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IONOTROPIC GELATION - A NOVEL METHOD TO PREPARE CHITOSAN NANOPARTICLES

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ABSTRACT

The encapsulation of drugs inside polymeric nanoparticles/microparticles is a strategy currently employed in the search for new and more effective therapies. The use of biocompatible and biodegradable polymers gives several advantages to these formulations. Protection of the active principals against the action of environmental and physiological agents, the reduced number of doses and a subsequent decrease in drug-related adverse effects, and increased bioavailability are some of these advantages. This review outlines the ionotropic gelation method for the preparation of chitosan-based nanoparticulate drug delivery systems published over the past decade. From a literature survey, it was found that research activities on chitosan-based micro/nanoparticles having various drugs for various therapeutic applications have increased at the rapid rate. Hence ionotropic gelation method can use to arrange these chitosan nanoparticles as it is incredibly simple and having many advantages then other methods.

KEYWORDS

Chitosan, Nanoparticles and Ionotropic gelation.

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INTRODUCTION

Polymer nanoparticles are particles of less than 1 mm diameter that are prepared from natural or synthetic polymers. Nanoparticles have become an important area of research in the field of drug delivery because they have been extensively used to deliver drugs, genes, diagnostics, and vaccines into specific cells or tissues. The strategy of using nanoparticles as a carrier system for drug and gene delivery has attracted increasing interest. The important target of many pharmaceutical delivery systems is to deliver the drug to the specific cell types and is successful only when the drug through

its delivery vehicle is internalized into cells. Owing to their small size, prolonged circulation time, and sustained drug release profile, nano-sized polymeric nanoparticles bearing drugs have received an increasing amount of attention for their ability to improve the efficacy of potent drugs. It has been reported that nano-sized drug carriers composed of natural and synthetic polymers maintain a prolonged circulation time in the body by avoiding the reticuloendothelial system (RES), as such reduced liver and spleen uptake has been exploited in cancer therapies[1].

Recently, polymer nanoparticles have been widely used as a carrier for drug delivery. Among them, much attention has been paid to the nanoparticles made of biodegradable polymers such as chitosan (CS) which has feature like good biocompatibility, biodegradability, and novel drug release behavior. Chitosan nanoparticles are potential delivery systems for vaccines, genes, and anticancer agents. It has been reported that chitosan nanoparticles are having little particle size and enhanced zeta potential. Chitosan may be a polysaccharide, same in structure to cellulose. Both are made by linear h-(1Y4)-linked monosaccharides[2-7]. However, an important difference to cellulose is that chitosan is mainly composed of 2-amino-2-deoxy-h-d-glucan combined with glycosidic linkages. The primary amine groups contribute special properties that make chitosan very useful in pharmaceutical applications. As compared to many other natural polymers, chitosan has a positive charge and is mucoadhesive. Therefore, it is used extensively in drug delivery applications. Chitosan is made from the deacetylation of chitin, a naturally occurring and abundantly available (in marine crustaceans) biocompatible polysaccharide.

However, applications of chitin are limited compared to chitosan because chitin is structurally similar to cellulose, but chemically inert. Acetamide group of chitins can be converted into amino group to give chitosan, which is carried out by treating chitin with concentrated alkali solution. Chitin and chitosan show long-chain polymers having molecular mass up to several million Daltons. Chitosan is relatively reactive and can be produced in various forms such as powder, paste, film, fiber, etc[8-11].

MOST USED SPECIES FOR IG

A search in Pub Med database for articles containing ionotropic gelation as a keyword (by March 2020) shows 559 occurrences. Of this total, 83.18% (465) correspond to articles containing chitosan or alginate (312 for chitosan, 217 for alginate, 64 for both). Together or separately, these are the most commonly used polymers in IG procedures. Other polymeric species such as gellan gum, fibrin, collagen, gelatin, hyaluronic acid, dextran, pectin and carboxymethyl cellulose have also been used[12].

Chitosan

CS is a natural polysaccharide that is obtained from alkaline deacetylation of chitin. The percentages of Ⱳ(1-4)-2-amino-2-deoxy-Ⱳ-D-glucan and Ⱳ(1-4)-2-acetamido-2-deoxy-Ⱳ-D-glucan monomers in the polymer chain determine the degree of deacetylation of CS.1 Since 2001, CS has been approved by the US Food and Drug Administration as a GRAS substance. Its biocompatibility, absence of immunogenicity, muco-adhesion and no skin irritation are among the properties that justify CS applications in biomedicine[13]. In the presence of diluted acids, CS solubility increases, and protonation of the amine groups in the polymer molecules occurs. This situation favors its electrostatic interactions with negatively charged species. A high number of polyanions have been used, but non-toxic TPP is possibly the most employed anionic cross linker to produce the gelation of CS.8 The CS: TPP ratio and the interactions between the two molecules are critical for the formation of nanoparticles/microparticles[14]. According to the work of Koukaras *et al*, smaller particles can be synthesized using a CS: TPP ratio near to 4:1 (w/w)[15]. This proportion is in agreement with the results obtained by our group for the synthesis of CS-TPP nanoparticles with sizes around 200nm.

Wu *et al* used this system for the production of nanoparticles with diameters between 10 and 500nm. They encapsulated anti-cancer agent

resveratrol and synthesized stable nanoparticles that were efficiently incorporated by a tumoral cell line[16].

Similar results were obtained by other authors in the encapsulation of insulin[17], magnetic ferrofluids[18], doxorubicin[19] and ascorbyl palmitate[20] Looking for better and reproducible results of IG formulations, the technique has been studied in terms of nanoparticle mono-dispersion[21], the conditions needed to reduce nanoparticle sizes[22] and sustained drug release kinetics[23]. The use of other anionic species interacting with CS has also been assayed. This is the case of sodium sulfate[24], our own experience with magnesium sulfate and CS to obtain nanoparticles and microparticles by IG (data not shown), and the interaction of CS with another polymer: sodium alginate[25].

Alginate

Sodium alginate (commonly alginate) is a natural polysaccharide which have the biodegradable and biocompatible character, composed of ᵾ-D-mannuronic acid and ᵾ-l-glucuronic acid[26]. Alginate is soluble in water and, in solution, forms a reticulated structure in which its anionic acid groups can react with divalent or polyvalent cations to form insoluble networks[25]. Salts of calcium and zinc have been reported for cross linking with alginate[27,28] in the synthesis of nanoparticles and microparticles. Electrostatic interactions between CS and alginate lead to the formation of CS-alginate beads. These particles, obtained by IG, have a CS core and a CS-alginate surface. Some researchers report that, in order to increase the stability of the interaction, the classic IG procedure could be complexed with the addition of a salt. This way, there could be a pre-gelation phase, between one of the polymers and a salt, such as calcium chloride, and a polyelectrolyte complexation phase, in which the second polymer is added to the reaction[29,30]. According to Patil *et al*, this two-phase IG procedure contributes to the synthesis of more stable CS-alginate particles[25]. This system has been used for the encapsulation of doxorubicin and insulin, among others[31-33]. For applications of the IG method in the encapsulation of drugs, physical cross linking between CS and alginate could be preferable. The interactions between these complex polymers give more stability to the particles than when small anionic species are used with CS. Apparently, large anionic molecules buffer the solution and prevent the hydrolysis of CS protective chains[34], although an adequate selection of the storage conditions, and the characteristics of the encapsulated molecule, may also be of influence in the long term.

PROCEDURE FOR IONOTROPIC GELATION METHOD

The use of complexation between oppositely charged macromolecules to arrange CS nanoparticles has attracted much attention because the strategy is extremely simple and mild. To avoid the possible toxicity of reagents and other undesirable effects reversible physical cross-linking by electrostatic interaction should be used. Tripolyphosphate (TPP) could also be a polyanion, which could interact with the cationic CS by electrostatic forces. After Bodmeier *et al*, demonstrated the preparation of TPP–CS complex by dropping CS droplets into a TPP solution, many researchers have investigated its pharmaceutical usage. Within the ionic gelation method, CS is dissolved in aqueous acidic solution to induce the cation of CS. This solution is then added drop wise under constant stirring to polyanionic TPP solution. The chitosan molecules have abundant NH3 group which can react with negatively charged phosphoric ions of TPP to make cross-linked chitosan nanoparticles. During the process of cross-linking and hardening process water was extruded from the particles, which can help in sustaining the discharge of drug. Three types of phenomena were observed: solution, aggregation and opalescent suspension while preparing the nanoparticles. The last stage indicates the completion of the method.

Insulin loaded CS nanoparticles are prepared by mixing insulin with TPP solution and then adding this to CS solution under constant stirring. Two sorts of CS within the variety of hydrochloride salt (Seacure R 210 Cl and Protasan R 110 Cl), varying in their relative molecular mass and degree of deacetylation, were utilized for nanoparticle preparation. For both sorts of CS, TPP

concentration was adjusted to urge a CS/TPP ratio of 3.6:1. Chitosan nanoparticles thus obtained were within the size range of 300–390 nm with a positive surface charge ranging from +34 to +45 mV. Using this method, insulin loading was modulated reaching the values up to 55%. Efficiency of the strategy was dependent upon the deacetylation of CS, since it involves the gelation of protonated amino groups of CS[1,35,36,37].

There are many ongoing investigations, which demonstrate the improved oral bioavailability of peptide and protein formulations. Bioadhesive polysaccharide CS nanoparticles would appear to further enhance their intestinal absorption. Yan *et al*. prepared the insulin-loaded CS nanoparticles by ionotropic gelation of CS with TPP anions. Particle size distribution and zeta potential were determined by photon correlation spectroscopy. The flexibility of CS nanoparticles to the pharmacological bioavailability of insulin was investigated by monitoring the plasma glucose level of alloxan-induced diabetic rats after the oral administration of various doses of insulin-loaded CS nanoparticles. The positively charged, stable CS nanoparticles showed particle size within the range of 250-400nm. Insulin association was up to 80%. The *in vitro* release experiments show initial burst effect, which is pH-sensitive. The CS nanoparticles enhanced the intestinal absorption of insulin to a greater extent than the solution of CS *in vivo*. After administration of 21 I.U/kg insulin within the CS nanoparticles, hypoglycemia was prolonged over 15 h. The common pharmacological bioavailability relative to S.C injection of insulin solution was up to 14.9%[2,4,6,8.]

STABILITY OF IONIC GELATION OBTAINED NANOPARTICLES

Drug delivery applications are the main destination of the particles obtained by IG. This method produces nanoparticles/microparticles in aqueous dispersion, which can also be dried[38] to obtain powder formulations. Both presentations require stability of the carrier, which depends on factors such as the surface electrostatic charge and the pH of the solution, which can be altered during long-time storage[39]. The ionic strength, reagent concentrations and the anionic-cationic species ratio employed in the particle preparation directly affect the stability of the particles[40]. The use of nonionic stabilizers during the synthesis of the particles, added to one of the ionic species of the reaction, and also vacuum freeze-drying of the nanoparticles/microparticles, are to be considered effective factors against charge and zeta potential variability due to long-term storage[32]. For preparations in dispersion, storage temperatures around 4°C are preferable over 25°C. Some polymeric particles can be thermo-responsive and suffer a pH variation at 25°C. This could lead to a reduced stability and to the loss of other important properties, e.g. the antimicrobial character[41].

It is also important to consider that particles loaded with macromolecules or drugs can be more stable than polymer-salt beads, because of stronger interactions between the drugs and the gel network[42]. On the other hand, when storing polymer-based particles in powder form, their sensitivity to hydrolysis caused by environmental humidity must be prevented. The resistance of the specific polymer, and drug, to the freeze-drying process should also be considered.

ADVANTAGES OF IONOTROPIC GELATION METHOD[1,5,7]

- The method is very economic and simple
- The method requires less equipment and time
- To avoid the possible toxicity of reagents and other undesirable effects reversible physical cross-linking by electrostatic interaction is used.
- No use of organic solvent.

DISADVANTAGES OF IONOTROPIC GELATION METHOD[7]

- The only disadvantage of TPP/CS nanoparticles is their poor mechanical strength.

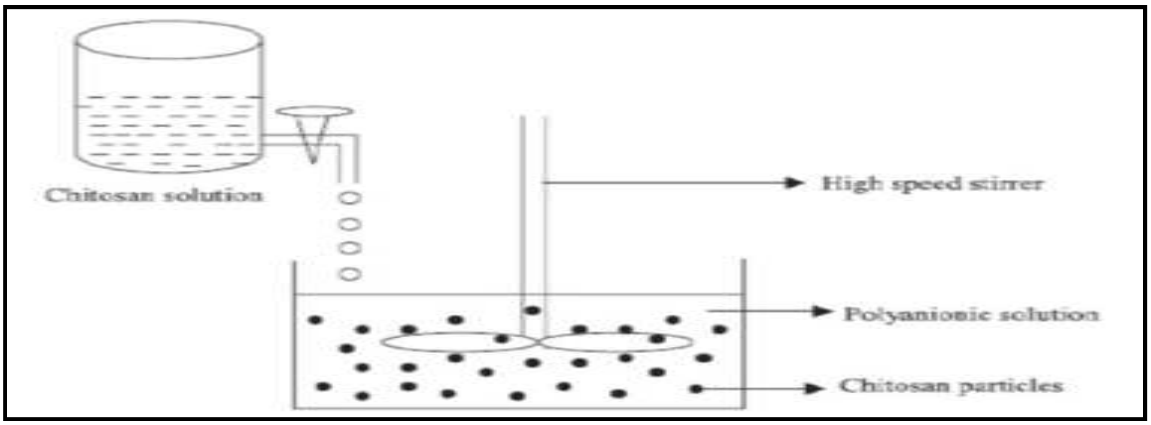

Figure No.1: Schematic representation of preparation of chitosan nanoparticles by ionotropic gelation method

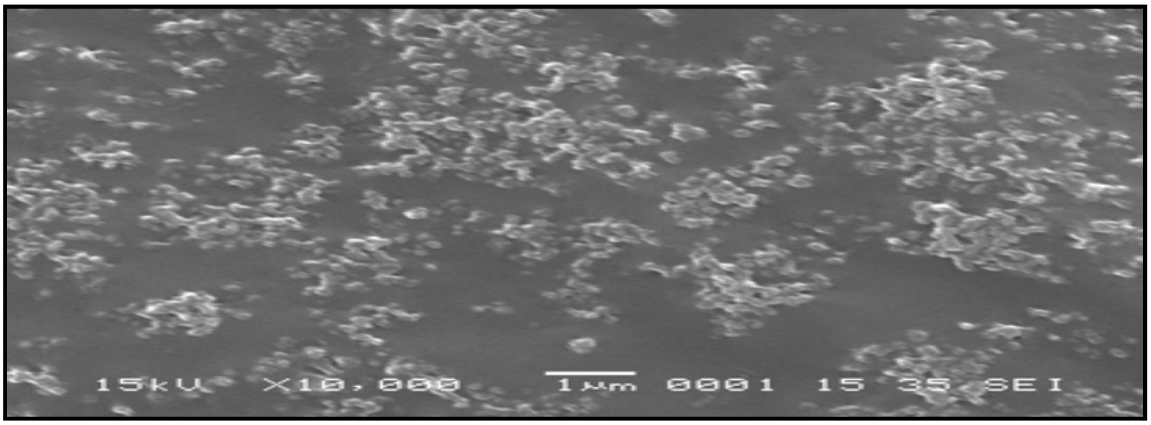

Figure No.2: Chitosan nanoparticles prepared by ionotropic gelation method

CONCLUSION

The advantages of nanoparticles and microparticles obtained by IG position this method amongst those for election for biological uses. Biocompatible polymers and reagents guarantee a preliminary interest in the formulation that must be confirmed with *in vitro* and *in vivo* experiments. The characterization of the particles in terms of size, shape, zeta potential and drug release provide an accurate knowledge of the system and allows its long-term behaviour to be estimated. Higher biological specificity of the formulation is sometimes desirable to ensure correct drug targeting. This can be obtained by a site-specific functionalization of the particles, after the synthesis, or using a previously modified polymer. Commonly applied strategies involve the activation of carboxyl groups in the polymeric chains and their interaction with amine groups of proteins (e.g. antibodies)[43].

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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