Review Article

Chitosan Based Nanoparticles in Drug Delivery

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ABSTRACT: Nanoparticles have gained considerable attention in recent years as one of the most promising drug delivery systems owing to their unique potentials via combining the different characteristics of hydrophobicity and hydrophilicity with a nanoparticle (e.g., very small size). Several polymeric nanoparticulate systems have been prepared and characterized in recent years, based on both natural and synthetic polymers, each with its own advantages and drawbacks. Among the natural polymers, chitosan has been studied extensively for preparation of nanoparticles. Chitosan nanoparticles have been reported with different characteristics with respect to drug delivery. This review presents various types of chitosan based nanoparticles in drug delivery.

Introduction

In recent years, significant efforts have been devoted to use the potentials of nanotechnology in drug delivery since it offers a suitable means of site-specific and/or time-controlled delivery of small or large molecular weight drugs and other bioactive agents [Brigger et al., 2002, Panyam et al., 2003, Moghimi et al., 2001, Lockman et al., 2002, Min et al., 2008, Cho et al., 2008, Ragusa et al., 2007, Kingsley et al., 2006, Nahar et al., 2006]. Pharmaceutical nanotechnology focuses on formulating therapeutically active agents in biocompatible nano forms such as nanoparticles, nanocapsules, micellar systems, and conjugates. These systems offer many advantages in drug delivery, mainly focusing on improved safety and efficacy of the drugs, e.g. providing targeted delivery of drugs, improving bioavailability, extending drug or gene effect in target tissue, and improving the stability of therapeutic agents against chemical/enzymatic degradation [Moghimi et al., 2001]. The nanoscale size of these delivery systems is the basis for all these advantages [Vinogradov et al., 2002]. By a general definition, nanoparticles vary in size from 10 to 1000nm. The drug is dissolved, entrapped, encapsulated or attached to a nanoparticle matrix and depending upon the method of preparation, nanoparticles, nanospheres or nanocapsules can be obtained. Nanocapsules are vesicular systems in which the drug is confined to a cavity surrounded by a boundary structure, e.g., polymeric, while nanospheres are matrix spherical systems in which the drug is physically and uniformly dispersed [Sahoo et al., 2003]. Several types of nanoparticulate systems have been attempted as potential drug delivery systems, including biodegradable polymeric nanoparticles, polymeric micelles, solid nanoparticles, lipid-based nanoparticles, e.g., Solid lipid nanoparticles (SLN), nanostructured lipid carriers (NLC) and lipid drug conjugates (LDC), nanoliposomes, inorganic nanoparticles, dendrimers, magnetic nanoparticles, Ferrofluids, and quantum dots. This review presents various types of chitosan based nanoparticles in drug delivery.

Chitosan-based nanoparticles

Chitosan, of (1-4)-2-amino-2-deoxy β-D-glucan, is a deacetylated form of chitin, an abundant polysaccharide present in crustacean shells. Even though the discovery of chitosan dates back to 19th century, it has only been over the last two decades that this polymer has received attention as a material for biomedical and drug delivery applications. The accumulated information about the physicochemical and biological properties of chitosan led to the recognition of this polymer as a promising material for drug delivery and, more specifically for the delivery of macromolecules [Patel et al., 2007, Kato et al., 2003, Prabaharan et al., 2006, George et al., 2006, Sharma et al., 2006, Bernkop-Schnürch et al., 2001, Van der Lubben et al., 2001]. From a technical point of view, it is extremely important that chitosan is hydro-soluble and positively charged. These properties enable this polymer to interact with negatively charged polymers, macromolecules, and even with certain polyanions upon contact in aqueous environment. These interactive forces and the resulting sol-gel transition stages have been exploited for nano-
encapsulation purposes [Min et al., 2008, Shutava et al., 2006, Fan et al., 2006, Pandey et al., 2005]. On the other hand, chitosan has the special possibility of adhering to the mucosal surfaces within the body, a property leading to the attention to this polymer in mucosal drug delivery [Sharma et al., 2006, Van der Lubben et al., 2001, Lehr et al., 1992]. The potential of chitosan for this specific application has been further enforced by the demonstrated capacity of chitosan to open tight junctions between epithelial cells though well organized epithelia [Janes et al., 2001, Artursson et al., 1994, Borchard et al 1996, Schipper et al., 1996, Schipper et al., 1997, Schipper et al., 1999]. The interesting biopharmaceutical characteristics of this polymer are accompanied by its well documented biocompatibility and low toxicity [Knapczyk et al 1989, Hirano et al., 1990, Hirano et al., 1989, Bersch et al., 1985]. Many articles on the potential of chitosan for pharmaceutical applications have already been published [Dodane et al., 1998, Paul et al., 2000]. Therefore, our purpose is to focus on the specific features and applications of the chitosan-based nanoparticulate systems prepared and characterized to date for delivery of macromolecular compounds such as peptides, proteins, antigens, oligonucleotides, and genes.

1. Chitosan-based nanoparticles with covalent crosslinks

The earliest works on chitosan-based nanomaterials predominantly involved chemical crosslinking within polymer chain. Watzke and Dieschbourg [1994] formed chitosan-silica nano composites by reacting tetraethoxysilane with hydroxyl groups on the chitosan monomers. However, it was not attempted to associate pharmaceutically active agents to the prepared polymer network. Ohya et al., [1994] was the first to present data involving chitosan nanospheres for drug delivery applications. Using a water-in-oil (w/o) emulsion method followed by glutaraldehyde crosslinking of the chitosan amino groups, the group produced nanospheres loaded by 5-fluorouracil (5-FU), an anticancer drug. Since 5-FU derivatives in formulations also contained a terminal amine, indiscriminate glutaraldehyde addition bound the active agent to the polymer as it did between chitosan chains, causing drug immobilization rather than encapsulation. These studies demonstrated the feasibility of synthesizing stable, reproducible nano sized chitosan particles which could entrap and deliver drugs [Janes et al., 2001].

2. Chitosan-based nanoparticles with ionic cross-links

As mentioned, the cationic nature of chitosan has been conveniently exploited for the development of particulate drug delivery systems. Aside from its complexation with negatively charged polymers, an interesting property of chitosan is its ability to gel upon contact with special poly anions, a process referred to as ‘ionotropic gelation’. This gelation process is due to the formation of inter and intra cross-linkages between/within polymer chains, mediated by the poly anions. More recently, chitosan NPs have been developed based on the ionotropic gelation of chitosan with tripolyphosphate (TPP), for drug encapsulation [Shirashi et al., 1993, Calvo et al., 1997, Shu et al., 2000, Kawashima et al., 1985, Dung et al, 2007, Gun et al., 2005]. This simple and straightforward technique involves the addition of an alkaline phase (pH = 7–9) containing TPP into an acidic phase (pH = 4–6) containing chitosan. NPs are formed immediately upon mixing of the two phases through inter and intra molecular linkages created between TPP phosphates and chitosan amino groups. Insulin-loaded chitosan NPs have been prepared by mixing insulin with TPP solution and then adding the mixture to chitosan solution under constant stirring [Fernandez-Urrusuno et al 1999]. Chitosan NPs thus obtained were within size range of 300-400nm with a positive surface charge ranging from + 54 to + 25mV. Using this method, insulin loading was optimized reaching the loading efficiency of up to 55%. There are many ongoing investigations, which demonstrate the improved oral bioavailability of peptide and proteins upon undergoing this loading procedure. In these studies, it is claimed that the bioadhesions property of chitosan NPs further enhances the intestinal absorption of the drug. Pan et al [2002] prepared insulin-loaded chitosan NPs by ionotropic gelation of chitosan with TPP anions. The ability of chitosan NPs to enhance the intestinal absorption of insulin and the relative bioavailability of insulin was investigated by monitoring the plasma glucose level in alloxan-induced diabetic rats after oral administration of various doses of insulin-loaded chitosan NPs. The positively charged, stable chitosan NPs showed particle sizes within the range of 250-400nm with insulin association ratio of up to 80%. The in vitro release experiments indicated an initial burst phase which was pH-sensitive. The chitosan NPs enhanced the intestinal absorption of insulin to a greater extent than the aqueous solution of chitosan in vivo. After administration of 21.1U/kg insulin loaded in the chitosan NPs, hypoglycemia was prolonged over 15h. The average bioavailability relative to the subcutaneous injection of free insulin solution was up to 14.9%. Xu et al., [2003] have studied different formulations of chitosan NPs produced by the ionic gelation of TPP and chitosan. Transmission electronic microscopy (TEM) indicated particle diameters ranging between 20 and 200nm with spherical shapes.
3. Chitosan-based nanoparticles prepared by desolvation method

The use of desolvating agents for the synthesis of chitosan particles originally emerged from the microencapsulation studies. Berthold et al., [1990] first proposed the use of sodium sulfate as a precipitating agent to form chitosan particles. Dropwise addition of sodium sulfate into a solution of chitosan and polysorbate 80 (used as a stabilizer for the suspension) under both stirring and ultrasonication, desolvated chitosan in a particulate form. Although the investigators called the resulting suspensions microspheres, the precipitated particles were at micronano interface (900±200 nm). Drug encapsulation was not reported, but the group demonstrated that by virtue of the positive charge on the particle surface, they were able to absorb significant amounts (up to 30% loading) of the hydrophilic amionic corticosteroid, prednisolone sodium phosphate to the particle surface. A variation of this technique was later employed for the controlled release of antineoplastic proteoglycans for immunostimulation [Tian et al., 1999]. Following glutaraldehyde crosslinking of the nanoparticles, stable particles between 600 and 700nm were obtained. Unfortunately, the necessity for glutaraldehyde forbids the application of this formulation toward the delivery of therapeutically active macromolecules. Chitosan-DNA NPs have been prepared using the complex coacervation technique [Dodane et al, 1998, Ray et al 1999]. At the amino-to-phosphate groups' ratio between 3 and 8 and the chitosan concentration of 100mcg/ml, the particle size was optimized to 100-250nm range with a narrow distribution. The chitosan-DNA NPs could partially protect the encapsulated plasmid DNA from nuclease degradation.

4. Chitosan-based nanoparticles prepared by emulsion-droplet coalescence method

Emulsion-droplet coalescence method, introduced by Tokumitsu et al., [1999], utilizes the principles of both emulsion cross-linking and precipitation. In this method, instead of cross-linking the stable droplets, precipitation is induced by allowing coalescence of chitosan droplets with sodium hydroxide droplets. A stable emulsion containing aqueous solution of chitosan along with the drug to be loaded is produced in liquid paraffin. At the same time, another stable emulsion containing chitosan aqueous solution containing sodium hydroxide is produced in the same manner. When, finally, both emulsions are mixed under high speed stirring, droplets of each emulsion would collide at random and coalesce, thereby precipitating chitosan droplets to provide small solid particles. In this study, Tokumitsu et al., [1999] prepared gadopentetic acid loaded chitosan NPs by this method using 100% deacetylated chitosan, with the mean particle size of 452nm and drug loading efficiency of 45%.

5. Chitosan-based nanoparticles prepared by reverse micellar method

Reverse micelles are thermodynamically stable liquid mixtures of water, oil, and surfactant. Microscopically, they are homogenous and isotropic structures consisting of aqueous-in-oil droplets separated by surfactant-rich films. NPs prepared by conventional emulsion polymerization methods are not only large (N200 nm), but also possess a broad size range. Preparation of ultrafine polymeric NPs with narrow size distribution could be achieved by using reverse micellar medium [Leong et al., 1982]. Aqueous core of the reverse micellar droplets can be used as a 'nanoreactor' to prepare such particles. Since the size of this highly monodispersed and narrow size range reverse micellar droplets usually lies between 1 and 10nm [Maitra et al., 1984], they are among the promising NPs interested in drug delivery studies. Since micellar droplets in Brownian motion are in liquid medium, they undergo continuous coalescence followed by re-separation on a time scale that varies between milliseconds and microseconds [Luisi et al., 1988]. The size, polydispersity and thermodynamic stability of these droplets are maintained in the system by a rapid dynamic equilibrium. In this method, the surfactant is dissolved in an organic solvent to prepare reverse micelles. To this, aqueous solutions of chitosan and drug are added gradually with constant vortexing to avoid any turbidity. The aqueous phase is regulated in such a way as to keep the entire mixture in an optically transparent microemulsion phase. Additional amount of water may be added to obtain NPs of large sizes. To this transparent solution, a cross-linking agent is added with constant stirring overnight. The maximum amount of drug that can be dissolved in reverse micelles varies from drug to drug and has to be determined by gradually increasing the amount of drug until the clear dispersion is transformed into a translucent solution. The organic solvent is, then, evaporated to obtain the micellar transparent drug mass. The remaining material is dispersed in water and then, by adding a suitable salt, the surfactant precipitates out. The mixture is, then, subjected to centrifugation. The supernatant solution is decanted, which contains the drug-loaded NPs. The aqueous dispersion is immediately dialyzed through a dialysis membrane for about 1h and the liquid is lyophilized to drug powder. Mitra et al., [2001] have encapsulated doxorubicin-dextran conjugate in chitosan NPs, using this method.
6. Chitosan-based nanoparticles prepared by self-assembly via chemical modification

The self-assembly of chemically modified chitosan into NPs has been investigated for the delivery of macromolecules [Yu et al., 2006, Ichikawa et al., 2005, Ohya et al., 1999, Uchegbu et al., 1998], Lee et al., 1998b, Lee et al. 1998a]. Fractional conjugation of polyethylene glycol, PEG, via an amide linkage to soluble chitosan was shown to yield self-aggregation at basic pH [Ohya et al., 1999]. These aggregates could trap insulin following incubation in phosphate buffer saline (PBS), likely due to the electrostatic interactions between the unconjugated chitosan monomers and the anionic residues of the protein. Depending on the degree of PEGylation, aggregate sizes between 5 and 150nm can be obtained. The degree of PEGylation also influences the release rate, as more extensively PEGylated aggregates release insulin more rapidly.

However, it is difficult to draw conclusions based upon this data, as loading levels for the respective PEG formulations were not reported. An interesting approach leading to the formation of chitosan vesicles has been developed by Uchegbu et al., [1998]. They linked palmitic acid to modified glycol chitosan chains, thus producing an amphiphilic polymer, which, upon mixing with cholesterol, formed nanovesicles approximately 300-600nm in size. These vesicles demonstrated good hemocompatibility, and stability in serum and bile salt. Moreover, the vesicles were able to encapsulate bleomycin, a chemotherapeutic agent. The loading process was performed via an ammonium sulfate gradient which drove the peptide into the vesicles. Lee et al., [1998] have investigated the effects of conjugating chitosan with deoxycholic acid in their attempts to design a new carrier for DNA delivery. Attachment of this hydrophobic moiety to soluble chitosan was found to have substantial effects on its aqueous stability, and the resulting amphiphilic macromolecule formed self-assemblies of self-aggregates upon sonication. The group has reported the ability of these self-aggregates to associate with DNA and transfect in vitro. Further work is currently underway aiming at gaining a better understanding of the arrangement of the deoxycholic microdomains imbedded within the chitosan aggregates [Lee et al. 1998a].

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