DIFFERENT TECHNIQUES USED FOR THE PREPARATION OF NANOPARTICLES USING NATURAL POLYMERS AND THEIR APPLICATION

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ABSTRACT

Over the past three decades, there has been a considerable research interest in the area of developing drug delivery using nanoparticles (NPs) as carriers for small and large molecules. Targeting delivery of drugs to the diseased lesions is one of the most important aspects of drug delivery system. They have been used *in vivo* to protect the drug entity in the systemic circulation, restrict access of the drug to the chosen sites and to deliver the drug at a controlled and sustained rate to the site of action. Various polymers have been used in the formulation of nanoparticles for drug delivery research to increase therapeutic benefit, while minimizing side effects. In order to see functionality and toxicity of nanoparticles in various food and drug applications, it is important to establish procedures to prepare nanoparticles of a controlled size.Natural polymers have been classified into polysaccharides and proteins. Chitosan is a natural polymer obtained by deacetylation of chitin.After cellulose chitin is the second most abundant polysaccharide in nature.It is biologically safe,non toxic,biocompatible and biodegradable polysaccharide.Chitosan nanoparticles 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. Alginate nanoparticles is a anionic polysaccharide taht it has been widely used in drug delivery. Its merits for making particles of less than 100 nm for gene delivery. The benefits of protein nanoparticles are: nontoxicity, stability for long duration, non-antigen also posse biodegradability (Muller et al., 1996; Weber et al., 2000). In fact protein is biopolymer, which is commonly used for preparation of nano structured molecules for drug delivery. In this articles various techniques have been discussed in detail for the preparation of nanoparticles using natural polymers chitosan,alginate and proteins such as albumin,gelatin,legumin.

Keywords: Nanoparticles, Deacetylation, Alginate nanoparticles.

INTRODUCTION

Nanoparticles are sub nanosized colloidal structures composed of synthetic or semisynthetic polymers.Nanospheres are solid core spherical particulates which are nanometric in size.They contain drug embeded within the matrix or adsorbed on to the surface.Nanocapsules are vesicular system in which drug is essentially encapsulated with in the central volume surrounded by an embryonic polymeric sheath.In nanocrystals drug is mainly encapsulated in the solution system.[1]

Natural hydrophilic polymers:-.Natural hydrophilic polymers are studied because of their intrinsic biodegradability and biocompatibility.Natural polymers are classified as proteins and polysaccharides.Proteins are gelatin, albumin,lecitin,legumin and vicillin.Polysaccharides are alginate,dextran, chitosan and pollulan.[2]

Preparation methods and applications of chitosan nanoparticles

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 fungi. The primary unit in the chitin polymer is 2-deoxy-2-(acetylamino) glucose. These units combined by β-(1,4)glycosidic linkages, forming a long chain linear polymer. 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 acid. It is insoluble in phosphoric and sulfuric acid. Chitosan is available in a wide range of molecular weight and degree of deacetylation . Molecular weight and degree of deacetylation are the main factors affecting the particle size, particles formation and aggregation.[3,4]

SPECIFICATIONS & CHARACTERISTICS OF PHARMACEUTICAL-GRADE CHITOSAN

The pharmaceutical requirements for chitosan include: a white or yellow appearance (powder or flake), particle size < 30 m, density between 1.35 and 1.40 g/cm3, a pH of 6.5 to 7.5, moisture content < 10%, residue on ignition <0.2%, protein content <0.3%, degree of deacetylation 70% to 100%, viscosity <5 cps, insoluble matter <1%, heavy metals (As) <10 ppm, heavy metals (P**b) <10 ppm,** and no taste and smell.[5,6]

Ionotropic gelation

Chitosan NP prepared by ionotropic gelation technique was first reported by Calvo and has been widely examined and developed by Janes. The mechanism of chitosan NP formation is based on electrostatic interaction between amine group of chitosan and negatively charge 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 was then added and nanoparticles were spontaneously formed under mechanical stirring at room temperature. The size and surface charge of particles can be modified by varying the ratio of chitosan and stabilizer.

Microemulsion method

Chitosan NP prepared by microemulsion technique was first developed by Maitra .This technique is based on formation of chitosan NP 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. Nanoparticles 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 conjugate with glutaraldehyde. The organic solvent is then removed by evaporation under low pressure. The yields obtained were the cross-linked chitosan NP and excess surfactant. The excess surfactant was then removed by precipitate with CaCl2 and then the precipitant was removed by centrifugation. The final nanoparticles suspension was dialyzed before lyophilyzation. 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 alter 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.
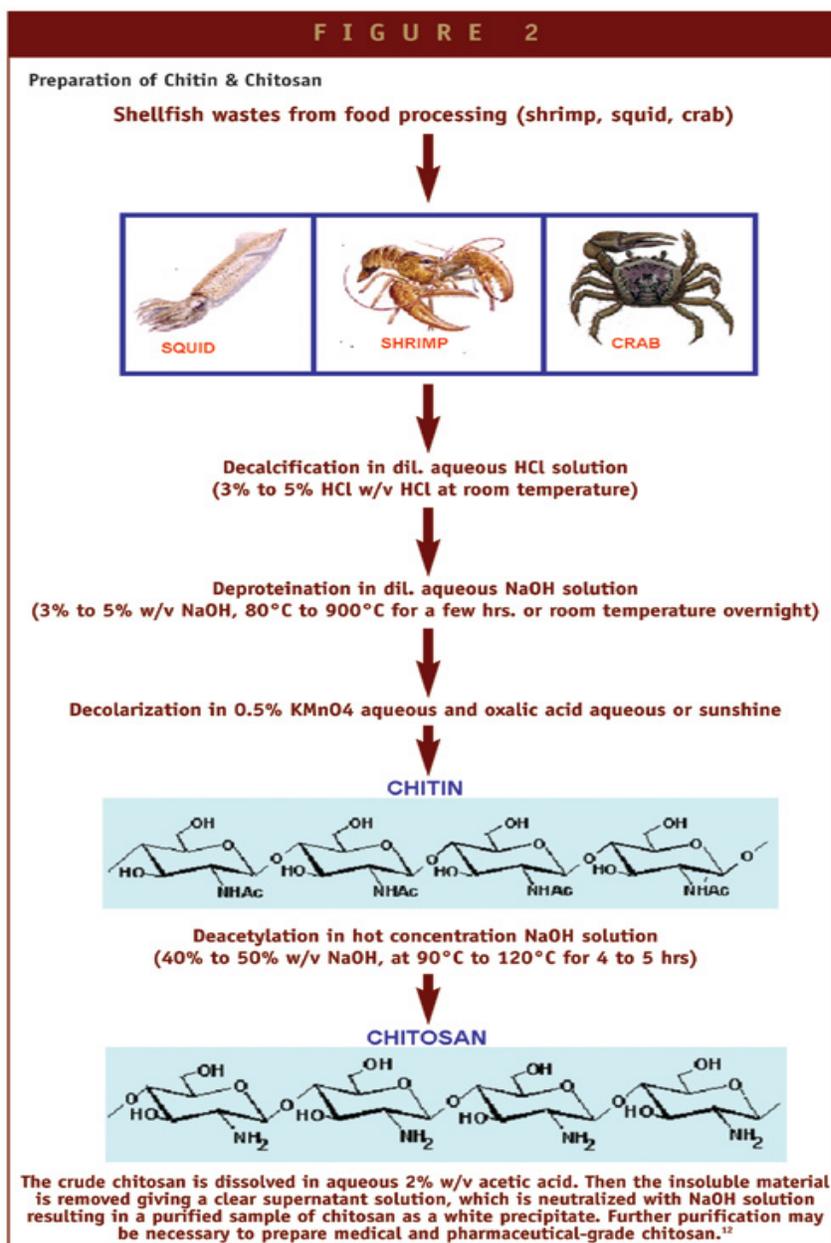
Emulsification solvent diffusion method

El-Shabouri reported chitosan NP prepared by emulsion solvent diffusion method, which originally developed by Niwa *et al.*employing PLGA .This method is based on the partial miscibility of an organic solvent with water. An o/w emulsion is obtained upon injection an organic phase into chitosan solution containing a stabilizing agent (i.e. poloxamer) under mechanical stirring, followed 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 nanoparticles. 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 nanoparticle preparation.

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 hydrophilicity.Several cationic polymers (i.e. gelatin, polyethylenimine) also possess this property. Generally, this technique offers simple and mild preparation method without harsh conditions involved. The nanoparticles spontaneously formed after addition of DNA solution into chitosan dissolved in acetic acid solution, under mechanical stirring at or under room temperature. The complexes size can be varied from 50 nm to 700 nm.

FIGURE 2

Preparation of Chitin & Chitosan

Shellfish wastes from food processing (shrimp, squid, crab)

SQUID SHRIMP CRAB

Decalcification in dil. aqueous HCl solution
(3% to 5% HCl w/v HCl at room temperature)

Deproteination in dil. aqueous NaOH solution
(3% to 5% w/v NaOH, 80°C to 900°C for a few hrs. or room temperature overnight)

Decolarization in 0.5% KMnO4 aqueous and oxalic acid aqueous or sunshine

CHITIN

Deacetylation in hot concentration NaOH solution
(40% to 50% w/v NaOH, at 90°C to 120°C for 4 to 5 hrs)

CHITOSAN

The crude chitosan is dissolved in aqueous 2% w/v acetic acid. Then the insoluble material is removed giving a clear supernatant solution, which is neutralized with NaOH solution resulting in a purified sample of chitosan as a white precipitate. Further purification may be necessary to prepare medical and pharmaceutical-grade chitosan.[12]

Applications of chitosan nanoparticles

Parenteral administration:- Following intravenous injection, many nanoparticle systems including chitosan NP exhibited a marked tendency to accumulate in a number of tumors. One possible reason for the phenomenon may involve the leakiness of tumor vasculature. Doxorubicin loaded chitosan NP showed regression in tumor growth and enhance survival rate of tumor-implanted rats after IV administration. In addition, chitosan NP less than 100 nm in size have been developed which showed to be RES evading and circulate in the blood for considerable amount of time.Delivery of antiinfectives such as antibacterial, antiviral, antifungal and antiparasitic drugs, is another common use of nanoparticles. The low therapeutic index of antifungal drugs, short half-life of antivirals and the limited ability of antibiotics to penetrate infected cells in intracellular compartments make them ideal candidates for nanoparticle delivery. Thus, it has been suggested that nanoparticles should improve the therapeutic efficacy while decreasing the toxic side effects of these drugs. In theory, chitosan NP are very attractive carrier system for these drugs as they offer many advantages such as hydrophilic surface particles, nano-size of less than 100 nm. However, to the best of my knowledge, chitosan NP as a tool to deliver these drugs have not yet been examined.[7]

Peroral administration:- The idea that nanoparticles might protect labile drugs from enzymatic degradation in the gastrointestinal tract (GIT) leads to the development of nanoparticles as oral delivery systems for macromolecules, proteins and polynucleotides.Among polymeric nanoparticles, chitosan NP showed to be attractive carriers for oral delivery vehicle as they promote absorption of drug. The mucoadhesive properties of chitosan are due to an interaction between positively charged chitosan and negatively charge of mucin which provide a prolonged contact time between the drug and the absorptive surface, and thereby promoting the absorption. Chitosan mucoadhesion is also supported by the evidence that chitosan increases significantly the half time of its clearance. Furthermore, *in vitro* studies in Caco-2 cells have shown that chitosan is able to induce a transient opening of tight junctions thus increasing membrane permeability particularly to polar drugs, including peptides and proteins. [8]

Non-viral gene delivery vectors:-Chitosan is a cationic polymer with extremely low toxicity. It showed significantly lower toxicity than poly-L-lysine and PEI. Additionally, it enhances the transport of drug across cell membrane as discussed earlier. Chitosan as a promising gene delivery vector was first proposed by Mumper. Chitosan mediates efficient *in vitro* gene transfer at nitrogen to phosphate (N/P) ratio of 3 and 5. At these ratios, small chitosan-DNA complexes can be prepared in the range of 50-100 nm with a positively surface charge of approximately +30 mV. Sato *et al.* found that *in vitro* chitosan-mediated transfection depends on the cell type, serum concentration, pH and molecular weight of chitosan. Hela cells were efficiently transfected by this system even in the presence of 10% serum. In contrast, chitosan have not been able to transfect HepG2 human hepatoma cells and BNL CL2 murine hepatocytes. The transfection efficiency was found to be higher at pH 6.9 than that at pH 7.6.

Ocular administration

Among mucoadhesive polymers explored now, chitosan has attracted a great deal of attention as an ophthalmic drug delivery carrier because of its absorption promoting effect. Felt *et al.* found that chitosan solutions prolonged the cornea resident time of antibiotic in rabbits. The same effects were also observed employing chitosan NP as demonstrated by De Campos *et al.* that chitosan NP remained attached to the rabbits' cornea and conjunctiva for at least 24 hr.In addition, De Campos *et al.* found that after ocular administration of chitosan NP in rabbits, most of drug were found in extraocular tissue, cornea and conjunctiva, while negligible drug were found in intraocular tissues, iris/ciliary body and aqueous humor. Together, these results suggested that chitosan NP showed to be attractive material for ocular drug delivery vehicle with potential application at extraocular level.[9,10]

Non-viral gene delivery vectors

Among the polymers used to form vaccine nanoparticles, chitosan is one of the most recently explored and extensively studied as prospective vaccine carriers. Its absorption promoting effect is believed to improve mucosal immune response. Illum *et al.* successfully developed chitosan vaccines containing influenza, pertussis and diphtheria antigens for nasal delivery. They demonstrated that these vaccines produced a significant antibody level in mice, both serum and secretoryIgA .Despite the potential carrier for mucosal delivery vaccine, chitosan has also been reported to act as an adjuvant for systemic vaccine delivery such as increasing the accumulation and activation of macropharges and polymorphonuclear cells. Activation of macropharges is initiated after uptake of chitosan. Furthermore, chitosan has also been widely explored as the application for DNA mucosal vaccines. For instance, a chitosan-based DNA flu vaccine has been developed by Illum *et al.* This system showed high antibody level in mice after intranasal administration. [11,12]

Preparation and Applications of Alginate nanoparticles

Preparation of alginate nanopartcles by desolvation technique

Alginate nanoparticles were prepared using a desolvation method.Alginate powder was added to distilled water. Ethanol solution was added continuously or intermittently into 1% alginate solution at pH 7 under stirring at 700 rpm at room temperature until the solution became just turbid. In continuous addition method ethanol was added continuously in the solution with rate addition about 1.0 to 2.0 ml per min and for intermittent method 2ml of ethanol was added for every 5 min interval.

Preparation of Alginate Nanoparticles by counter ion induced aggregation

Alginate nanoparticles were prepared by the principle involving cation induced controlled gelification of alginate. Briefly, calcium chloride (0.5 ml, 18 mM) was added to 9.5 ml of sodium alginate solution (0.06% w/v) containing drug. The initial ratio of drug : polymer was 7.5:1 w/w. Two ml of chitosan solution (0.05% w/v) was added followed by stirring for 30 min and the mixture was kept at room temperature overnight. Drug loaded nanoparticles were recovered by centrifugation at 19000 rpm for 30-45 min and washed thrice with distilled water to obtain the final pellet. Separate formulations were prepared for each drug.

Applications of Alginate nanoparticles

Alginate Nanoparticles as Antituberculosis Drug Carriers:- Alginate (a natural polymer) based nanoparticulate delivery system was developed for frontline ATDs (rifampicin, isoniazid, pyrazinamide and ethambutol).Alginate nanoparticles were prepared by the controlled cation induced gelification method and administered orally to mice. The drug levels were analysed by high performance liquid chromatography (HPLC) in plasma/tissues. The therapeutic efficacy was evaluated in *M. tuberculosis* H37Rv infected mice. High drug encapsulation efficiency was achieved in alginate nanoparticles, ranging from 70%-90%. A single oral dose resulted in therapeutic drug concentrations in the plasma for 7-11 days and in the organs (lungs, liver and spleen) for 15 days. In comparison to free drugs (which were cleared from plasma/organs within 12-24 h), there was a significant enhancement in the relative bioavailability of encapsulated drugs. In TB-infected mice three oral doses of the formulation spaced 15 days apart resulted in complete bacterial clearance from the organs, compared to 45 conventional doses of orally administered free drugs.[13]

Efficient gene transfection using chitosan– alginate core-shell nanoparticles:-

Reverse microemulsion was used as a template to fabricate chitosan–alginate coreshell nanoparticles encapsulated with enhanced green fluorescent protein (EGFP)-encoded plasmids. The average size of DNA-entrapped nanoparticles measured by dynamic light scattering was increased proportionally, with the N/P ratios ranging from 5 to 20. These alginate-coated chitosan nanoparticles

endocytosed by NIH 3T3 cells trigged swelling of transport vesicles which render gene escape before entering digestive endolysosomal compartment and concomitantly promote gene transfection rate. Results showed that DNA-encapsulated chitosan–alginate nanoparticles with average size of 64 nm (N/P ratio of 5) could achieve the level of gene expression comparable with the one obtained by using polyethyleneimine–DNA complexes.[14,15]

Gelatin

Gelatin is one of the protein materials that can be used for the production of nanoparticles. It is obtained by controlled hydrolysis of the fibrous, insoluble protein, collagen, which is widely found as the major component of skin, bones and connective tissue . In terms of nanopharmaceutics, gelatin was already considered as interesting biodegradable base material in the early days of particle development. The interest was based on the facts that gelatin is biodegradable, non-toxic, easy to crosslink and to modify chemically and has therefore an immense potential to be used for the preparation of colloidal drug delivery systems such as microspheres and nanoparticles. Other advantages are: it is inexpensive, can be sterilized, is not usually contaminated with pyrogens and possesses relatively low antigenicity. Un fortunately, formulations containing gelatin in the outer layer (hard and soft gelatin capsules) are prone to inter or intramolecular cross-linking of gelatin with time, temperature and humidity. Because of this tendency, the very use of gelatin in pharmaceutical formulations has been put to question. On the other hand, the material is used widely despite efforts to replace it with other substances. Addition of a chemical cross-linker like glutaraldehyde, gives gelatin stability, shape and a raised circulation time *in vivo* as compared to unmodified particles, and release is a function of cross-linking density of these nanoparticles. This structural change improves the performance, properties and characteristics of gelatin like insolubility at high temperatures, reduced swelling in water and less permeability to cell membranes.Two different gelatins, A and B with different isoelectric points (IEP), are formed following either acid or base hydrolysis, respectively. Gelatin type A is derived from acid processed collagen, while type B is obtained by alkaline collagen treatment, resulting in a difference in isoelectric points, being 7 – 9 for gelatin type A and 4 – 5 for gelatin type B. Characteristic features of gelatin are the high content of the amino acids glycine, proline (mainly as hydroxyproline) and alanine. Gelatin molecules contain repeating sequences of glycine, proline and alanine amino acid triplets, which are responsible for the triple helical structure of gelatin. The primary structure of gelatin offers many possibilities for chemical modification and covalent drug attachment. This can be done either within the matrix of the particles or on the particle surface only. In the first case, chemical modifications have to be done to the gelatin macromolecules before nanoparticles are formed, in the latter case the particle surface is used. These properties, combined with the high potential of nano-sized delivery systems make gelatin-based nanoparticles a promising carrier system for drug delivery.[16]

Preparation and Applications of Gelatin nanoparticles

Phase separation in aqueous medium:-Coester and coworkers, reported a new two step desolvation method for manufacturing gelatin nanoparticles.After the first desolvation step, lower molecular gelatin fractions present in the supernatant were removed by decanting.The high molecular weight fractions present in the sediment were dissolved and then desolvated again at Ph 2.5.In the second step centrifugation and redispersion methods were used to purify particles so obtained.

pH Induced aggregation:- Gelatin and tween 20 were dissolved in aqueous phase and pH of solution adjusted. The clear solution so obtained was heated to 40 degrees followed by its quenching at 4 degrees for 24 hours and subsequently left at ambient temperature for 48 hours. The sequential temperature treatment resulted into a colloidal dispersion of aggregated gelatin. The aggregates were finally cross-linked using glutaraldehyde as a cross linking agent. The nanospheres resulted are of 200nm average size with uniform dispersity.The optimum pH for ideal and uniform preparation of gelatin nanosphere was 5.5-6.5.pH below 5.5 produce no aggregation. Above 6.5 produce uncontrolled aggregation led to formation of larger nanospheres

Applications of gelatin nanoparticles

Non-viral gene delivery vectors:-Cationized gelatin nanoparticleshave shown the potential of being a new effective carrier for non-viral gene delivery. The major benefit of gelatin nanopaticle is not only the very low cell toxicity, but also their simple production combined with low cost. Native gelatin nanoparticles were prepared by two step desolvation technique.Inorder to bind DNA by electrostatic interaction onto the surface of the particles,the quarternary amine cholamine was covalently coupled to the particles. The modified nanoparticles were loaded with different amounts of plasmid in varying buffers and compared to polyethyleneimine -DNA complexes as a gold standard. Transfection ability of the loaded nanoparticles was tested on B16F10 cells. Additionally the cell toxicity of the formulation was monitored. Different setups resulted in efficient gene delivery displayed by exponential increase of gene expression. The gene expression itself occurred with a certain delay after transfection. In contrast to PEI complexes cationized gelatin nanoparticles almost did not show any significant cytotoxic effects.[17]

Treatment of BladderCancer

 Ze Lu, Teng-Kuang Yeh, reported that Paclitaxel-loaded gelatin nanoparticles were prepared using the desolvation method, and their physicochemical and biological properties were characterized. The size of the particles ranged from 600 to 1,000 nm and increased with the molecular weight of the gelatin polymer. Under optimal conditions, the yield was >80%, and the drug loading was 0.7%. Wide-angle X-ray diffraction analysis showed that the entrapped paclitaxel was present in an amorphous state, which has higher water solubility compared with the crystalline state. Identical, rapid drug release from nanoparticles was observed in PBS and urine, with 90% released at 37℃ after 2 hours. Treatment with a protease (*i.e.*, Pronase) rapidly degraded the nanoparticles, with half-lives of 23.8 minutes, 0.6 minute, and 0.4 minute in the presence of 0.01, 0.05, and 0.25 mg/mL Pronase, respectively. The paclitaxel-loaded nanoparticles were active against human RT4 bladder transitional cancer cells;the IC_{50} paclitaxel-equivalent concentrations were nearly identical to those of aqueous solutions of paclitaxel, *i.e.*, ~30 nmol/L (equivalent to ~25 ng/mL) for 2-hour treatments and ~4 nmol/L for 96-hour treatments.[18,19,20]

Albumin

Albumin is an attractive macromolecular carrier and widely used to prepare nanospheres and nanocapsules, due to its availability in pure form and its biodegradability, nontoxicity and nonimmmunogenicity . Both Bovine Serum Albumin or BSA and Human Serum Albumin or HSA have been used. As a major plasma protein, albumin has a distinct edge over other materials for nanoparticle preparation. On the other hand, albumin nanoparticles are biodegradable, easy to prepare in defined sizes, and carry reactive groups (thiol, amino, and carboxylic groups) on their surfaces that can be used for ligand binding and/or other surface modifications and also albumin nanoparticles offer the advantage that ligands can easily be attached by covalent linkage. Drugs entrapped in albumin nanoparticles can be digested by proteases and drug loading can be quantified. A number of studies have shown that albumin accumulates in solid tumors making it a potential macromolecular carrier for the site-directed delivery of antitumor drugs.[21]

Preparation and applications of BSA nanoparticles

Cross linking in w/o emulsion:- The method involves the emulsification of bovine serum albumin (BSA/Human serum albumin (HAS) or protein aqueous solution in oil using high pressure homogenization. The water in oil emulsion so formed is then poured into preheated oil. The suspension in preheated oil maintained above 100 degrees is held stirred for a specific time in order to denature and aggregate the protein contents of aqueous pool completely and to evaporate water. Protenacious

subnanoscopic particles thus formed where the size of the internal phase globule mainly determines the ultimate size of particulates**.**

Phase seperation in aqueous medium-

BSA nanoparticles were prepared using a desolvation method .BSA powder was added to distilled water. desolvating agent was added continuously or intermittently into 1% BSA solution at pH 7 under stirring at 700 rpm at room temperature until the solution became just turbid. In continuous addition method desolvating agent was added continuously in the solution with rate addition about 1.0 to 2.0 ml per min and for intermittent method 2ml of desolvating agent was added for every 5 min interval.

Simple coacervation technique:- It was implemented for preparation of BSA nanoparticles (lazko et al., 2004). Anhydrous ethyl alcohol was added to 150 ml BSA (5 mg/l in 10 mM Tris/HCl contained 0.02% sodium azide, pH 7.5) till the solution became turbid then 150 μl of 25% glutaraldehyde was added for cross linking. The reaction was continued at room temperature (24°C). Ethanolamine was added to block the non-reacted aldehyde functional group. Also Tween-20 was added at a final concentration of 0.01% (v/v) to stabilize the preparation. Large aggregates were eliminated by centrifuge (50,000 g, 30 min, 4°C). The supernatant was dialyzed and subsequently micro and ultrafiltrated through a 0.2 μm acetate membrane and polyvinylchloride copolymer membrane with cut off 300 kDa, respectively. The concentration of BSA determined with coomassie blue reagent. The size distribution and shape of BSA nanoparticles were determined by scanning electronic microscope .

Applications of BSA nanoparticles

Preparation of Aspirin loaded albumin nanoparticles

Saikat Das, Rinti Banerjee has reported that aspirin loaded albumin nanoparticles are prepared by coacervation method. Aspirin is a common anti-inflammatory and anti platelet agent widely used for various conditions. Albumin being both bioacceptable and biodegradable has a distinct advantage as a vehicle of drug delivery. By varying aspirin albumin ratios from 0.06 to 1.0 stable nanoparticles of sizes 46.8 nm to 190.8 nm respectively with low polydispersity can be obtained. Photon Correlation Spectroscopy (PCS) and Transmission Electron Microscopy (TEM) of the samples were done to characterize the nanoparticles. Drug encapsulation measured by UV spectroscopy varied from 30% to 80% for different ratios of aspirin: albumin. *In vitro* release study was conducted across a Spectrapor-membrane (cut off 3500 Da) precluding albumin. In contrast to simple drug solution, whose concentration peaks with in ½ to 1 hour, nanoparticle formulation releases aspirin at a sustained rate for prolonged duration (50% total cumulative percentage at the end of 20 hours, 90% at 72 hrs).[22]

Interferon-γ loaded albumin nanoparticles

S. Segura, C. Gamazo**,** studied the activity of IFN-γ, when it was either adsorbed or loaded in albumin nanoparticles. *Brucella abortus* infected macrophages and infected BALB/c mice were selected as the models to test the therapeutic potential of these cytokine delivery systems in view of well-established role of IFN-γ activated macrophages for control of *Brucella spp.* infections. Whereas the encapsulation of IFN-γ inside the matrix of nanoparticles completely abrogated its activity, adsorbed IFN-γ increased 0.75 log the bactericidal effect induced by RAW macrophages activated with free IFN-γ, along with a higher production of nitric oxide. In infected BALB/c-mice,adsorbed IFN-γ on nanoparticles was also more active than free cytokine for reducing the number of bacteria in the spleens and the effect was mediated by an increased ratio of IFN-γ (Th1) to IL-4 (Th2) secreting cells. Overall, albumin nanoparticles would be suitable as carriers to target IFN-γ to macrophages and, thus, potentiate their therapeutic activity**.**[23]

Gliadin and legumin

The use of nanoparticles is of interest for bioadhesion purposes because these pharmaceutical dosage forms have a large specific surface, which is indicative of a high interactive potential with

biological surfaces. For biological applications, vegetal particles have been derived from protein, such as gliadin extracted from gluten of wheat and vicillin or legumin extracted from pea seeds. Their potential appears to be large, especially in the targeting of active principles. Gliadin appears to be a suitable polymer for the preparation of mucoadhesive nanoparticles capable of adhering to the mucus layer. It has been used as a nanoparticle material owing to its versatile biodegradability, biocompatibility, and natural origin. Its hydrophobicity and solubility permit the design of nanoparticles capable of protecting the loaded drug and controlling its release.Gliadin nanoparticles (GNP) have shown a great tropism for the upper gastrointestinal regions, and their presence in other intestinal regions has been shown to be very low. This high capacity to interact with the mucosa may be explained by gliadin composition. In fact, this protein is rich in neutral and lipophilic residues. Neutral amino acid can promote hydrogen bonding interaction with the mucosa whereas the lipophilic components can interact within biological tissue by hydrophilic interaction. The related protein gliadin possessing an amino and disulphide groups on the side chain has a good probability of developing bonds with mucin gel. Legumin is also one of the main storage proteins in the pea seeds (*Pisum sativum* L.) Legumin is an albuminous substance that resembles casein and functions as the source of sulfur-containing amino acids in seed meals.The molecules of this protein have the capacity of binding together to form nanoparticles after aggregation and chemical cross-linkage with glutaraldehyde.Legumin nanoparticles can be prepared by phase separation in aqueous medium by modification of pH.[24,25]

REFERENCES

1. Kreuter J. Nanoparticles. In: *Colloidal Drug Delivery Systems,* edited by Kreuter, J. New York: Marcel Dekker, 1994.p. 261-276.
2. Mueller, R. H.. Colloidal Carriers for Controlled Drug Delivery and Targeting, Boston: CRC Press, 1991. p. 379.
3. Illum, L. Chitosan and its use as a pharmaceutical excipient. *Pharm. Res.* 1998; 15:1326-1331.
4. Muzzarelli RAA, Baldassare V, Conti F, Gazzanelli G, Vasi V, Ferrara P, Biagini G.The biological activity of chitosan: ultrastructural study. Biomaterials. 1988;8:247- 252.
5. Miyazaki S, Ishii K, Nadai T. The use of chitin and chitosan as drug carriers. Chem Pharm Bull. 1981;29:3067-3069.
6. Kurita K. Chemical modifications of chitin and chitosan. In: Muzzarelli RAA, Jeuniaux C, Gooday GW, eds. Chitin in Nature and Technology. Plenum: New York, NY. 1986:287- 293.
7. Bender, von Briesen, Kreuter J, Duncan I. B, and Rubsamen-Waigmann H. Efficiency of nanoparticles as a carrier system for antiviral agents in human immunodeficiency virus-infected human oncocytes/macrophages *in vitro*. *Antimicrob. Agents Chemothe;*1996;.40: 1467 1471.
8. Luessen H. L, de Leeuw B.J, Langemeyer M.W, de Boer A.B, Verhoef J.C, and Junginger H.E. Mucoadhesive polymers in peroral peptide drug delivery. VI.Carbomer and chitosan improve the intestinal absorption of the peptide drug buserelin in vivo. *Pharm. Res.* 1996; 13: 1668-1672.
9. Fernandez-Urrusuno,. Calvo R.P, Remunan-Lopez C, Vila-Jato J.L, and Alonso M.J. Enhancement of nasal absorption of insulin using chitosan nanoparticles. *Pharm. Res.* 1999; *16:* 1576-1581.
10. Dyer, A. M, Hinchcliffe M, Watts P, Castile J, Jabbal-Gil I, Nankervis R, Smith A, and Illum L. Nasal delivery of insulin using novel chitosan based formulations: a comparative study in two animal models between simple chitosan formulations and chitosan nanoparticles. *Pharm. Res.* 2002;19:998-1008.
11. Erbacher, Zou P.S, Steffan A.M, and Remy J.S. Chitosan-based vector/ DNA complexes for gene delivery: biophysical characteristics and transfection ability. *Pharm.Res.* 1998; 15: 1332-1339.
12. Guang Liu, W. and K. De Yao. Chitosan and its derivatives a promising non- viral vector gene transfection. *J. Control. Release*2002; Vol. 83, pp. 1-11.
13. Qurrat-ul Ain, Sharma S, Khuller GK, Garg SK. Alginate based oral drug delivery system for tuberculosis: pharmacokinetics

and therapeutic effects. *J Antimicrob Chemother* 2003; 51: 931-8

14. Yi YM, Yang TY, Pan WM. Preparation and distribution of 5-fluorouracil ^{125}I sodium alginate-bovine serum albumin nanoparticles. *World J Gastroenterol* 1999; 5: 57-60.

15. Zhang ChuangNian, Wang Wei, Wang ChunHong. Cytotoxicity of liver targeted drug-loaded alginate Nanoparticles. *Sci China Ser B-Chem* Sep. 2009 ; 52(9); 1382-1387.

16. Coester,C.J.,Langer,K., Von Briesen., gelatin nanoparticles by two step desolvation- A new preparation method,surface modifications and cell uptake.J.microencapsule. 2000,17(2); 187-193.

17. Conrad coester,Klaus Zwiorek., gelatin nanoparticles as a new and simple gene Delivery system.J Pharm.pharmaceut Sci 2004 ;7(4):22-28.

18. Ze Lu, Teng-Kuang Yeh, Max Tsai, Paclitaxel-Loaded Gelatin Nanoparticles for Intravesical Bladder Cancer Therapy Clinical Cancer Research *2004;Vol. 10,November 15, 7677–7684,*

19. Roth BJ. Preliminary experience with paclitaxel in advanced bladder cancer. Semin Oncol 1995;22:1–5.

20. Au JL, Kalns J, Gan Y, Wientjes MG. Pharmacologic effects of paclitaxel in human bladder tumors. Cancer Chemother Pharmacol 1997;41:69 –74.

21. Merodio M,. Arnedo A, Renedo M.J. Irache J.M, Ganciclovir-loaded albumin nanoparticles:characterization and in vitro release properties, European J of Pharmceutical Sciences, (2001); Vol.12(3), 251

22. Saikat Das, Rinti Banerjee and Jayesh Bellare Aspirin Loaded Albumin Nanoparticles by Coacervation: Implications in Drug Delivery *Trends Biomater. Artif. Organs, 2005; Vol 18 (2): January203-212.*

23. SiguraS,GamazoC;Interferon-g loaded albumin nanoparticles:invitro and invivo activity against Brucella abortus . 2007 *January*.

24. Ezpeleta I, Irache JM, Stainmesse S, Chabenat C, Gueguen J, Popineau Y, Orecchioni AM Gliadin nanoparticles for the controlled release of all-trans-retinoic acid. Int. J. Pharm. (1996); 131: 191-200.

25. Ezpeleta I, Irache JM, Stainmesse S, Chabenat C, Popineau Y,Orecchionic AM Preparation of Ulex europaeus lectin-gliadin nanoparticles conjugates and their interaction with gastrointestinal mucus. Int. J. Pharm. (1999);191: 25-32.