST. 11(3):51-66.
69. Roy K, Mao HQ, Huang SK, Leong KW. (1999). Oral gene delivery with chitosan–DNA nanoparticles generates immunologic protection in a murine model of peanut allergy. Nat med. 5(4):387-91.
70. Mao HQ, Roy K, Troung-Le VL, Janes KA, Lin KY, Wang Y, *et.al*. (2001). Chitosan-DNA nanoparticles as gene carriers: synthesis, characterization and transfection efficiency. J control release. 70(3):399-421.136
71. Prabhakaran M, Mano JF. (2004). Chitosan-based particles as controlled drug delivery systems. Drug deliv. ;12(1):41-57.
72. Mohammed MA, Syeda J, Wasan KM, Wasan EK. (2017). An overview of chitosan nanoparticles and its application in non-parenteral drug delivery. Pharm;9(4):53.
73. Onishi H, Machida Y. (1999). Biodegradation and distribution of water-soluble chitosan in mice. Biomater. ;20(2):175-82
74. Yuan ZX, Sun X, Gong T, Ding H, Fu Y, Zhang ZR. (2007). Randomly 50% N-acetylated low molecular weight chitosan as a novel renal targeting carrier. J drug target. 15(4):269-78.
75. Wilson B, Samanta MK, Santhi K, Kumar KP, Paramakrishnan N, Suresh B. (2008). Targeted delivery of tacrine into the brain with polysorbate 80-coated poly (n-butylcyanoacrylate) nanoparticles. Eur J Pharm and Biopharm. ;70(1):75-84.
76. Rajalakshmi R, Indira MY, Aruna U, Vinesha V, Rupangada V, Moorthy SB. (2014). Chitosan nanoparticles-An emerging trend in nanotechnology. Int J Drug Deliv. 6:204-9.
77. Ogawara KI, Yoshida M, Higaki K, Kimura T, Shiraishi K, et.al. (1999). Hepatic uptake of polystyrene microspheres in rats: effect of particle size on intrahepatic distribution. J Control Release. 59(1):15-22.
78. Kato Y, Onishi H, Machida Y. (2001). Biological characteristics of lactosaminated N-succinyl-chitosan as a liver-specific drug carrier in mice. J Control Release. 23;70(3):295-307.
79. Kodaira H, Tsutsumi Y, Yoshioka Y, Kamada H, Kaet.al. (2004). The targeting of anionized polyvinylpyrrolidone to the renal system. Biomater. 1;25(18):4309-15.

80. El-Kamel A, Sokar M, Naggar V, Al Gamal S. (2002). Chitosan and sodium alginate—Based bioadhesive vaginal tablets. AapsPharmsci. 4(4):224-30.
81. Sandri G, Rossi S, Ferrari F, Bonferoni MC, Muzzarelli C, Caramella C. (2004). Assessment of chitosan derivatives as buccal and vaginal penetration enhancers. Eur j pharm sci. 21(2-3):351-9.
82. Soane RJ, Frier M, Perkins AC, Jones NS, Davis SS, Illum L. (1999). Evaluation of the clearance characteristics of bioadhesive systems in humans. Int J Pharm. 178(1):55-65.
83. Ilium L. (1998). Chitosan and its use as a pharmaceutical excipient. Pharm Res. 15(9):1326-31.
84. Barthelmes J, Perera G, Hombach J, Dunnhaupt S, Bernkop-Schnürch A. (2011). Development of a mucoadhesive nanoparticulate drug delivery system for a targeted drug release in the bladder. Int J Pharm. 416(1):339-45.
85. Bernkop-Schnürch A, Dunnhaupt S. (2012). Chitosan-based drug delivery systems. Eur J Pharm biopharm;81(3):463-9.
86. Aiedeh K, Taha MO. (2001). Synthesis of iron-crosslinked chitosan succinate and iron-crosslinked hydroxamated chitosan succinate and their in vitro evaluation as potential matrix materials for oral theophylline sustained-release beads. Eur J Pharm Sci. 13(2):159-68.
87. Langoth N, Kahlbacher H, Schoffmann G, Schmerold I, Schuh M, Franz S, Kurka P, Bernkop-Schnurch A. (2006). Thiolated chitosan: design and in vivo evaluation of a mucoadhesive buccal peptide drug delivery system. Pharm res. 23(3):573-9.