IJMPR 2019, 3(2), 21-28

International Journal of Modern Pharmaceutical Research

www.ijmpronline.com

ISSN: 2319-5878
IJMPR
Research Article

SJIF Impact Factor: 3.458

FORMULATION AND OPTIMIZATION AND *IN-VITRO* RELEASE KINETICS OF 5-FLUOROURACIL LOADED CHITOSAN NANOPARTICLES

Shravan L. Nargund*[1], C. S. R. Lakshmi[1], Amit Kumar Das[1], Shachindra L. Nargund[1]

Nargund College of Pharmacy, Banashankari 3rd Stage, Bangalore-560085.

Received on: 16/02/2019
Revised on: 06/03/2019
Accepted on: 27//03/2019

***Corresponding Author**
Shravan L. Nargund
Nargund College of Pharmacy, Banashankari 3rd Stage, Bangalore-560085.

ABSTRACT

In the present study, 5-fluorouracil-loaded-Chitosan nanoparticles are prepared by ionic gelation method. The experimental design is done by COST (changing one variable at one time) method. The concentration of chitosan is varied from 2 to 14mg/ml which is dissolved in a range of acetic acid(1-7%) and sodium tripolyphosphate concentration used as a crosslinker is varied from 2 to 14mg/ml. The drug 5 Fluorouracil (5-FU) is dissolved in the double distilled water. First sodium tripolyphosphate and Drug solution is prepared and added to chitosan solution drop by drop under magnetic stirrer at 500rpm. Nanoparticles were formed by the result of electrostatic interactions between amines of chitosan and phosphates of sodium tripolyphosphate. The particle size, zeta potential, surface morphology and polydispersity index of the 5-FU loaded chitosan nanoparticles were investigated by scanning electron microscopy (SEM), Dynamic light scattering (DLS) and Fourier transform infrared spectrometry (FTIR). The percentage of drug encapsulation efficiency and *in-vitro* drug release of the 5-FU loaded chitosan nanoparticles was investigated by UV Spectroscopy. The drug loaded chitosan nanoparticles are spherical in shape with a size distribution range of 100 to 150 nm in diameter. The zeta potential of the chitosan nanoparticles was in the range of 5 to 15 mV, The drug encapsulation efficiency of 5-FU loaded chitosan nanoparticles was 27.05±3.2%.

KEYWORDS: 5-Fluorouracil, nanoparticles, biodegradable nanoparticles, *in-vitro* release studies and Ionic gelation method.

INTRODUCTION

Chitosan is a linear polymer composed of β-(1–4)-2-amino-2-deoxy-D-glucopyranose units. Being a polycationic, nontoxic, biodegradable, and biocompatible polymer, chitosan has attracted much attention and has wide applications in biotechnology, pharmaceutical, textile, food, cosmetics, and agricultural industries.[1,2] Chitosan has achieved much interest compared with chitin and cellulose due to the presence of primary amine groups present in its repeating units. The presence of primary amine groups makes chitosan an excellent cell transfectant. Like polyethyleneimine, chitosan exhibits a "proton sponge" effect, which refers to the swelling behaviour of the polymer on encountering an acidic pH inside the cell's endosome, making it an efficient carrier for therapeutic molecules.[3-5]

Drug delivery research is clearly moving from the micro-scale to the nano-size scale. Nanotechnology is therefore emerging as a field in medicine that is expected to elicit significant therapeutic benefits. The development of effective nanoparticulate delivery systems capable of carrying a drug specifically and safely to a desired site of action is one of the most challenging tasks of pharmaceutical formulation investigators. They are attempting to reformulate and add new indications to the existing blockbuster drugs to maintain positive scientific outcomes and therapeutic breakthroughs.[6] 5-Flourouracil (5-FU or 5-fluoro-2,4-pyrimidinedione) is an antimetabolite of pyrimidine analogue type, with a broad spectrum activity against solid tumors (of gastrointestinal tract, pancreas, ovary, brain, breast, etc). Due to its structure, 5-FU interferes with nucleoside metabolism and can incorporate into RNA and DNA, leading to cytotoxicity and cell death.[7] Like many other chemotherapeutic drugs, 5-FU also has limitations that include a short biological half-life due to rapid metabolism, toxic side effects on bone marrow and the gastrointestinal tract, and non-selective action against healthy cells. Thus, it has been suggested that chitosan nanoparticles might prevent the side effects induced by 5-FU.

Previously Suter et. al. have worked on Poly-lactoglycolic acid loaded nanoparticles which gave a particle size of 150-200 nm and also gave 80% cell toxicity effect but the use of biodegradable polymers was taken as a challenge.[23] In another study Ramesh C. N. et. al. prepared 5-FU loaded chitosan nanoparticles which also gave a particle size of 100-150 nm it gave a release up to only 60% after 8hrs.[17] The present work

will focus on use of biodegradable nanoparticles loaded with drug and give a sustained release effect which will help to optimize all the different factors used in the formulation.

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. 5-Fluorouracil from nice Chemicals, India. Double distilled water was used throughout the study. All other reagents were of analytical grade unless otherwise stated.

a- Preparation of 5-Fluorouracil loaded Chitosan nanoparticles by ionic gelation method

Chitosan nanoparticles were prepared by the ionotropic gelation method. Blank nanoparticles were obtained by the addition of an aqueous solution of sodium tripolyphosphate to a chitosan solution in acetic acid. Both sodium tripolyphosphate and chitosan were used at varying concentrations. Sodium tripolyphosphate was added to chitosan solution by flush method and stirred for 2 h at 500 rpm. 5-FU loaded nanoparticles were obtained by the above described procedure and the ratios of chitosan and sodium tripolyphosphate were varied. 5-Fluorouracil were incorporated in the chitosan solution prior to the formation of nanoparticles.

Nanoparticles were collected by cooling centrifugation at 18000 rpm for 50 min at 4°C. The Chitosan drug loaded nanoparticles pellet was suspended in double distilled water using ultrasonication for 3 min. The colloidal suspension was pre-frozen at -20°C for 24 h. Drug loaded nanoparticles were freeze dried (-60°C) for 12 h by lyophilization and dry powder of nanoaprticles were obtained. The concentration of chitosan, sodium tripolyphosphate, acetic acid and pH was varied to check their effect on particle size, zeta potential, polydispersity index, encapsulation efficiency, *in-vitro* drug release studies and surface morphology.[8]

b- 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 Fourier transformation 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. Procedure: 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.

c- Physical characterization of 5-Fluorouracil loaded Chitosan nanoparticles

1- 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. Dynamic light-scattering measurements were done at 25 °C with an angle detection of 90° and 173°.

2- Determination of Zeta-potential (ζ)

The zeta (ζ) potential of 5-FU 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.

3- Determination of encapsulation efficiency (EE)

The drug content in the prepared 5-FU loaded Chitosan nanoparticles was calculated by the difference between the total amount of 5-Fluorouracil added during the preparation and the amount of drug present in the supernatant after centrifugation. The 5-FU present in the supernatant was determined spectrophotometrically by reading the absorbance at 265.6 nm using UV–vis spectrophotometer Shimadzu 1800, Kyoto, Japan. Encapsulation efficiency was determined by the following formulae,

$$E.E.(\%)=\frac{W_t - W_u}{W_t} \times 100$$

Where, Wt, weight of initial drug; Wu, weight of un-encapsulated drug.

4- Morphology of ChtNPs

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.

d- Invitro Dissolution of chitosan loaded 5 Fluorouracil nanoparticles

In-vitro release studies of 5-FU loaded nanoparticles were performed by mesh basket dissolution paddle in type 2 USP *in-vitro* dissolution apparatus. The nanoparticles equivalent to 5mg of 5-FU were filled in the basket and dipped in a 200ml beaker containing 1.2N Hydrochloric acid buffer. The entire system was placed in a larger beaker (250ml) containing distilled water used as outer jacket to maintain the temperature of medium at 37.5°C. At predetermined periods, 2ml of the medium was removed and the amount of 5-FU was analyzed by Spectrophotometric method at 265nm. The release experiments were carried out in triplicate. The control nanoparticles without 5-fluorouracil were treated similarly and used as blanks for the measurements.[15]

1. **Formulation with different ranges of Acetic acid concentration containing 4mg/ml chitosan, 10mg 5-Fluorouracil, 2mg/ml sodium tripolyphosphate and at pH 6:-**

Table 1: Formulation with different range of acetic acid concentration.

Formulation	Concentration of acetic acid (%)
A1	1
A2	2
A3	3
A4	4
A5	5
A6	6
A7	7

2. **Formulation with different ranges of chitosan concentration containing 3% acetic acid, 10mg 5-Fluorouracil, 2mg/ml sodium tripolyphosphate and at pH 6:-**

Table 2: Formulation with different range of Chitosan concentration.

Formulation	Amount of Chitosan(mg/ml)
C1	2
C2	4
C3	6
C4	8
C5	10
C6	12
C7	14

3. **Formulation with different ranges of Sodium TPP containing 3% acetic acid, 4mg/ml Chitosan, 10mg 5-Fluorouracil and at pH 6.**

Table 3: Formulation with different range of Sodium TPP concentration.

Formulation	Amount of Sodium TPP(mg/ml)
T1	2
T2	4
T3	6
T4	8
T5	10
T6	12
T7	14

4. **Formulation different ranges of pH containing 3% acetic acid, 4mg/ml Chitosan, 10mg 5-Fluorouracil and 2mg/ml Sodium tripolyphosphate**

Table 4: Formulation at different pH range.

Formulation	pH
P1	1
P2	2
P3	3
P4	4
P5	5
P6	6

RESULTS AND DISCUSSION

The compatibility studies carried out for 5-FU with chitosan and sodium tripolyphosphate using Fourier transform infrared spectroscopy (FTIR) showed no interactions inferring to be compatible. The best concentration for different ranges acetic acid concentrations is A3 which contains 3% acetic acid. 3% acetic acid gave a particle size of 476.9±2.1nm, zeta potential of 1.7±0.2mV, PDI 0.958±0.03 and the particles appeared to be smooth surfaced and globular with no agglomeration. It gave an encapsulation efficiency of 27.05±3.2%. Chitosan concentrations ranging from 0.2% to 1% were used and nanoparticles were prepared. The best concentration for different Chitosan concentrations is C2 which contains 4mg/ml chitosan in 3% acetic acid. The surface morphology of C2 appeared to be smooth and spherical with no agglomeration. The best concentration for different ranges of sodium tripolyphosphate is T1 which contains 2mg/ml sodium tripolyphosphate.

The surface morphology of T1 appeared to be smooth and spherical with no agglomeration. The analysis of surface morphology gave very interesting results. The surface was smoother and spherical shaped and the particles were found equidistant from each other as the pH increased from 1 to 6. Similarly better results were shown at lower concentrations of chitosan and Sodium Tripolyphosphate, as the concentration increased the particles started to agglomerate. The concentration of acetic acid also played an important role at lower concentrations the particles were found to be smooth and spherical shaped and are equidistant but agglomerated as the concentration increased.

The Zeta potential of the formulations varied a lot in different trials. In acetic acid trials the zeta potential reduced with the increase in the concentration of acetic acid. The zeta potential was highly cationic at lower pH and as the pH increased the zeta potential was reduced to neutral charge. The chitosan concentration and sodium TPP concentration did not play a significant role in modification of zeta potential. In most of the trials particle size gave a linear graph.

The particle size increased with the increase in the concentration of acetic acid, chitosan, sodium tripolyphosphate and pH. Hence the optimized formulation have lesser concentration of acetic acid, chitosan, sodium tripolyphosphate and pH. The encapsulation efficiency increased with the increase in the acetic acid concentration. The best concentration was found to be A3 containing 3% acetic acid with an encapsulation efficiency of 27.05±3.2%. The increase in concentrations of chitosan lead to the increase in the percentage of encapsulation efficiency but after 8mg/ml concentration of chitosan there was a lag phase. The percentage encapsulation efficiency in different concentrations of sodium tripolyphosphate showed an initial rise at 2mg/ml and further reduced with increase in the concentration of sodium tripolyphosphate.

The different range of pH also showed the similar results as that of sodium tripolyphosphate with initial rise followed by a lag phase at higher pH. The particle size increasing with slight increase in pH. The polydispersity index decreased with the increase in pH, the encapsulation efficiency initially showed poor results at lower pH and increased with the increase in pH and the zeta potential was high at lower pH and slowly decreased with the increase in pH. Based on consideration of all the parameter the optimized formulation was at pH6. The *in-vitro* drug release studies proved that a sustained release effect is seen with total drug release of 87% at 8hrs. The kinetic studies showed that the release pattern followed first order kinetics hence proving to have a sustained release effect.

Based on all the parameters, particle size, zeta potential, polydispersity index and percentage encapsulation efficiency the optimized formulation was Trial A3.C2,T1 and P6 containing chitosan concentration of 4mg/ml in 3% acetic acid and 2mg/ml sodium TPP prepared at optimized pH 6 gave best results. The particles were found to be very smooth and spherical with no agglomeration.

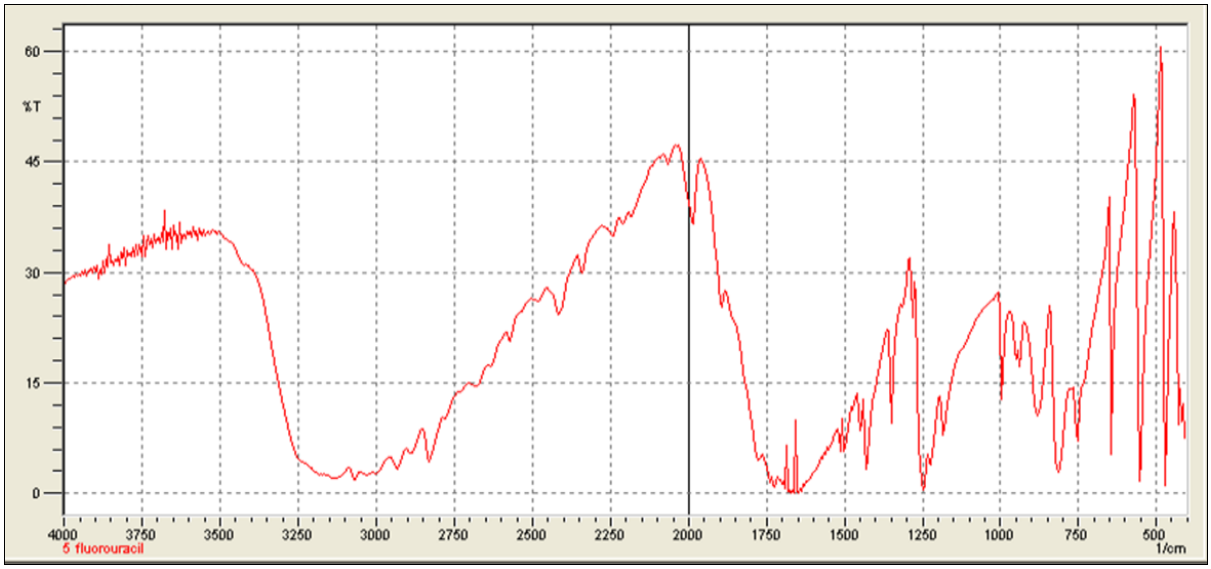


Figure 1: IR spectra of 5-Fluorouracil.

Figure 1: Combined IR spectra of 5-Fluorouracil and sodium tripolyphosphate.

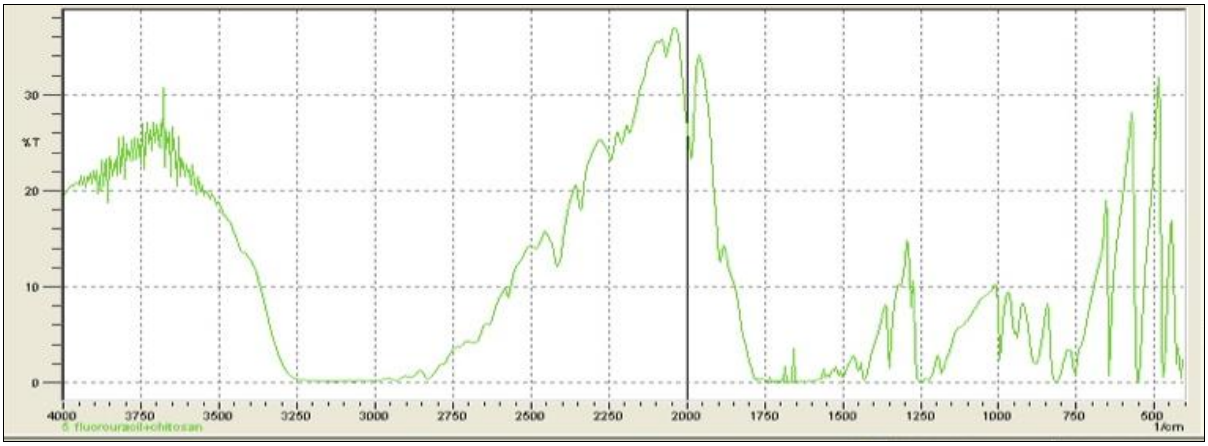


Figure 2: Combined FTIR (Fourier transform Infrared spectroscopy) spectra of 5-Fluorouracil and Chitosan.

Table – IR: peaks chart of 5-Fluorouracil with functional group.

Sr. No.	Functional group	Theoretical(cm^{-1})	Practical(cm^{-1})
1	C=C	1500-1600	1550
2	C=O	1690-1730	1700
3	F	700-800	750
4	NH	3000-3300	3200

b- Physical characterization of 5-Fluorouracil loaded chitosan nanoparticles.

1- Particle size graph of optimized formulation

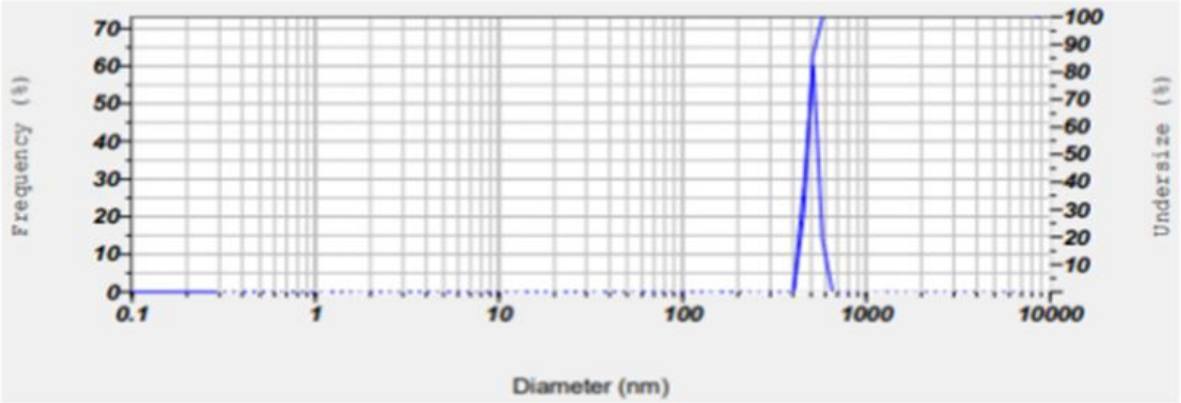


Figure 3: Particle size graph of optimized formulation.

2-Determination of ζ-potential

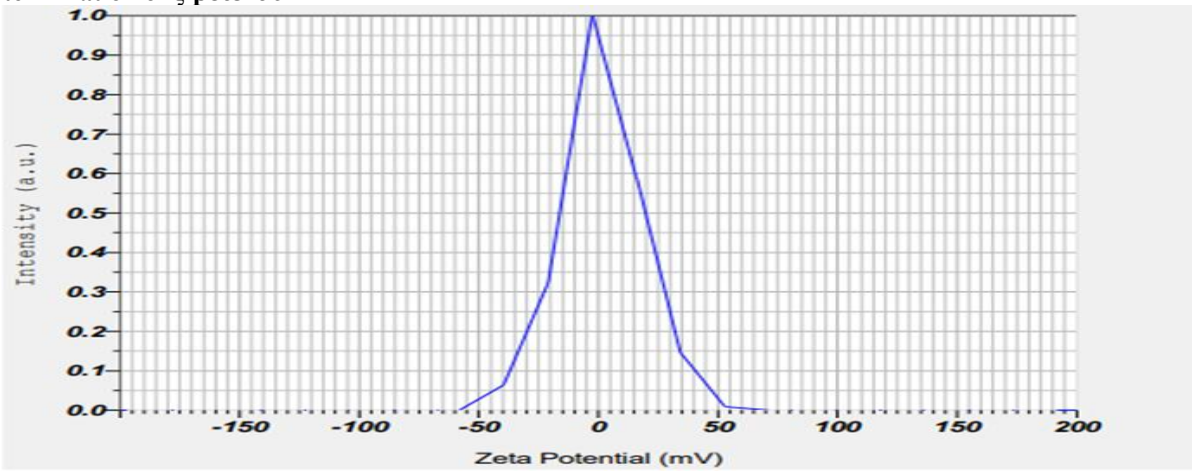


Figure 4: Zeta potential of different range of acetic acid concentration.

5. Morphology of 5-Fluorouracil loaded chitosan nanoparticles.

SEM images of optimized formulation

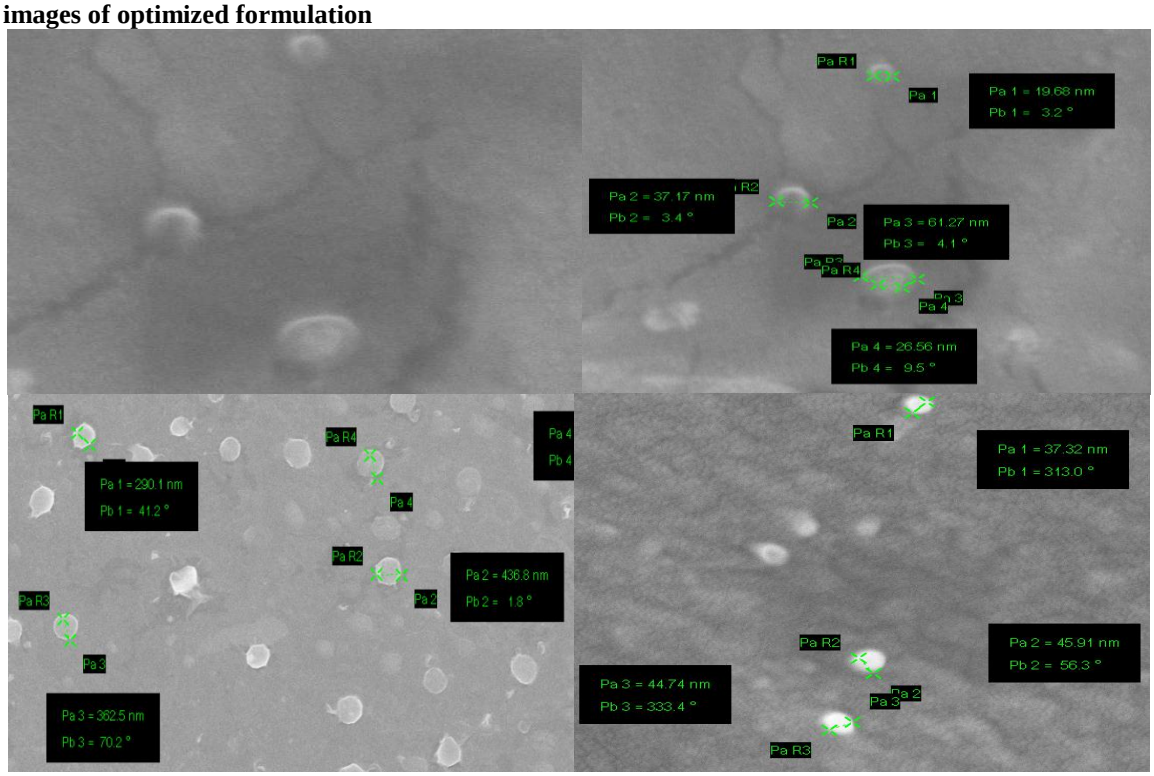
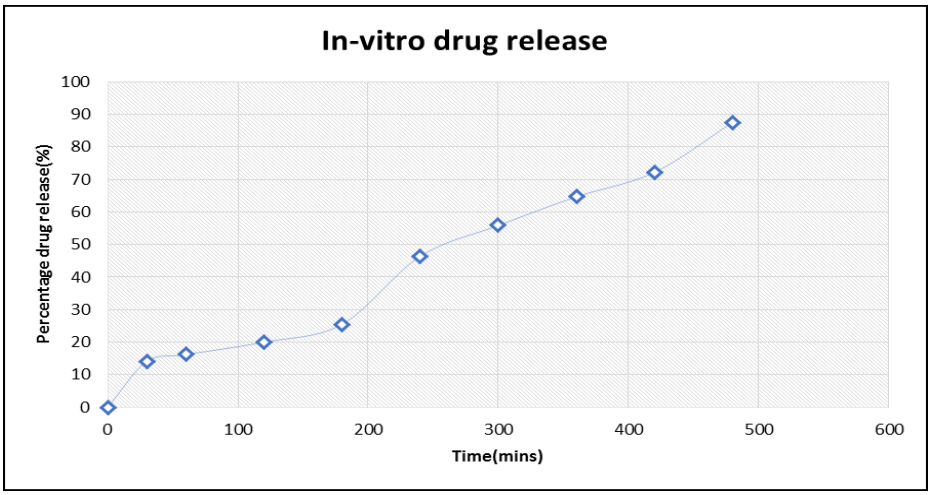


Figure 5: SEM images of optimized formulation at 50,000-1,00,000X magnification and current varied from 5-15kV.

5-Fluorouracil loaded chitosan nanoparticles *in-vitro* Drug release.

Table 5: 5-Fluorouracil loaded chitosan nanoparticles *in-vitro* Drug release in the 1.2pH Hydrochloric acid.

Time (mins)	Percentage drug release (%)
30	14.05
60	16.17
120	20.01
180	25.49
240	46.4
300	55.88
360	64.7
420	72.22
480	87.58



Graph 1: 5-Fluorouracil loaded chitosan nanoparticles *in-vitro* Drug release graph.

5-Fluorouracil loaded chitosan nanoparticles

Table 6: Analysis of the release kinetics for 5 Fluorouracil loaded chitosan nanopartricles.

Model	R^2
Zero order	0.9755
First order	0.8872
Higuchi	0.9178

Effect of different concentrations of Chitosan, acetic acid, sodium TPP and pH on particle size, PDI, zeta potential and % encapsulation efficiency of all formulation:-

Table 7: Compilation of effect of different concentrations of Chitosan, acetic acid, sodium TPP and pH on particle size, PDI, zeta potential and % encapsulation efficiency.

Trial no.	Particle size(nm)	PDI	Zeta potential(mV)	% Encapsulation efficiency
A1	317.6±3.8	1.325±0.12	7.0±1.3	13±1.2
A2	476.1±4.2	0.979±0.4	0.0±0.4	19±2.3
A3	**476.9±2.1**	**0.958±0.03**	**1.7±0.2**	**27.05±3.2**
A4	614.9±4.5	0.701±0.31	-2.1±3	25±4.1
A5	1400.8±14.5	0.902±0.12	5.6±2.4	23±5.3
A6	334.9±12.3	1.117±0.22	4.1±0.5	15±2.3
A7	697.8±8.6	0.604±0.32	7.9±3.2	28.32±7.1
C1	379±7.6	1.337±0.2	1.9±0.5	15.75±2.2
C2	**476.9±2.1**	**0.958±0.03**	**1.7±0.2**	**27.05±3.2**
C3	613.4±7.6	1.142±0.42	0.0±0.6	21.41±4.2
C4	885±9.1	0.554±0.32	-2.52±0.45	20.32±5.4
C5	831.2±13.2	0.851±0.12	2.4±0.6	25.71±3.2
C6	746.1±5.4	1.159±0.4	3.4±1.2	32.57±6.4
C7	1790.7±16.2	0.719±0.21	2.0±0.53	20.5±7.3
T1	**476.9±2.1**	**0.958±0.03**	**1.7±0.2**	**27.05±3.2**
T2	715.1±6.5	1.399±0.23	2.4±0.45	9.87±2.4
T3	750.7±9.3	0.738±0.11	-0.4±0.65	18.36±5.3
T4	1414.9±4.7	1.312±0.42	0.3±0.53	16.24±6.3
T5	2339±6.4	1.101±0.53	0.7±0.6	9.877±3.3
T6	889.7±9.2	0.701±0.74	1.0±2.3	23.91±8.2
T7	3283.3±17.4	0.747±0.64	4.8±0.54	7.77±4.2
P1	38±45.6	7.545±4.1	-1.2±1.3	29.95±8.2
P2	211±5.2	1.382±0.5	25.7±2.1	13.30±4.2
P3	421±3.2	0.835±0.52	16±3.2	1.55±5.6
P4	305±6.2	0.737±0.3	4.3±3.4	24.73±2.4
P5	672±4.4	0.515±0.3	3.4±1.6	25.71±4.2
P6	**476.9±2.1**	**0.958±0.03**	**1.7±0.2**	**27.05±3.2**

CONCLUSION

The preparation of 5-FU loaded chitosan nanoparticles by Ionic gelation method by cross linking with sodium TPP gave promising results. Several formulations prepared showed significant effect on the physical and morphological characteristics of the nanoparticles formed. The FTIR analysis showed that all the excipients were compatible with the drug. The best formulation was found to contain 3% acetic acid, 4mg/ml chitosan and 2mg/ml sodium TPP prepared at optimized pH 6. The optimized formulation gave a particle size of 476.9±2.1nm, zeta potential of 1.7±0.2mV, PDI 0.958±0.03 and encapsulation efficiency of 27.05±3.2%. This was concluded based on COST (changing one single variable at a time) optimization for encapsulation efficiency, surface morphology, zeta potential and PDI during experimentation. The *in-vitro* release studies proved the nanoparticles to give a sustained release effect and follow first order kinetics as desired. Preliminary results in this study suggest that this new 5-FU formulation can be effectively screened for *in-vivo* analysis.

ACKNOWLEDGEMENTS

We thank **Vision Group of Science and Technology, Government of Karnataka** and Nargund college of Pharmacy, Bangalore for their immense support in accomplishing the work. We also want to thank Rajiv

Gandhi University of health Sciences, bangalore for their support in the completion of the research work.

REFERENCES

1. Li Q, Dunn ET, Grandmasion EW, Goosen MFA. Applications and properties of chitosan. In: Goosen MFA, editor. Applications of Chitin and Chitosan. Lancaster: Technomic Publishing Company, Inc., 1992; 3–29.
2. Ravi-Kumar MNV. A review of chitin and chitosan applications. React Funct Polym, 2000; 46: 1–27.
3. Boussif O, Lezoualch F, Zanta MA, Mergny MD, Scherman D, et al. A versatile vector for gene and oligonucleotide transfer into cells in culture and in-vivo – polyethylenimine. Proc Natl Acad Sci U S A. 1995; 92: 7297–7301.
4. Cho YW, Kim JD, Park K. Polycation gene delivery systems: escape from endosomes to cytosol. J Pharm Pharmacol, 2003; 55: 721–734.
5. Ishii T, Okahata Y, Sato T. Mechanism of cell transfection with plasmid/ chitosan complexes. Biochim Biophys Acta, 2001; 1514: 51–64.
6. Yashwant P, Deepak T Drug delivery nanoparticles formulation and characterization. Drugs and Pharmaceutical Science Series, informal health care USA, 2009; 191: 1-30.
7. Li S, Wang A, Jiang W, Guan Z Pharmacokinetic characteristics and anticancer effects of 5-Fluorouracil loaded nanoparticles. BMC cancer, 2008; 8: 103.
8. R. C. Nagarwal, P. N. Singh, Shrikant, P. Maiti, J. K. Pandit. Chitosan Nanoparticles of 5-Fluorouracil for Ophthalmic Delivery: Characterization, in-Vitro and in-Vivo Study. *Chem. Pharm. Bull.* 2011*; 5*9: 272-8.
9. B. Reddy, B. Manjula, T. Jayaramudu, E. R. Sadiku, P. A. Babu, S. P. Selvam. 5Fluorouracil Loaded Chitosan–PVA/Na MMT Nanocomposite Films for Drug Release and Antimicrobial Activity. *Nano-Micro Lett*., 2016; 8: 260-9.
10. A. Raj, R. Jonisha, B. Revathi, E. Jayalakshmy. Preparation and characterization of BSA and chitosan nanopartices for sustainable delivery system for quercetin. *Journ of Applied Pharmaceutical Science*, 2015; 5: 1-5.
11. Y. D. Reddy, D. Dhachinamoorthi, K. B. Chandrasekhar. Formulation and in vitro evaluation of antineoplastic drug loaded nanoparticles as drug delivery system. *African Journal of Pharmacy and Pharmacology*, 2013; 7: 1592-1604.
12. M. Malhotra, C. L. Catherine, T. Duchesneau, S. Saha, S. Prakash. A novel method for synthesizing Pegylated chitosan nanoparticles: strategy, preparation, and in vitro analysis. *International Journal of Nanomedicine*, 2011; 6: 485-94.
13. Y. M. Choudhari, S. V. Detane, S. S. Kulthe, C. C. Godhani, N. N. Inamdar, S. M. Shirolikar, L. C. Borde, V. K. Mourya. Low molecular weight palmitoyl chitosan: Synthesis, characterization and nanoparticle preparation. *Adv. Mat. Lett.*, 2012; 3: 487-92.
14. L. Liang, J. Ping, C. Ming, Z. Guoliang, Z. Fengbao. 5-Fluorouracil loaded self-assembled pH-sensitive nanoparticles as novel drug carrier for treatment of malignant tumors. *Chinese J. Chem. Eng*, 2006; 14: 377-82.
15. P. S. Kumar, N. Punniamurthy. Formulation development and characterization of curcumin loaded solid lipid nanoparticles for improved aqueous solubility and bioavailability. The Pharma Innovation Journal, 2017; 4: 07-11.
16. H. Kouchakzadeh, S. H. Makarem, S.A. Shojaosadati. Simultaneous loading of 5-florouracil and SPIONs in HSA nanoparticles: Optimization of preparation, characterization and in vitro drug release study. *Nanomed. J*, 2016; 3: 35-42.
17. M. Kadagi, N.B. Shridhar, J. S Sanganal, A.R. Phani, H. D. Narayanaswamy, S. Isloor, S.G. Ramachandra. Synthesis, Characterisation of 5-Fu Loaded Chitosan Nanoparticles. *Global journal for research analysis*, 2014; 3: 114-6.
18. U. Aruna, R. Rajalakshmi, Y.M. Indira, V. Vinesha, M. Sushma, K.R. Vandana, N. Vijay. Role of chitosan nanoparticles in cancer therapy. *International Journal of Innovative Pharmaceutical Research*, 2013; 4: 318-24.
19. K. S. Oh, K. Kim, B. D. Yoon, H. J. Lee, D. Y. Park, E. Kim, K. lee, J. H. Seo, S. h. Yuk. Docetaxel loaded multilayer nanoparticles with nanodroplets for cancer therapy. *International Journal of Nanomedicine*, 2016; 11: 1077-87.
20. C. Benz, E. Cadman, J. Gwin, W. Terrence, J. Amara, A. Eisenfeld, P. Dannies. Tamoxifen and 5-Fluorouracil in breast cancer: cytotoxic synergism in Vitro. *Cancer research*, 1983; 43: 5298-303.
21. A. Maghsoudi, S. A. Shojaosadati, E. V. Farahani. 5-Fluorouracil loaded BSA nanoparticles: Formulation optimization and In Vitro release study. *AAPS PharmSciTech*. 2008; 9: 1092-6.
22. V. S. Shenoy, R. P. Gude, R. S. R. Murthy. In vitro anticancer evaluation of 5-fluorouracil lipid nanoparticles using B16F10 melanoma cell lines. *International Nano Letters*, 2013; 3:36.
23. P. S. Sutar, V. G. Joshi. Preparation and characterisation of 5-fluorouracil loaded PLGA nanoparticles for colorectal cancer therapy. *UJPBS*, 2013; 1: 52-8.
24. T. Mallamma, B. S. Thippeswamy, D. R. Bharathi, Snehalatha, T. S. Nagaraja, R. Yogananda, T. Vyjayanthimala, M. K. Mahanthesha. Formulation and evaluation of 5-flurouracil loaded HSA nanoparticle for controlled drug delivery. *International Journal of Advanced Research*, 2013; 7: 23-30.