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Research Article

FORMULATION AND EVALUATION OF TAMOXIFEN CITRATE LOADED CHITOSAN NANOPARTICLES

Shravan L. Nargund*, C.S.R. Lakshmi, Shachindra L. Nargund, L.V.G. Nargund

Department of Pharmaceutics, Nargund College of Pharmacy, 2nd main, Dattatreyanagar, Hoskerehalli, Banashankari 3rd stage, Bangalore-85, INDIA.

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ABSTRACT

Chitosan loaded Tamoxifen citrate nanoparticles were prepared by using Ionic gelation method. Sodium tripolyphosphate is used as a cross linker. The concentration of the polymer and cross linker were varied to find their effect on the particle size, Zeta potential, percentage encapsulation efficiency and surface morphology. The polymer concentration was varied from 0.2% to 1.6% and sodium tripolyphosphate concentration was varied from 0.04% to 0.2%. The compatibility studies of various excipients were conducted by using Fourier transform infrared spectroscopy and all the excipients were found compatible. The best formulations were found to contain 0.8% Chitosan and 0.08% sodium tripolyphosphate which gave a particle size of 315±2.8 nm, zeta potential of 53.7±0.6 mV, PDI 0.818±0.12 and the particles appeared to be slightly rough and spherical with no agglomeration. It gave an encapsulation efficiency of 64.51±2.5 %.This optimized formulation was taken up for further in vitro and in vivo drug release studies.

KEYWORDS: *Ionic gelation, Nanoparticles, Percentage encapsulation efficiency.*

INTRODUCTION

Cancer is one of the most deadliest diseases the modern world has experienced. Cancer is the leading cause of death worldwide, accounting for 7.6 million deaths (around 13% of all deaths) in the year 2008. Lungs, stomach, liver, colon and breast cancer causing the most cancer deaths every year. About 70% of all cancer deaths occurred in low and middle income countries. Deaths from cancer worldwide are projected to rise to over 11 million in 2030 [4]. The main reasons for the difficulties in cancer treatment are targeting cancer stem cells (CSCs) is difficult, drug resistance properties of cancer stem cells make them immune to anticancer drugs, Lack of cancer epigenetic profiling and specificity of existing epidrugs, problems associated with cancer diagnosis make it difficult to treat, Unavailability of effective biomarkers for cancer diagnosis and prognosis, limitations of conventional chemotherapeutic agents and metastasis poses a huge problem in the cancer treatment. Based on all the above statistics and the challenges faced in the treatment of cancer has led us to study and explore in formulating an effective product which will lead us to achieve our goal to overcome the difficulties in the treatment of cancer [5]. According to N. R. Ravikumar and coworkers who worked on chitosan drug loaded nanoparticles and reported that the physical parameters highly influence the size, surface charge and morphology of the nanoparticles. The approach of nanoparticles has been proven to be widely successful due to its enhanced bioavailability and targeted approach inturn reducing the adverse effects due to reduction in dose [6]. The stability of such combinational nanoparticles have been proved by Clavlo et. al. These are developed as targeted nano formulation for delivery of anti cancer drug and checking the effect of variation of various parameters in the formulation on the physical characteristics of the nanoparticles. Further cytotoxicity studies are conducted to prove the efficacy of the formulation.

***Corresponding author:**

Shravan L. Nargund

Department of Pharmaceutics,

Nargund College of Pharmacy, 2nd main,

Dattatreyanagar, Hoskerehalli, Banashankari 3rd stage,

Bangalore-85, INDIA.

** E-Mail: shravan.nargund@gmail.com*

MATERIALS AND METHODS

Chitosan (low molecular weight) from Sigma (St. Louis, MO, USA), Sodium Tripolyphosphate from sigma (St. Louis, MO, USA), Acetic acid from NICE (India) were purchased. Tamoxifen Citrate was a gift sample from Torrentz pharma, India. Double distilled water was used through- out the study. All other reagents were of analytical grade unless otherwise stated.

Preparation of Chitosan-TPP Tamoxifen citrate nanoparticles by ionic gelation method:

Chitosan Nanoparticles were produced based on an Ionic gelation of tripolyphosphate and chitosan. Tamoxifen citrate loaded Chitosan nanoparticles were formed by the slow drop wise addition of tamoxifen citrate dissolved in methanol (5 mg/ml) to chitosan solution and followed by the addition of sodium tripolyphosphate solution in a drop wise manner. Tamoxifen citrate loaded Chitosan nanoparticles formed were concentrated by centrifugation at 15000 rpm for 45 min. The concentration of chitosan, Sodium tripolyphosphate and acetic acid was varied to check their effect on particle size, zeta potential, polydispersity index, encapsulation efficiency and surface morphology [1].

Standard calibration curve of Tamoxifen citrate:

Preparation of Standard Calibration Curve of Tamoxifen citrate in 0.02N hydrochloric acid Tamoxifen citrate equivalent to 10 mg was transferred into a 10 ml volumetric flask. 5 ml of methanol was added and the volume made up with 0.02N hydrochloric acid to dissolve Tamoxifen citrate. This gives stock- I solution of 1000 µg/mL. 1 ml of the stock-I solution was transferred into a 10 ml volumetric flask and diluted with of 0.02 N hydrochloric acid up to the mark to give Stock-II solution of 100 µg/mL. From this stock-II solution serial dilutions were made to obtain solutions of the drug in the concentration ranging from 2, 4, 6, 8 and 10µg/mL. The absorbance of the solutions was measured

at 234.5 nm and 275 nm using UV- visible spectrophotometer. A graph of concentration Vs absorbance was plotted. Preparation of Tamoxifen citrate stock solution (1000 µg/mL) in pH 6.8 Phosphate buffer and tamoxifen citrate equivalent to 10 mg was transferred into 10 ml volumetric flask. 5 ml of methanol was added and the volume was made up with phosphate buffer to obtain a solution having a pH of 6.8. This gives the stock-I solution of Tamoxifen citrate containing1000 µg/mL. 1 ml of the stock-I solution was transferred into a 10 ml volumetric flask and diluted with of 6.8 pH Phosphate buffer up to the mark to give Stock-II solution, containing 100 µg/mL of Tamoxifen citrate. From Stock-II solution serial dilutions were made to obtain solutions of the drug in the concentration ranging from 2, 4, 6, 8 and 10 µg/mL. The absorbance of the solutions was measured at 234.5 nm and 275 nm using UV- visible spectrophotometer. A graph of concentration Vs absorbance was plotted [3].

Drug-excipient compatibility study by FTIR:

Compatibility of the drug with the excipients was determined by subjecting the physical mixture of the drug and the polymers of the main formulation to infrared absorption spectral analysis (FTIR). Any changes in chemical composition of the drug after combining it with the polymers were investigated with I.R. spectral analysis. Weighed amount of drug (1 mg) and excipients were mixed with 100 mg of potassium bromide (dried at 40-50°C). The mixture was taken and compressed under 10-ton pressure in a hydraulic press to form a transparent pellet. The pellet was scanned by IR spectrophotometer.

Particle size distribution:

The nanoparticle size was determined by dynamic light scattering using Horiba SZ100 nanoparticle size analyzer. It's a routine method to determine the mean hydrodynamic diameter and the particle size distribution (polydispersity index, PDI = 22/Γ 2) of the nanoparticles. The dynamic light-scattering measurements were done at 25 °C with an angle detection of 90° and 173°.

Determination of ζ-potential:

The zeta (ζ) potential of Tamoxifen citrate loaded Chitosan nanoparticles was measured from the mobility of the electrons of nanoparticles using laser doppler electrophoresis (Horiba SZ100). The measurements were carried out at 25°C in a carbon electrode cell.

Determination of encapsulation efficiency (EE):

The drug content in the prepared Tamoxifen citrate loaded Chitosan nanoparticles was calculated by the difference between the total amount of tamoxifen added during the preparation and the amount of drug present in the supernatant after centrifugation. The tamoxifen present in the supernatant was determined spectrophotometrically by reading the absorbance at 272 nm using UV–Vis spectrophotometer, Shimadzu 1800, Kyoto, Japan.

Morphology of Chitosan Nanoparticles:

Scanning electron microscopy (SEM) was performed using a ESEM Quanta 200, FEI operating between 5 and 20 kV with a magnification of 10 to 100 K and scan speed of 5 to 12. The samples were deposited on silicon wafers, dried at room temperature, and coated with a gold layer using a Cressington sputter-coater with a rotary planetary-tilt stage, along with a thickness controller. These prepared samples were further subjected to imaging.

RESULTS AND DISCUSSION

The compatibility studies were carried out for tamoxifen citrate with chitosan and sodium tripolyphosphate using Fourier Transform Infrared Spectroscopy. No interactions were observed and they were found to be compatible. Several trials were conducted with different concentrations of acetic acid, chitosan and sodium tripolyphosphate to check their effect on particle size, encapsulation efficiency, polydispersity index, zeta potential and surface morphology. Acetic acid concentrations ranging from 0.2% to 1.4% were used to prepare nanoparticles. The best concentration was found to be 0.8% acetic acid. 0.8% acetic acid gave a particle size of 196 ±5.3 nm, zeta potential of 43.2±0.6 mV, PDI 1.187±0.37 and the particles appeared to be smooth surfaced and globular with no agglomeration. It gave an encapsulation efficiency of 60.84±4.3%. Chitosan concentrations ranging 0.2% to 1.6% were used and nanoparticles were prepared. The best concentration was found to be 0.8% and 1.2%. 0.8% chitosan gave a particle size of 315±2.8 nm, zeta potential of 53.7±0.6 mV and PDI 0.818±0.12 and the particles appeared to be slightly rough but spherical with no agglomeration. It gave an encapsulation efficiency of 60.84±4.3 %. 1.2% chitosan gave a particle size of 239±6.6 nm, zeta potential of 56.3±0.4 mV and PDI 2.591±0.53 and the particles appeared to be smooth and spherical with no agglomeration. It gave an encapsulation efficiency of 64.51±2.5 %. Sodium tripolyphosphate concentrations of 0.04%, 0.08%, 0.12%, 0.16% and 0.2% were used and nanoparticles were prepared. The best concentrations were 0.08% and 0.16%. 0.08% sodium TPP gave a particle size of 315±2.8 nm, zeta potential of 53.7±0.6 mV and PDI 0.818±0.12 and the particles appeared to be slightly rough and spherical with no agglomeration. It gave an encapsulation efficiency of 60.84±4.3 %. 0.16% of sodium tripolyphosphate gave particles of size 284±2.8nm, zeta potential of 45.3±0.7mV and of PDI 0.983±0.13 and the particles appeared to be smooth and globular with no agglomeration. It gave an encapsulation efficiency of 51.05±3.6%. There are several other formulations which showed very promising surface morphology with 0.16% Sodium tripolyphosphate, 0.8% Chitosan and 0.8% Acetic acid. The particles formed were smooth and globular but due to less encapsulation efficiency so the formulation containing 0.08% sodium tripolyphosphate which gave a highest encapsulation efficiency and spherical smooth particles were optimized. Chitosan concentration of 1.2% in 0.8% acetic acid and 0.08% sodium tripolyphosphate also gave good results with high encapsulation efficiency but this was also not considered as optimized formulation as the PDI of the prepared nanoparticles was very high. The particles were found to be very smooth and spherical with no agglomeration still due to the less concentration of such particles in the sample during imaging this formulation was not considered as an optimize formulation.

Formulations:

1. **Different range of Acetic acid concentration:**

Table No. 1: Formulation with different range of acetic acid concentration

Formulation	Concentration of acetic acid	Amount of Chitosan(3ml)	Amount of Tamoxifen(1ml)	Amount of Sodium TPP(1.2ml)
A1	0.2N	0.8%	5mg	0.08%
A2	0.4N	0.8%	5mg	0.08%
A3	0.6N	0.8%	5mg	0.08%
A4	0.8N	0.8%	5mg	0.08%
A5	1N	0.8%	5mg	0.08%
A6	1.2N	0.8%	5mg	0.08%
A7	1.4N	0.8%	5mg	0.08%

2. Different range of chitosan concentration:

Table No. 2: Formulation with different range of Chitosan concentration

Formulation	Concentration of acetic acid	Amount of Chitosan(3ml)	Amount of Tamoxifen(1ml)	Amount of Sodium TPP(1.2ml)
C1	0.8N	0.2%	5mg	0.08%
C2	0.8N	0.4%	5mg	0.08%
C3	0.8N	0.6%	5mg	0.08%
C4	0.8N	0.8%	5mg	0.08%
C5	0.8N	1%	5mg	0.08%
C6	0.8N	1.2%	5mg	0.08%
C7	0.8N	1.4%	5mg	0.08%
C8	0.8N	1.6%	5mg	0.08%

3. Different range of Sodium TPP:

Table No. 3: Formulation with different range of Sodium TPP concentration

Formulation	Concentration of acetic acid	Amount of Chitosan(3ml)	Amount of Tamoxifen(1ml)	Amount of Sodium TPP(1.2ml)
S1	0.8N	0.8%	5mg	0.04%
S2	0.8N	0.8%	5mg	0.08%
S3	0.8N	0.8%	5mg	0.12%
S4	0.8N	0.8%	5mg	0.16%
S5	0.8N	0.8%	5mg	0.20%

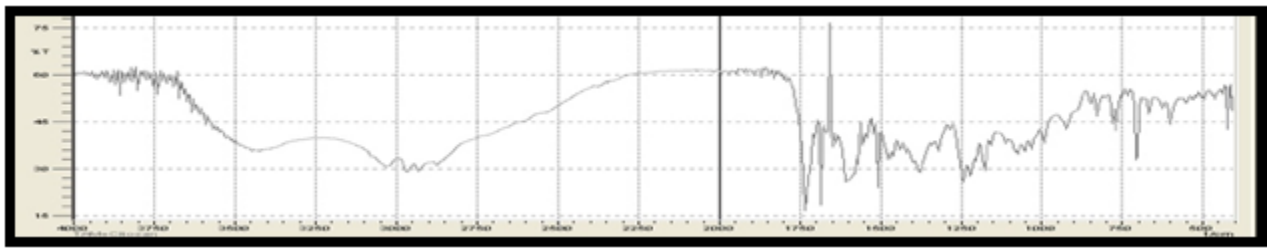


Fig. 1: Combined IR spectra of Tamoxifen citrate and chitosan

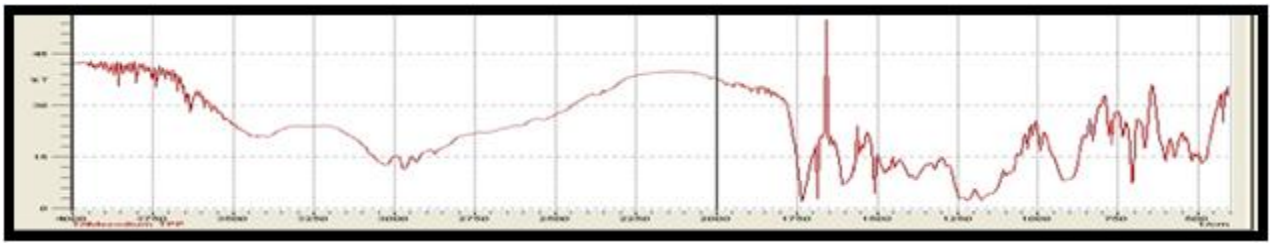
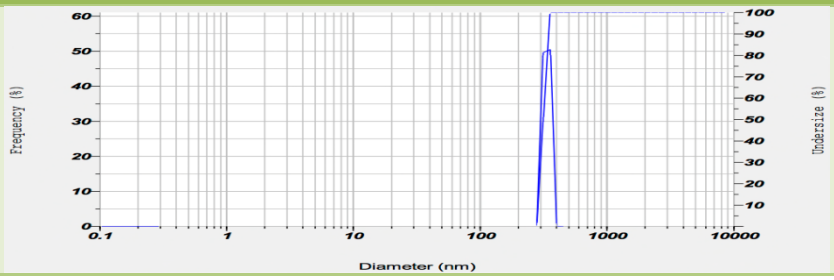


Fig. 2: Combined IR spectra of Tamoxifen citrate and sodium Tripolyphosphate

Table No. 4: Particle size and PDI of optimized formulation

Trial no.	Graph	Average particle size(nm)	PDI
A4		196±5.3	1.187±0.37

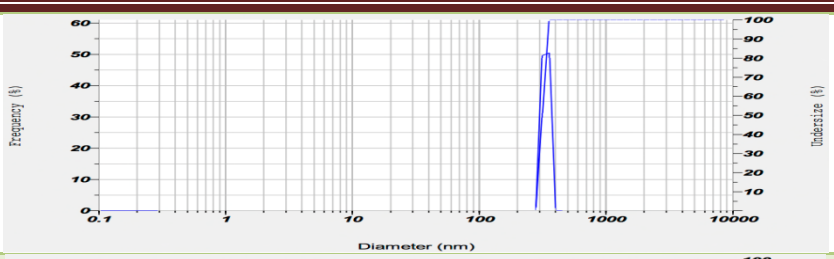
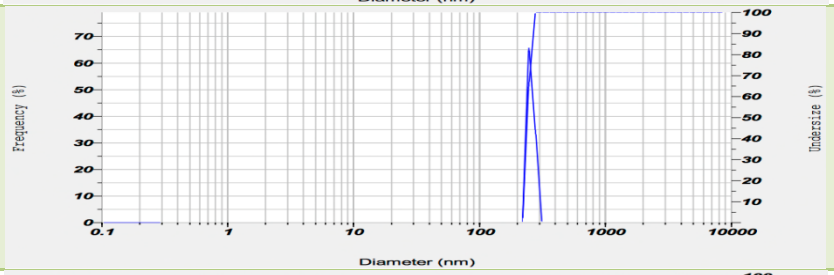
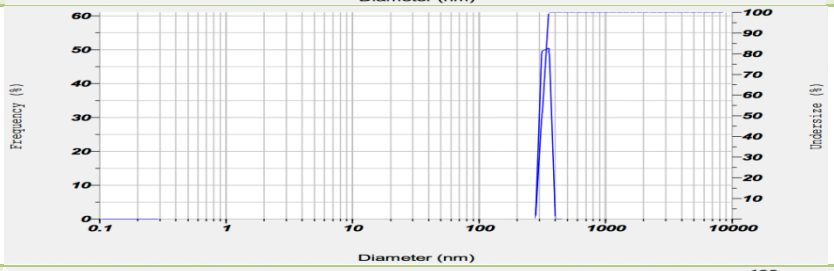
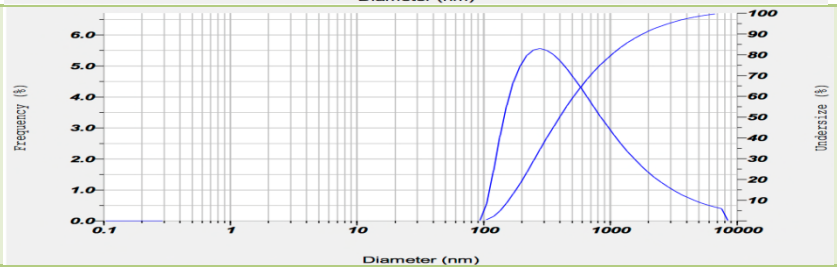
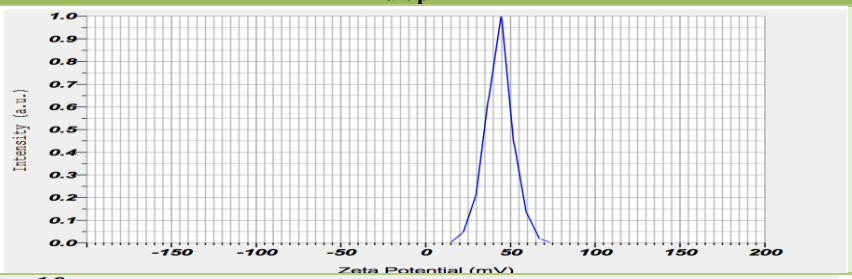
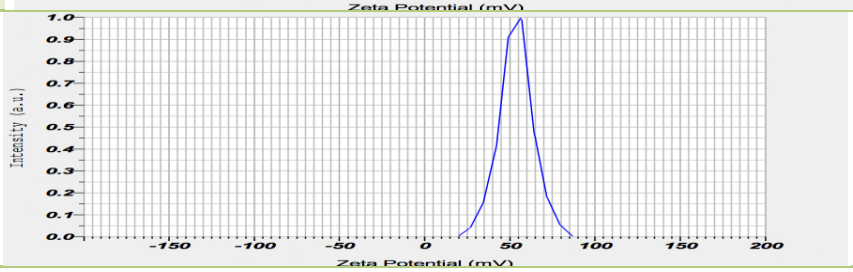
C4		315.6±2.8	0.818±0.12
C6		239.9±6.6	2.591±0.53
S2		315.6±3.2	0.818±0.17
S4		284.5±2.8	0.983±0.13

Table No. 5: Zeta potential of optimized formulations

Trial no.	Graph	Zeta potential(mV)
A4		43.2±0.6
C4, S2		53.7±0.6

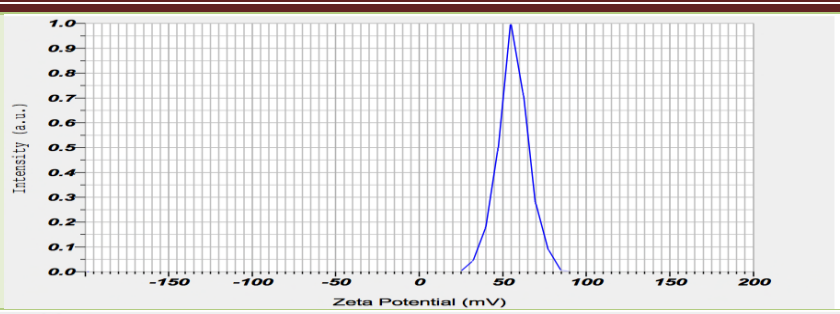
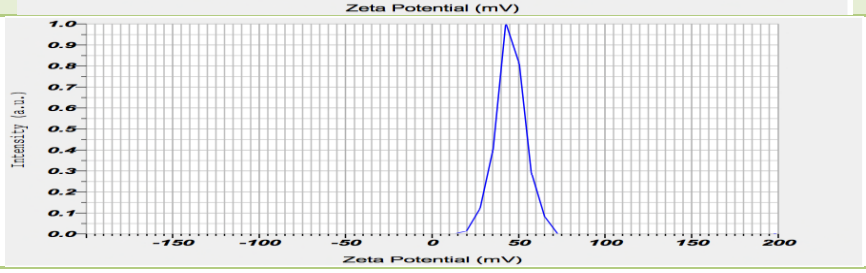
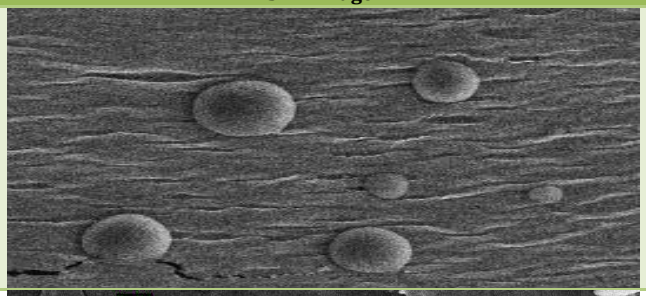
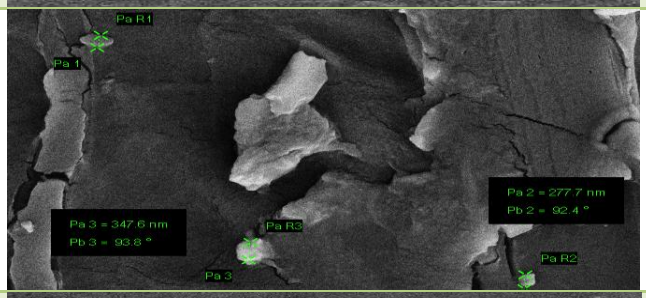
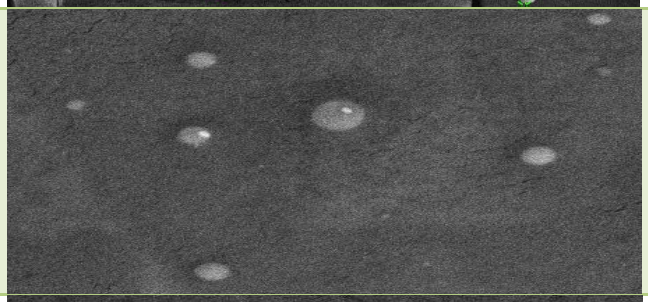
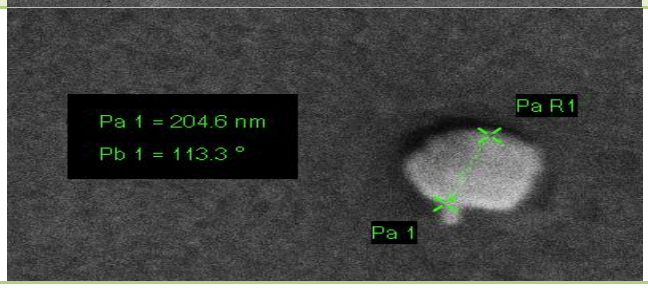
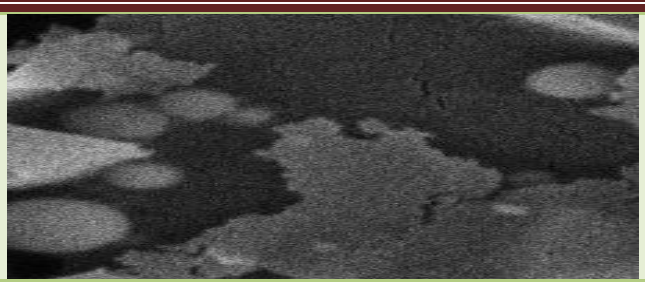
C6		56.3±0.4
S4		45.3±0.7

Table No. 6: Surface morphology of optimized formulation

Trial No.	SEM Image	Particle Size	Surface Morphology
A4		200-300	Smooth, globular, no agglomeration
C4		200-300	Slightly rough, spherical, no agglomeration
C6		300-400	Smooth, spherical, no agglomeration.
S2		200-450	Smooth spherical, no agglomeration.

S4		80-200	Smooth globular, no agglomeration

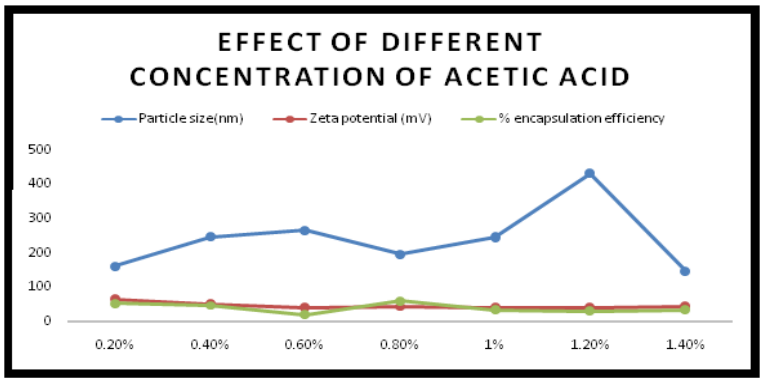


Fig. 3: Graphical representation of effect of different concentration of acetic acid on particle size, zeta potential and encapsulation efficiency

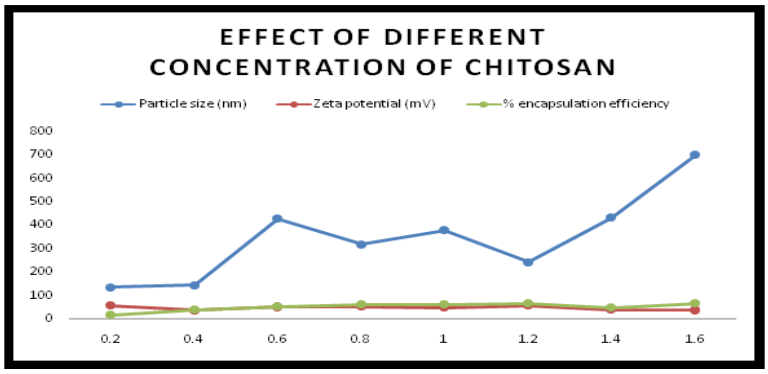


Fig. 4: Graphical representation of effect of different concentration of Chitosan on particle size, zeta potential and encapsulation efficiency

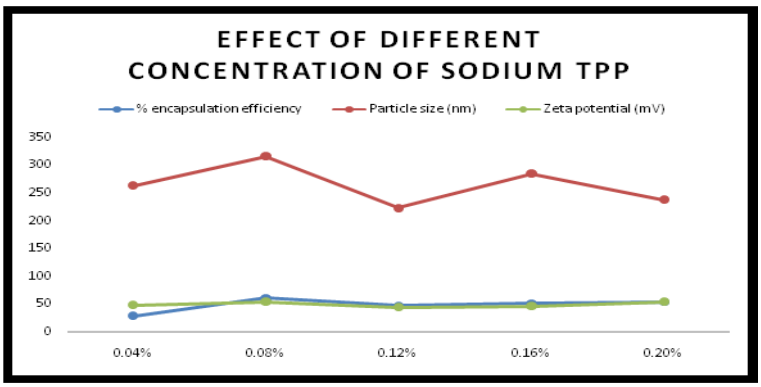


Fig. 5: Graphical representation of effect of different concentration of Sodium TPP on particle size, zeta potential and encapsulation efficiency

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