International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
IJCS 2019; 7(2): 1530-1537
© 2019 IJCS
Received: 19-01-2019
Accepted: 20-02-2019

D Kanjana
Central Institute for Cotton Research, Regional Station, Coimbatore, Tamil Nadu, India

Correspondence
D Kanjana
Central Institute for Cotton Research, Regional Station, Coimbatore, Tamil Nadu, India

Preparation and characterization of Nanoformulated zinc fertilizer by using biopolymer and their effects on cotton

D Kanjana

Abstract
Nanoformulated zinc fertilizer with or without zein polymer coating was synthesized as a novel slow or controlled release fertilizer through ionic gelation process. Results of the synthesized nanofertilizer size ranging between 70 – 86 nm for Zn loaded CS/TPP nanoparticle whereas size of zinc loaded CS/TPP nanoparticle with zein was 80 to 300 nm. Shape of the Zn loaded chitosan nanoparticle with or without zein coating was spherical with smooth surface and homogenous in diameter. Surface charge, *ie*., zeta potential of zinc loaded CS/TPP nanoparticle with or without zein coating was found to be positively charged. A preliminary pot culture experiment was also carried out to determine the effects of foliar application of newly synthesised nanoformulated zinc fertilizer (50 ppm) on 30 day old cotton (var: suraj) plants. A significant improvement was recorded on plant height, number of leaves, no.of squares and root length by application of nanoformulated zinc with chitosan and zein (0.1%) in 7 weeks old plant.

Keywords: Chitosan, zein, zinc, cotton, nanofertilizer, foliar spray

1. Introduction

Zinc is one of the essential micronutrients which required in smaller quantities for normal plant growth and complete their life cycle. In India, Zinc is now considered the fourth most important yield limiting nutrient after nitrogen (N), phosphorus (P) and potassium (K) (Adhikari *et al.* 2016) [1]. Zinc (49%) is the most deficient micronutrient in Indian soils as compared to other nutrients *viz.* boron (33%), molybdenum (13%), iron (12%), manganese (5%) and copper (3%) (Singh, 2009) [20]. Not only Indian soils are very poor for micronutrient but also micronutrient use efficiency is very low (2-5%) due to some means of losses. But Zinc is very important for chlorophyll synthesis, carbohydrate formation, pollen production, fertilization and biomass production (Kaya & Higgs, 2002) [10]. An experiment crop Cotton (*Gossipium spp*.) 'White gold', one of the most important commercial crops playing a key role in economic, political and social affairs of the country was used in the present investigation. Zinc plays a vital role in flower enhancement, boll formation and good fibre quality of cotton. In addition, zinc is an integral component of many enzyme structures and is the only metal to be represented in all six enzyme classes viz., oxidoreductase, transferases, hydrolases, lyases, iso merases and ligases (Auld, 2001) [2]. Normally micronutrient fertilizers are applied through foliar spray than soil application because smaller quantities of micronutrients cannot be applied over the entire field uniformly and also nutrient uptake by plant get reduced due to poor root ability, fixation and precipitation behaviour of the soil. Therefore, to increase the use efficiency of zinc and enhance the efficacy of the nutrient on leaf surface, smart delivery system is crucial, nowadays. Nanotechnology is an emerging technology in the world and has the potential to revolutionize the agriculture with new concepts like smart delivery system. Nanoencapsulation is considered as one of the unique technique to produce the nanoparticle with diameters ranging from 1-1000 nm (Mohanraj & Chen, 2006) [14] for increasing the bioavailability, solubility and retention time of bioactive compounds (Shenoy & Amiji, 2005) [19]. Polymer-containing nanoparticles are attractive because of their process feasibility and repeatability, lower cost, controlled/sustained release property, subcellular size and biocompatibility (Panyam & Labhasetwar, 2003) [15]. Recently, Chitosan, the cationic polysaccharide obtained by partial alkaline deacetylation of chitin, the second most abundant natural polymer and as a component of the exoskeletons of shrimp and crustacean shells has

Drowned tremendous attention among researchers in various fields like biomedical and agriculture due to its versatile properties such as biodegradability, biocompatibility and low toxicity (Gan *et al.* 2005) [7]. Likewise, polyanion *viz.*, Tripolyphosphate (TPP) has the quick gelling capability and non-toxic property (Gan & Wang, 2007) [8]. Because of these beneficial characteristics, increasing attention has been drawn to prepare the chitosan nanoparticles by ionic gelation method in which, cationic chitosan interacts with anionic tripoly phosphates (Calvo *et al.* 1997) [3]. These biopolymers till date considered as most active nano encapsulation material for providing efficient delivery with lowering toxicity and increasing bioavailability as well as achieving the long term controlled release of nutrient. Zein is a water insoluble corn protein, which can be used for coating the micronutrients loaded chitosan/TPP nanoparticles to enhance the encapsulation efficiency and controlled release of bioactive compounds.

2. Materials and Methods

2.1 Materials

Low molecular weight Chitosan (CS) with 75-85% deacetylation degree (Batch No: MKBK4182V), Penta sodium tripolyphosphate (TPP), Acetic acid (>99.7%) and Zinc sulphate hepta hydrate ($ZnSO_4.7H_2O$) were purchased from Sigma-Aldrich Chemical Co. Ltd. Zein was obtained from Himedia Laboratories Pvt. Ltd and then Methanol was purchased from SD Fine chemical Ltd.

2.2 Preparation of zinc loaded CS/TPP nanoparticles with or without zein coating

In the first step, chitosan (0.1%) solution was prepared by dissolving 0.1g of chitosan in 100 ml of 1% acetic acid under magnetic stirring (400 rpm) for 30 mins at room temperature. The same concentration of chitosan can be used for preparation of TPP solution *ie.*, 0.1% (0.1 g TPP in 100 ml of deionised water). According to Calvo *et al.* (1997) [3] ionic gelation process, TPP solution was added drop wise to the CS solution and stirred (400 rpm) for 30 mins at room temperature to obtain the blank nanoparticles *ie.*, CS/TPP nanoparticles. For preparation of zinc loaded CS/TPP nanoparticles, zinc fertilizer ($ZnSO_4.7H_2O$) 0.5% *ie.*, recommended dose of fertilizer for cotton was added slowly by drop wise to the CS solution and then TPP solution was also added drop wise to the mixture with mild stirring (400 rpm) for another 30 mins. This is considered as zinc loaded CS/TPP nanoparticle without zein coating. Different concentrations *viz.*, 0.1, 0.2 and 0.3% zein solution were prepared by dissolving 0.1, 0.2 and 0.3 g zein in 100 ml of 90% methanol by using magnetic stirrer at 500 rpm for 45 mins. These various concentrated zein solution was added into the mixture of zinc loaded CS/TPP nanoparticles to produce 1:1, 1:2 and 1:3 ratio of zinc loaded CS/TPP nanoparticles with zein coating. Finally, CS/TPP (blank) nanoparticles, zinc loaded CS/TPP nanoparticles and Zinc loaded CS/TPP with zein coating were centrifuged at 10,000 rpm for 15 mins at room temperature. This process was done for 2 to 3 times by adding distilled water and discarded the supernatant solution. Then the gel type samples were collected to dry it in Lyophilizer and used for further characterization of nanoparticles.

2.3. Characterization of zinc loaded CS/TPP nanoparticles with or without zein coating

Particle size distribution and zeta potential of blank (CS/TPP), zinc loaded CS/TPP and zein coated (1:1, 1:2 and 1:3 ratio) zinc loaded CS/TPP nanoparticles were measured by using Particle Size Analyser (HORIBA SZ-100) and Zeta analyser (HORIBA SZ-100) respectively. PSA works on the principle that when a beam of light (laser) is scattered by a group of particles, the angle of light scattering is inversely proportional to particle size (*ie.*, the smaller particle size, the larger the angle of light scattering). In the Zeta analyser, laser light is divided into input light (scattered by particles) and reference light (by the modulator), these two beams are interfere in the prism which are detected as digital signal. Accurately, 0.5 mg sample was dispersed in 10 ml of Millipore water and sonicated for 30 mins and then samples were taken into the cuvette for measuring the particle size and zeta potential.

2.3.1. Scanning Electron Microscopy (SEM) Analysis

SEM was extremely useful for the determination of topology by scanning it with high energy beam electrons in raster scan pattern. Morphological structures of chitosan (CS), zinc loaded CS/TPP nanoparticle in with or without zein were obtained by using SEM (Quanta 250, FEI, Netherlands) and Field Emission Scanning Electron Microscopy (FESEM) instruments. For taking the images, samples were dusted on conductive carbon tapes and mounted on specimen stubs and then coated with a thin conductive gold layer using a sputter coater.

2.3.2 Transmission Electron Microscopy (TEM) Analysis

Confirmation of size and shape of the nanoparticles, TEM measurements was carried out using drop coating method in which a drop of solution containing nanoparticles was placed on the carbon coated copper grids and kept under vacuum desiccation for overnight before loading them onto a specimen holder. TEM images of the sample were taken using a FEI Tecnai microscope.

2.3.3 X-Ray Diffraction (XRD) Analysis

The crystal structure and size confirmation were examined by X Ray Diffractometer (XRD) analysis. XRD analyses of zinc loaded CS/TPP nanoparticle and zein coated nanoparticles were carried out by thin film mode of XRD using 'X' pert PRO MRD model of PAN alytical system operated at 20 kV voltages and a current of 15 mA with Cu Kα radiations. Powder form of the sample was placed tightly in a sample holder and analysis was carried out at 25 ^{0}C with degree of 2θ.

2.3.4 Fourier Transform Infrared (FTIR) Analysis

The FTIR spectrophotometer (BRUKER) was used to measure the changes in chemical structure of the chitosan (CS), zinc loaded CS/TPP nanoparticle and zein coated nanoparticles. The lyophilized samples were ground into homogeneous powders and mixed with IR transparent material KBr in the ratio of 2:1 by using mortar and pestle for 30 minutes. Then the mixture was converted into pellets by pressing the prepared mixture with a hydraulic/ hand press into a hard disk. Ideally, 0.5-1 mm thick of the pellet was placed in a transmission holder and scanned.

2.4 Encapsulation and loading efficiency of nanoparticles

The encapsulation efficiency (EE) of the nanoparticle was defined as the amount of drug entrapped into the nanoparticles (Hu *et al.* 2008 [9]; Luo *et al.* 2010 [12]). Likewise loading efficiency (LE) was defined as ratio of amount of nutrient bound in the nanoparticle to amount of zinc nutrient loaded nanoparticle produced. Here the

Encapsulation Efficiency and Loading Efficiency (LE) was calculated as follows

Encapsulation efficiency (%) =

$$\frac{\text{Amount of zinc nutrient bound in the Nanoparticles}}{\text{Total amount of zinc nutrient used for nanoparticle roduction}} \times 100$$

Loading efficiency (%) =

$$\frac{\text{Amount of zinc nutrient bound in the Nanoparticles}}{\text{Amount of zinc loaded nanoparticle produced}} \times 100$$

The amount of zinc nutrient bound in the nanoparticle was calculated by deviation of zinc content in free solution (supernatant solution during synthesis process) which was obtained by using Atomic Absorption spectrophotometer from total amount of zinc fertilizer used for nanoparticle production. In the LE, amount of zinc loaded nanoparticle produced was derived from final weight of the powdered lyophilized sample.

2.5 Zinc foliar uptake studies by SEM

One attempt has been tried for studying the efficiency of foliar uptake of zinc loaded chitosan nanoparticles. For this, synthesized zinc loaded chitosan/ TPP nanoparticle in with or without zein coating sample was prepared for 100 ppm concentration and sonicated for 30 mins and then the solution was sprayed (25 ml / pot) on 30 day old cotton leaves. After 1 hr spray, leaf sample (4th leaf from top) was taken and fixed in 100% methanol for 10 mins, followed by 100% dry ethanol for 30 mins. After finishing the chemical fixation, the dehydrated leaf sample was used for field emission scanning electron microscopic (FESEM) analysis (Talbot & White, 2013) [22].

2.6 Effect of foliar spray of nanoformulated zinc fertilizer on growth of cotton

2.6.1 Physico chemical characteristics of soil used for the study

The surface soil sample (0-15cm) used for pot culture experiment was collected from Central Institute for Cotton Research, Regional Station, Coimbatore (located at 11^0 N 77^0 E and 427.6 m above mean sea level). The collected soil samples were air dried and sieved through 2 mm sieve for analysing the physico chemical characteristics of the soil.

2.6.2 Pot culture experiment

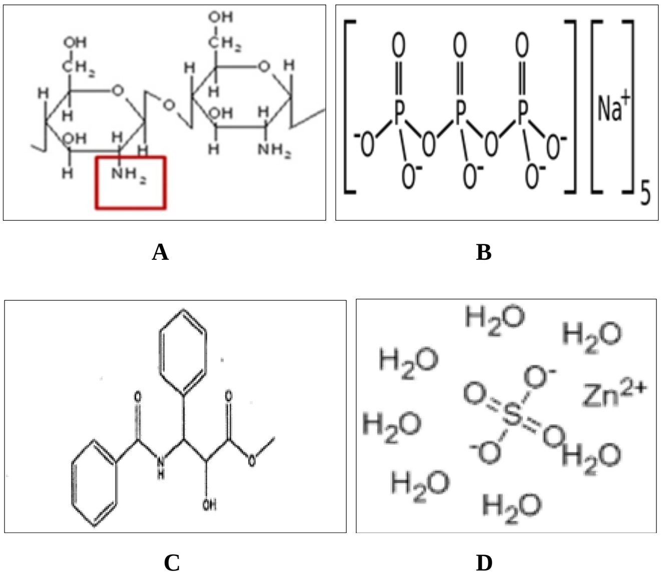
The seeds of cotton (*Gossypium hirsutum)* variety Suraj were obtained from The SIMA Cotton Development and Research Association, Coimbatore and dibbled three seeds in each pot of air dried soil. The pots, 30 cm height and 11 cm width were filled with surface soils of Research farm (CICR, Regional Station, Coimbatore). The soil samples were air dried for 48 hrs, sieved through <2 mm sieve, thoroughly mixed and then filled the pots with 10 kg of the soil each. After germination, one plant per pot was maintained throughout. Proper agronomic and plant protection management was done to all the treated plants for their maximum growth expression.

The experiment was carried out with six treatments *viz.*, T1 – Control, T2 – Normal $ZnSO_4$, T3 –Zinc loaded chitosan nanoparticles, T4 - Zinc loaded chitosan nanoparticles with zein 0.1%, T5 – Zinc loaded chitosan nanoparticles with zein 0.2%, T6 – Zinc loaded chitosan nanoparticles with zein 0.3% and three replicates. No fertilizer was applied as basal in any of the treatments. The above treatment samples from T2 - T6 except control were prepared for 50 ppm concentration and sonicated for 30 mins and then sprayed (25 ml per pot) on 30 days old cotton (suraj) plants. For assessing the slow releasing behaviour of the encapsulated samples, data on plant height and number of leaves were taken on 20 days after sprayed cotton plants.

3. Results and Discussion

3.1 Synthesis and Characterization of zinc loaded CS/TPP nanoparticles with or without zein coating

In this study, zinc loaded chitosan/TPP nanoparticles were synthesised with or without zein coating by using chemical synthesis approach. According to Calvo *et al.* 1997[3], nanoparticles were prepared by coacervation, which is a spontaneous phase separation process arising from electrostatic interaction, when oppositely charged molecules are mixed together. The success of the process is largely dependent on intermolecular linkages created between the negatively charged phosphatic groups of TPP with that of positively charged amino groups of CS (Shah *et al.* 2009) [18]. This similar principle has been used in the medical field for very long time to encapsulate and controlled release of peptides (Gan & Wang, 2007) [8], proteins (Sun & Wan, 2007) [21], insulin (Lin *et al.* 2007) [11] and trace elements (Luo *et al.* 2010) [12]. As far as my knowledge, this is the first report for encapsulation of zinc sulphate fertilizer in CS/TPP nanoparticles. The molecular structures of chitosan (CS), sodium tri poly phosphate (TPP), zein and zinc sulphate hepta hydrate are given in Fig.1.



A B

C D

Fig 1: Molecular structure of Chitosan (A), TPP (B), Zein (C) and $ZnSO_4.7H_2O$ (D)

The nanoencapsulated zinc fertilizer sample was characterized for size, shape, zeta potential and molecular interaction *etc* by using the equipments like particle size analyser, scanning electron microscopy, transmission electron microscopy, X ray diffraction analysis and fourier transform infra-red analysis. The average particle size *ie.,* intensity mean diameter was measured for zinc loaded chitosan/TPP nanoparticles which produced one peak at 103.2 nm. But, zinc loaded CS/TPP nanoparticle with zein coating had two peaks, one is at less than 500 nm and another peak is at more than 500 nm. So, the average mean diameter is 709 nm (Fig 2). Similarly, Luo *et* *al.* (2011) [13] reported that particle size of complex molecule α-tocopherol (TOC)/Zein- chitosan (CS) increased from around 200 to 800 nm which indicates denser complex formation at greater concentrations.

Surface charge *ie*., zeta potential of zinc loaded CS/TPP nanoparticle was found to be positively charged (+48.5). This indicated that the stability of nanoencapsulated zinc fertilizer was better with slight agglomeration. After coating of zinc loaded CS/TPP nanoparticles with zein polymer, the similar better stability was attained due to positively charged zeta potential (+ 36.7 mV). In the medical field, Duan *et al.* (2010) [4] studied the synthesis of CS coated curcumin nanoparticles with high zeta potential and they concluded that positive surface charge of nanoparticles could prolong the retention of drug in the blood compartment as well as provide sustained release of the drug.

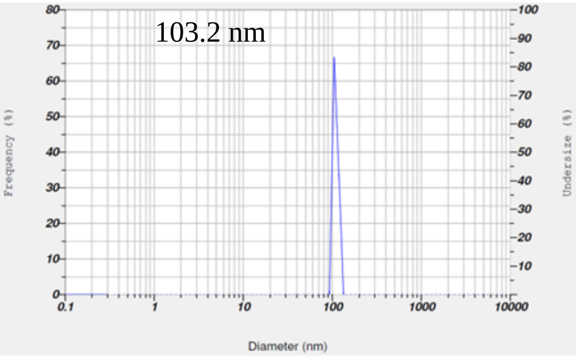
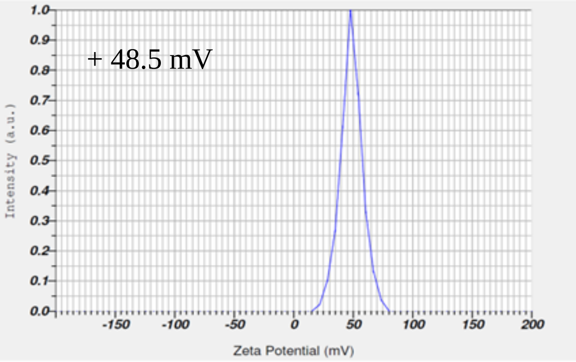
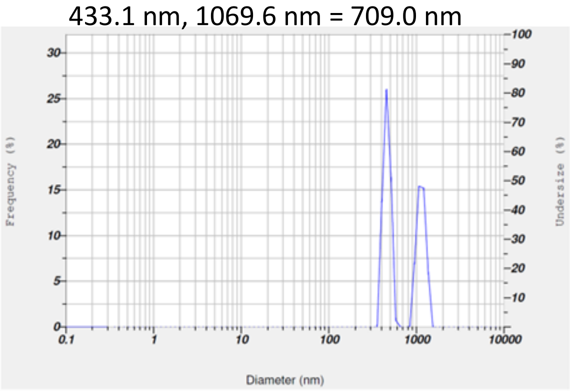
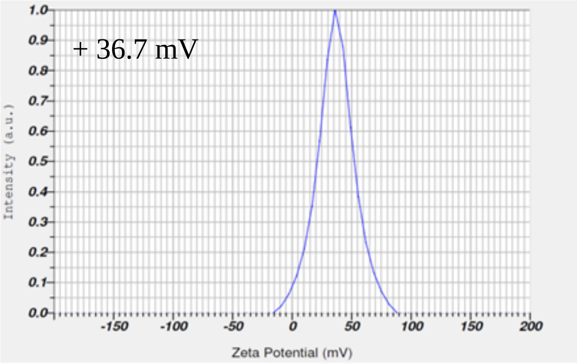
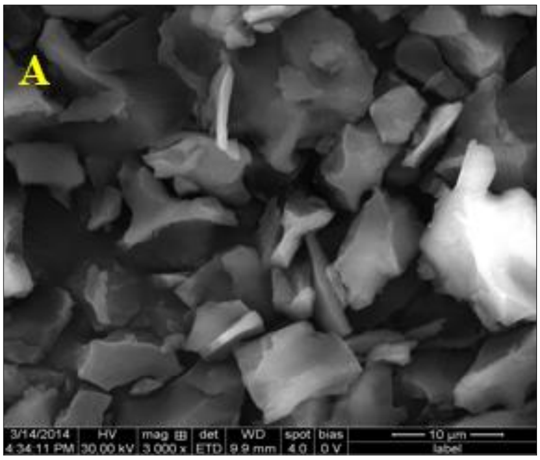
Average particle size	**Zeta potential**
Zinc loaded CS/TPP	
	
Zinc loaded CS/TPP with Zein	
	

Fig 2: Average particle size and zeta potential of nanoparticles

The morphological structures of chitosan (Fig.3A) and zinc loaded CS/TPP nanoparticle size in Scanning Electron Microscopy was 70-86 nm *ie*., within < 100 nm, known as nanoscale level (Fig 3B). It was also confirmed with TEM analysis (Fig. 4). The nanoparticle was smooth in surface, spherical in shape, much smaller and homogenous in diameter but the nanoparticles were in slight agglomerated condition, which might be due to positively charged zeta potential. Similarly nanoparticle size of zinc loaded CS/TPP nanoparticle with zein 0.1% coating was 80 to 300 nm (Fig.3C). In 0.2% zein coating, the nanoparticle size was 100 – 500 nm (Fig.3D). In 0.3% zein coating, the nanoparticle size was 200 – 600 nm (Fig. 3E). Nanoparticles of Zinc loaded chitosan nanoparticle with zein coating were also smooth in surface, spherical in shape and not homogenous and it varied from 80 to 600 nm or more. This data clearly indicated that particle size was increased in increased concentration of double coated zein polymer. This was in Agreement with Estaca *et al.* (2012) [5] and Podaralla & Perumal (2012) [16].



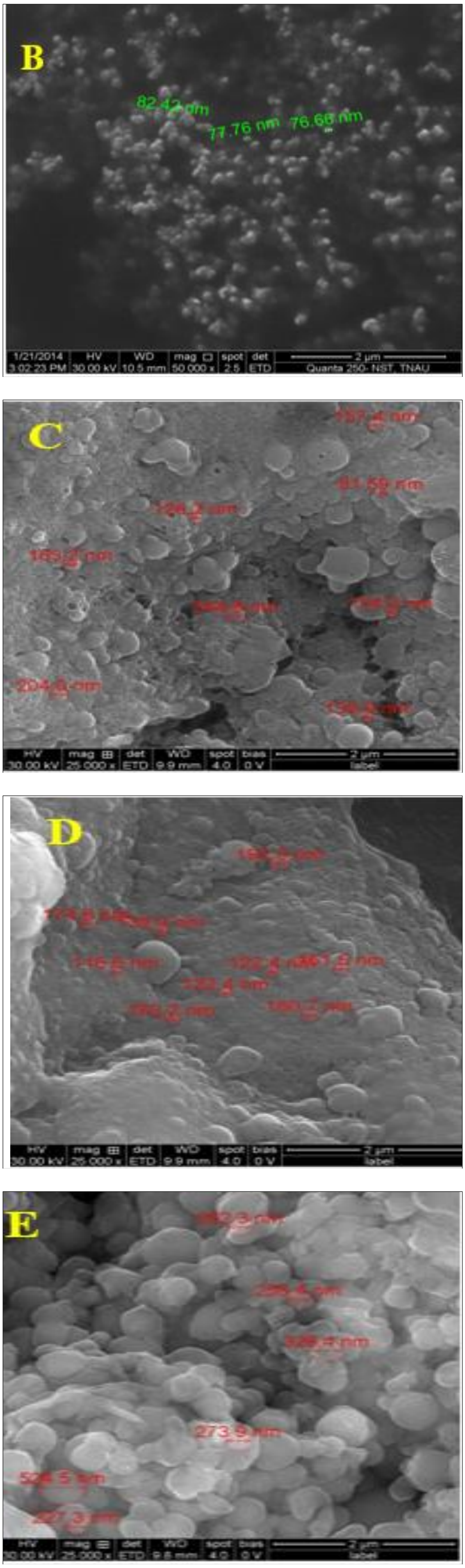


Fig 3: Scanning Electron Microscopy (SEM) photographs of CS (A), Zinc loaded CS /TPP NP's (B), Zinc loaded CS/TPP NP's with Zein 0.1% (C), 0.2% (D), 0.3% (E)

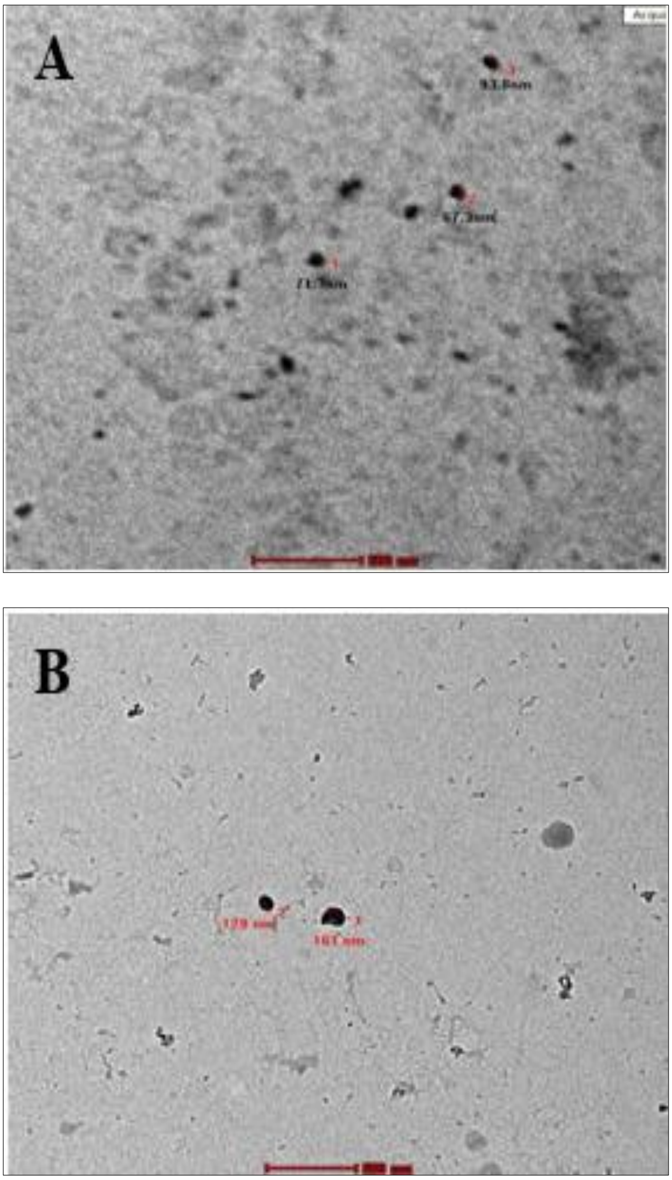


Fig 4: Transmission Electron Microscopy (TEM) photographs of Zinc loaded CS/TPP NP's (A) and Zinc loaded CS/TPP nanoparticle with Zein 0.1% (B)

Ramya *et al.* (2012) [17] revealed that XRD patterns of pure chitosan exhibited two characteristics of distinct crystalline peaks at 10° and 20°. This is because of presence of plenty of –OH and –NH2 groups in the chitosan structure, which could form stronger inter and intramolecular hydrogen bonds and the chitosan structure has certain regularity to form crystalline regions. In the zinc loaded CS/TPP nanoparticles, peak of chitosan at $2\theta = 10^\circ$ became weak and disappeared. But sharp peak was developed at $2\theta = 26^\circ$ (Fig.5A). This result confirmed that there was a strong interaction between zinc molecule and chitosan nanoparticle. The unit cell dimension of the zinc loaded CS nanoparticle was 35 nm *ie.*, grain size. Likewise, in the zinc loaded CS nanoparticles with zein, peaks were at $2\theta = 24^\circ$ uniformly in both 0.1 (Fig. 5B) and 0.2% (Fig. 5C) zein concentration, which showed the strong interaction between zein and zinc loaded CS nanoparticles. Also there was a slight significant peak shift at $2\theta = 42^\circ$ which might be due to additional coating of zein compound.

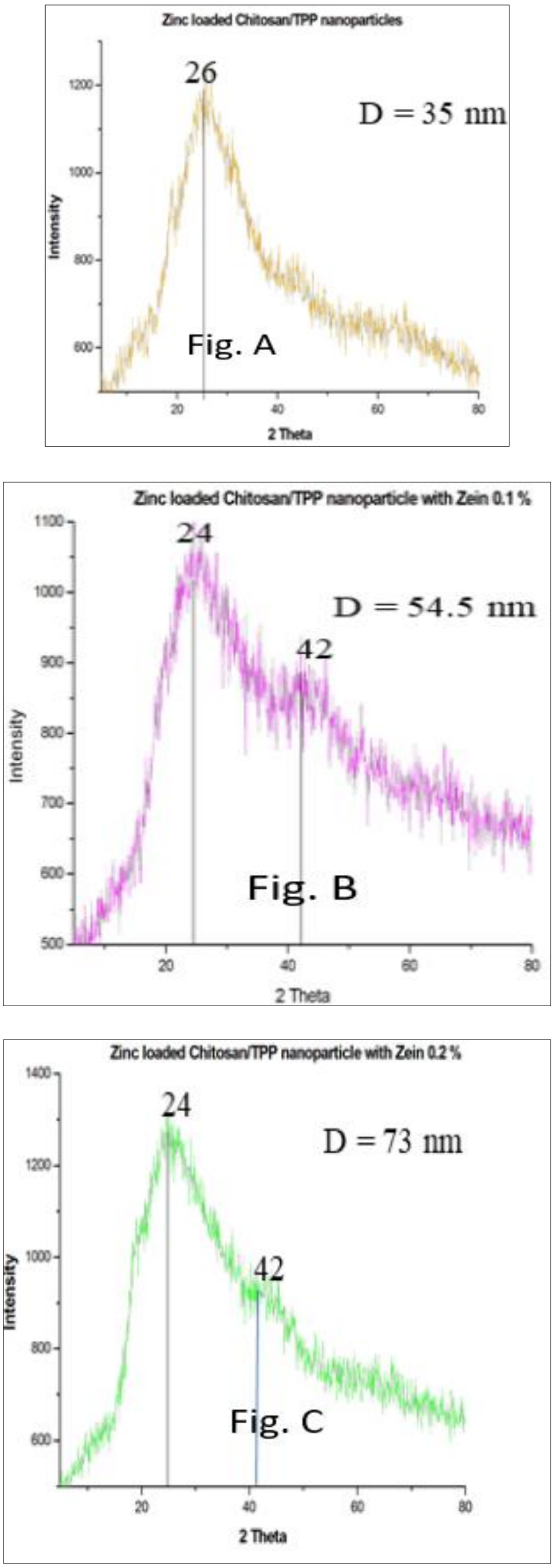
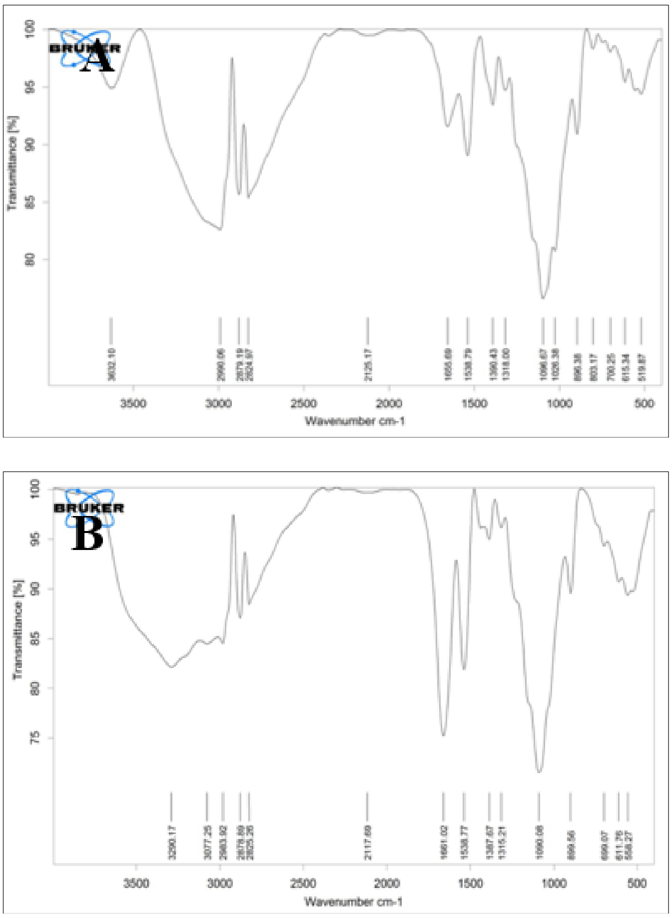


Fig 5: X Ray Diffraction (XRD) graph of Zinc loaded CS/TPP nanoparticle (A), Zinc loaded CS/TPP nanoparticle with Zein 0.1% (B), 0.2% (C)

Luo *et al.* (2010) [12] revealed that IR spectra of pure chitosan showed six characterization peaks at 3358.52, 1648.61, 1586.59, 1418.88, 1375.24 and 1025.94 cm^{-1} which were thought to be O-H stretch, C = O stretching from amide I, N-H bending and C-N stretching from amide II, $-CH_2$ bending, $-CH_3$ symmetrical deformation and skeletal vibration of C-O stretching respectively. The intermolecular interaction of zinc loaded CS/TPP nanoparticle in with or without zein was characterized by FTIR. It was observed that the spectrum of zinc loaded CS/TPP nanoparticle in with or without zein was different from that of pure chitosan spectra and highlighted from the wavenumber of 500 to 3500 cm^{-1}. A small peak of 3632 cm^{-1} due to O – H stretch, strong absorption band at 2990 cm^{-1} due to symmetric $–CH_2$ stretching vibration attributed to pyranose ring, smaller peaks of 1655 and 1538 cm^{-1} due to C=O stretching (amide I) and NH stretching amide II and also sharp peak at 1096 cm^{-1} due to C-O stretching vibrations were present in zinc loaded CS/TPP nanoparticle (Fig. 6A). Likewise, peaks at 3290, 3408 and 3296 cm^{-1} in zinc loaded CS/TPP nanoparticle with zein coating of 0.1% (Fig. 6B), 0.2% (Fig. 6C), and 0.3% (Fig. 6D), respectively were assigned to O-H stretch, which became wider and flatter due to enhanced hydrogen bonding (Wu, Yang, Wang, Hu, & Fu, 2005). In all the zein coating percentage, similar peaks of 2878 cm^{-1} were raised due to symmetric -CH2 stretching vibration attributed to pyranose ring. A sharp peak was aroused in all the zein coating viz., 0.1% (1661 and 1538 cm^{-1}), 0.2% (1657 and 1538 cm^{-1}) and 0.3% (1658 and 1537 cm^{-1}) which might be due to C=O stretching (amide I) and NH stretching amide II. But the sharp peak rose at 1090 cm^{-1} was reduced by increasing the concentration of zein coating. Compared with pure chitosan peaks, zinc loaded CS/TPP in without and with zein peaks showed the difference *ie.*, peak shifting which indicated that electrostatic interaction was happened between the molecules. The complex deformation of the molecule represented that the newer synthesized nanoparticle was achieved.



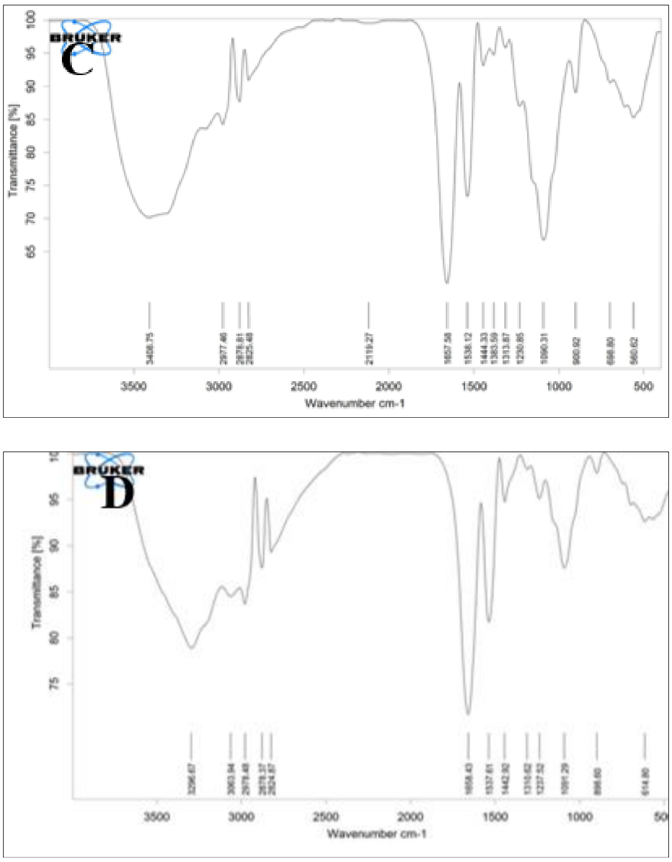


Fig 6: FTIR spectrum of Zinc loaded CS/TPP nanoparticle (A), Zinc loaded CS/TPP nanoparticle with Zein 0.1% (B), 0.2% (C), 0.3% (D)

3.2. Encapsulation and loading efficiency of nanoparticles

Encapsulation efficiency was higher in zinc loaded chitosan nanoparticle in with (85.50%) or without zein (88.81%). Similarly, loading efficiency was very poor in with zein (2.44%) than without zein coating (15.54%). This result concluded that both the encapsulation and loading efficiency were increased in zinc loaded CS/TPP nanoparticles than zinc loaded CS/TPP nanoparticle with zein (Table 1). Eventhough the encapsulation efficiency was same (85.5%) in both lower and higher concentration of zein coating, but the loading efficiency was highly decreased in higher concentration of zein (0.2%) coating as compared to lower concentration of zein (0.1%) coating. And also there was a positive correlation between encapsulation efficiency and loading efficiency *ie.*, when the encapsulation efficiency was higher, loading efficiency was also higher. Reversely, Luo, *et al.* 2010) [12] reported that the encapsulation efficiency was decreased when loading concentration of selenite increased in chitosan solution. So, confirmation study is required to find out the relationship between encapsulation and loading efficiency of zinc in chitosan/TPP nanoparticles.

Table 1: Encapsulation and Loading Efficiency of zinc loaded CS/TPP nanoparticle in with or without zein

Nanoparticles	Encapsulation Efficiency (%)	Loading Efficiency (%)
Zinc loaded CS/TPP nanoparticle	88.8	15.54
Zinc loaded CS/TPP NP's + Zein 0.1%	85.5	2.44
Zinc loaded CS/TPP NP's + Zein 0.2%	85.5	1.37

3.3 Effect of foliar spray of nanoformulated zinc fertilizer on growth of cotton

3.3.1 Physico chemical characteristics of soil used for the study

The experimental soil sample was alkaline in nature (pH 8.5), non-saline (0.28 dSm^{-1}) with moderate CEC (19.5 Cmol (p^{+}) kg^{-1} and organic carbon (0.60%) content. The available nutrient status of the soil was low for available N (168 kg ha^{-1}), high for available P (28.5 kg ha^{-1}) and K (590 kg ha^{-1}).

The results of the pot experiment (Fig. 7 and Fig 8) showed that application of zinc loaded CS/TPP nanoparticle with zein (0.1%) increased the plant height (15 cm), number of leaves (13.3), number of squares (8.2), root length (14.5 cm), shoot fresh weight (8.2 g/plant) and root fresh weight (1.6 g/plant) on 20 days after spray or 50 days after sowing (DAS) which was followed by zinc loaded CS/TPP with zein 0.2%. The Zn loaded CS/TPP with zein 0.3% was statistically on par for all the above parameters with Zn loaded CS/TPP nanoparticle in without zein. The increased parameters may be not only due to crop growth ages but also slow releasing of zinc nutrient from encapsulated material. The normal recommended dose of zinc fertilizer in the form of $ZnSO_4$ recorded the lowest plant height (11.8 cm), number of leaves (7.5), number of squares (2.0), root length (12.7 cm) shoot weight (4.7 g/plt) and root weight (0.6 g/plt) on 50 DAS which was more or less on par with control.

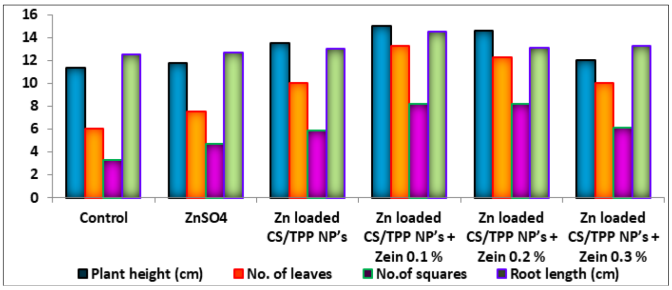


Fig 7: Effect of foliar spray of normal and nanoformulated zinc fertilizer (50 ppm) on cotton plant growth characters at 50 DAS

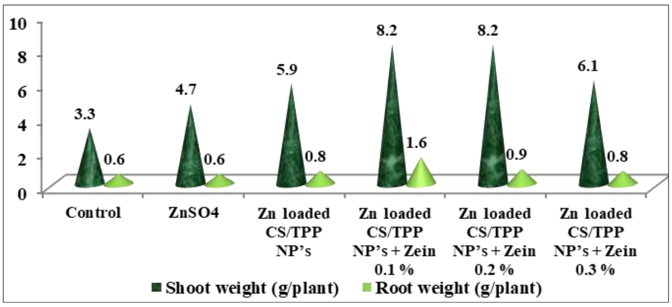


Fig 8: Effect of foliar spray of normal and nanoformulated zinc fertilizer (50 ppm) on dry shoot and root weight of cotton at 50 DAS

4. Conclusion

A new nanoencapsulated zinc fertilizer with controlled release property was prepared, it possessed two or three layer structures in which water soluble zinc sulphate fertilizer *ie.*, the core was encapsulated with chitosan/TPP nanoparticles (two layers) and zein polymer (three layers). The newly synthesized nanofertilizer size was ranging between 70 – 86 nm for Zn loaded CS/TPP nanoparticle whereas size of zinc loaded CS/TPP nanoparticle with zein was 80 to 300 nm. Both of the nanoparticles shape was spherical with smooth surface and homogenous in diameter and then surface charge *ie.*, zeta potential was found to be positively charged. The pot culture experimental results showed that application of 50 ppm concentration of nanoencapsulated zinc with chitosan and zein (0.1%) increased the plant height (15 cm), number of leaves (13.3) and root length (14.5 cm) of 7 weeks old cotton plant as compared to nanoencapsulated zinc with chitosan but without zein, normal recommended dose of zinc fertilizer ($ZnSO_4$) and control.

5. Acknowledgement

Support was provided by Central Institute for Cotton Research, Nagpur (Indian Council of Agricultural Research, New Delhi, India).

6. References

1. Adhikari T, Kundu S, Subba Rao A. Zinc delivery to plants through seed coating with nano-zinc oxide particles. Journal of Plant Nutrition. 2016; 39: 136-146.
2. Auld DS. Zinc coordination sphere in biochemical zinc sites. Biometals. 2001; 14:271-313.
3. Calvo P, Lopez CR, Vila Jato JL, Alonso MJ. Novel hydrophilic chitosan polyethylene oxide nanoparticles as protein carriers. Journal of Applied Polymer Science. 1997; 63:125-132.
4. Duan JH, Zhang YD, Han SW, Chen YX, Li B, Liao MM *et al*. Synthesis and in vitro/in vivo anti-cancer evaluation of curcumin-loaded chitosan/poly(butyl cyanoacrylate) nanoparticles. International journal of pharmaceutics. 2010; 400:211-220.
5. Estaca JG, Balaguer MP, Gavara R, Munoz PH. Formation of zein nanoparticles by electro hydrodynamic atomization: Effect of the main processing variables and suitability for encapsulating the food coloring and active ingredient curcumin. Food Hydrocolloids. 2012; 28:82-91.
6. Fernández V, Eichert T. Uptake of hydrophilic solutes through plant leaves: current state of knowledge and perspectives of foliar fertilization. CRC Critical Reviews in Plant Sciences. 2009; 28:36-68.
7. 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: Biointerfaces. 2005; 44:65-73.
8. Gan Q, Wang T. Chitosan nanoparticle as protein delivery carrier—Systematic examination of fabrication conditions for efficient loading and release. Colloids and Surfaces B: Biointerfaces. 2007; 59:24-34.
9. Hu B, Pan CL, Sun Y, Hou ZY, Ye H, Hu B *et al.* Optimization of Fabrication Parameters to Produce Chitosan−Tripolyphosphate Nanoparticles for Delivery of Tea Catechins. Journal of Agricultural and Food Chemistry. 2008; 56:7451-7458.
10. Kaya C, Higgs D. Response of tomato (Lycopersicon esculentum L.) culture at low zinc. Scientific Horticulture. 2002; 93:53-64.
11. Lin YH, Mi FL, Chen CT, Chang WC, Peng SF, Liang HF *et al.* Preparation and Characterization of Nanoparticles Shelled with Chitosan for Oral Insulin Delivery. Biomacromolecules. 2007; 8:146-152.
12. Luo Y, Zhang B, Cheng WH, Wang Q. Preparation, characterization and evaluation of selenite-loaded chitosan/TPP nanoparticles with or without zein coating. Carbohydrate Polymers. 2010; 82:942-951.
13. Luo Y, Zhang B, Whent M, Yu L, Wang Q. Preparation and characterization of zein/chitosan complex for encapsulation of α-tocopherol, and its in vitro controlled release study. Colloids and Surfaces B: Biointerfaces. 2011; 85:145-152.
14. Mohanraj VJ, Chen Y. Nanoparticles– A Review. Tropical Journal of Pharmaceutical Research. 2006; 5:561-73.
15. Panyam J, Labhasetwar V. Biodegradable nanoparticles for drug and gene delivery to cells and tissue. Advanced Drug Delivery Reviews. 2003; 55:329-347.
16. Podaralla S, Perumal O. Influence of Formulation Factors on the Preparation of Zein Nanoparticles. AAPS Pharm SciTech. 2012; 13:919-927.
17. Ramya R, Sudha PN, Mahalakshmi J. Preparation and Characterization of Chitosan Binary Blend. International Journal of Scientific and Research Publications. 2012; 2:1-9.
18. Shah S, Pal A, Kaushik VK, Devi S. Preparation and characterization of Venlafaxine hydrochloride loaded chitosan nanoparticles and invitro release of drug. Journal of Applied polymer science. 2009; 112:2876-2887.
19. Shenoy DB, Amiji MM. Poly (ethylene oxide) - modified poly (-caprolactone) nanoparticles for targeted delivery of tamoxifen in breast cancer. International Journal of Pharmaceutics. 2005; 293:261-270.
20. Singh MV. Micronutrient nutritional problems in Soils of India and Improvement for Human and Animal Health. Indian journal of fertilizers. 2009; 5:11-16.
21. Sun Y, Wan A. Preparation of nanoparticles composed of chitosan and its derivatives as delivery systems for macromolecules. Journal of Applied Polymer Science. 2007; 105:552-561.
22. Talbot MJ, White RG. Methanol fixation of plant tissue for scanning electron microscopy improves preservation of tissue morphology and dimensions. Plant methods. 2013; 9:1-7.
23. Wu Y, Yang WL, Wang CC, Hu JH, Fu SK. Chitosan nanoparticles as a novel delivery system for ammonium glycyrrhizinate. International journal of pharmacology. 2005; 29:235-245.