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Synthesis and characterization of Chitosan / TPP encapsulated curcumin nanoparticles and its antibacterial efficacy against colon bacteria

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Abstract
The present study was designed to encapsulate curcumin by nanocoating of biodegradable polymer chitosan to enhance its stability and bioavailability and to compare its antibacterial efficacy with standard drug vancomycin against colon bacteria. Chitosan / TPP layer capsule was obtained by ionic gelation method through electrostatic interaction by mixing of equimolar concentration of both the compounds in which a known amount of curcumin was incorporated. The resulting nanoparticles were characterized by various instrumental techniques. Size of Chitosan / TPP protected curcumin nanoparticles were obtained between 135.2 nm and 72 nm by particle size analyzer and TEM analysis respectively. Morphology of nanoparticles was found to be spherical in shape by optical and fluorescence microscopes. Stability of the nanoparticles was obtained by zeta potential measurement as +12.9 mV. Antibacterial efficacy was evaluated by Antibiotic sensitivity test (ABST) by measuring the zone of inhibition scale and MIC value which was recorded as 12.5 µg / ml and 20 µg / ml for both Gram positive *S. aureus* and Gram negative *E. Coli* bacteria respectively.

Keywords: Chitosan (Chi), curcumin (cur), vancomycin (vanco), sodium tri poly phosphate (TPP) and *Escherichia coli* (*E. coli*)

Introduction
Curcumin is a hydrophobic golden yellow crystalline diphenolic compound which is present in the rhizome of a perennial herb *Curcuma longa*, commonly known as turmeric which belongs to family Zingiberaceae. Chemical structure of curcumin consists of bis-α, β-unsaturated β-diketone, commonly known as diferuloyl methane (Deka *et al*., 2016) [1]. There are several reports available for its various biological activities such as anti-bacterial (Mun *et al*., 2013) [2], antiviral, antiprotozoal, antifungal, antiinflammatory, antioxidant and anticarcinogenic activities (Pandit *et al*., 2015 and Moghadamtousi *et al*., 2014) [3, 4]. Despite the presence of various biological effects, its use is limited as therapeutic agent at clinical level, because of its low solubility in water, less bioavailability and higher rate of metabolism and excretion. As curcumin is hydrophobic in nature its solubility in different solvents other than water such as in acetone-1 mg/ml, ethanol - 10 mg/ml, dimethylsulfoxide -25 mg/ml, acetic acid, 0.1M NaOH, 10mM Na2CO3, acetone and water 0.1 mg/ml, and also soluble in dimethyl formamide and surfactants like polysorbate (tween) 20, 60 and 80 – 0.2 – 2 mg/ml concentration (Carvaldh *et al*., 2015 and Inchai *et al*., 2015) [5, 6].
To improve the bioavailability and solubility of curcumin, various approaches have been undertaken. It includes use of liposomal carrier for curcumin, use of piperine like adjuvants, curcumin-phospholipid complex. Recent studies have shown that reduction in the size of particles in nano form improved water solubility and bioavailability with increased stability (Ravichandran 2013) [7]. Curcumin and nanocurcumin has been reported for its anticancer properties and many researchers reported its anticancer potential against colon cancer (Chuah *et al*., 2013) [8]. Colon cancer mainly occurs due to environmental factors rather than genetic factors and like change in life style, food habits and microbial load in the gastrointestinal tract. In a recent study by Gold *et al.* 2004 [9] it was reported that colonic neoplasia was found in 39% patients, invasive colorectal cancer was seen in 7% of adults and eight patients among adult patients have shown malignant lesions inside the gastrointestinal tract and these results along with the previous available data supports that there is strong association of bacterial

infection and occurrence colonic cancer.

Nanoparticle (NP) based delivery system is gaining attention as novel drug carriers and their use is rapidly increasing due to their higher therapeutic potential (Hickey *et al*., 2015) [10]. Different types of polymeric materials are available which can be used for synthesis of biodegradable NPs, including poly (glycolic acid), polysaccharides, poly (methyl-methacrylate), polyacrylamide, polycaprolactone, polylactic acid, proteins and polypeptides (Soppimath *et al*., 2001) [11]. Polysaccharides, the most popular polymeric NP materialsare advantageous for targeted drug delivery (Liu *et al*., 2008) [12]. Chitosan is polysaccharide present naturally, it is a hydrophilic cationic polysaccharide which obtained from alkaline deacetylation of chitin which is present in the outer skeleton of insects and crustaceans like, crabs and shrimp. It is biodegradable, biocompatible nontoxic in nature, and positive charge makes it suitable for mucoadhesion with negatively charged mucus membrane (Paz *et al*., 2011) [13]. In this study our aim was to make a stable biocompatible and biodegradable nano carrier by mixing equimolar concentration of polycationic chitosan with polyanionic TPP through ionic gelation method to carry curcumin with enhanced antibacterial efficacy.

Materials and Methods

Chemicals and materials

Chitosan (90% deacetylated) was procured from Sisco Research Laboratories Pvt. Ltd., sodium tripolyphosphate pentabasic (TPP) in the form of powder (technical grade, 85%), Vancomycin and curcumin from Sigma–Aldrich were purchased. Ethanol was purchased from Merck (India). Nutrient broth and Nutrient agar and inhibition zone scale were purchased from HiMedia, India. All other chemicals used in study were of analytical grade and deionized water was used for all experimental protocols.

Methods

Synthesis of Chi-TPP encapsulated curcumin nanoparticels

Curcumin was dessolved in ethanol at 0.1% (1mg/ml) concentration, as it is insoluble in water. Chitosan was dissolved in 0.1M acetic acid, at different concentration (0.1%, 1%, 2%). Sodium tri poly phosphate (TPP) was dissolved in deionized water. TPP solution (1%) was added dropwise manner to the chitosan at 1:1, Chi:TPP ratio and few drops of glutaraldehyde were added (for cross linking of particles) under constant magnetic stirring at 1000 rpm for 30 minutes. Nanocapsules were formed spontaneously by ionic gelation mechanism, between chitosan and TPP then finally curcumin was added to Chi /TPP solution with continuous stirring for 2 hours followed by sonication at 20000 Hertz, for 15 minutes (Cur:Chi:TPP-5:2:2 ratio). Final solution was centrifuged at 12,000 rpm for 15 minutes, 4˚C to remove the non-entrapped particles and pellet was resuspended in deionized water and converted to powder form by freeze drying.

Characterization of nanoparticles

The size, morphology and stability of the synthesized nanoparticles were characterized by using the techniques such as Optical microscopy, fluorescence microscopy, Particle size analysis, Zeta Potential measurement and Transmission Electron Microscopy (TEM) analysis.

Structure of nanoparticles under optical and fluorescence microscope

Initial structure, shape and morphology of the nanoparticles with formation of capsule were observed at 100x oil immersion under light biological microscope. Nanoparticles were smeared on glass slide and observed after covering with cover slip. As curcumin is an auto florescence compound with excitation and emission range of 425 nm and 470 nm respectively. So based on this quality curcumin nano particles images had been taken.

Particle size measurement

The particle size distribution (Z-average) and polydispersity index (PDI) of cubosomal dispersions were determined by dynamic light scattering using nanoparticle analyzer SZ-100 (nano partica HORIBA Scientific). Samples were diluted (10-fold) with deionized water and measured in triplicates.

Zeta potential measurement

Zeta potential measurement was performed in the particle size analyzer SZ-100 (nano parrtica HORIBA Scientific) instrument operated in the electrophoretic light scattering mode (ELS). The dispersion and re-dispersed lyophilized samples were placed in standard glass cuvettes and the zeta potential was measured at 23°C with a scattering angle of −14.06° using a helium neon laser of wavelength 658 nm. Three measurements of 1 min each were performed to calculate the mean value of zeta potential.

Transmission Electron Microscopy (TEM) Study

Transmission electron microscopy was used to confirm the size, shape and dispersion of nanoparticles. The samples for observation by TEM were prepared by ultrasonication of particles by dispersing in acetone and a drop of stable solution was taken on TEM grids and dried at 37˚C for 15-30 minutes, and then grid was inserted into the sample block of the TEM and pictures has been captured.

Antibiotic Sensitivity Test (ABST)

Curcumin nanoparticles were analysed for antibacterial effect by using Kierby Bauer disc diffusion method (Moghadamtousi *et al*., 2014) [4].

Antibacterial effect was observed against gram positive and gram negative bacteria. Gram positive bacteria *Staphylococcus aureus* ATCC No. 6538 were procured and streaked on nutrient agar and gram negative bacteria *E. coli*. were obtained by streaking bacterial culture on EMB agar with the help of sterile loop for selective growth of bacteria. Bacterial samples were inoculated in nutrient broth (100 µl sample + 9900 ml broth) and incubated at 37ºC for 24 hours. Bacterial growth was diluted with sterile water and turbidity was matched with 0.5 McFarland standard, which indicate 1-2×10^8 CFU/ml bacterial load. After 24 hours growth period bacterial turbidity was diluted and matched with 0.5 McFarland standard and then streaked with sterile swab on Muller Hinton Agar. After that discs of different concentrations of nanoparticles were placed on MHA media and plates were kept for incubation in 5 % CO_2 atmosphere at 37°C for 18 – 24 hrs. For comparing effects on bacterial culture, standard drug vancomycin was used as it is known for effective against colon bacteria but *E. coli*. are resistant, so vancomycin was used at different concentrations (10 – 30 µg /

ml). After 24 hours zone of inhibition was measured using Hi Antibiotic Zone Scale. Minimum inhibitory concentration (MIC) was measured by comparing the growth of bacteria at different concentration of nanoparticles with normal curcumin and vancomycin.

Statistical Analysis

Statistical analyses were performed by using GraphPad Prism version 5.00 (SanDiego, California) software. Differences in mean values were considered statistically significant at $^{***}P \leq 0.001$, $^{**}P \leq 0.01$, $^{*}P \leq 0.05$ (Snedecor and Cochran 1989) [14]. The results were expressed as mean ± S.E.M and data were analyzed by two-way ANOVA followed by Bonferroni test using GraphPad Prism version 5.00 (San Diego, California, USA).

Results

Synthesis of Chi-TPP encapsulated curcumin nanocomposites

The nano composites were prepared by ionic gelation method by interaction of the cationic charge of Chitosan amino group and anionic charge of TPP and glutaraldehyde were used for cross linking of polymer with curcumin nanoparticles and were used for further studies.

Characterization of nanoparticles

Structure of nanoparticles under optical and flourescence microscope

The nanoparticles were observed under the conventional light microscope at 100 X oil immersion magnification for initial capsule formation (Figure, A – 1). All particles were spherical in shape. After addition of curcumin, nanocomposite size was very small for characterization by optical microscopy. Curcumin nanoparticles emission was seen as bright green-yellow florescence under microscopy. (Figure, A– 2). Reason behind auto florescence of curcumin was reported as the electrons present in the curcumin molecule absorbed the ultraviolet light and gained energy and moved to the excitation state and when fell down to the resting state it emitted visible light and shown green-yellow colour florescence. Formation of spherical shape nanoparticles were visualised at 100 X under florescence microscope.

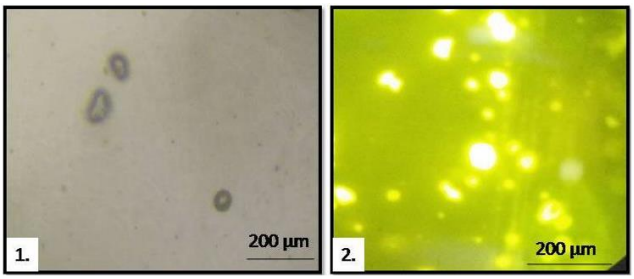


Fig A: Microscopic images of nanoparticles – Nanoparticles were observed as spherical in shape.
Fig 1: Optical microscope image.
Fig 2: Fluorescence microscope image.

Particle size measurement

The mean particle size was measured for the ratios of Chi-TPP 1:1. To overcome and minimize the error, the particle size analysis for each sample was taken as in triplicates (n=3). For Chi-TPP capsule size was found to be 17.33 nm and after addition of curcumin as nanocapsule size was found to be 135.2 nm (Figure, B – 3).

Zeta potential measurement

The zeta potential is a characteristic measure for the stability of the nanoparticles by interaction of the different charged particles. Here we recorded that pectin and curcumin zeta potential was – 41.2 mV and +1 mV respectively which indicates highly unstable compounds. After encapsulation by Chi-TPP their stability increased towards positive charge maximum upto +12.9 mV. This indicates that there is interaction between positive amino group of Chi and negative carboxyl group of TPP (Figure, B – 4).

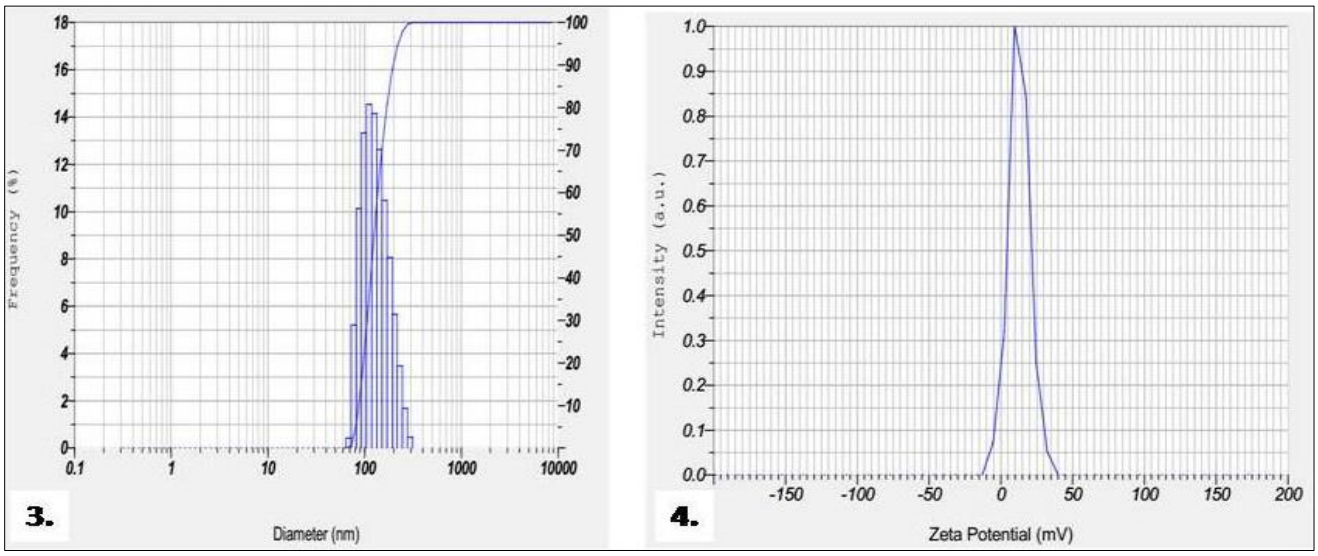


Fig B: Particle size analysis and zeta potential measurement.
Fig 3: Particle size of nanocurcumin was recorded as 135.2 nm.
Fig 4: Zeta potential of nanocurcumin was recorded as + 12.9 Mv.

Transmission Electron Microscopy (TEM) Study

TEM images of Chi–TPP incorporated incorporated nanocomposite shown in figure, C – 5. These particles observed as spherical in shape and evenly dispersed. Chi-TPP were observed as hollow spherical nanoparticles and curcumin nanocomposites appeared as dark solid spherical particles indicating entrapment of curcumin inside hollow

spherical capsule. Average particle size was recorded as 72 ± 3.12 nm.

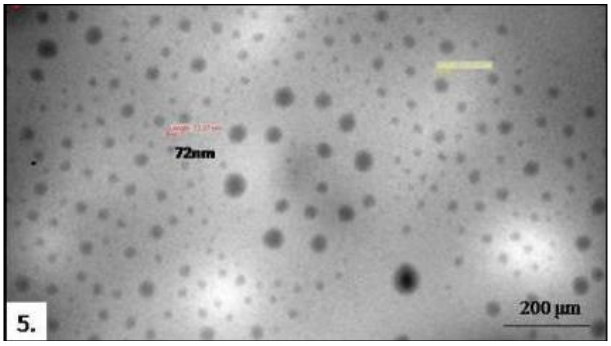


Fig C-5: Morphology and size of nanocurcumin by TEM imaging. Nanocurcumin particles were spherical in shape and size was observed as 72 ± 3.12 nm.

ABST (Antibiotic Sensitivity Test)

Antibacterial efficacy of nanoparticles was analyzed against Gram positive (*S. aureus*) and Gram negative (*E. coli*.) bacteria. Curcumin treated bacterial culture of *E. coli* and *S. aureus* showed zone of inhibition of 12.66 ± 0.66 nm and 14.17 ± 0.44 nm respectively. Vancomycin showed no zone of inhibition against *E.coli* which indicate that bacteria were resistant for vancomycin. However it showed 19.00 ± 1.52 nm zone of inhibition against *S. aureus* indicating that inhibitory capacity was higher for Gram positive bacteria than Gram negative bacteria. Cur-Chi-TPP nanoparticles showed significantly (P<0.001) increased zone of inhibition as compared to curcumin alone with the value 18.33 ± 0.73 nm and 20.5 ± 0.29 nm for both *E. coli.* and *S. aureus* bacteria respectively (Figure, D – 6 & 7). In case of *E. coli* nanoparticles has shown significantly higher (P<0.001) zone of inhibition than vancomycin. However in case of *S. aureus*, there was no significant difference (P>0.05) between them indicating that Cur-Chi-TPP was equally effective as vancomycin for gram positive bacteria (Graph – 1). MIC value was recorded as per growth inhibition of both the bacteria and it was found to be as 12.5 µg / ml and 20 µg / ml for both Gram positive (*S. aureus*) and Gram negative (*E. Coli*) respectively.

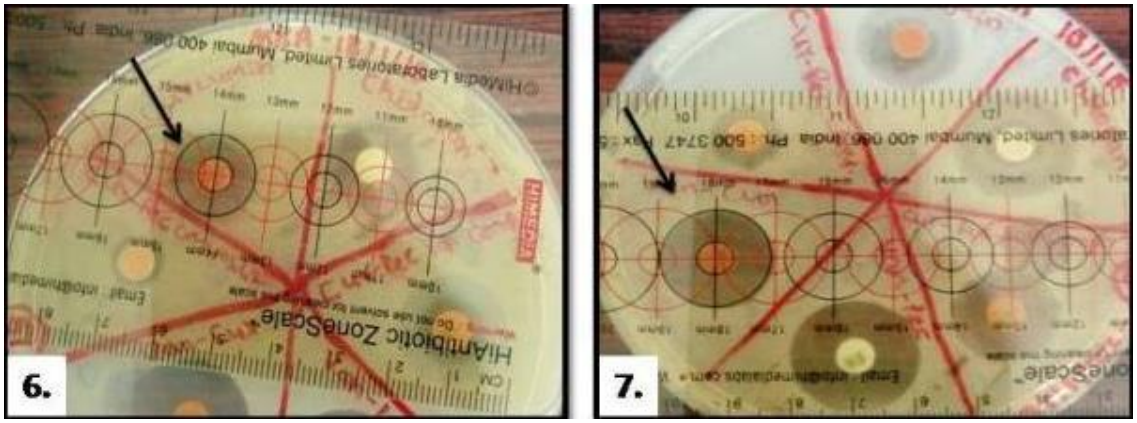
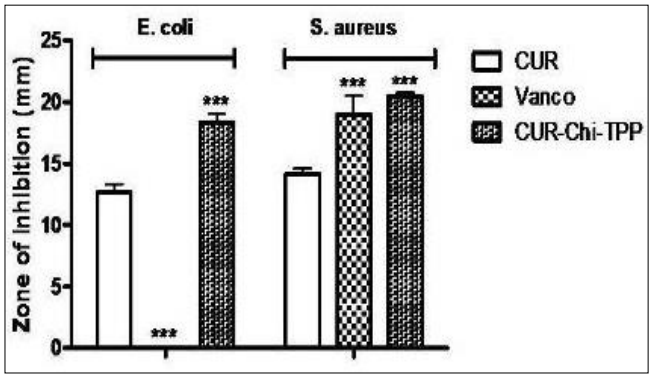


Fig D: Antibacterial efficacy of nanocurcumin against
Fig 6: *E. Coli*. Bacteria.
Fig 7: S. aureus bacteria.



Graph 1: Zone of inhibition of nanocurcumin compared with normal curcumin, and standard drug vancomycin significant at ***P ≤ 0.001

Discussion

In present study synthesis of Chi – TPP encapsulated nanocurcumin was done by ionic gelation method. In this method both positively and negatively charged particles interacted to each other and cross linking by TPP provided highly stable nanocapsule which was limiting factor for curcumin delivery in previous litratures. Similar method was shown by Sujima *et al*. for preparation of nanocapsule for plant extract *Gymnemna sylvestre* for their study (Sujima *et al*., 2016) [15].

Regarding morphology and shape of nanoparticles, we have observed under optical and fluorescence microscope they have found to be spherical in shape and it was further confirmed by the TEM analysis in which curcumin was encapsulated inside Chi –TPP hollow capsule and appeared as solid spherical evenly distributed nanoparticles. For TEM analysis (Papadimitriou and Bikiaris, 2009) [16] also found that nanoparticles were spherical in shape, outer core – shell structure arranged in concentric rings which looked like onion structure. Present study revealed that size of particles by zeta sizer and TEM varied between 72 nm and 135.2 nm. In a previous study conducted by (Elzatahry and Eldin 2008) [17] also reported that at different ratios of Chi-TPP smaller ratios produced smaller size particles with linearity in increasing of size by increasing the Chi-TPP weight ratio, and reported that Chi-TPP ratio 4:1 produced nanoparticles of size less than 300 nm. Zeta potential for Cur-Chi-TPP was + 12.9 mV which shows stability of particles. In a study by (Maruyama *et al*. 2016) [18] reported that herbicide shown increased stability after encapsulating with Chi-TPP from +15 mV to + 26 mV which was due to the interaction of herbicide with polymeric chain of Chi-TPP capsule.

Conclusion

Curcumin a potent anticancer and antibacterial drug was encapsulated inside the layer of Chi-TPP by ionic gelation method with increased water solubility, improved stability and higher antibacterial capacity. Antibacterial activity of curcumin and Chi-TPP has been reported by many researchers in various previous studies. In our study we demonstrate that nanoparticles has been effective against both gram positive and gram negative bacteria. Study revealed that gram positive bacteria were more sensitive than gram negative bacteria. Cur-Chi-TPP has shown significant increased zone of inhibition than curcumin and vancomycin for gram negative *E. coli* bacteria While for *S. aureus,* both vancomycin and Cur-Chi-TPP have expressed similar antibacterial efficacies with no significant differences.

References

1. Deka C, Aidew L, Devia N, Buragohainb AK, Kakati DK. Synthesis of curcumin-loaded chitosan phosphate nanoparticle and study of its cytotoxicity and antimicrobial activity. Journal of biomaterials science, polymer edition. 2016; 27(16):1659-1673.
2. Mun SH, Joung DK, Kim YS, Kang OH, Kim SB, Seo YS *et al*. Synergistic antibacterial effect of curcumin against methicillin-resistant Staphylococcus aureus. Phytomedicine. 2013; 20(8-9):714-718.
3. Pandit RS, Gaikwad SC, Agarkar GA, Gade AK, Rai M. Curcumin nano particles: physico-chemical fabrication and its in vitro efficacy against human pathogens. 3 Biotech. 2015; 5(6):991-997.
4. Moghadamtousi SZ, Kadir HA, Hassandarvish P, Tajik H, Abubakar S, Zandi K. A Review on Antibacterial, Antiviral, and Antifungal Activity of Curcumin. BioMed Research International. 2014, 186864.
5. Carvaldh DDM, Takeuchi KP, Geraldine RM, Mdura CJD, Tdrres MCL. Production, solubility and antioxidant activity of curcumin nanosuspension Food Sci. Technol, Campinas. 2015; 35(1):115-119.
6. Inchai N, Ezure Y, Hongwiset D, Wimonwat SY. Investigation on solubility and stability of curcumin in aqueous polysorbate micelle. Proceedings of 31st The IIER International Conference, Bangkok, Thailand, 2015. ISBN: 978-93-85465-65-9.
7. Ravichandran R. Studies on Dissolution Behaviour of Nanoparticulate Curcumin Formulation. Advances in Nanoparticles. 2013; 2(1):51-59.
8. Chua LH, Billa N, Roberts CJ, Burley JC, Manickam S. Curcumin-containing chitosan nanoparticles as a potential mucoadhesive delivery system to the colon. Pharmaceutical Development and Technology. 2013; 18(3):591-599.
9. Gold JS, Bayar S, Salem RR. Association of Streptococcus bovis Bacteremia with Colonic Neoplasia and Extracolonic Malignancy. Arch Surg. 2004; 139(7):760-765.
10. Hickey JLS, Williford JM, Mao HQ. Control of polymeric nanoparticle size to improvetherapeutic delivery. J Control. Release. 2015; 219:536-547.
11. Soppimath KS, Aminabhavi TM, Kulkarni AR, Rudzinski WE. Biodegradable polymeric nanoparticles as drug delivery devices. J Control. Release. 2001; 70:1-20.
12. Liu Z, Jiao Y, Wang Y, Zhou C, Zhang Z. Polysaccharides-based nanoparticles as drugdelivery systems, Adv. Drug Deliv. Rev. 2008; 60:1650-1662.
13. Paz LCED, Resin A, Howard KA, Sutherland DS, Wejse PL. Anti microbial Effect of Chitosan Nanoparticles on *Streptococcus mutans* Biofilms. Applied and environmental microbiology. 2011; 77(11):3892-3895.
14. Snedecor GW, Cochran WG. *Statistical Methods, Eighth Edition*, Iowa State University Press, 1989.
15. Sujima AA, Sahi SV, Venkatachalam P. Synthesis of Bioactive Chemicals Cross-linked Sodium Tripolyphosphate (TPP) - Chitosan Nanoparticles for Enhanced Cytotoxic Activity against Human Ovarian Cancer cell Line (PA-1). J Nanomed Nano technol. 2016; 7(6):1000418.
16. Papadimitriou S, Bikiaris D. Novel self-assembled core–shell nanoparticles based on crystalline amorphous moieties of aliphatic copolyesters for efficient controlled drug release. Journal of Controlled Release. 2009; 138(2):177-184.
17. Elzatahry AA, Eldin MSM. Preparation and characterization of metronidazole loaded chitosan nanoparticles for drug delivery application. Polym. Adv. Technol. 2008; 19(12):1787-1791.
18. Maruyama CR, Guilger M, Pascoli M, Jose NB, Abhilash PC, Fraceto LF *et al*. Nanoparticles Based on Chitosan as Carriers for the Combined Herbicides Imazapic and Imazapyr. Scientific reports. 2016; 6:19768.