Nano -Polysaccharides at Drug Delivery Systems

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Abstract **- Nanomaterials have great potential applications in medicine, food science and technology. As natural biomaterials are quite abundant in nature and biodegradable, they are favoured over synthetic polymer based materials. In natural polymers, polysaccharides are known as a renewable, cheap and nontoxic raw material. So for preparation of nanoparticles, polysaccharides are particularly considered. Natural polysaccharides shown outstanding merits in the field of drug delivery. So they attracted many researchers to work on them for drug delivery applications. In the preparation of nanometeric carriers, polysaccharides, in particular, considered as the most promising material. The aim of this review is to study the developments in the preparation of nanoparticles from polysaccharides. Mainly the mechanisms used so far to prepare nanoparticles from polysaccharides are: Crosslinking – covalent & ionic, polyelectrolyte complex (PEC), and the self-assembly of hydrophobically modified polysaccharides. [1]**

Keywords **— Starch copolymers, Chitosan copolymers, redox initiator, depolymerisation.**

I. INTRODUCTION

In nano drug delivery systems, nanoparticles are used to deliver drugs or biomolecules as nanometeric carriers. These nanometeric carriers are of size below 1000 nm, also include sub-micro particles. Carriers are having various morphologies, including nanodrugs, nanoliposomes, nanomicelles, nanocapsules, and nanospheres etc. [3,4].

There are many outstanding advantages of nanodrug delivery systems like [3]: (1) the ultra – tiny size make it to pass through the smallest capillary vessels (2) they can penetrate to target organs such as liver, spleen, lung, spinal cord and lymph through cells and tissue gap; (3) can prolong their duration in blood stream by avoiding rapid clearance through phagocytes; (4) controlled release properties could be achieved due to their sensitivity to temperature, pH, and/or ion and biodegradability; (5) they can reduce toxic side effects and improve the utility of drugs; etc. [1]

The ideal nanopolymeric carriers should be: Water soluble, Non-toxic, Non-immunogenic, Biodegradable, Biocompatible, Inexpensive, Easy to synthesize and characterize. Ideally nanoparticle delivery systems should be: Non-toxic, Stable after administration, Able to lyophilize, Applicable to a broad category of drugs; small molecules, proteins and polynucleotides, Reproducible and stable, Simple and inexpensive to manufacture and scale-up. There preparation should not require organic solvents, high shear forces or heat. [5]

In nanodrug delivery system, biomolecules or drugs can be absorbed on the exterior surface or can be entrapped into interior structures of nanoparticles. Presently, nanoparticles are widely used to deliver drugs, vaccines, proteins, polypeptides, nucleic acids, genes and so on. Over past few years, huge potential has been shown by nanodrug delivery systems in pharmaceutical, medical and biological applications [6].

For the preparation of nanoparticles applicable in drug delivery, only biodegradable and biocompatible polymers can be used. Many polymeric materials have been applied, including poly (acrylic acid) family, polysaccharides (particularly chitosan), polycaprolactone, poly (glycolic acid), poly (lactic acid), proteins or polypeptides (such as gelatin), etc. for preparing nanoparticles for drug delivery, polysaccharides are most popular among the polymeric materials to prepare. [1]

II. POLYSACCHARIDES

Polysaccharides are the polymers of monosaccharides [3].The complex Polysaccharides are made from chains of monosaccharides joined with each other by glycosidic bonds. Polysaccharides are some of the main structural elements of animals and plants. These Polysaccharides also play key role in the plant energy storage (starch, paramylon, etc.) [7]. In nature, polysaccharides have various resources from plant origin (e.g. pectin, guar gum), algal origin (e.g. alginate), animal origin (chitosan, chondroitin), and microbial origin (e.g. dextran, xanthan gum), [3,8]. General formula of Polysaccharides is $Cx(H2O)y$ where value of x is found between 200 and 2000. In Polysaccharides the repeating units are usually six-carbon monosaccharides, and so the general formula can also be represented as $(C6H10O5)n$ where $40 \leq n \leq 3000$.[7]

Natural polysaccharides are biodegradable, hydrophilic, non-toxic, safe, highly stable, cheaper and have abundant resources in nature. Polysaccharides have wide variety in properties and diversity in structure due to varying chemical composition, a wide range of molecular weight (MW) and a most one, large number of reactive groups. According to polyelectrolyte, there are two classes of polysaccharides: polyelectrolytes and nonpolyelectrolytes. Polyelectrolytes can be further classified as positively charged polysaccharides (e.g. chitosan) and negatively charged polysaccharides (e.g. alginate, heparin, hyaluronic acid, pectin, etc.). [1]

There are large number of reactive groups on polymeric chains provide opportunity to modify chemically and biochemically to produce derivatives. Hydrophilic groups such as hydroxyl, carboxyl and amino groups are found on most of natural polysaccharides, which could form non-covalent bonds and inturnbioadhesion with biological tissues (mainly epithelia and mucous membranes) [9]. For example, starch, chitosan, alginate, etc. are good bioadhesive materials. To improve absorbance of loaded drug, such bioadhesive polysaccharides should be used to prepare nanoparticle carriers, which can prolong the residence time. All these merits endow polysaccharides a promising future as biomaterials. When natural polysaccharides are used for drug delivery, it becomes safer and non-toxic. So polysaccharides

and their derivatives have potential application as nanoparticle drug delivery systems [8,10-12].

There are several methods developed for preparation of nanoparticles. Some are: emulsification solvent evaporation method, solvent diffusion method, self-assembly of hydrophobically modified, dialysis method and other methods. The selection of method depends on factors, such as, particle size, particle size distribution, area of application and etc. [1]

III. NANO POLYSACCHARIDES PREPARATION

Mechanisms of Nanoparticles formation:

The charge density on polysaccharide polymeric chain is one of the most important factor. When the polysaccharide chain is having high charge density, the chains are extended conformation (I). But when the charge density is lower, the chains collapse to make compact sphere. Such coil globule transition is due to the attraction and repulsion interaction between the polymer segments. Polysaccharide like chitosan is a weak base. Its pKa value of the D-glucosamine residue is about 6.2- 7.0. So its solubility at neutral and alkaline pH values is lower. The amino groups of chitosan are positively charged in acidic medium, resulting in a highly charged polyelectrolyte polysaccharide.

According to charges, there are four types of nanoparticles coils possible to form: Polycationic chains (II), polyanionic chains (V), polyampholytic chains (IV), or uncharged chains (III). These cross-linked polysaccaride nanoparticles are prepared by chemical modification of linear chains (I) with crosslinkers. [13]

When crosslinkers like dicarboxylic acids are used to react with polysaccharides, it gives polycationic polysaccharide nanoparticles (II). In these reactions, the cross-linking takes place less than 100% stoichiometric ratio. In case of chitosan, polycations are formed by protonation of free amino groups in acidic media, whereas the carboxyl groups were bound covalently. Chitosan particles can act as bioadhesive cationic polyelectrolytes. [13]

Most polysaccharides contains positive charge on it, and so it has a strong electrostatic interaction with negatively charged molecules, like DNA and RNA. So they are considered good candidates for drug- or gene delivery systems. These electrostatic interactions provide stability and protection from degradation.

When all the functional groups of polysaccharide are covalently bound, the stoichiometric ratio of crosslinking becomes 100%. It gives uncharged nanoparticles (III) in aqueous media. [13]

When the polysaccharides crosslinked with carboxylic acid like citric acid, polyanion (V) or polyampholyte (IV) cross-linked nanoparticles are produced. Citric acid acts as a difunctional cross-linking agent used in excess. So free carboxyl groups are available after cross-linking. When these free groups are deprotonated in neutral and alkaline media, partially negatively charged particles produced and the stoichiometric ratio of cross-linking found less than 100%. [13]

Polyampholyte nanoparticles colloid dispersion are unstable, and so it precipitated. Polyanions are prepared by condensation reaction between polysaccharides and crosslinkers. All groups of the polysaccharide chain are bound covalently but functional groups of crosslinker are available. Stoichiometric ratio of cross-linking of 100%. [13]

When the colloid dispersion prepared with lower concentrations of cross-linkers, the solubility found more and the solutions were either clear or opalescent. With increased concentration of crosslinker, the particles become compact and solution opalescent. [13]

Solubility of the polysaccharide nanoparticles is related to the hydrophilic character of the cross-linking agents and the ratio of free functional groups of the polysaccharide chain. The more hydrophilicity of crosslinkers used, more solubility of the polysaccharide particles. [13]

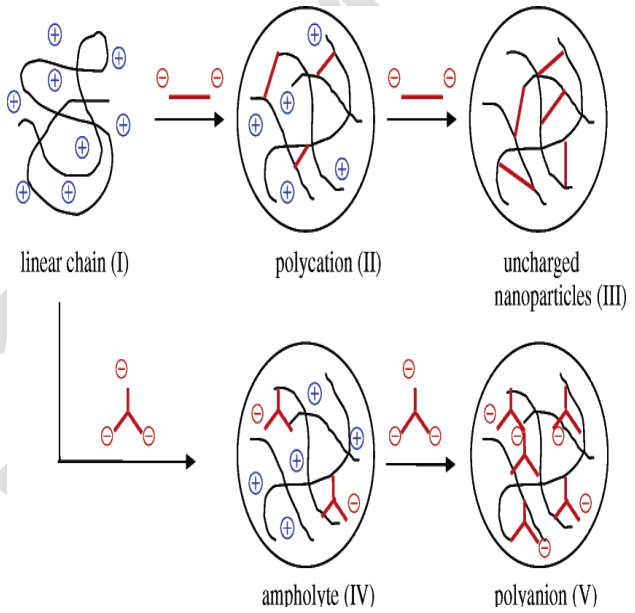

Fig.1. Schematic diagram of structure of cross-linked polyelectrolytes.

Alonso et al. [14] and Prabaharan et al. [15] have discussed the preparation and application of polysaccharide nanoparticle carriers. Now a days many versatile nanoparticle carriers made from polysaccharide has emerge. Nanoparticles can be prepared by many methods. According to structural characteristics, there are four mechanisms: (1) covalent crosslinking, (2) ionic crosslinking, (3) polyelectrolyte complexation, and (4) self-assembly of hydrophobically modified polysaccharides. [1]

3.1. Covalently crosslinked polysaccharide nanoparticles

It is the first method developed for preparation of polysaccharide nanoparticles. Nanoparticles of chitosan were made by this method. Chitosan based nanoparticles prepared by crosslinking with Glutaraldehyde, but toxicity of Glutaraldehyde is limiting its application in drug delivery. So it is required to use biocompatible crosslinkers. There are many such crosslinkers suggested by researchers like natural di- and tricarboxylic acids, such as citric acid, tartaric acid, malic acid, succinic acid, etc. Some water-soluble condensation agents like carbodiimide, also used as biocompatible crosslinkers for chitosan nanoparticles [16,17]. To prepare chitosan nanoparticles, the pendant amino groups of chitosan and the carboxylic groups of natural acids will react by condensation reaction. By this method different

types of nanoparticles can be formed like polycations, polyanions, and polyampholyte. The average size of the particles, depends on the pH, in the swollen state, found in the range of 270–370 nm. The prepared nanoparticles found stable in aqueous media at any pH, either acidic, neutral, or mild alkaline conditions.[1].

There are several methods used to prepare nanopolysaccharides by covalent crosslinking like: thermal cross-linking, [18] spraydrying, [19] solvent evaporation, [20] reverse micellar,[21] or emulsion crosslinking. [22]

To produce a crosslinkedpolysachharides suspension, a well- known technique spraydrying is used. In this method fine dispersed droplets are dried in a hot air stream followed by the addition of a cross-linking agent. [13]

In thermal cross-linking, crosslinking reactions are taking place at high temperature. [13]

3.2. Ionicallycrosslinked polysaccharide nanoparticles

This mechanism has some advantages over covalent crosslinking like preparation conditions are mild and procedure is simple. For both polycationic and polyanionic charged polysaccharides, opposite change ions of low MW can work as crosslinkers. Tripolyphosphate (TPP) is one such widely used polyanioncrosslinker. Chitosan nanoparticles crosslinked with TPP was developed first time. [23,24] TPP contains multivalent anions and it is non-toxic. Ionic interaction between negatively charged counterions of TPP and positively charged amino groups of chitosan will form a gel. [25]

Xu et al. [26] prepare nanoparticles from water soluble derivative of chitosan. They ionicallycrosslinked N-(2-hydroxyl) propyl-3-trimethyl ammonium chitosan chloride by the reaction between glycidyl-trimethyl-ammonium chloride and chitosan.[1].many researchers reported formation of nanoparticles by ionic interaction between chitosan derivatives and other ions for different drug delivery systems, for cosmetic and pharmaceutical applications.[27-31]

3.3. Polysaccharide nanoparticles by polyelectrolyte complexation (PEC)

Intermolecular electrostatic forces between oppositely charged polymeric compounds are responsible for the formation of Polyelectrolyte complexation (PEC) of polysaccharides.[1].

Theoretically any polyelectrolyte could react with polysaccharides to fabricate PEC nanoparticles. With adjustment of molecular weight, nanoparticles of polysaccharide- based PEC can be formed.

But only water soluble and biocompatible polymeric components are used for PEC nanoparticles, to avoid undesirable properties. From natural polycationic polysaccharides, only chitosan satisfies the conditions. For the formation of PEC with polysaccharides, there are many negative polymer components available like peptides, polyacrylic acid family, etc. [1]

3.4. Self-assembly of hydrophobically modified polysaccharides

When hydrophilic polymeric chains are grafted with hydrophobic segments, amphiphilic copolymers are synthesized. When these amphiphiles comes in contact with an aqueous environment, to minimize interfacial free enrgy, its hidrophillic component will aggregates the hydrophobic moieties through intra- or intermolecular associations and form micelles or micelle like shape. Depending on the hydrophilic/hydrophobic constituents, the micelles exhibit core-shell structure of less than microsize. It will show some unique characteristics, like thermodynamic stability, small hydrodynamic radius and unusual rheological feature. In this nanopolymers, the hydrophobic domain, surrounded by a hydrophilic outer shell, serves as preservatory for various hydrophobic drugs. So they are proved as best carriers for hydrophobic drugs. [1]

Generally, these hydrophobic molecules are cyclic or linear hydrophobic molecules, hydrophobic drug, polyacrylate family, etc. [1]

Methods for preparation of nanoparticles from polymerization of monomers

For preparation of polysaccharide nanoparticles, methods developed are mostly based on microparticle technology. Mainly used methods are: ionotropic gelation, microemulsion, emulsification solvent evaporation, emulsification solvent diffusion and polyelectrolyte complex. The most widely developed methods are ionotropic gelation and self assemble polyelectrolytes. These methods are comparatively simple, not using organic solvents or high shear force. [32]

Ionotropic gelation: nanoparticles are formed by electrostatic interaction between functional groups of polysaccharides and crosslinking agent or another component. Positively charged polysaccharide functional group interact with negatively charged functional groups of polyaniniccrosslinker like tripolyphosphate in emulsion. This process is a water/oil emulsion polymerization method. It is simple and mild preparation technique out of all other methods in the aqueous environment.[33] In this technique, the size of the nanoparticles prepared depends on the size of droplets in emulsion. However, the size of the particles is large, and they have a broad size range. Narrow size distribution ultrafine polymeric nanoparticles can be Prepared with narrow size distribution could be achieved by using reverse micellar medium. [13] Calvo et al developed & reported Chitosan NP first time prepared by ionotropic gelation technique.

Coacervation is also ionotropic gelation method, [34] which also avoids the use of toxic organic cross-linking agents. [13]

Microemulsion method

Microemulsions are also known as Reverse micelles. These emulsion usually form spontaneously, single optically isotropic and thermodynamically stable systems. Microemulsions can solubilize both aqueous and oil-soluble compounds. Microemulsions have ultralow interfacial tension and large interfacial area. They are made from liquid mixture of water, oil, and surfactant. [Preparation and Characterization of Chitosan-Based Nanoparticles]

Depending on the hydrophilic–lypophilic balance (HLB) value of the used surfactant and proportion of various components microemulsions can be classified as water-in-oil (W/O), oil-in-water (O/W) emulsions. There is one intermediate structural type, bicontinuous emulsions. These can turn reversibly from one type to the other.

In this technique, the nanoparticles of polysaccharides form in the aqueous core of reverse micellar droplets and subsequently cross-linked. Maitra et al. prepared & reported Chitosan NP by microemulsion technique first time.

Emulsification solvent evaporation method

Emulsification solvent evaporation is the most widely employed technique to prepare nanoparticles of polymers. [35] In the conventional methods, two main strategies are being used for the formation of emulsions: the preparation of single emulsions, e.g., oil-in-water (o/w) or double-emulsions, e.g., (water-in-oil)-in-water, (w/o/w).

In single emulsification solvent evaporation process, volatile organic solvents like ethyl acetate, chloroform, dichloromethane, etc are used as solvent. These solvents are immiscible with water, creates oil – in – water emulsion. These solvents will dissolve hydrophobic surfactants also. To reduce problem of organic solvent droplets coalescence, the droplet size is reduced by continuous stirring, or microfluidization by using sonication and/or homogenizer.

In double emulsion process, hydrophilic solvents are used. Aqueous solution of active component is added in organic phase to create primary emulsion. Which is now added in aqueous phase (external water phase), results in w/o/w double emulsion. To keep the droplet size much lower, first aqueous phase needs large qty of emulsifier than second one. To solidify nanoparticles formed, the solvents are either evaporated or extracted. [13]

In o/w emulsion, the size of nanoparticles produced depend not only on concentration of surfactive polymer in the aqueous phase, but also depends on zeta potential, hydrophilicity, and drug loading, Homogenization intensity and duration, Type and amounts of emulsifier, polymer and drug, Particle hardening (solvent removal) profile [36].

Solvent diffusion method

Solvent diffusion method is also known as spontaneous emulsification. It is a modified version of solvent evaporation method. In this method, pharmaceutically acceptable partially water soluble solvents are used. So high-pressure homogenizers are not required to handle emulsions. [37,38] Water soluble solvents can diffuse in aqueous phase and so offer advantage of reduction in particle size with increase in concentration. Due to differences in the polymer-solvent and water–solvent interactions, the type of solvent and polymer affect the formation of nanoparticles. Solvents like DMSO, DMF, acetone, THF and pyridine are used in this method. Due to lower miscibility of organic solvents in water, large amount of water is used. Due to diffusion of organic solvent molecules into water, nanoparticles formed. It shows high percentage of drug entrapment. This method was developed by Niwaet al.to prepare PLGA nanoparticles. El-Shabouri prepared & reported chitosan NP by emulsion solvent diffusion method first time. Harsh processing conditions (e.g., the use of organic solvents) and the high shear forces are major drawback of this method.

Polyelectrolyte complex (PEC)

Cationic charged polymer and plasmid DNA forms Polyelectrolyte complex by self-assembly. So this technique is also known as self-assembled polyelectrolyte. Due to the charge neutralization of both components, hydrophilicity of the complex decreases. Several cationic polymers (i.e. gelatin, polyethylenimine) also possess this property. This technique is simpler and mild, no harsh conditions involved. DNA-Chitosan nanoparticles spontaneously formed when DNA solution added into acetic acid solution of chitosan. The complexes size can be varied from 50 nm to 700 nm. When hydrophilic polymeric chains are grafted with hydrophobic segments, amphiphilic copolymers are formed. Usually, these hydrophobic molecules can be divided into linear, cyclic hydrophobic molecules, hydrophobic drug, polyacrylate family, etc.

3. Modified polysaccharides (MP) for preparation of their nanoparticles

Natural biopolymers offer advantages like, biodegradability, biocompatibility and available from replenish able natural resources and, therefore leading to ecological safety. Recently, many researchers working on modification of various polysaccharides and prepared their derivatives for biodegradable nanoparticles. These nanoparticles have shown ability to control the drug release and also offer drug protection.

Polysaccharides have a number of positive characteristics such biodegradability, biotolerability, receptor interaction through specific sugar moieties, protein rejecting ability and abundance of functional groups for modification or functionalization [39]. Amphiphillic polysaccharides consisting of hydrophilic and hydrophobic fragments. They can form self-assembled nanoparticles and show unique physicochemical characteristics such as a thermodynamic stability and nanoparticle structure. The amphiphilic character imparted upon polysaccharides after hydrophobic modification gives them a wide and interesting use spectrum, for instance as emulsion stabilizers, rheology modifiers [40,41], surface modifiers for liposomes and nanoparticles [42] and as drug delivery vehicles [43,44].

4. Medical applications of polysaccharide-based nanoparticles

Owing to their unique potentials, polysaccharides have made special place in nano drug delivery systems. Many research groups are working with different polysaccharides in this area.

Applications of chitosan nanoparticles

Parenteral administration

The smallest blood capillary is approximately 4 μm diameter, so Nano-sized polysaccharide particles can be administered intravenously. Nanoparticles size less than 100 nm tend to have a prolonged circulation time, while larger particles are rapidly taken up by the reticuloendothelial

system (RES) in the liver, spleen, lung and bone marrow. Compare to positively-charged or neutral particles, negatively-charged particles are eliminated faster. It is expected that that nanoparticles should decrease the toxic side effects of drugs while improving the therapeutic efficacy. Theoretically, chitosan NP of size less than 100 nm offer advantage such as hydrophilic surface, and so are very attractive carrier system [11,12].

Peroral administration

Polysaccharides like chitosan contains positive charge, which interact with negative charge of mucin results in mucoadhesive properties. Which in turn prolong contact time between the drug and the absorptive surface, and thereby promoting the absorption of drug. As they promote absorption of drug, polysaccharide NP became attractive carriers for oral delivery. Recent studies have shown that polysaccharide like protonated soluble chitosan, in its uncoiled configuration, only can trigger the opening of the tight junctions, thereby, facilitating the paracellular transport of hydrophilic compounds. This property implies that polysaccharide like chitosan would be effective as an absorption enhancer only in a limited area of the intestinal lumen where the pH values are below or close to its pKa.

Non-viral gene delivery vectors

Polysaccharides are cationic polymers with extremely low toxicity than PEI and poly-L-lysine. Additionally, it enhances the transport of drug across cell membrane.

The transfection efficiency of polysaccharide like chitosan found higher at acidic pH than that at neutral pH. It is due to protonated amine groups of chitosan facilitate the binding between complexes and negatively charged cell surface.

4. Characterization of polysaccharide-based nanoparticle

Numerous properties of materials depend on the size and internal structure of their constituents.

Nanomaterials, due to the particle size in the range of nanometers, exhibit properties that are unique and qualitatively different from those of large-size particles. Biomaterials posses internal nanostructure, they are biocompatible and biodegradable therefore they are favoured over synthetic polymer based materials. [46] Particle size is the greatest important characteristics of nanoparticles. It is challenging also. Some methods for the determining particle size are [45]

a. Photon-correlation spectroscopy.
b. Dynamic light scattering.
c. Brownian motion and light scattering properties.
d. Scanning or transmission electron microscopy (SEM or TEM).

One can understand in vivo distribution, biological fate, toxicity and targeting ability of these delivery systems. In addition, they can influence drug loading, drug release and stability of the nanoparticles. SEM, TEM, AFM are also used to confirms size, shape and dimensions of nano particles. We prepare nano particles of chitosan, starch, and their copolymers. Details of synthesis explained somewhere else. The size, shape and dimensions in SEM micrographs are shown here.

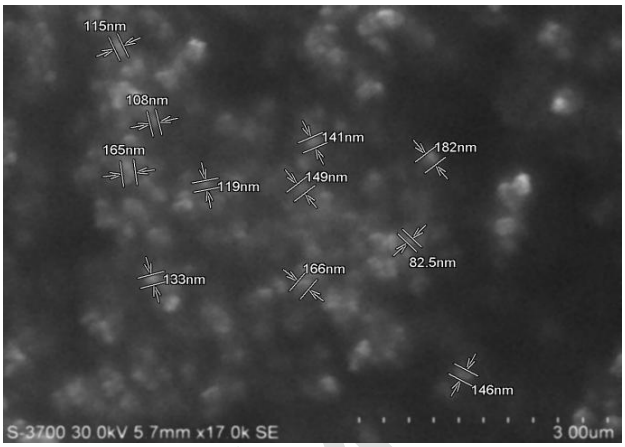

Fig. 2 SEM images of chitosan/starch nano particles (APS3%)

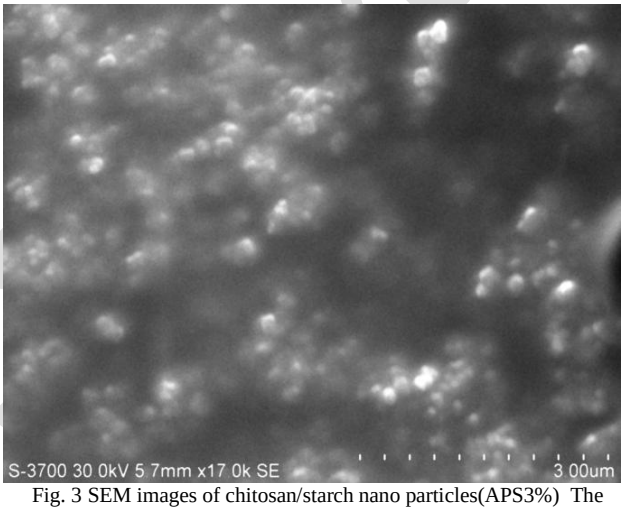

Fig. 3 SEM images of chitosan/starch nano particles(APS3%) The morphology of nanoparticles was observed at 3 kV using a scanning electron microscope (SEM; S-4200, Hitachi, Japan).

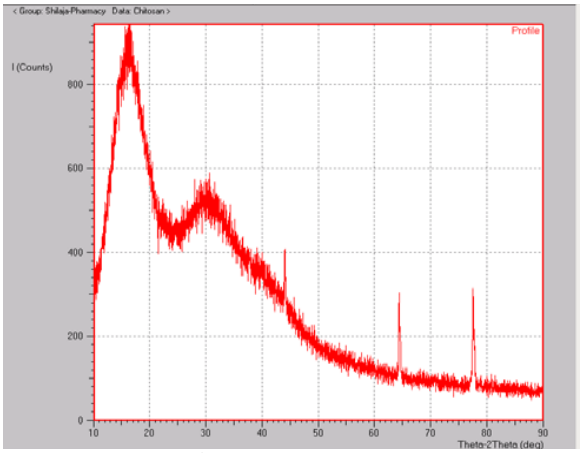

Fig. 4XRD for Chitosan/ starch nanoparticles

Physical status of chitosan/starch nanoparticles: An X-ray diffractometer (Philips, Xpert-Pro, The Netherlands) was used to determine the physical status of chitosan/ starch in the nanoparticles. The diffraction angle (2θ) was recorded from 3° to 80° with a scanning speed of 5°/minute. CuKa radiation was used as the X-ray source at 40 kV and 30 mA. Particle size of chitosan/ starch is also confirmed by X-ray diffraction study. Particles are found be crystalline in nature.

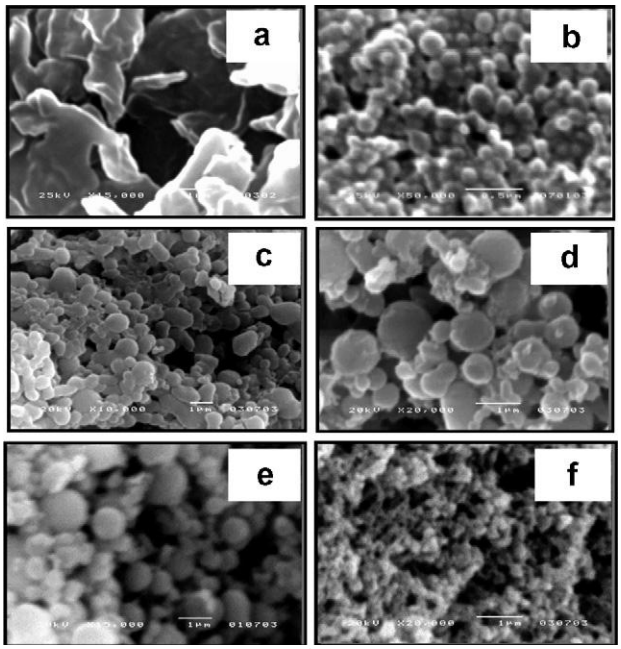

Fig.5. SEM photographs at 25kV of (a) chitosan (15,000·), (b) chitosan (50,000·), (c) starch (10,000·), (d) starch (20,000·), (e) chitosan/ starch copolymer PECs (15,000·), and (f) chitosan/ starch copolymer PECs (20,000·).

IV. CONCLUSIONS

To improve performance and scope of nanodrug delivery systems, one should focus on: (1) proper selection of carrier materials either single or combination, for desired drug release profile; (2) improvement in targeting ability by surface modification of nanoparticles; (3) the optimization of the nanoparticles characteristics to increase their application in clinics, their drug delivery capability and industrial production; (4) understanding in vivo interaction of blood, targeting tissues, organs, etc with nanoparticles.

REFERENCES

[1] Zonghua Liu et al, "Polysaccharides-based nanoparticles as drug delivery systems", Advanced Drug Delivery Reviews 60 (2008) 1650–1662.
[2] T. Jung, et al, "Biodegradable nanoparticles for oral delivery of peptides: is there a role for polymers to affect mucosal uptake" Eur. J. Pharm. Biopharm. 50 (2000) 147–160.
[3] C. Pinto Reis, R.J. Neufeld, A.J. Ribeiro, F. Veiga, Nanoencapsulation I. Methods for preparation of drug-loaded polymeric nanoparticles, Nanomedicine 2 (2006) 8–21.
[4] A Krishna Sailaja*, P Amareshwar, P Chakravarty, "Chitosan nanoparticles as a drug delivery system", RJPBCS, (1)3, 474-484.
[5] L. Illum, "Nanoparticulate systems for nasal delivery of drugs: a real improvement over simple systems" J. Pharm. Sci. 96 (2007) 473–483.
[6] Aminabhavi, T. M., Balundgi, R. H. & Cassidy, P. E. (1990). A Review on Biodegradable Plastics. Polymer-Plastics Technology and Engineering 29(3): 235-262.
[7] V.R. Sinha, R. Kumria, Polysaccharides in colon-specific drug delivery, Int. J. Pharm. 224 (2001) 19–38.
[8] J.W. Lee, J.H. Park, J.R. Robinson, Bioadhesive-based dosage forms: the next generation, J. Pharm. Sci. 89 (2000) 850–866.
[9] A. Rubinstein, Natural polysaccharides as targeting tools of drugs to the human colon, Drug Dev. Res. 50 (2000) 435–439.
[10] T.F. Vandamme, A. Lenourry, C. Charrueau, J. Chaumeil, The use of polysaccharides to target drugs to the colon, Carbohydr. Polym. 48 (2002) 219–231.
[11] C. Lemarchand, R. Gref, P. Couvreur, Polysaccharide-decorated nanoparticles, Eur. J. Pharm. Biopharm. 58 (2004) 327–341.
[12] MagdolnaBodnar, John F. Hartmann, Janos Borbely, "Preparation and Characterization of Chitosan-Based Nanoparticles", Biomacromolecules 2005, 6, 2521-2527.
[13] K.A. Janes, P. Calvo, M.J. Alonso, "Polysaccharide colloidal particles as delivery systems for macromolecules", Adv. Drug Deliv. Rev. 47 (2001) 83–97.
[14] M. Prabaharan, J.F. Mano, "Chitosan-based particles as controlled drug delivery systems", Drug Deliv. 12 (2005) 41–57.
[15] M. Bodnar, J.F. Hartmann, J. Borbely, Preparation and characterization of chitosan based nanoparticles, Biomacromolecules 6 (2005) 2521–2527.
[16] M. Bodnar, J.F. Hartmann, J. Borbely, Nanoparticles from chitosan, Macromol. Symp. 227 (2005) 321–326.
[17] Orienti, I.; Aiedeh, K.; Gianasi, E.; Bertasi, V.; Zecchi, V. J. Microencapsulation 1996, 13, 463-472.
[18] He, P.; Davis, S. S.; Illum, L. Int. J. Pharm. 1999, 187, 53-65.
[19] Lim, S. T.; Martin, G. P.; Berry, D. J.; Brown, M. B. J. Controlled Release 2000, 66, 281-292.
[20] Mitra, S.; Gaur, U.; Ghosh, P. C.; Maitra, A. N. J. Controlled Release, 2001, 74, 317-323.
[21] Jameela, S. R.; Jayakrishnan, A. Biomaterials 1995, 16, 769-775.
[22] Berger, J.; Reist, M.; Mayer, J. M.; Felt, O.; Peppas, N.; A.; Gurny, R. Eur. J. Pharm. Biopharm. 2004, 57, 19-34.
[23] Khor, E.; Lim, L. Y. Biomaterials 2003, 24, 2339-2349.
[24] Suh, J. K. F.; Matthew, H. W. T. Biomaterials 2000, 21, 2589-2598.
[25] Crini, G. Prog. Polym. Sci. 2005, 30, 38-70.
[26] Brack, H. P.; Tirmizi, S. A.; Risen, W. M. Polymer 1997, 38, 2351-2362.
[27] Noble, L.; Gray, A. I.; Sadiq, L.; Uchegbu, I. F. Int. J. Pharm. 1999, 192, 173-182.
[28] Shu, X. Z.; Zhu, K. J. Int. J. Pharm. 2000, 201, 51-58.
[29] Betigeri, S. S.; Neau, S. H. Biomaterials 2002, 23, 3627-3636.
[30] Krajewska, B. Enzyme Microb. Technol. 2004, 35, 126-139.
[31] WareeTiyaboonchai, Chitosan Nanoparticles : A Promising System for Drug Delivery, Naresuan University Journal 2003; 11(3): 51-66.
[32] Kim, S. E.; Park, J. H.; Cho, Y. W.; Chung, H.; Jeong, S. Y.; Lee, E. B.; Kwon, I. C. J. Controlled Release 2003, 91, 365-374.
[33] Berthold, A.; Cremer, K.; Kreuter, J. J. Controlled Release 1996, 39, 17-25.
[34] Vanderhoff, J., El Aasser, M. &Ugelstad, J. (1979).Polymer emulsification process. In US Patent, Vol. 4,177,177.
[35] Zambaux, M., Zambaux, X. F., Gref, R., Maincent, P., Dellacherie, E., Alonso, M., Labrude, P. & Vigneron, C. (1998). Influence of experimental parameters on the characteristics of poly(lactic acid) nanoparticles prepared by a double emulsion method. Journal of Controlled Release 50: 31-40.
[36] Allemann, E., Quintanar-Guerrero, D., Fessi, H. &Doelker, E. (1998). Preparation techniques and mechanisms of formation of biodegradable nanoparticles from preformed polymers. Drug Development and Industrial Pharmacy 24(12): 1113-1128.
[37] Leroux, J. C., Allemann, E., Doelker, E. &Gurny, R. (1995). New Approach for thePreparation of Nanoparticles by an Emulsification-Diffusion Method. European Journal of Pharmaceutics and Biopharmaceutics 41(1): 14-18.
[38] Couvreur, P., Lemarchand, C. &Gref, R. (2004). Polysaccharide-decorated nanoparticles. European Journal of Pharmaceutics and Biopharmaceutics 58(2): 327-341.
[39] Chen, X. G., Lee, C. M. & Park, H. J. (2003a). OM emulsification for the self-aggregation and nanoparticle formation of linoleic acid-modified chitosan in the aqueous system. Journal of Agricultural and Food Chemistry 51: 3135-3139.
[40] Durand, A., Rouzes, C., Leonard, M. &Dellacherie, E. (2002). Surface activity and emulsification properties of hydrophobically modified dextrans. Journal of Colloid and Interface Science 253(1): 217-223.
[41] Vyas, S. P. &Sihorkar, V. (2001). Potential of polysaccharide anchored liposomes in drug delivery, targeting and immunization. Journal of Pharmacy and PharmaceuticalSciences 4(2): 138-158.
[42] Rodrigues, J. S., Santos-Magalhaes, N. S., Coelho, L. C. B. B.,

Couvreur, P., Ponchel, G. &Gref, R. (2003). Novel core (polyester)-shell(polysaccharide) nanoparticles: protein loading and surface modification with lectins. Journal of Controlled Release 92: 103- 112.

[43] Leonard, M., Rouzes, C., Durand, A. &Dellacherie, E. (2003). Influence of polymeric surfactants on the properties of drug-loaded PLA nanospheres. Colloids and Surfaces B-Biointerfaces 32(2): 125-135.

[44] Labhasetwar, V., Panyam, J., Dali, M. A., Sahoo, S. K., Ma, W. X., Chakravarthi, S. S.,Amidon, G. L. & Levy, R. J. (2003). Polymer degradation and in vitro release of a model protein from poly(D,L-lactide-co-glycolide) nano- and microparticles. Journal of Controlled Release 92(1-2): 173-187.

[45] J Szymońska1, M Targosz-Korecka, F Krok, Characterization of starch nanoparticles, Journal of Physics: Conference Series 146 (2009) 012027.