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(Review Article)

Chitosan Nanospheres As Potential Carrier Delivery of Pharmaceutical API's

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ABSTRACT

Chitosan is a biodegradable natural polymer. Chitosan nanospheres have gained more attention as drug delivery carriers because of their better stability, low toxicity, simple and mild preparation method, and providing versatile routes of administration. Chitosan nanospheres are used to provide controlled release of many drugs, improve the dissolution of poorly soluble drugs and to improve the bioavailability of degradable substances such as protein or enhance the uptake of hydrophilic substances across the epithelial layers. Their sub-micron size is not only suitable for parenteral application, but also applicable for mucosal routes of administration, i.e., oral, nasal, and ocular mucosa, which are non-invasive routes. This review is an insight into the exploitation of the various properties of chitosan to encapsulate drug. Various techniques used for preparing chitosan nanospheres and evaluation of these nanospheres and different categories of drug used as chitosan nanospheres have also been reviewed. Chitosan is a versatile polymer with wide applications range which is being used widely in marketed formulation potentially.

Key Words: Chitosan, Nanospheres, Drug delivery carriers, Controlled release of drug, Cross-linking.

INTRODUCTION

The efficacy of many drugs is often limited by their potential to reach the site of therapeutic action. In most cases (conventional dosage forms), only a small amount of administered dose reaches the target site, while the majority of the drug distributes throughout the rest of the body in accordance with its physicochemical and biochemical properties. Therefore, developing a drug delivery system that optimizes the pharmaceutical action of a drug while reducing its toxic side effects in vivo is a challenging task. One approach is the use of colloidal drug carriers that can provide site or targeted drug delivery combined with optimal drug release profiles. The submicron drug delivery system for drug targeting was developed. Polymeric nanoparticles, which possess a better reproducibility and stability profiles than other carrier like (microparticle, liposome,) have been proposed as alternative drug carriers that overcome many of these problems. Nanoparticles are solid colloidal particles with diameters ranging from 1-1000 nm. Polymers used to form nanoparticles can be both synthetic and natural polymers¹.

There are two types of nanoparticles depending on the preparation process:

Nanospheres and nanocapsules.

Nanospheres have a monolithic-type structure (matrix) in which drugs are dispersed or adsorbed onto their surfaces.

Nanocapsules exhibit a membrane-wall structure and drugs are entrapped in the core or adsorbed onto their exteriors (fig.1)².

CHITOSAN

Chitosan is a modified natural carbohydrate polymer prepared by the partial N-deacetylation of chitin, a natural biopolymer derived from crustacean shells such as crabs, shrimps and lobsters. Chitosan is also found in some microorganisms, yeast and fungi³. The primary unit in the chitin polymer is 2-deoxy-2-(acetyl-amino) glucose. These units combined by β - (1, 4) glycosidic linkages, forming a long chain linear polymer (fig.2). Although chitin is insoluble in most solvents, chitosan is soluble in most organic acidic solutions at pH less than 6.5 including formic, acetic, tartaric, and citric acid⁴.

Commercially, chitosan is available in the form of dry flakes, solution and fine powder. It has an average molecular weight ranging between 3800 and 2,000,000 and is from 66 to 95% deacetylated. Particle size, density, viscosity, degree of deacetylation, and molecular weight are important characteristics of chitosan which influence the properties of pharmaceutical formulations based on chitosan. Chitosan has been shown to possess mucoadhesive properties due to molecular attractive forces formed by electrostatic interaction between positively charged chitosan and negatively charged mucosal surfaces. These properties may be attributed to:

- (a) Strong hydrogen bonding groups like -OH, -COOH
- (b) Strong charges
- (c) High molecular weight
- (d) Sufficient chain flexibility and

(e) Surface energy properties favouring spreading into mucus.

Properties such as biodegradability, low toxicity and good biocompatibility make it suitable for use in biomedical and pharmaceutical formulations, e.g. it is used for hypobilirubinaemic and hypocholesterolemic effects, antacid and antiulcer activities, wound and burn healing properties immobilization of enzymes and living cell and in ophthalmology. Among pharmaceutical applications it has been used as a vehicle for directly compressed tablets, as a disintegrant, as a binder, as a granulating agent, in ground mixtures, as a drug carrier for sustained release preparations as well as a co-grinding diluent for the enhancement of dissolution rate and bioavailability of water insoluble drugs⁵.

CHITOSAN NANOSPHERES

The use of nanospheres-based therapy allows drug release to be carefully used to the specific treatment site through the choice and formulation of various drug-polymer combinations. The total dose of medication and the kinetics of release are the variables, which can be manipulated to achieve the desired result. Using innovative encapsulation technologies and by varying the copolymer ratio, molecular weight of the polymer, etc., nanospheres can be developed into an optimal drug delivery system which will provide the desired release profile. Nanospheres based systems may increase the life span of active constituents and control the release of bioactive agents. Being small in size, nanospheres have large surface to volume ratios and can be used for controlled release of insoluble drugs. Extensive research is being carried out to exploit chitosan as a drug carrier to attain the desirable drug release profile. Nanospheres made up of biodegradable carrier such as chitosan have advantage of providing steric interference in the systemic circulation³. Chitosan nanospheres are used to provide controlled release of many drugs and to improve the bioavailability of degradable substances such as protein or enhance the uptake of hydrophilic substances across the epithelial layers. These nanospheres are being investigated both for parenteral and oral drug delivery⁵.

Nanospheres prepared from water-insoluble polymers are involved heat, organic solvent or high shear force that can be harmful to the drug stability. Moreover, some preparation methods such as emulsion polymerization and solvent evaporation are complex and require a number of preparation steps that are more time and energy consuming. In contrast, water-soluble polymers offer mild and simple preparation methods without the use of organic solvent¹. Among water-soluble polymers available, chitosan is one of the most extensively studied. This is because chitosan possesses some ideal properties of polymeric carriers for nanospheres such as biocompatible, biodegradable, nontoxic, and inexpensive. Furthermore, it possesses positive charge and exhibits absorption enhancing effect. These properties render chitosan a very attractive material as a drug delivery carrier^{1,5}.

PREPARATION OF CHITOSAN NANOSPHERES

Reacting chitosan with controlled amounts of multivalent anion results in crosslinking between chitosan molecules. The crosslinking may be achieved in acidic, neutral or basic environments depending on the method applied. This

crosslinking has been extensively used for the preparation of chitosan nanospheres.

Ionotropic Gelation

The mechanism of chitosan nanospheres formation is based on electrostatic interaction between amine group of chitosan and negatively charged group of polyanion such as tripolyphosphate. This technique offers a simple and mild preparation method in the aqueous environment. First, chitosan can be dissolved in acetic acid in the absence or presence of stabilizing agent, such as poloxamer, which can be added in the chitosan solution before or after the addition of polyanion. Polyanion or anionic polymers were then added and nanospheres were spontaneously formed under mechanical stirring at room temperature (fig.3). The size and surface charge of particles can be modified by varying the ratio of chitosan and stabilizer⁶.

Premix Membrane Emulsification Technique

The chitosan aqueous solution was used as a dispersed phase, and the mixture of liquid paraffin and petroleum ether containing emulsifier was used as a continuous phase. The coarse emulsions were first prepared by low-speed stator homogenization and then poured into the premix reservoir. Nanodroplets were achieved by extruding the coarse emulsions through the SPG (Shirasu porous glass) membrane with a high pressure. The nanodroplets were further cross-linked to obtain chitosan nanospheres. The results showed that the chitosan nanospheres from 300 nm to 1.85 μm were successfully prepared by premix membrane emulsification by changing the pore size of the membrane and the polydispersity index could be as low as 0.027 under optimized conditions and it is a potential technique to prepare size-controllable uniform chitosan nanospheres with fast production^{7,8}.

Microemulsion Method

This technique is based on formation of chitosan nanospheres in the aqueous core of reverse micellar droplets and subsequently cross-linked through glutaraldehyde. In this method, a surfactant was dissolved in n-Hexane. Then, chitosan in acetic solution and glutaraldehyde were added to surfactant/hexane mixture under continuous stirring at room temperature. Nanospheres were formed in the presence of surfactant. The system was stirred overnight to complete the cross-linking process, which the free amine group of chitosan conjugates with glutaraldehyde. The organic solvent is then removed by evaporation under low pressure. The yields obtained were the cross-linked chitosan nanospheres and excess surfactant. The excess surfactant was then removed by precipitate with CaCl_2 and then the precipitant was removed by centrifugation. The final nanospheres suspension was dialyzed before lyophilization. This technique offers a narrow size distribution of less than 100 nm and the particle size can be controlled by varying the amount of glutaraldehyde that alters the degree of cross-linking. Nevertheless, some disadvantages exist such as the use of organic solvent, time-consuming preparation process, and complexity in the washing step^{1,9}.

Emulsification Solvent Diffusion Method

This method is based on the partial miscibility of an organic solvent with water. An o/w emulsion is obtained upon injection of an organic phase into chitosan solution containing

a stabilizing agent (i.e. poloxamer) under mechanical stirring, follow by high pressure homogenization. The emulsion is then diluted with a large amount of water to overcome organic solvent miscibility in water. Polymer precipitation occurs as a result of the diffusion of organic solvent into water, leading to the formation of nanospheres. This method is suitable for hydrophobic drug and showed high percentage of drug entrapment. The major drawbacks of this method include harsh processing conditions (e.g., the use of organic solvents) and the high shear forces used during nanospheres preparation¹⁰.

Polyelectrolyte Complex (PEC)

Polyelectrolyte complex or self-assemble polyelectrolyte is a term to describe complexes formed by self-assembly of the cationic charged polymer and plasmid DNA. Mechanism of PEC formation involves charge neutralization between cationic polymer and DNA leading to a fall in hydrophobicity as the polyelectrolyte component self-assembles. Several cationic polymers (i.e. gelatine, polyethylenimine) also possess this property. Generally, this technique offers simple and mild preparation method without harsh conditions involved. The nanospheres spontaneously formed after addition of DNA solution into chitosan dissolved in acetic acid solution, under mechanical stirring at or under room temperature¹¹. The complexes size can be varied from 50 nm to 700 nm.

Reverse Micellar Method

Reverse micelles are thermodynamically stable liquid mixtures of water, oil and surfactant. Macroscopically, they are homogeneous and isotropic, structured on a microscopic scale into aqueous and oil microdomains separated by surfactant-rich films. One of the most important aspects of reverse micelle hosted systems is their dynamic behaviour. Preparation of ultrafine polymeric nanoparticles with narrow size distribution could be achieved by using reverse micellar medium¹². Aqueous core of the reverse micellar droplets can be used as a Nano reactor to prepare such particles. Since the size of the reverse micellar droplets usually lies between 1 and 10 nm¹³. These droplets are highly monodispersed; preparation of drug-loaded nanospheres in reverse micelles will produce extremely fine particles with a narrow size distribution. The size polydispersity and thermodynamic stability of these droplets are maintained in the 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 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 micro emulsion phase. Additional amount of water may be added to obtain nanospheres of larger size. To this transparent solution, a cross-linking agent is added with constant, and cross-linking is achieved by 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 microemulsion is transformed into a translucent solution. The organic solvent is then evaporated to obtain the transparent dry mass. The material is dispersed in water and then adding a suitable salt precipitates the surfactant out. The mixture is then subjected to centrifugation. The supernatant solution is decanted,

which contains the drug-loaded nanospheres. The aqueous dispersion is immediately dialyzed through dialysis membrane for about 1 h and the liquid is lyophilized to dry powder (Fig.4).

Emulsion-Droplet Coalescence Method

The novel emulsion-droplet coalescence method, which utilizes the principles of both emulsion cross-linking and precipitation. However, in this method, instead of cross-linking the stable droplets, precipitation is induced by allowing coalescence of Chitosan droplets with NaOH droplets. First, a stable emulsion containing aqueous solution of Chitosan along with drug is produced in liquid paraffin oil and then, another stable emulsion containing Chitosan aqueous solution of NaOH is produced in the same manner. When both emulsions are mixed under high-speed stirring, droplets of each emulsion would collide at random and coalesce, thereby precipitating Chitosan droplets to give small size particles of chitosan nanospheres (Fig.5)¹⁴.

EVALUATION OF CHITOSAN NANOSPHERE

Estimation of Amount of Drug Incorporated to Chitosan Nanospheres

A known quantity of drug loaded nanospheres from each batch is dispersed in 100 ml of normal saline and sonicated for half an hour. The solution is centrifuged at 10000rpm for 15 mins, and the absorbance is determined using UV spectrophotometer with plain chitosan nanospheres as reagent blank¹⁵.

Determination of Particle Size

An aqueous dispersion of the nanospheres is finely spread over a gold-coated stub and is dried by keeping in a desiccator. The dried film of the nanospheres is given a 25 nm thick gold layer and is observed by Scanning Electron Microscope (Fig.6). The sizes of minimum of 50 particles are checked for their size distribution to determine the average particle size and size range¹⁶.

Study on In-vitro Drug Release

A quantity of nanospheres of drug is taken in a 250ml conical flask and to it 100ml of suitable buffer is added. Then the flask is kept in a shaker cum incubator and shaker is adjusted to 40-50 horizontal stokes / min at 37°C. 2ml of drug release medium is withdrawn at various time intervals while replacing it with fresh 2ml of normal saline. The samples are centrifuged and filtered. From the filtrate 1ml of the sample is withdrawn and diluted to 10 ml with normal saline and the drug content is analysed by UV Spectrophotometer¹⁵.

In-vivo Biodistribution Studies

In vivo distribution studies are carried out using Wistar rats weighing 100 to 150 g and are divided into 3 groups containing 3 animals each. The three groups namely group I are treated with free drug, group II are treated with drug loaded nanospheres and group III treated with solvent control. On the first day the mice of group I are treated with free drug of 3.6mg/200g of rats through intravenous route. The similar concentration of drug loaded nanospheres is administered to group II and suitable buffer as solvent control for group III. After 18h of injection, animals are sacrificed, then blood was taken and plasma is separated out,

and also different organs like liver, lung, kidney and spleen are extracted out and homogenized in suitable buffer saline followed by centrifugation. Supernatant of the homogenized tissue is analysed by HPLC to estimate the bio distribution of the drug administered¹⁸.

Zeta Potential

The zeta potential of the nanospheres reflecting their charge is measured with Brookhaven's Zeta Plus apparatus (Brookhaven Instruments Corporation). The electrophoretic mobility of drug loaded chitosan nanospheres, in suitable buffer is determined. The zeta potential is calculated using the Huckel Approximation for small particles in low dielectric constant medium¹⁹.

Determination of Kinetics of Drug Release

In order to predict and correlate the release behaviour of the drug from the polymer matrix it is necessary to fit in vitro release data in to a suitable model. Hence the dissolution data are fitted according to the well-known exponential equation, which is often used to describe the drug release behaviour from polymeric system. The equation, which is used to describe drug release mechanism is:

$$m_t / m_\infty = k t^n$$

Where m_t / m_∞ is the fraction release of the drug, 't' is the release time, 'k' is the constant. Which indicates the properties of the macromolecular polymer system, and 'n' is the release exponent indicative of the mechanism of release. The 'n' value is used for the analysis of drug release mechanism from the drug-loaded nanospheres²⁰.

DIFFERENT CATEGORIES OF DRUG USED AS CHITOSAN NANOSPHERES

Nanospheres prepared using chitosan are being extensively investigated for various classes of drugs. The findings of these research studies are summarized in the following sections.

Anticancer Drugs

Fluorouracil (5-FU)

Chitosan nanospheres were prepared by modifying the reverse micelle medium. 400 μ l of 0.1% w/v chitosan solution dissolved in acetic acid was added to 40 ml of 0.04 M sodium bis (2-ethylhexyl) sulfosuccinate (AOT) solution in n-hexane with continuous stirring at room temperature. Evaporated the solvent off in a rotary evaporator and dry the mass in 20 ml of Tris-HCl buffer (pH 8.0) by sonication. Added 4 ml of 30% CaCl_2 solution to precipitate the surfactant as calcium salt of diethylhexylsulfosuccinate. The precipitate was pelleted by centrifugation at 6000 rpm for 15 min at 4 °C. The cake of Ca was dissolved in 10 ml n-hexane and washed two to three times with 1 ml of Tris-HCl buffer. The phase-separated aqueous layer was drained out and centrifuged. Dialyzed the total aqueous dispersion of nanospheres and lyophilized. Reverse micellar method used to prepare F-Chitosan nanospheres. 200 μ l of 5-Fluorouracil (10 mg/ml) added after addition of chitosan solution in 6% v/v acetic acid¹⁷.

Cytarabine

Cytarabine nanospheres were prepared by ionic gelation method with an objective of improving its intracellular

targeting and thereby targeting the cancer cells. To 3ml of 0.4% chitosan gel, Cytarabine 0.50mg/ml was added and stirred and then 1.2ml (0.5%w/v) of Tripolyphosphate solution which is the cross linking agent was added. Chitosan Nanospheres formed spontaneously upon addition of aqueous Tripolyphosphate solution to chitosan solution under high speed rate of 3000 rpm using high speed stirrer for 1hr. The resulting chitosan particle suspension were subsequently centrifuged four times for 15 minutes cycles at 10,000 rpm and washed with distilled water and freeze dried. The chitosan molecules has abundant NH_3 group which can react with negatively charged phosphoric ions of TPP to form cross-linked chitosan nanospheres. During the process of cross-linking and hardening process water was extruded from the particles, which may help in sustaining the release of drug. The particle size of the nanospheres was determined using Scanning Electron Microscopy (SEM) and the average particle size for drug loaded nanoparticles was found to be 466.45 ± 5.32 nm. Cytarabine is being released is a non-Fickian anomalous diffusion mechanism, which is drug release during dissolution test is controlled by all diffusion, erosion and swelling mechanism¹⁵.

Mitoxantrone-loaded BSA

Bovine serum albumin (BSA) and chitosan (CS) nanospheres of Mitoxantrone (MTO) were comparatively evaluated in terms of tissue distribution, acute toxicity and therapeutic efficiency against breast cancer and its lymph node metastases. After local injection in rats, MTO nanospheres showed a slower elimination rate and a much higher drug concentration in lymph nodes compared with MTO solution, and a lower drug concentration in other tissues. There was no observed acute toxicity to the main tissues of Kunming mice after local injection of MTO-BSA-NS. Mild toxicity to liver and lung was observed for MTO-CS-NS, but, for MTO solution, severe toxicity to liver and lung and much lower number of white blood cells were observed. Human MCF-7 breast cancer in nude mice and animal model of P388 lymph node metastases in Kunming mice were applied to investigate the therapeutic efficiency. The inhibition rate of the nanospheres against breast cancer was much higher than that of MTO solution, and lymph node metastases were efficiently inhibited by the nanospheres, especially MTO-BSA-NS²¹.

Proteins

Insulin

The Insulin-loaded chitosan nanospheres were evaluated in vivo in rats in order to investigate its potential to transport insulin, a model protein, to the deep lung, where it is absorbed into systemic circulation. This was prepared by ionotropic gelation and characterized for morphology, size, zeta potential, and association efficiency and loading capacity. Afterwards, the Nanospheres were co-spray dried with mannitol resulting in a dry powder with adequate aerodynamic properties for deposition in deep lungs. The assessment of the plasmatic glucose levels following intratracheal administration to rats revealed that the Insulin-loaded chitosan nanospheres induced a more pronounced and prolonged hypoglycaemic effect compared to the controls²².

Antiviral

Acyclovir

Acyclovir loaded chitosan nanospheres were prepared by ionic gelation of chitosan solution with sodium Tripolyphosphate (0.25%) prepared in the presence of Tween 80 (0.5%) as a re-suspending agent to prevent aggregation, at ambient temperature while stirring. Compatibility study of drug with the polymer was determined by FTIR Spectroscopy. The results of the DSC thermogram suggested that there was no chemical interaction between acyclovir and chitosan. The size of the nanospheres was analysed by Transmission electron microscopy (TEM). The Encapsulation efficiency and loading capacity of the nanospheres weredetermined by the separation of nanospheres from the aqueous medium containing non associated acyclovir by cold centrifugation at 12000g for 30 minutes. The amount of free acyclovir in the supernatantwas measured by UV method at 253 nm.The release profile of acyclovir from nanospheres has shown a slow controlled release following zero order kinetic with non Fickian mechanism. The results demonstrated the effective use of acyclovir loaded chitosan nanospheres as a controlled release preparation for treatment of ocular viral infections²³.

Others

A/H1n1 Influenza Vaccine

Chitosan nanospheres prepared by ionic gelation method. 0.9 mL chitosan nanospheres (0.5 mg/ml) with different molecular weight (20 kDa, 30 kDa and 300 kDa) were mixtured 0.1 ml (3Rg) H1N1 antigen containing 128 antigens for 30 min at room temperature. Then, the mixture was centrifuged at 12,000 rpm, 4oC for 10 min. The amount of free antigen (HA) in the supernatant was determined by hem agglutinin assay. Loading efficiency and loading capacity of chitosan nanoparticles were calculated according to following formulas:

$$LE \text{ (Loading efficiency)} = \frac{\text{Total HA} - \text{Free HA}}{\text{Total Antigen}} \times 100\%$$

$$LC \text{ (loading capacity)} = \frac{\text{Total HA} - \text{Free HA}}{1 \text{ mg Chitosan nanospheres dry weight}}^{24}$$

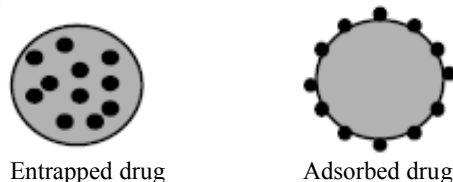
Isoniazid

First line antitubercular drug, isoniazid was loaded in chitosan Nanospheres in order to enhance bioavailability and to reduce dose frequency. Chitosan was dissolved in acetic acid aqueous solution at various concentrations; Drug was dispersed in above Chitosan solution kept over magnetic stirrer at room temperature for a period of 30 minute. The Tripolyphosphate aqueous solution with various concentrations added drop wise to the above solution. Followed by sonication for 5 min. The resulting Chitosan nanoparticles suspension was centrifuged at 16,000 rpm for 30 min. After freeze drying the Nanoparticles were collected. Zeta potential shows good positive potentials. It shows good encapsulation efficiency. And good release profile follows first order release kinetics. In this study, we have encapsulated Isoniazid which has not been formulated in a drug delivery system yet. Moreover, this work can be considered as the first step for further studies on the application of Isoniazid loaded-chitosan nanospheres and a promising step for the possible targeted delivery to the Lungs¹⁹.

CONCLUSION

Chitosan nanosphere was found to be asuitable and potential natural carrier in terms of their particle size, drug loading capacity, invitro release characteristics and invivo bio distribution study. Chitosan nanospheres are used to provide controlled release of many drugs, improves the dissolution of poorly soluble drugs and to improve the bioavailability of degradable substances such as protein or enhance the uptake of hydrophilic substances across the epithelial layers. Hence it may be used as an alternative and cheaper carrier in therapy for reduction dose and reducing dose related systemic toxicities, in turn reduced the cost of therapy.

Nanospheres



Nanocapsules

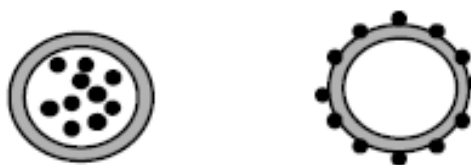


Fig. 1: Various types of drug loaded nanoparticles

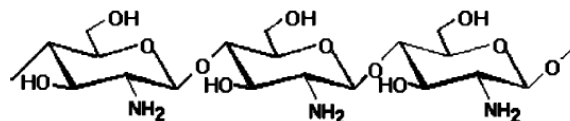


Fig. 2: Structure of chitosan.

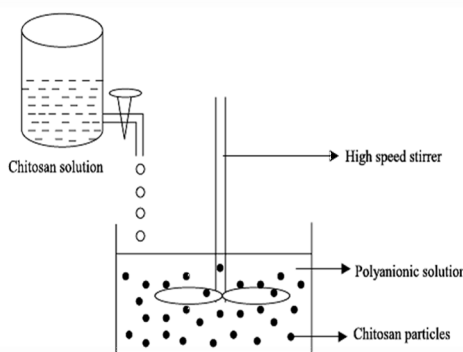


Fig.3: Schematic representation of preparation of chitosan nanospheres by ionic gelation method.

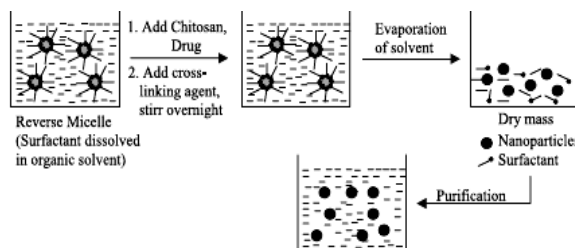


Fig.4: Schematic representation of preparation of chitosan nanosphere by reverse micellar method.

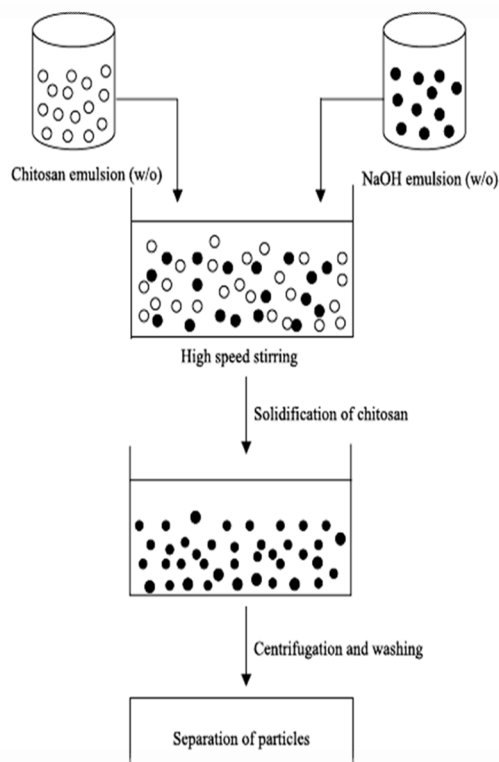


Fig. 5: Schematic representation of preparation of chitosan Nanosphere by emulsion-droplet coalescence method.

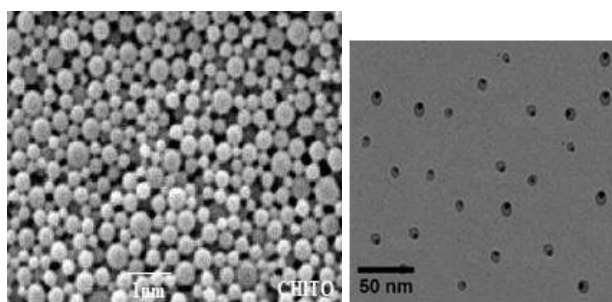


Fig.6: SEM of Drug Loaded nanospheres ¹⁷

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