

# Optimization and effects of physico-chemical parameters on synthesis of chitosan nanoparticles by ionic gelation technique

Pankaj Shard<sup>1\*</sup>, Aruna Bhatia<sup>2</sup>, Dipika Sharma<sup>2</sup>

\*Corresponding author:

Pankaj Shard

<sup>1</sup>Department of Biotechnology, Punjabi University Patiala, Punjab, India 147002

<sup>2</sup>Department of Biosciences, Asian Institution Sirhind Road Patiala, Punjab, India 147002

## Abstract

Biopolymers are used as vehicles for the carrying drugs to their site of action. These polymers are less toxic and can even protect the drug entity from degradation in physiological conditions of the body. Large number of techniques are employed to prepare the nanoformulation of these polymers but ionic gelation is of great interest because no harsh and incompatible chemicals are used during the process. Chitosan nanoparticles are good drug carriers because of their good biocompatibility and biodegradability. As a new drug delivery system they have attracted attention due to their applications in loading protein, drugs etc. In the present research ionic gelation technique have been optimized for the preparation of chitosan nanoparticles. Various physico-chemical parameters of Nanoparticles such as size, Zeta potential and poly dispersity index were evaluated under different process parameters.

In current study ionic gelation technique had been optimized for the preparation of chitosan nanoparticles. We have evaluated the effect of various physico-chemical parameters such as type of polyanion, Sonication, surfactant etc and their effect on size and zeta potential of nanoparticles had been studied.

A novel Nanoparticle system composed of low molecular weight chitosan was successfully prepared in the present study by simple ionic-gelation techniques under aqueous-based conditions. It was observed that there was considerable effect of various physico-chemical parameters on size, zeta potential and polydispersity index of Nanoparticles prepared. Result shows that the size of Nanoparticles decreases with the increase in concentration of Chitosan. Polyvinyl alcohol was found to be the best surfactant because it was very effective in decreasing the surface tension without increasing viscosity. Intermittent sonication during the process decreases the size of Nanoparticles considerably. Controlled use of polyanions under different conditions can develop negatively charged Nanoparticles that can be used for delivery of positively charged drugs and therapeutic molecules.

**Keywords:** Chitosan, Poly Vinyl alcohol, Ionic Gelation technique, polydispersity index

## Introduction

Nanotechnology facilitates innovative biomedical solutions at molecular level. Drug delivery is one of the rising innovations of Nanoscience [1]. The use of recombinant proteins as therapeutics is known but these proteins and peptides have very limited storage stability, high immunogenicity and low efficacy due to lack of proper delivery systems [2]. Besides this proteins and peptides are poorly absorbed after oral administration due to low permeation across intestinal epithelium and susceptibility to enzymatic degradation. Many of the drugs are poorly absorbed across the mucosal surfaces but encapsulation of these drugs in biopolymeric carriers can prevent the degradation of these drugs in hostile environment

of gastro intestinal tract [3]. Nanoparticles modify the pharmacokinetics and pharmacodynamics of drug and protects drugs from hydrolytic enzymes in gastrointestinal tract. [4]. Hydrophilic nanoparticles have received considerable attention to deliver wide variety of therapeutic drugs, peptides, oligonucleotide and genes by intravenous, oral and mucosal administration[5]. NPs have gained much attention because they have mucoadhesive properties, biodegradable, less toxic, higher loading capacity, enhanced interaction sites due to large surface to volume ratio and long shelf life. A wide variety of matrices are being used for the preparation of nanoparticles such as Chitosan[6] Cyclodextrins[7], Ceramics[8], Emulsions[9], Quantum dots[10], Gold shell nanoparticles[11]. Some nanomaterials, such as ultra fine TiO<sub>2</sub>, Ultra fine cadmium oxide, Single walled carbon nanotubes, Ultrafine nickel, Single walled carbon nanotubes & silver



nanoparticles may be toxic to living systems [12-16] and may cause oxidative stress and some are reported to be potent carcinogens[17]. Since metallic nanomaterials may have toxic effects hence non-toxic and biodegradable nanomaterials are the materials of choice. Chitosan has received considerable attention as a material for the formation of NPs[18] as it helps in controlled drug release, increases drug solubility and stability, enhances drug loading efficiency, reduces toxicity, biocompatible and biodegradable [18] mucoadhesive, efficient intestinal permeation enhancer[19, 20]. CS is copolymer containing  $\beta$  (1,4)-2-acetamido-D-glucose and  $\beta$ -(1,4)-2-amino-D-glucose unit. Plant extract loaded Chitosan Nanoparticles were found to have immunorestorative properties [21]. It possesses positive charge due to presence of surface amine groups. It actively binds negatively charged sialic acid residues on mucous membranes and enhances drug release profile of the drug. It was also reported to enhance intestinal permeation across intestinal epithelial cell line Caco-2 [22]. The principle behind the mechanism of formation of NPs includes the electrostatic interaction between both oppositely charged polysaccharides with the ability of CS to undergo a liquid gel transition because of its ionic interaction with poly anions such as Trisodium polyphosphate [24]. It is used as excellent protein delivery system [23], gene delivery systems [24] and carrier for nasal vaccines [25]. The residual amine groups present on the surface of Chitosan after gelation, interact with negatively charged drugs.

Hence in present study, Chitosan has been employed for formulation of nanoparticles and effect of various physico-chemical parameters on mean diameter of nanoparticles were studied. The charge of drug affects considerably the uptake of the drug in acidic environment produced by tumor cells due to excessive consumption of oxygen. Negatively charged drugs such as camptothecin isup taken by tumor tissue efficiently but weakly basic and positively charged drugs were excluded [26]. Hence the present study was also done to optimize ionic gelation process for chitosan nanoparticle formulation and to broaden the application of CS NP's in delivery of both positively and negatively charged drugs by altering surface charge using different poly anions.

## Materials and methods

### Optimization of process parameters for preparation of Chitosan Nanoparticles

In present study, four different methods differing in various physico chemical aspects had been used for the preparation of Chitosan Nanoparticles. Ionic gelation technique was used for preparation of NPs. The method followed was a modification of the Janes *et al.*[27] The effect of various physico chemical parameters on the characteristics Chitosan Nanoparticles obtained were studied. These process parameters affects the mean diameter and zeta potential of the CS particles obtained.

Method 1: 0.17 % (W/V) chitosan was dissolved distilled water acidified with 2% acetic acid. 2% tween 80 was added as a surfactant to prevent aggregation of nanoparticles. The solution

was stirred over magnetic stirrer at 500 rpm for 48 hours without ultrasonication. After 48 h sodium sulphate was added slowly to the solution with continued stirring for 1 h to precipitate Chitosan nanoparticles from the solution. Temperature was maintained at 37 C during the process.

### Lyophilization of Chitosan nanoparticles

The CS NPs prepared by method 1 were lyophilized. 1% mannitol was used as cryoprotectant. During primary drying process solvent is first removed by sublimation by maintaining the temperature below eutectic temperature of nanoparticles suspension. Primary drying is followed by secondary drying process to remove residual moisture to prevent growth of microorganism and chemical reactions. Lyophilized cultures were stored at 4° C in deep freezer for a month.

Method 2: Chitosan (0.05% w/v) was dissolved in 2% acetic acid and solution was stirred using magnetic stirrer at 25°C. Prepared 0.1% (w/v) sodium hexametaphosphate solution in distilled water and added it in chitosan solution at a rate of 1 mL/min till the transmittance of the suspension reaches up to 75% at an operating wavelength of 480 nm. The whole solution was sonicated for 20 mins. 1% PVA was added to stabilize the particle suspension. The Chitosan Nanoparticles were characterized by diffraction light scattering using Zeta Sizer (Malvern Co.)

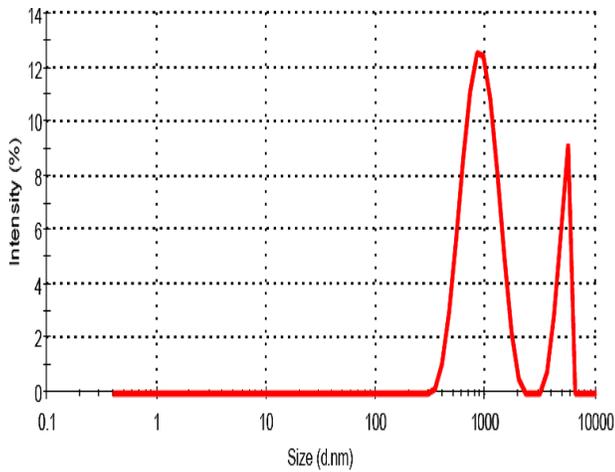
Method 3: 0.1 % (W/V) chitosan was dissolved in distilled water containing 2% Acetic acid. The resulting solution was agitated using magnetic stirrer at 500 rpm at 37°C for 4h. Chitosan Nanoparticles were precipitated with 0.5 % Sodium hexametaphosphate. Sodium hexametaphosphate was added drop wise with uninterrupted stirring till the transmittance of the solution becomes 75%. Ultrasonication was not done during entire process. The nano particle suspension was stabilized by drop wise addition of 0.5% (W/V) polyvinyl alcohol as surfactant.

Method 4: 0.1 % (W/V) chitosan was dissolved in distilled water containing 2% Acetic acid. The resulting solution was stirred gently over magnetic stirrer at 500 rpm at 37°C for 4h. Chitosan nano particles were precipitated with 0.5 % Pentasodium Tripolyphosphate (TPP). TPP was added drop wise with uninterrupted stirring till the transmittance of the solution becomes 75%. Ultrasonication was not done during entire process. The nano particle suspension was stabilized by drop wise addition of 0.5% (W/V) polyvinyl alcohol as surfactant.

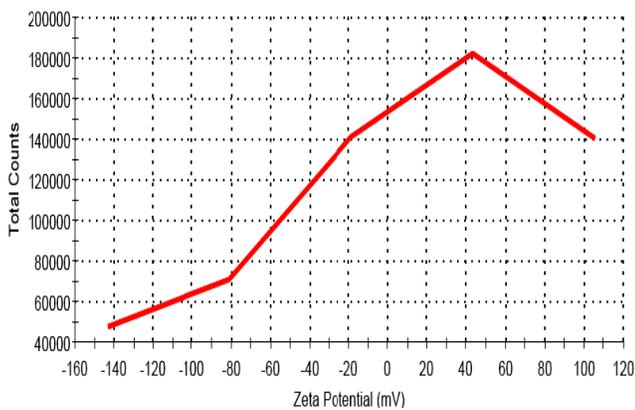
## Results and Discussion

Method 1: The average size of Chitosan nanoparticles was found to be 1275 nm (Figure 1) with Polydispersity Index of 0.474. The Zeta Potential was recorded to be +16.7 mV (Figure 2)





**Figure 1:** Size of Chitosan Nanoparticles obtained by method 1

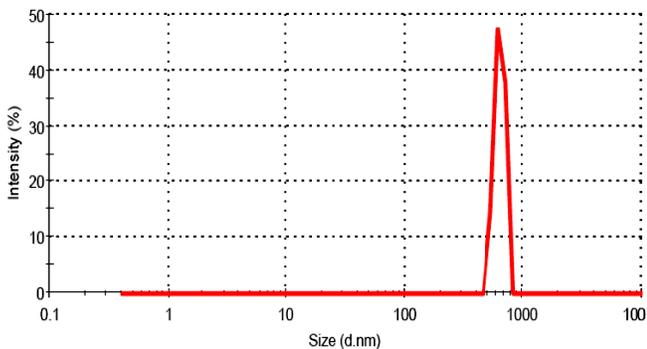


**Figure 2:** Zeta potential of NPs came out to be +16.7 mV

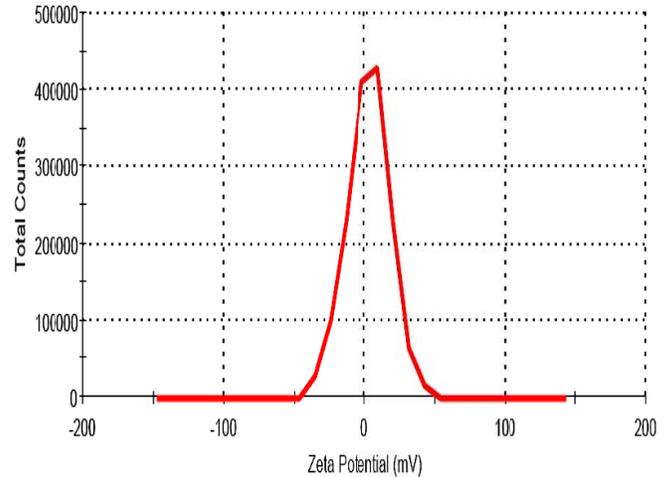
The positive Zeta Potential (16.7 mV) is may be due to presence of unreacted NH<sub>2</sub> groups available on Chitosan surface for attachment to other negatively charged molecules.

### Effect of Lyophilization on size of NPs prepared by method 1

No considerable change in size of CS NPs has been seen before and after Lyophilization (figure 3).



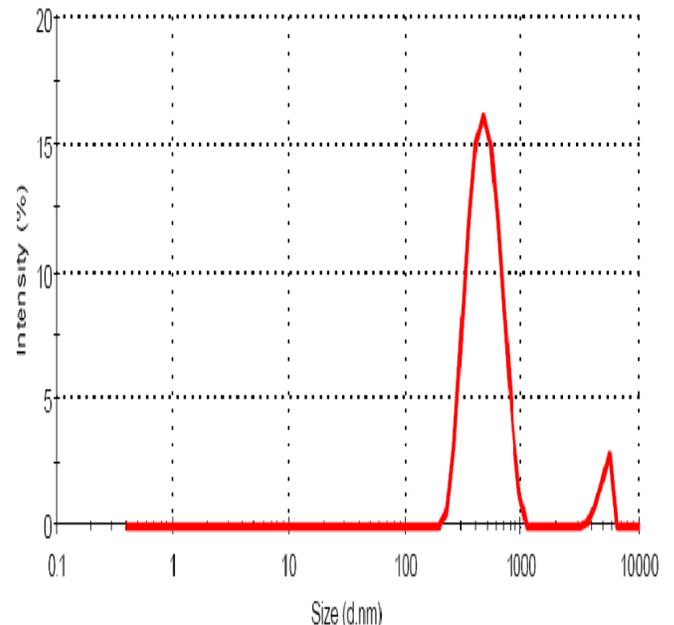
**Figure 3:** Effect of Lyophilization on size of NPs



**Figure 4:**Effect of Lyophilization on Zeta potential of NPs.

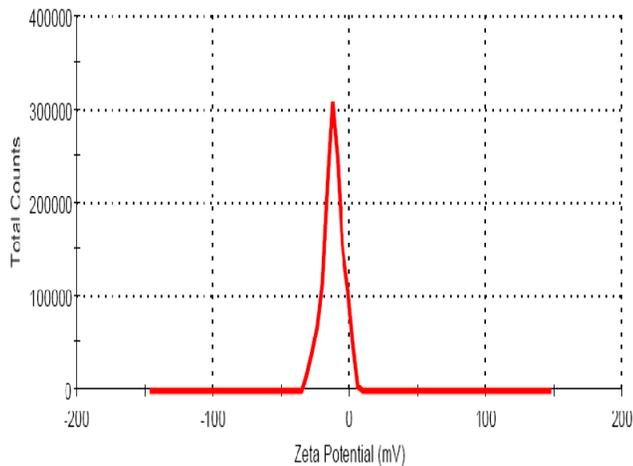
An increase of 100nm in size was observed but zeta potential is nearly constant as depicted in figure 4.

METHOD 2:The size of nanoparticles was found to be 487 nm as shown in figure 5.



**Figure 5:** Size of Chitosan Nanoparticles by method 2.

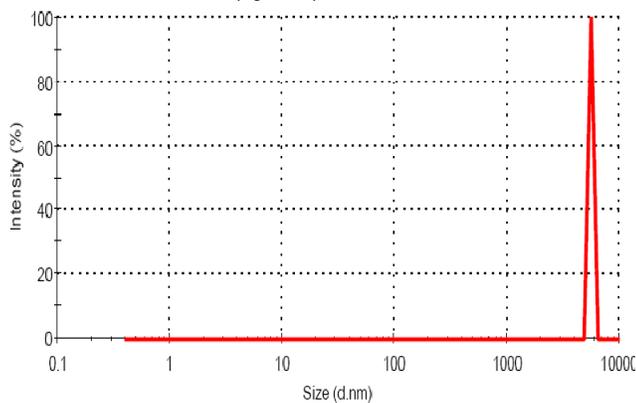




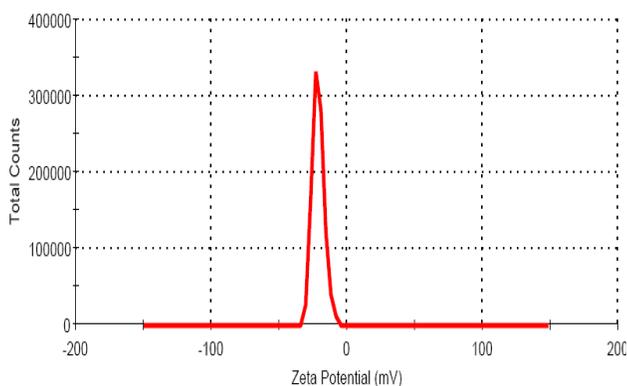
**Figure 6 :** Zeta Potential of Chitosan Nanoparticles by method 2:

and Zeta potential of the chitosan nanoparticles was recorded to be -12.2 mV which is found to be quite significant (figure 6) The polydispersity index was 0.334 showing monodisperse nature of Nanoparticles.

METHOD 3: The size of nanoparticles was found to be 6711 nm (figure 7) and Zeta potential of the chitosan nanoparticles was recorded to be -21.1 mV (figure 8).



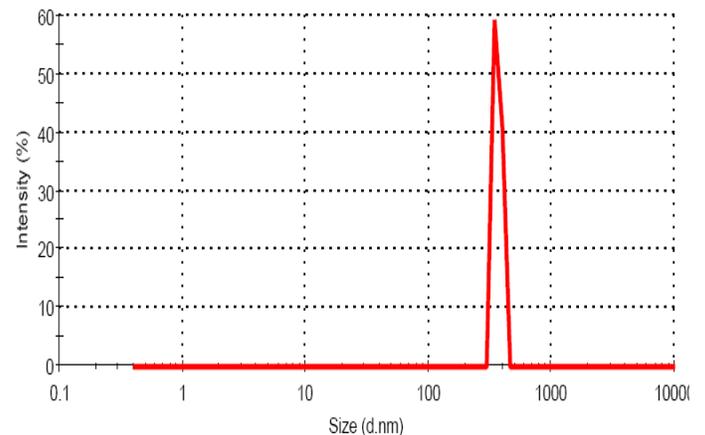
**Figure 7 :** Size of Chitosan Nanoparticles obtained by method 3:



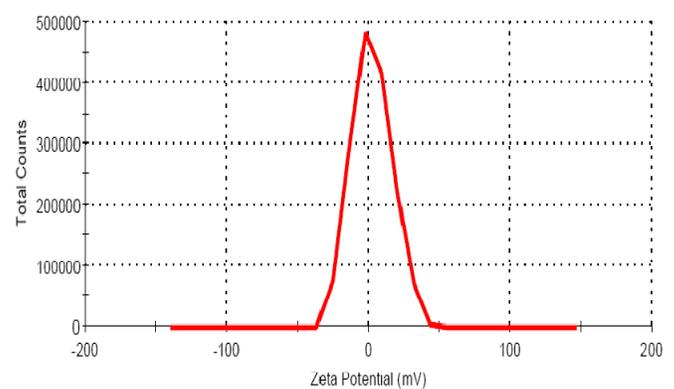
**Figure 8 :** Zeta Potential of Chitosan Nanoparticles obtained by method 3

The negative zeta potential was observed may be due to negatively charged phosphate groups in Sodium hexametaphosphate. The polydispersity index was 0.454 showing that maximum nanoparticles are having the size of 6711 nm and process results are within significant limits.

METHOD 4: The size of nanoparticles was found to be 5621 nm (figure 9) and Zeta potential of the chitosan nanoparticles was recorded to be +2.34 mV (figure 10).



**Figure 9 :** Size of Chitosan Nanoparticles obtained by method 4



**Figure 10 :** Zeta Potential of Chitosan Nanoparticles obtained by method 4

The polydispersity index was found to be 1.000 showing the process exceeds the prescribed limit of PDI and hence process is insignificant because Chitosan Nanoparticles synthesized were polydisperse in nature and have very narrow utility as therapeutic carriers.

The various parameters such as concentration of CS, sonication, type of polyanion and surfactant used in the process have been studied in relation to the acceptability of CS NPs.

### Effect of Chitosan concentration

It has been found that with the increase in concentration of chitosan, the size of nanoparticles increases. 0.05% chitosan gave best results. This may be due to the fact that at higher



concentration CS molecules are present in close vicinity to each other and they got precipitated to form a large particle during precipitation by polyanion.

### Effect of polyanion

The zeta potential was dependent upon the type of polyanion used during the preparation of CS NPs. When sodium sulphate and TPP were used for precipitation of NPs, the zeta potential was recorded to be +16.7mV. The positive zeta potential is may be due to surface residual amine groups present in chitosan. But when sodium hexametaphosphate is used as polyanion for precipitation of Chitosan nanoparticles, the zeta potential was found to be -21.1mV. The negative zeta potential is may be due to the excessive phosphate groups remained un-interacted during the process.

### Effect of surfactant

PVA was found to be better surfactant as compared to Tween-80. It may be due to the fact that PVA was more efficient in reducing the surface tension. Besides this, Tween-80 was found to be more viscous than PVA.

### Effect of Sonication

Sonication affected the mean diameter of Chitosan NPs to considerable extent. It is possibly due to shear forces that reduced the mean diameter of Nanoparticles during the process.

### Effect on properties of Chitosan Nanoparticles upon long term storage in lyophilized form.

CS NPs were lyophilized and stored for one month at 4 °C. These nanoparticles were resuspended in phosphate buffer and Dynamic Light Scattering analysis revealed that no considerable change in size and zeta potential occurs after long term storage. Hence these Nanoparticles can be stored lyophilized but in aqueous suspension, they swell in size and degrade gradually.

## References

- [1]. Sahoo SK, Labhasetwar. Nanotech approaches to drug delivery and imaging. *Drug Discov Today*. 2003;8(24): 1112-1120
- [2]. Frokjaer S, Otzen DE. Protein drug stability: a formulation challenge. *Nature Rev Drug Discov*. 2005; 4: 298-306
- [3]. Wilding IW, Davis SS, O'hagen DT. Targeting of Drugs and vaccines to the gut. *Pharmacol. Ther*. 1994;62:97-124
- [4]. Sakuma S, Hayashi M, Akashi M. Design of nanoparticles composed of graft copolymers for oral peptide delivery. *Adv Drug Deliv Rev* 2011;47: 21-37
- [5]. Mcclean S, Processer E, O'Malley D, Clark N, Ramtoola Z, Brayden D. Binding and uptake of biodegradable polylactide micro and nanoparticles in intestinal epithelia. *Eur J Pharm Sci* 1998;6: 153-63
- [6]. Agnihotri SA, Mallikarjuna NN, Aminabhavi TM. Recent advances on chitosan based micro and nano particles in drug delivery. *J Control rel*. 2004;100:5-28
- [7]. Loftssora T. Cyclodextrins in ophthalmic drug delivery. *Adv Drug Deliv Rev*. 1998;36: 59-79
- [8]. Cherian AK, Rana AC, Jain SK. Self assembled carbohydrates stabilized nanoparticles for the potential delivery

## Conclusion

In the present study, attempts were made to optimize method for preparation of chitosan nanoparticles. Chitosan nanoparticles was successfully prepared in the study by a simple ionic-gelation method under aqueous-based conditions Results revealed that there is considerable effect of chitosan concentration, surfactant, Sonication and polyanion on the mean diameter and zeta potential of Nanoparticles obtained. Size of Nanoparticles stored for one month at low temperature increased upon Lyophilization to some extent but zeta potential remained the constant. Hence it is possible to store therapeutics for longer duration without the loss of efficacy of drugs. The negatively charged Chitosan Nanoparticles obtained can be employed for delivery of poorly absorbed positively charged drugs. Hence charge on Nanoparticles can be altered according to the nature of drug and application of Chitosan Nanoparticles can be broadened as drug delivery vehicles.

### Conflict of Interests

No conflict of Interests as declared by authors.

### Author's Contribution

PS designed and supervised the experiment and experimentation was done by PS and DS. PS did manuscript drafting formulated nanoparticles and characterization of nanoparticles such as particle Size ,zeta potential is also done by PS. AB directed the Study and drafting of manuscript by many revisions.

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- of drug delivery. *Ind Pharm.* 2000;26: 459-63
- [9]. Sarkar DK. Engineering of nanoemulsions for drug delivery. *Curr Drug Deliv.* 2005;2:297-310
- [10]. Wang L, Wang A: *J Hazard Mater.*2006; 136:930.
- [11]. Hirsch IR, Gobin A.M, Lowery AR, Tom F, Drezek RA, Halas NJ: Metal nanoshells. *Ann biomed Engg.* 2006; 34: 15-22
- [12]. Bermudez E, Mangum JB, Wong BA, Asgharian B, Hext PM, Waheit DB, Everitt JI : Pulmonary responses of mice, rats and hamsters to subchronic inhalation of ultrafine titanium dioxide particles. *Toxicol Sci.*2004;77:347-357
- [13]. Warheit DB, Webb TR, Sayes CM, Colvin VL, Reed KL. Pulmonary instillation studies with nanoscale TiO<sub>2</sub> rods and dots in rats: Toxicity is not dependent on particle size and surface area. *Toxicol Sci.* 2006; 91:227-236
- [14]. Takenaka S, Karg E, Kreyling W, Lentner B, Schulz H, Ziesenis A, Schramel P, Heyder, J: Fate and toxic effects of inhaled ultrafine cadmium oxide particles in the rat lung. *Inhalation Toxicol.* 2004; 16:83-92
- [15]. Serita F, Kyono H, Seki Y. Pulmonary clearance and lesions in rats after a single inhalation of ultrafine metallic nickel at dose levels comparable to the threshold limit value. *Ind. Health.* 1999; 37:353-363
- [16]. Hussain SM, Hess KL, Gearhart JM, Geiss KT, Schlager JJ: In vitro toxicity of Nanoparticles in BRL 3A rat liver cells. *Toxicol In Vitro.* 2005; 19: 975-983
- [17]. Kawanaka, Matsumoto E, Wang N, Tsuchiya Y, Yun SJ, Jiang ZW, Sakamoto K: Mutagenic activity of atmospheric ultrafine particles at a roadside site and a suburban site. *J. Health Sci.* 2006, 52:352-357
- [18]. Calvo P, Remunan-Lopez C, Vila-Jato JL, Alonso MJ: Novel hydrophilic chitosan-polyethylene oxide nanoparticles as protein carriers. *J. Appl. Polymer Sci.* 1997, 63: 125-132
- [19]. Wang JJ, Zeng ZW, Xiao RZ, Xie Tian, Zhou GL, Zhan XR, Wang SL. Recent advances of chitosan nanoparticles as drug carriers. *International J. of nanomed.* 2011, 6: 765-774
- [20]. Thanou M, Verhoef JC, Junginger HE. Oral drug absorption enhancement by chitosan and its derivatives. *Adv. Drug Deliv. Rev.* 2001; 52, 117-126.
- [21]. Bhatia A, Shard P, Chopra D, Mishra T. Chitosan nanoparticles as Carrier of Immunorestoratory plant extract: synthesis, characterization and Immunorestoratory efficacy. *Int. J. of Drug Del.* 2011; 3:381-385
- [22]. Kowapradit J, Opansopit P, Ngawhirunpat T, Apirakaramwong A, Rojanarata T, Ruktanonchai U, Sajomsang W. In vitro permeability enhancement in intestinal epithelial cells(caco-2)monolayer of water soluble quaternary ammonium chitosan derivatives. *AAPS Pharm Sci Tech.*2010;11(2):497-508
- [23]. Zhang HL, Wu SH, Tao Y, Zang LQ, and Su ZQ. Preparation and characterization of water soluble chitosan nanoparticles as protein delivery system. *J. of Nanomaterials,* 2010; 2010: 898910
- [24]. Gan Q, Wang T, Cochrane C, McCarron P. Modulation of surface charge, particle size and morphological properties of chitosan-TPP nanoparticles intended for gene delivery. *Colloids and Surfaces B: Biointerface.* 2005; 44, 65-73.
- [25]. Vila A, Sanchez A, Janes K, Behrens I, Kissel T, Jato JLV and Alonso MJ. Low molecular weight chitosan nanoparticles as new carriers for nasal vaccine delivery in mice. *European J. of Pharma and Biopharma.* 2004;57, 123-131.
- [26]. Adams DJ, Morgan LR: tumor physiology and charge dynamics of anti cancer drugs: Implications for Camptothecin based drug development. *Curr Med Chem.* 2011;18(9):1367-1372
- [27]. Janes KA, Calvo P, Alonso MJ. Polysaccharide colloidal particles as delivery systems for macromolecules. *Adv. Drug Deliv. Rev.* 2001;47: 83-97

