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Effects of some parameters on chitosan nanoparticles prepared by ionic gelation method

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Abstract
The present study included the process optimization of different method for preparation of Thiotepa loaded nanoparticles. Ionic gelation method of chitosan nanoparticle preparation was standardized and the physico-chemical characterization was done. Effects of parameters such as chitosan concentration, Chitosan to sodium tripolyphosphate ration and pH of the chitosan solution on the particle size distribution were investigated. The characterization parameter involved estimation of particle size which was 200.37±5.40 nm and PDI o 0.33±0.01 for the drug loaded chitosan nanoparticles. TEM images depicts smooth surface and spherical shape of the nanoparticles. The optimum condition to obtain the smallest nanoparticle were found to be 0.1% chitosan concentration, 4.6 pH of the chitosan solution and 3:1 ratio of chitosan to sodium tripolyphosphate.

Keywords: PDI, TEM, thiotepa

Introduction
Nanotechnology is a new science that works with the nanometer scale, and nanoparticles are one of its building blocks. Solid colloidal particles of the nanometer range, i.e., 10–1000 nm, are known as nanoparticles. Natural polymers like protein and polysaccharide, as well as synthetic polymers like polystyrene, can be used to make nanoparticles. Heat, chemical solvents, or strong shear force are used to make synthetic polymer nanoparticles, which might compromise drug stability. Nanoparticles made from natural polymers, on the other hand, have a moderate and easy manufacturing procedure that does not require the use of an organic solvent or a strong shear force (Agarwal *et al*., 2018) [1]
Chitin, a naturally abundant mucopolysaccharide that supports crustaceans, insects, and other organisms, is known to be composed of 2-acetamido-2-deoxy- β -D-glucose linked by a β (1,4) linkage. It's a nitrogenous polysaccharide that's white, rigid, and inelastic. Chitosan is an N-deacetylated chitin derivative. Chitosan is a polycationic and nontoxic polymer that has a wide range of uses in the food, pharmaceutical, and chemical sectors. The characteristics of chitosan can be influenced by a variety of factors, including molecular weight of polymer, degree of polymerization, degree of deacetylation, and particle size (Kumar, 2000) [4].
Chitosan nanoparticles (CNPs) can be easily prepared by the ionic gelation method. In this, electrostatic interaction occurs between the positively charged polymer and negatively charged polyanion like sodium tripolyphosphate (TPP). It leads to the formation of spherical shaped nanoparticles. Size and potential of the nanoparticles can be assessed by Dynamic lighting system (DLS). Morphology of the nanoparticles observed by Transmission Electron Microscope (TEM).

Materials and Methods
Thiotepa was obtained from was obtained from Sigma Aldrich Co. LLC, New Delhi. Low molecular weight chitosan powder (Molecular weight of 6000 D) having [85% degree of deacetylation was obtained from Sigma Aldrich, sodium tri-polyphosphate (TPP), acetic acid (analytical grade) was purchased from Merck (Mumbai, India).

Preparation of Chitosan Nanoparticles
They were prepared as was given by (Vaezifar *et al*., 2013) [5] with a slight modification. Low molecular weight chitosan powder was dissolved in 1% (w/v) concentration ofacetic acidaqueous solution under magnetic stirringfor a period of 4-6 hours. Likewise different concentrations of chitosan solution were prepared. By dropwise addition of 0.1N NaOH, the pH of the solutions was raised to 4.5–4.8.

A clear solution of sodium tripolyphosphate was obtained in distilled water by mixing 50 mg of asodium tripolyphosphate (TPP) powderin 50 ml of distilled water. Thiotepa was added in a particular ratio to the TPP solution, this solution was vortexed so as to make a uniform solution. Drug-TPP solution was then added dropwise into a chitosan solution while being agitated with a magnet stirrer at 700-800 rpm speed for specific time period. The solutions were taken in various ratios, such as 2:1, 3:1, 4:1 and 5:1, with former being the chitosan solution and the latter being the Drug-TPP solution. The solution is then given an ultrasonication bath. Three different kinds of solution were obtained i.e., a clear solution, colloidal solution and a flocculant solution. The resulting suspension was subsequently ultracentrifuged at 20,000 g for 25 min. The drug loaded chitosan nanoparticles were washed with deionised water to remove any impurity. Nanosuspension was then freeze dried for storage.
Temperature of the experiment was kept constant. Thus, the effects of chitosan concentration (CC), proportion of polymer to polyanion, strength of the solution and sonication timing on the particle size distribution were investigated.

Characterization of chitosan loaded drug nanoparticle
The prepared chitosan loaded thiotepa nanoparticles were characterized by the following method.

Dynamic Light Scattering (DLS) System: The average nanoparticle size and zeta potential was measured as described by Agnihotri *et al*. (2004) [2]. The zeta potential and particle size distribution of chitosan nanoparticles were determined using DLS with the Zetasizer Nano S (Malvern, UK). The testing was performed using nanoparticles dispersed in de-ionized distilled water at a scattering angle of 90° and a temperature of 25 °C (1 mg of nanoparticle sample was dissolved in 5 ml of distilled water and then sonication of the sample is done).

Transmission electron microscope (TEM): The morphology of the nanoparticles in suspension can be seen using a transmission electron microscope. The sample was imaged using a Hitachi H-7650 with a 40-120kV accelerating voltage; 0.2 nm resolution; magnification range 200x-200000x in high contrast (HC) mode; 4000x-600000x in high resolution mode (HR); tungsten and LaB_6 filaments; and a 1024*1024 pixels digital camera.

Results and Discussion
Chitosan concentration was very successful in forming nanoparticles. When the CS and TPP concentrations were suitable, the zone of opalescent nanosuspension, which indicated suspension of colloidal nanoparticles, was discovered. It can be noted that concentration of the chitosan solution, ratio of the polymer to polyanion and pH of the solution affects the nanoparticle formation.

Table 1: Particle size and PDI of thiotepa loaded nanoparticles for varying concentration at 5% level of significance

S. No.	Chitosan Concentration	Z- Average	PDI
1	1	1472.0±51.06	0.7630±0.07
2	0.5	543.4±28.64	0.6347±0.05
3	0.25	465.6±25.35	0.5977±0.04
4	0.1	212.1±3.05	0.3690±0.06

PDI- polydispersity index, ± - standard deviation

Table 2: Particle size and PDI of thiotepa loaded nanoparticles for varying formulation at 5% level of significance

S. No.	Formulations	Z- Average	PDI
1	(5:1) F1	1032.3±150.70	0.9877±0.01
2	(4:1) F2	944.0±48.84	0.7887±0.03
3	(3:1) F3	212.1±3.05	0.3690±0.06
4	(2:1) F4	307.3±34.44	0.4853±0.01

Table 3: Particle size and PDI of thiotepa loaded nanoparticles for varying pH at 5% level of significance

S. No.	pH	Z- Average	PDI
1	4	469.17±54.75	0.5877±0.02
2	4.6	200.37±5.40	0.3380±0.01
3	5	787.00±28.05	0.8827±0.05

It was observed that at a concentration of 0.1% (table no. 1) concentration of chitosan the nanoparticle size was found to be minimum. Formulation F3 (table no. 2) was found to be most suitable.

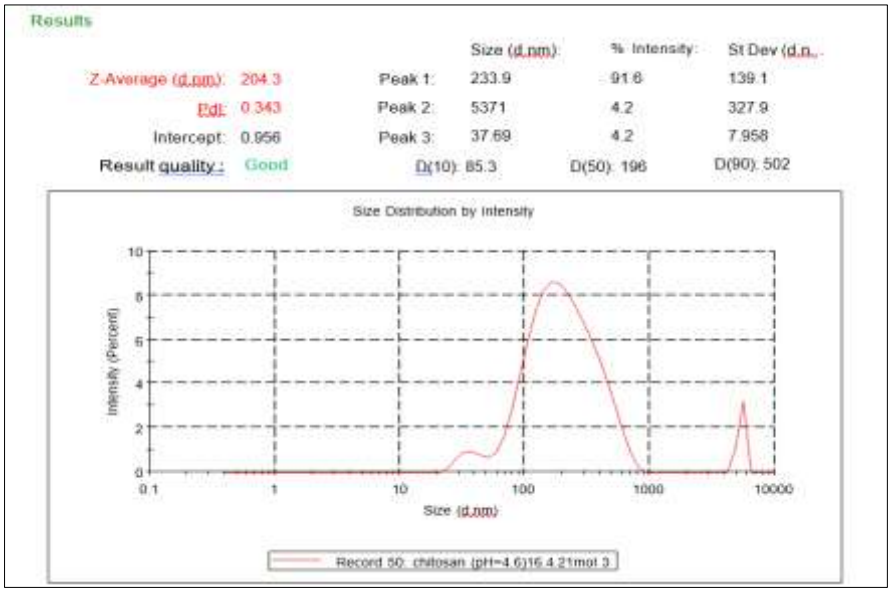

Fig 1: Particle size of the Chitosan loaded drug nanoparticle

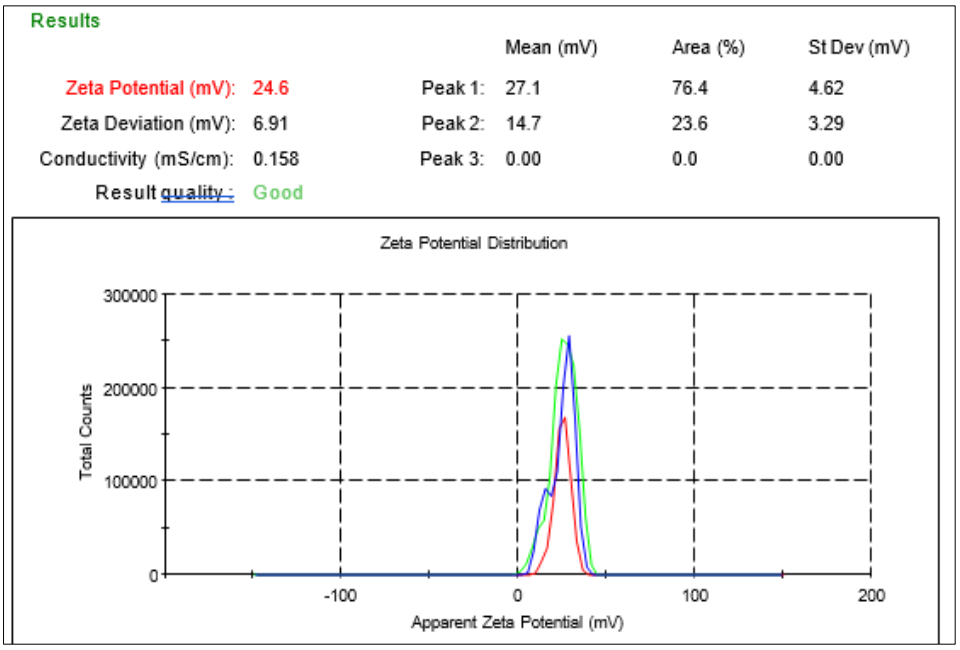

Fig 2: Zeta potential of the Chitosan loaded drug nanoparticle

At a pH of 4.6 (table no. 3) particle size and polydispersity index (PDI) were found be minimum. Similar findings were reported by Gharedaghi *et al*. (2012) [3], which stated that the pH of the buffer has a significant but non-linear influence on the size of chitosan nanoparticles generated using this approach Zeta potential represents the stability of the nanoparticle (Fig. 2), it was 24.6 mV on an average.

During the TEM analysis (Fig. 3), nanoparticles were found to have smooth surface and spherical shape, and nanoparticle size was around 200 nm. Similar reports were obtained by Vaezifar *et al*. (2013) [5].

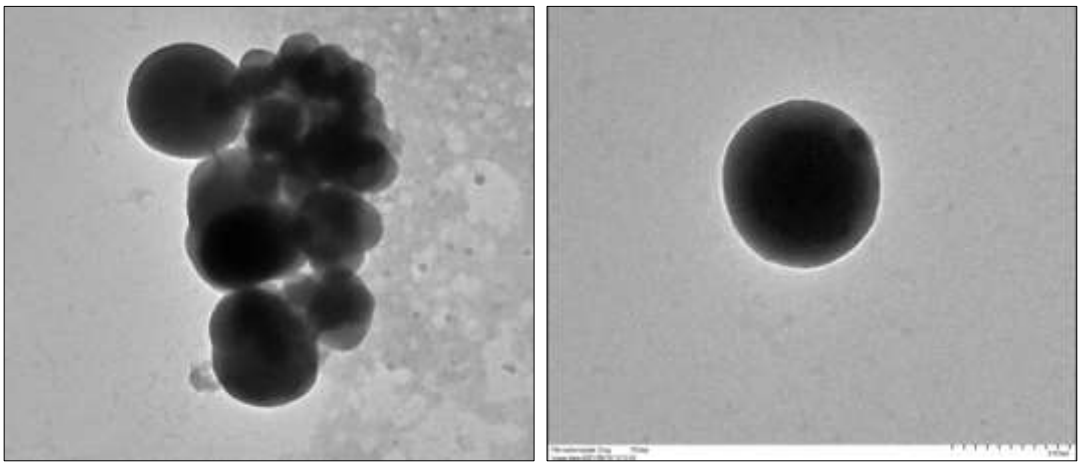

Fig 3: TEM analysis of the chitosan loaded drug nanoparticle

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