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A FORMULATION, APPRAISEMENT OF CAPECITABINE ABUNDANT CHITOSAN NANOPARTICLES USING TPP FOR NEOPLASMIC TARGETING

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abstract

Capecitabine is an orally-administered chemotherapeutic agent used in the treatment of advanced stage of colorectal cancers (CRC). The present research was to design and development of tumor targeted capecitabine loaded nanoparticles for CRC targeting to enhance bioavailability, reduce dose, minimize side effect, and sustain drug release for 48 hrs. Nanoparticle (NPs) tumor-targeted treatment is a major area of biomedical research. Capecitabine-loaded nanoparticles were prepared by Ionotropic gelation method were prepared with different ratios of Capecitabine and Chitosan-tripolyphosphate . It was employed in formulating and optimizing the nanoparticles to maximize entrapment efficiency and minimize particle size. The optimized nanoparticles were coated with polymer and were evaluated. Fourier transform infrared spectroscopy study revealed the compatibility of drug with excipients while differential scanning calorimetry study confirmed the complete drug entrapment in polymer matrix and scanning electron microscopy revealed spherical shape of nanoparticles. The release profile of capecitabine from chitosan nanoparticles was found to be pH dependent. In-vitro dissolution studies of polymer coated nanoparticles revealed negligible released in simulated gastric as well as intestinal fluid, followed by 94.4% released in simulated intestinal fluid (phosphate buffer pH 7.4) in 48 hrs. The optimized nanoparticles showed colon-specific controlled release properties, and thus could be effective for CRC treatment.

Key words: Capecitabine, ,chitosan, TPP, Nanoparticles (NPs), In-vitro, pH-dependent release, colorectal cancer (CRC).

Introduction

Colorectal cancer (CRC) is the third most prevalling cancer worldwise, In 2020.CRC caused 506,449 deaths in Asia,244,824 in Europ,63,987 in North America and 69,435 in Latin America the Caribbean.[1]. The morbidity is expected to increase up to 1.1 million worldwise by 2030,[2].CRC is usually seen inpatients under the age of 50 and it is associated with dietary characteristics and eating habits that contribute to bacterial dysbiosis the GIT and colon cancer [3].CRC is atype of malignancy that affected the large intestine and rectum region [4].Uncontrolled growth and spread of abnormal cell types in the body are characteristics of the disease known as cancer. One of the most feared diseases today, cancer is also one of the most prevalent and is occurring with growing frequency.[5] Because cancer is so common, all varieties of disease have one defining trait in common: unregulated growth that advances to limitless expansion. The primary cause of cancer is an imbalance between normal cells growth maturation and proliferation. Chromosome change is its primary characteristic, and it is mostly brought on by changes in the affected cells' genomes. Cancer is an out-of-control growth pattern that starts in the patient's bio-system. [6] A group of cells that exhibits uncontrolled growth (division to the maximum extent feasible), incursion (intrusion on and decimation of neighbouring tissues), and infrequently metastasis (transfer to distant parts of the body via lymph or blood) is said to be malignant. The area of medicine known as oncology is concerned with cancer prevention, research, diagnosis, and treatment. Even infants can develop cancer, although the risk of the majority of mixed bags increases with age. People have a lot of questions concerning the biology, analysis, causes, and prevention methods of this disease. Almost everywhere in the body can be the source. [7].Chitosan nanoparticles have been intensively investigated as a novel drug carrier due to their extensive advantages, such as good biocompatibility, biodegradability, and nontoxic properties [8,9]. Chitosan NPs are therefore required to increase the effectiveness of anticancer medication delivery. Capecitabine is universally used as an antineoplastic agent in gastrointestinal cancer. It gets distributed throughout the body tissue and fluids, crosses the blood brain barrier and appears in the cerebrospinal fluid and disappears from the plasma [10]. In fact, one needs only to change the size of particles rather than their composition to vary the properties. This is a revolution in science called Nano Revolution [11].Nanoscience is concerned with understanding the properties of the nanoscale materials and nanotechnology deals with exploiting these properties to create new devices and systems with novel properties and functions due to their size. Nanoparticles range from 100nm down to the size of atoms i.e. approx. 0.2 nm because at this scale, the properties of materials can be very different from those at a larger scale [12]

MATERIALS AND METHODS :

Material : Capecitabine was obtained as a gift sample from Sigma-Aldrich (Mumbai, India). Sodium tripolyphosphate was obtained as a gift sample from Krishna chemical,Vadodara, Chitosan obtained as a gift sample from Balaji drugs,Surat,Gujrat,India, Folic acid was obtained as a gift sample from Suvidhan Laboratories, Baroda, Gujrat, India and all organic solvents were of analytical grade.

Preformulation Studies :

Determination of purity of APIs :

Determination of Melting point : Melting point of the drug sample was determined by capillary method using digital melting point apparatus (REMI C-30BL), where the temperature at which the drugs started to melt and it completely melted was recorded[13]

Determination of Solubility : Solubility of the drug was determined by the saturation equilibrium method. Excess quantity of capecitabine was added to the 10 ml volumetric flask, and then volume was made up to the 10 ml mark with Milli-Q water, and then mixture was placed in an incubator shaker overnight to get a saturated solution of drug in Milli-Q water. The next day, undissolved drugs were separated from the solution by filtering the mixture from whatman filter paper. Supernatant was diluted appropriately with Milli-Q water, and the absorbance was determined using UV-visible spectrophotometers at 215 nm, where Milli-Q water was used as a reference solvent. The concentration of the drug was calculated from the standard calibration curve of the drug taken in Mill-Q water. Using the same method, solubility of capecitabine was also determined in phosphate buffer solution pH 7.4 and 1% v/v acetic acid.

Determination of λmax of capecitabine :

Capecitabine stock solution (1000 µg/ml) in phosphate buffer pH 7.4 was made. To create a stock solution with a concentration of (100 µg/ml), this solution was properly diluted with the same solvent. The resulting solution was scanned in a UV-Visible spectrophotometer between 200 and 400 nm. For capecitabine, it revealed a maximum at 215 nm in phosphate buffer pH 7.4. In the same way, λmax was also determined in water and 1% v/v acetic acid as a requirement to assess the solubility of capecitabine in a later stage

Standard calibration curve :

In a volumetric flask, 25 mg of capecitabine was dissolved in 25 ml of phosphate buffer, pH 7.4, to create a stock solution with a concentration of 1000 µg/ml. 10ml of the stock solution was diluted with 100 ml to produce 100µg/ml. To create a solution of 5 to 40 g/ml, this solution (100µg/ml) was further diluted with phosphate buffer pH 7.4. Using a UV-visible spectrophotometer, the absorbance of each solution was

measured at 215 nm, with phosphate buffer pH 7.4 serving as the control. The range of 5 to 40 g/ml was used to create the standard curve.

Compatibility study :

Drug - Excipient compatibility study :

FTIR study : T FT-IR spectral analysis was carried out for pure Capecitabine, excipient blend (LMW chitosan and sodium tripolyphosphate) and for a physical mixture of Capecitabine with the excipients, by using IR- Prestige-21Shimadzu make, Japan. The analysis was performed twice. The initial study was done at the beginning of the experiment and the final one was done after storing the samples in the humidity chamber at 40±2°C and 75± 5%RH for a period of 6 months .Each of the samples were crushed with KBr (1%w/w nanoparticles) to form pellets by applying pressure at 600 kg/cubic cm and were scanned between 4000-400 cm^{-1} with a resolution of 4 cm^{-1} and at a speed of 2mm/second.

DSC study : DSC was performed for pure capecitabine and its excipients using Pyris Diamond TG/DTA, PerkinElmer (SINGAPORE).About 20 mg of the sample was placed on a standard shallow platinum crucible. Platinum crucible with alpha alumina powder was used as the reference. The sample cell was heated at a uniform scan rate of 15°C/min from 30°C to 1000°C under a constant nitrogen purge of 150ml/min .Heat flow in mW was obtained as a function of sample temperature in degrees centigrade.

Preparation of Chitosan-TPP Nanoparticles :

Chitosan-tripolyphosphate nanoparticles were prepared by Ionotropic gelation method [14,15] (Calvo et al., 1997). Varying amounts of low molecular weight chitosan were weighed and dissolved in 50 ml aqueous solution of 1% w/v acetic acid by constant stirring at 350 rpm at room temperature, in a magnetic stirrer. After formation of a transparent solution, the pH was adjusted to 7.4 using 0.1N NaOH solution. 2mg of sodium tripolyphosphate (TPP) and 25 mg of Capecitabine were weighed and dissolved separately in 20 ml of Milli-Q water until a clear solution was obtained. For preparing blank nanoparticles, TPP solution was added drop wise to the chitosan solution, using a syringe needle under constant stirring (600 rpm) at room temperature. For preparing Capecitabine loaded nanoparticles, the Capecitabine solution was added drop wise to the chitosan solution using a syringe needle under constant stirring (600 rpm) at room temperature prior to the addition of TPP solution. Spontaneous formation of anopalescent suspension was observed and was left for stirring at room temperature, for 30 minutes. The nanoparticle suspension thus formed was sonicated for 20 minutes and was ultra-centrifuged at 12,000 rpm for 30 minutes at 4°C, to remove the non-entrapped drug. The supernatant was discarded and the wet pellets of chitosan-tripolyphosphate nanoparticles were collected. The pellets were freeze-dried and stored in an air tight closed container at 2-4°C. The formation of the nanoparticles was because of the interaction

between the negative groups of the TPP and the positively charged amino groups of chitosan (ionic gelation). The different formulation parameters of all the batches were tabulated in Table 1.

Evaluation of capecitabine NPs :

Particle Size, polydispersity index (PDI) and zeta potential[16,17] **:**

The determination of particle size, polydispersity index (PDI) and zeta potential of Capecitabine loaded nanoparticle dispersions were carried out by dynamic light scattering using Malvern Zetasizer following a 1/3 (v/v) dilution in Milli-Q water at 25°C.

% yield, %Entrapment Efficiency (%EE), and % drug content [8] **:**

Exactly 2ml of each Capecitabine loaded chitosan nanoparticle formulation was taken in separate Eppendorf micro centrifuge tubes of 2ml volume, with the help of a micropipette. The filled Eppendorf tubes were then subjected to centrifugation at a speed of 15000 rpm for 20 minutes at 4°C.The supernatant was collected after centrifugation and its absorbance was checked in UV- Visible Spectrophotometer, UV-2450 at 215 nm. The free drug concentration present in the supernatant was determined from the calibration curve of Capecitabine. The amount of free drug present in the supernatant was then subtracted from the amount of drug taken initially, to obtain the amount of bound drug present within the nanoparticles. For determining the entrapment efficiency of the nanoparticles,[18] the following equation was used:

$$\%\ EE = \frac{\textit{Amount of drug within the nanoparticle}}{\textit{Amount of drug added initially}} \times 100$$

Fourier transform infra-red (FT-IR) spectroscopy :

The FTIR spectral analysis was carried out for blank chitosan-tripolyphosphate nanoparticles and Capecitabine loaded nanoparticles (CS-NPs-8) by using IR- Prestige-21Shimadzu make, Japan. About 2 mg of each of the samples were crushed with KBr (1%w/w nanoparticles) to form pellets by applying pressure at 600 kg/cubic cm and were scanned between 4000-400 cm^{-1} with a resolution of $4cm^{-1}$ and at a speed of 2mm/second.

Differential Scanning Calorimetry (DSC) :

DSC was performed separately for blank chitosan-tripolyphosphate nanoparticles and Capecitabine loaded nanoparticles (CS-NPs-8) using Pyris Diamond TG/DTA, PerkinElmer (SINGAPORE).About 20 mg of the sample was placed on a standard shallow platinum crucible. Platinum crucible with alpha alumina powder was used as the reference. The sample cell was heated at a uniform scan rate of

15°C/min from 30°C to 1000°C under a constant nitrogen purge of 150ml/min .Heat flow in mW was obtained as a function of sample temperature in degrees centigrade.

Scanning Electron Microscopy (SEM) :

The surface morphology of freeze dried Capecitabine loaded nanoparticles (CS-NPs-8) were determined by scanning electron microscopy technique using Zeiss EVO®18, which was operated at an accelerating voltage of 20kV.Images of the samples were captured at different magnifications.[19]

Transmission Electron Microscopy (TEM) :

The morphological characteristics of the Capecitabine loaded nanoparticles (CS-NPs-8) were examined using a high resolution transmission electron microscope(200 KV HRTEM, Jeol make).A droplet of nanoparticle suspension was placed on a carbon-coated copper grid of 200 mesh (TED PELLA, INC) without being stained. Five minutes later, the excess liquid was removed by touching the edged of the copper grid with a piece of filter paper. The sample was then air-dried before observation by TEM. Images were captured at different magnifications.

In Vitro drug release study [20,21] **:**

In-vitro drug release from all 09 batches of CS-NPs was carried out by the dialysis bag diffusion method. A 4–5 cm long portion of the dialysis bag was made into a dialysis sac by folding and tying up one end of the bag with thread. It was then filled up with phosphate buffer pH 7.4 and examined for the leaks. The sac was then emptied, and NPs dispersion (equivalent to 10 mg of drug) was accurately transferred into the sac, which served as the donor compartments. The sac was once again examined for leak and then suspended in the glass beakers containing 50 ml phosphate buffer pH 7.4, which became the receptor compartment. Aliquots were taken at 1,2,3,4,5,6,7,8,12,24, and 48 hours and analyzed spectrophotometer at 215 nm. The cumulative percentage of drug release was calculated and graph plotted against time.

In vitro drug release mechanism by various kinetic models [22-27] **:**

The results of In vitro release profile obtained from all the formulations were plotted to know the mechanism of drug release. The data were treated according to zero order release, first order release, higuchi's, korsemeyer peppa's and Hixson Crowell model .

Stability study of optimized CS-NPs[28] :

The stability study was carried out for Capecitabine loaded optimized CS-NPs as per ICH guidelines. Nanoparticles of the optimized batch were placed in screw capped glass containers and stored at various ICH storage conditions.

RESULT AND DISCUSSION :

Determination of Melting point : Melting point of drug sample was determined 117-121^{0}C by digital capillary method point apparatus.

Determination of solubility : Solubility of Capecitabine in varous solvents were given in Table 2.

Determination of λmax of Capecitabine : The diluted stock solutions of Capecitabine, prepared in Milli-Q water, and phosphate buffer pH 7.4 stock solution in phosphate buffer pH 7.4, were analyzed between 200-400 nm and were measured after standing for 5 minutes at λ_{max} of each medium. λ_{max} of Capecitabine in Milli-Q water and pH 7.4 were found to be 215nm each show in Fig 1 and Fig 2.

Standard calibration curve : Determination of Standard calibration curves of Capecitabine in Milli-Q water and phosphate buffer pH 7.4 was given in Table 3 and Table 4. Standard calibration curves was plot concentration ((µg/ml) against absorbance at 215nm. (Fig 3 and Fig 4).

FTIR study : Fig 5.1 and Fig 5.2 represents the Fourier transform infra-red (FT-IR) spectrum of pure Capecitabine, excipient blend of chitosan + STPP and physical mixture of Capecitabine with the excipients, done at the beginning of the experiment and at the end of 6 months respectively. Since no significant changes were observed after comparing the FTIR spectra it could be assured that besides Capecitabine being compatible with the excipient blend both at the beginning of the experiment and after 6months, they were also stable individually.

DSC study : DSC analysis of Capecitabine with the excipient blend of chitosan and tripolyphosphate was also carried out to analyze the compatibility between Capecitabine and its excipients. It was found out from Fig 6 that Capecitabine, chitosan and TPP had retained its characteristic melting point at 123.72°C, 314.18°C and 602.56°C respectively, indicating that Capecitabine was compatible with both chitosan and sodium tripolyphosphate

Particle Size, polydispersity index (PDI) and zeta potential : The particle size, PDI and zeta potential of nanoparticle formulation (CS-NPs-8) were tabulated in Table 5. The average particle size of the nanoparticles ranged of 166.5 nm (CS-NPs-8) with a PDI of 0.346. CS-NPs-8 was selected as the optimized formulation owing to its uniform particle size and excellent PDI value. The zeta potential of CS-NPs-8 was observed to be + 37.4 mV, indicating the cationic property of Chitosan in the nanoparticles and also the stability of the suspension. The particle size distribution and zeta potential of CS-NPs-8 nanoparticle formulations were shown in Fig 7 and 8.

Percentage % yield, Entrapment Efficiency (%EE), and % drug content : The Entrapment efficiency (% EE) of each nanoparticle formulation was tabulated in Table 6. The entrapment efficiency (% EE) varied with increasing chitosan & TPP concentration. It was also observed that CS-NPs-8 had the maximum yield 78.90±1.11%, entrapment efficiency of 85.74±2.36% , and drug content 29.9±0.91%on the basis of which it was taken for further characterization. The effect of varying chitosan & TPP concentration on the Entrapment Efficiency (% EE) of nanoparticles was observed in Fig 9.

Fourier transform infra-red (FT-IR) spectroscopy : It was observed that the spectrum of Capecitabine loaded chitosan-tripolyphosphate nanoparticles (Fig 10) .The wave number range from 1500 cm-1 to

3600 cm^{-1} was different among the two spectra. The peak of 3523.5 cm^{-1}, became wider and flatter; indicating that hydrogen bonding was enhanced (Rodgers et al., 2006). The peaks of amide I and amide II in chitosan-TPP nanoparticles shifted to 1645.76cm-1 and 1557.67cm^{-1} respectively, due to electrostatic interaction between phosphoric groups of TPP and amino groups of chitosan in nanoparticles.

Differential Scanning Calorimetry (DSC) : Fig 11 represents the DSC curve of Capecitabine loaded nanoparticles (CS-NPs-8). Two endothermic peaks were observed at 59.22°C and 144.46°C. No peaks were observed near 300°C in Capecitabine loaded nanoparticles (CN-NPs-8). No polymorphism was observed due to the interaction between Capecitabine and the excipients used in the preparation. Since an endothermic peak was observed at 144.46°C, it could be concluded that Capecitabine was present in the nanoparticles in its crystalline form. The differences observed between the thermograms of pure polymers and the nanoparticles may indicate the presence of strong ionic interactions which could contribute to the formation of new structural entity with specific thermal characteristics.

Scanning Electron Microscopy (SEM) : Fig 12 and Fig 13 represents the SEM images of freeze dried Capecitabine loaded nanoparticles (CS-NPs-8) captured at different magnifications (6.00 KX and 10.00 KX). The increase in particle size after freeze drying could be attributed to agglomeration due to strong intra-molecular hydrogen bonding interactions between the nanoparticles, which became dominant over particle-particle repulsion, during freeze drying operation [18].

Transmission Electron Microscopy (TEM) : For the exploration of surface morphology, the transmission electron microscope study was performed on the prepared formulations of Capecitabine loaded nanoparticles (CS-NPs-8).This study revealed that particles were spherical in shape, smooth and had a non-porous surface as shown in Fig 14 and Fig 15. While TEM provide an actual diameter of NPs in dry state. It was also noticed that these nanoparticles had a deeper colour in core and surface indicating that these regions have higher electron density distribution. As TPP contains phosphorous element, which has a higher electron density than those elements of chitosan, so it can be inferred that chitosan has higher degree of cross-linking density with TPP in these regions. Capecitabine is assumed to be present at these deeper regions of cross-linking sites.

In Vitro drug release study : In-vitro drug release studies of capecitabine loaded nanoparticles were performed in pH 7.4 for 48 hrs in dissolution test apparatus. It was found that in vitro drug release of formulation CS-NPs-1 To CS-NPs-9 .Almost 1-10% of Capecitabine was released from the nanoparticles, during the initial burst release which lasted for a period of 1 hours. It was followed by a slower steady release rate of the drug which gradually became linear with time and commulative percentage drug release 70 % to 95% at end of 48 hour. Amongst the formulation CS-NPs-8 was found to be the best formulation as it release capecitabine 94.4% in a sustained manner with constant fashion over extended period at the end of 48 hours. It was followed by a slower steady release rate of the drug which gradually

became linear with time (Fig 16). The differences observed in the in-vitro release profile of Capecitabine in pH 7.4 was due to the property of Chitosan to swell in acidic media and its insolubility in alkaline media. This was because, with increase in concentration of the polymer, the nanosphere thickness increases as more than one chitosan molecule gets involved in the cross linking of a single particle. As a result the drug that gets embedded within the cross linked matrix of the polymer has to overcome a denser network of cross links to get dissolved in the dissolution fluid.

In vitro drug release mechanism by various kinetic models (CS-NPs-8) : Various kinetic models were employed to investigate drug release mechanism of the formulations using in vitro dissolution data. The in vitro release data were fitted to models representing Zero-order, First-order, Higuchi‘s square root of time, Korsmeyer-Peppas and Hixson Crowell model to determine the correlation coefficient, slope and intercept values (Fig 17 to Fig 21). Various parameters of the model equations were tabulated in Table 7. From the values of the correlation coefficients, the best fitted data can be predicted. The curve fitting of the release data was carried out mainly by regression analysis. In spherical matrices, if $n \leq 0.43$, a Fickian (case I), if $0.43 < n < 0.85$, a non-Fickian and if $n \geq 0.85$, a case-II (zero order) drug release mechanism dominates. The maximum correlation coefficient has been considered as statistical parameter to designate the function with the best fit to the data. The examination of correlation coefficient (R^2) values indicated that the drug release followed Korsmeyer-Peppas model, because the correlation coefficient value obtained for this model was the highest as compared to the R^2 values of zero-order, first-order and Higuchi‘s model. This means that the release of Capecitabine from the polymeric nanoparticles followed diffusion kinetics. The data are supportive to the findings that a water soluble drug incorporated in the swellable matrix device is mainly released by diffusional mechanism. To characterize the type of diffusion, log cumulative % drug release Vs. log time (Korsmeyer-Peppas model) and Wo-Wt Vs time, for different Capecitabine loaded nanoparticle formulations.

Stability Study :

The accelerated stability studies were performed according to ICH guidelines for 3 months at 25^0 C ± 2^0C (60%± 5%RH) and 40^0 C ± 2^0C (75%± 5% RH), for a period of 90 days (Fig 22). Prepared nanoparticles were observed to be stable in varying temperature. Stability study was carried out on optimized nanoparticles. which are the samples were analyzed for physical appearance and percentage drug content study at regular interval of 15 days (Table 8).

Table 1: Formulation parameters

Sr. No.	Batch No.	pH	Chitosan: TPP	Drug (mg)
1	CS-NPs-1	7.4	0.5 : 0.50	25
2	CS-NPs-2	7.4	1.0 : 0.75	25
3	CS-NPs-3	7.4	1.5 :1.00	25
4	CS-NPs-4	7.4	2.0 : 1.25	25
5	CS-NPs-5	7.4	2.5 : 1.50	25
6	CS-NPs-6	7.4	3.0 : 1.75	25
7	CS-NPs-7	7.4	3.5 : 2.0	25
8	CS-NPs-8	7.4	4.0 : 2.25	25
9	CS-NPs-9	7.4	4.5 : 2.50	25

Table 2 : solubility of capecitabine in various solvents

Sr No.	Solvent	Solubility	Terms
1	Water	25±0.19 mg/ml	sparingly soluble
2	1% v/v acetic acid	28.24±0.08 mg/ml	sparingly soluble
3	Phosphate buffer solution pH-7.4	31.21±0.11 mg/ml	sparingly soluble

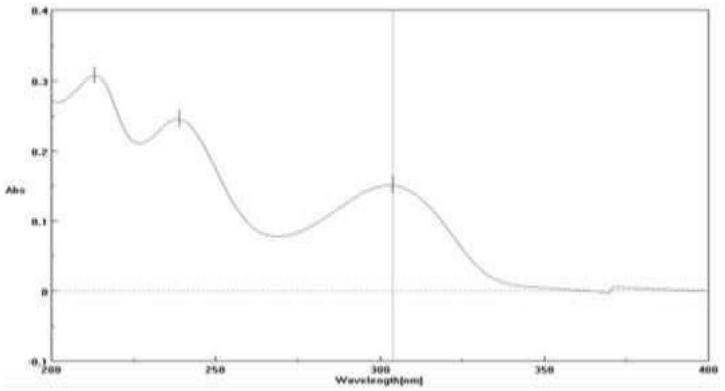

Fig 1 : Determination of λmax of Capecitabine in Milli-Q water.

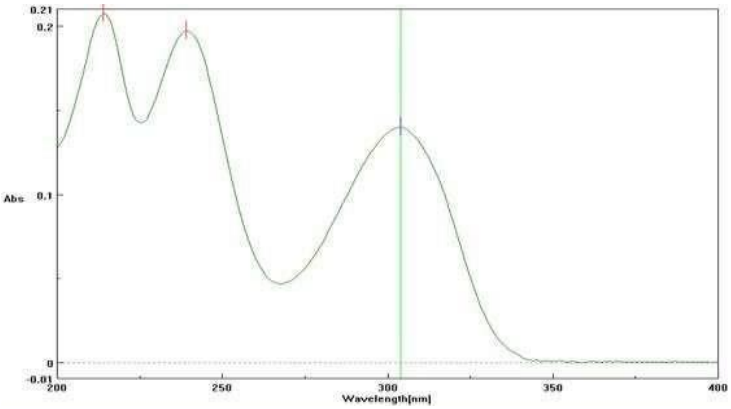

Fig 2 : Determination of λmax of capecitabine in phosphate buffer pH 7.4

Table 3 : Standard Calibration curve of capecitabine in Milli-Q water at λmax 215 nm

Sr. No.	Conc. (µg/ml)	Absorbance			Mean ± SD
		I	II	III	
1	5	0.098	0.099	0.098	0.098±0.000577
2	10	0.203	0.205	0.204	0.204±0.001
3	20	0.411	0.411	0.413	0.412±0.001155
4	30	0.709	0.703	0.71	0.707±0.003786
5	40	0.968	0.97	0.96	0.966±0.005292

Table 4 : Calibration curve of capecitabine in pH 7.4 at λmax 215 nm

Sr. No.	Conc. (µg/ml)	Absorbance			Mean ± SD
		I	II	III	
1	5	0.105	0.099	0.101	0.101±0.003055
2	10	0.221	0.224	0.223	0.222±0.002517
3	20	0.456	0.460	0.459	0.458±0.003
4	30	0.724	0.72	0.728	0.724±0.004
5	40	0.987	0.99	0.985	0.987±0.002517

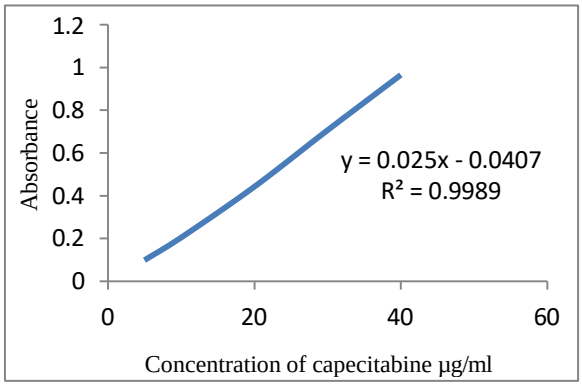

Fig 3: Standard Calibration curve of capecitabine in Milli-Q water at λmax 215 nm

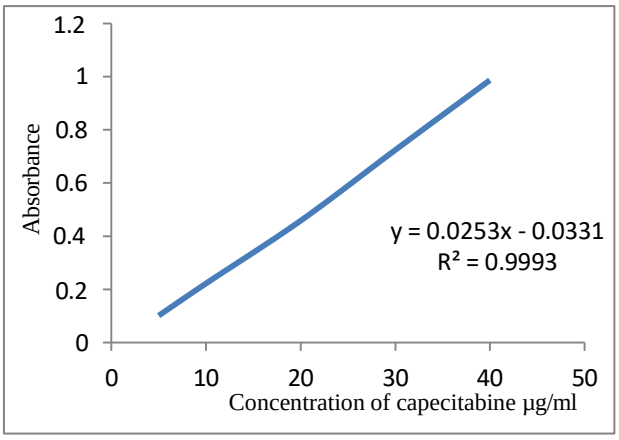

Fig 4: Standard Calibration curve of capecitabine in Phosphate Buffer pH 7.4 at λmax 215 nm

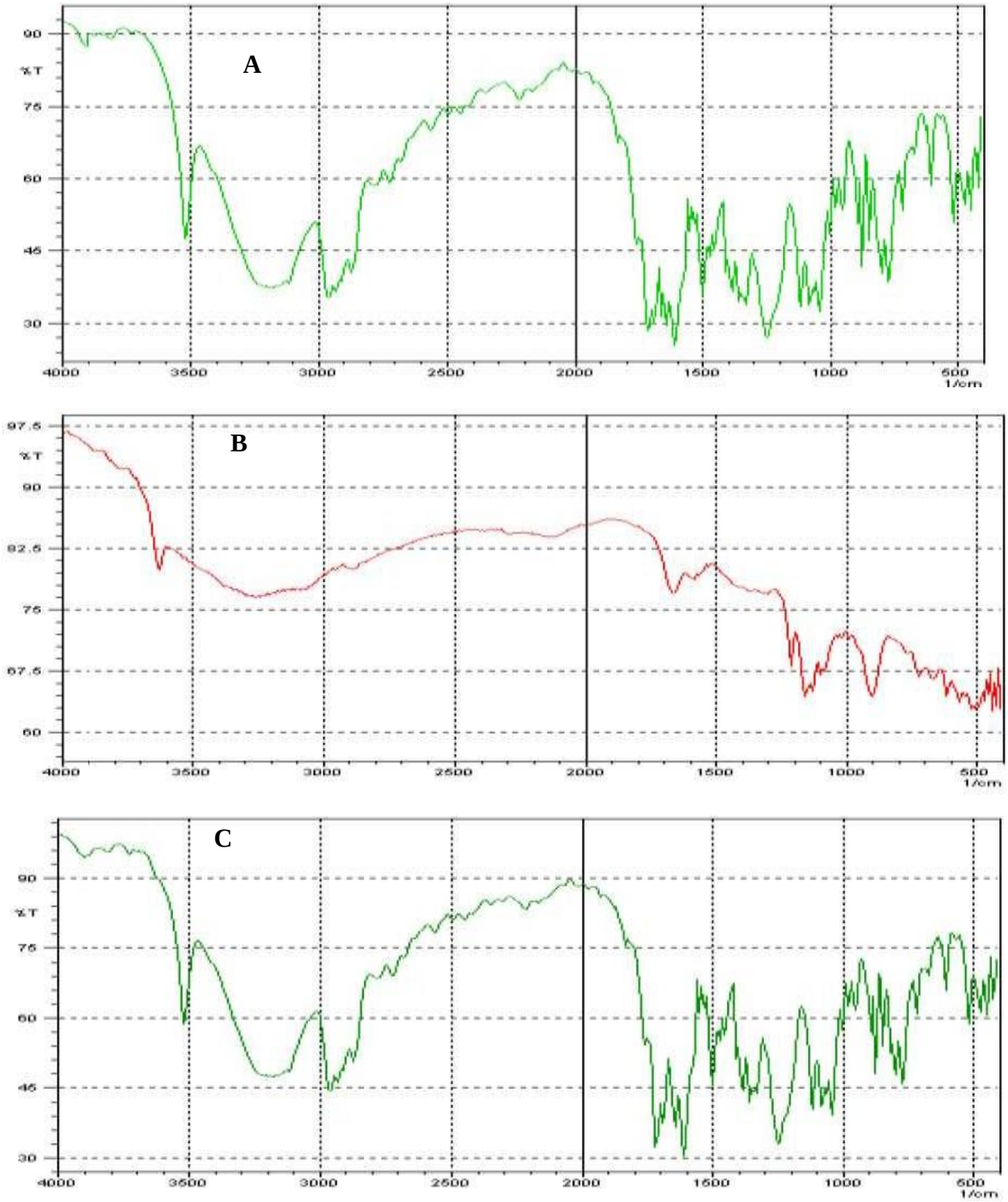

Fig 5.1 : Excipient compatibility study (I) Initial FTIR spectra study (A) Capecitabine (B) Chitosan+STPP (Excipient blend) (c) Capecitabine+excipients.

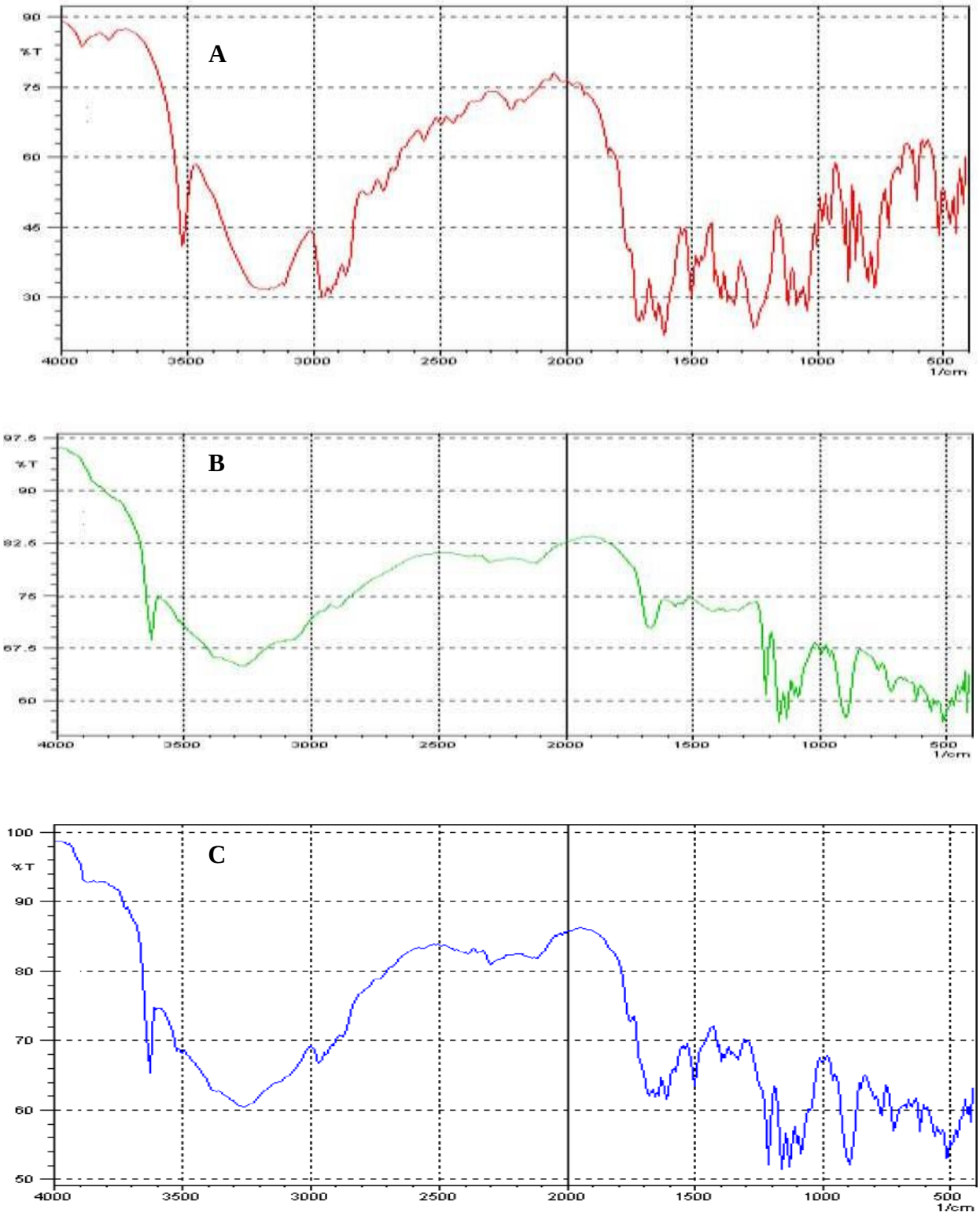

Fig 5.2 : **Excipient compatibility study (II) Final FTIR spectra study (A).Capecitabine (B) Chitosan+STPP (Excipient blend) (C) Capecitabine+excipients**

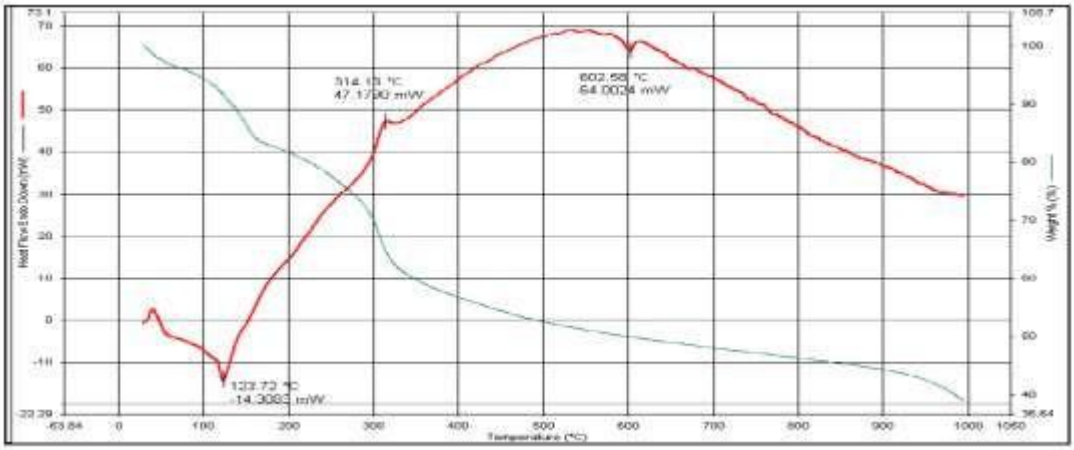

Fig 6 : DSC of Capecitabine with the excipient blend.

Table 5 : Particle size, PDI and Zeta Potential of Capecitabine nanoparticles.

Batch No.	Average particle size(nm)	PDI	Zeta Potential (mV)
CS-NPs -8	166.5	0.346	37.4

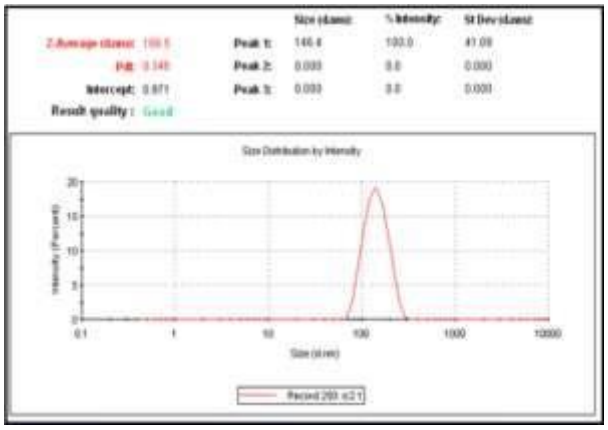

Fig 7 : .Particle size distribution of CS-NPs-8

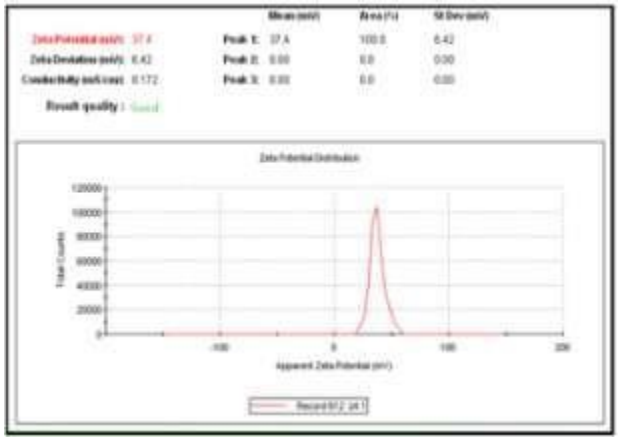

Fig 8 : .Zeta Potential of CS-NPs-8

Table 6 : % yield, % entrapment efficiency (EE), and % drug content of capecitabine CS-NPs

Batch No.	%Yield	%EE	%Drug content
CS-NPs -1	69.60±1.40	45.70±1.50	25.18±0.62
CS-NPs -2	66.38±1.21	57.94±1.25	27.2±0.76
CS-NPs -3	65.07±0.95	52.9±1.023	24.85±.54
CS-NPs -4	64.20±2.06	63.01±2.53	27.56±0.91
CS-NPs -5	75.10±1.30	80.15±1.32	28.67±1.04
CS-NPs -6	70.90±0.96	76.57±2.50	25.87±0.82
CS-NPs -7	59.17±2.50	60.39±2.21	24.66±0.85
CS-NPs -8	78.90±1.11	85.74±2.36	29.9±0.91
CS-NPs -9	73.06±0.93	81.19±3.21	23.03±0.43

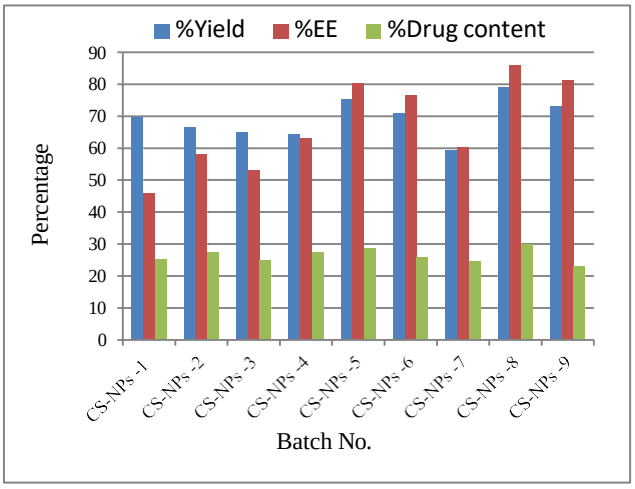

Figure 9 : Column diagram for % yield, %EE, and % Drug content in CS-NPs

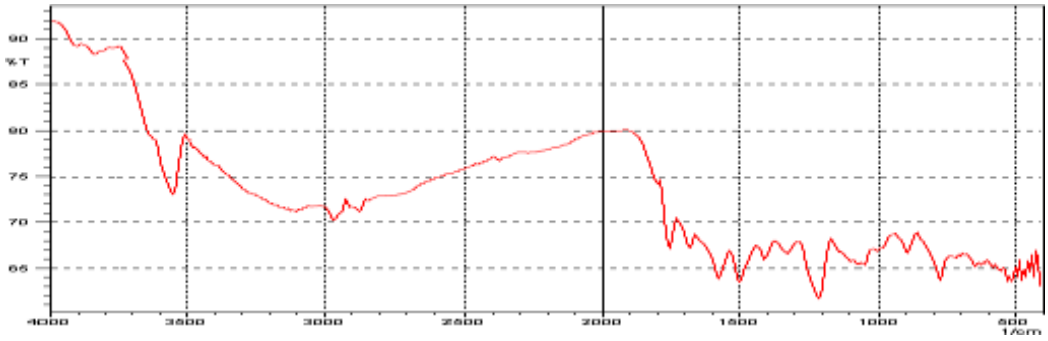

Fig 10 : FT-IR spectrum of Capecitabine loaded nanoparticles (CS-NPs-8).

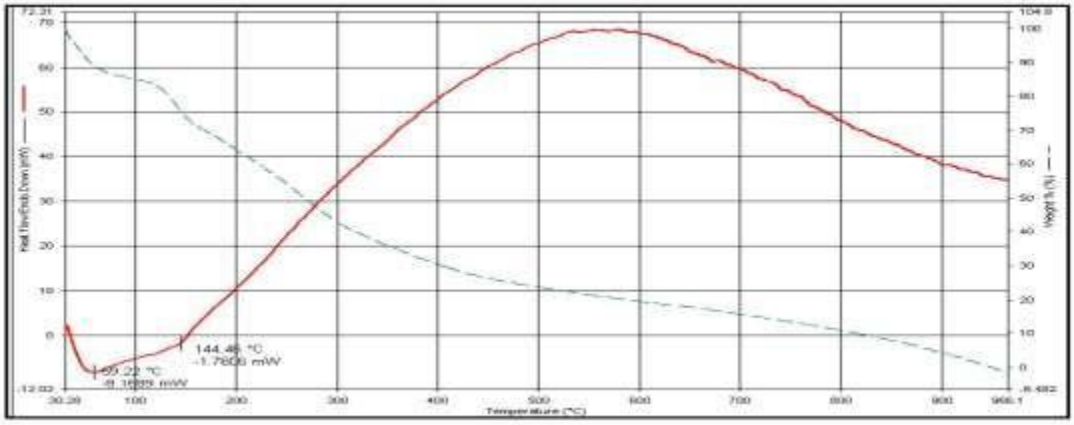

Fig 11 : DSC of Capecitabine loaded nanoparticles (CS-NPs-8).

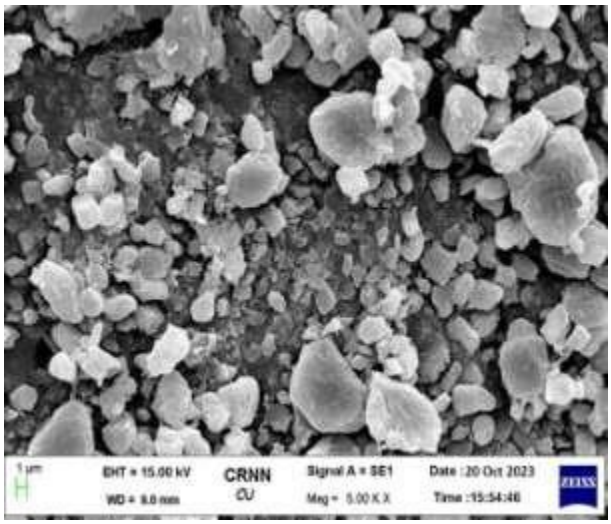

Fig 12 : SEM of Capecitabine loaded nanoparticles (CS-NPs-8).

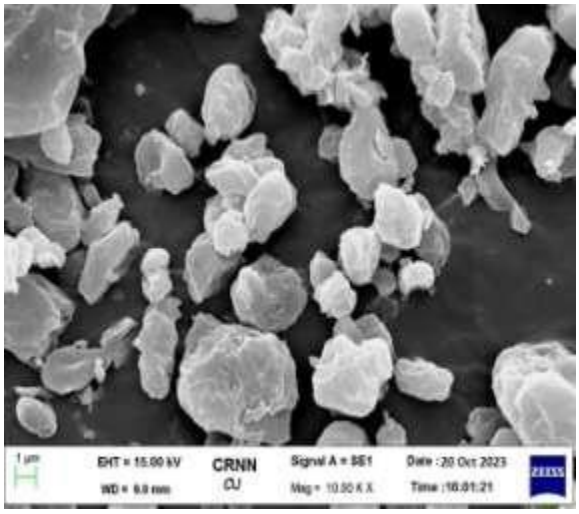

Fig 13 : SEM of Capecitabine loaded nanoparticles (CS-NPs-8).

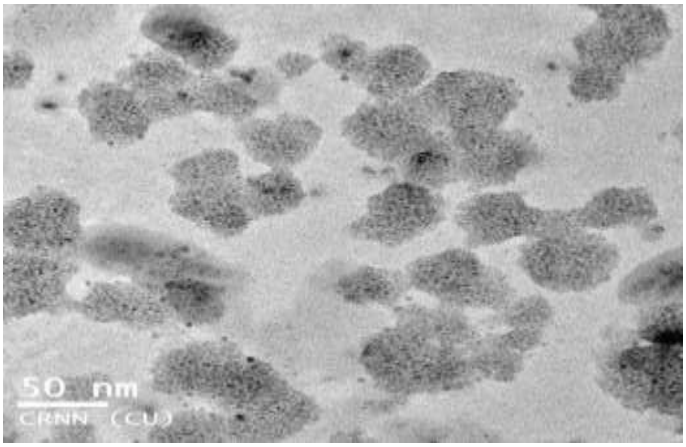

Fig 14: TEM of Capecitabine loaded nanoparticles (CS-NPs-8)

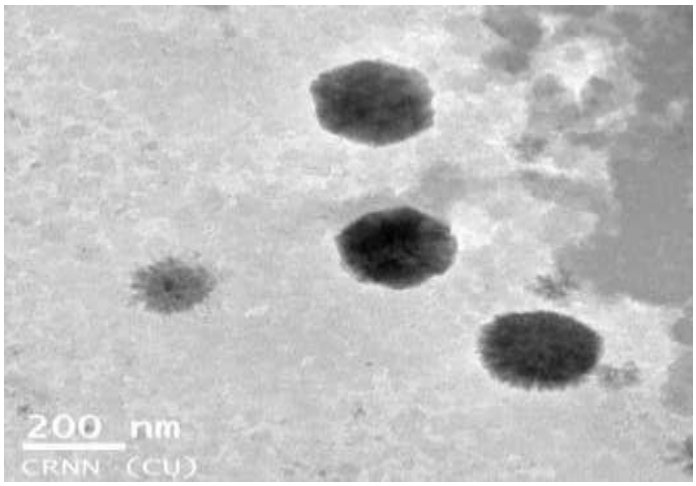

Fig 15 : TEM of Capecitabine loaded nanoparticles (CS-NPs-8)

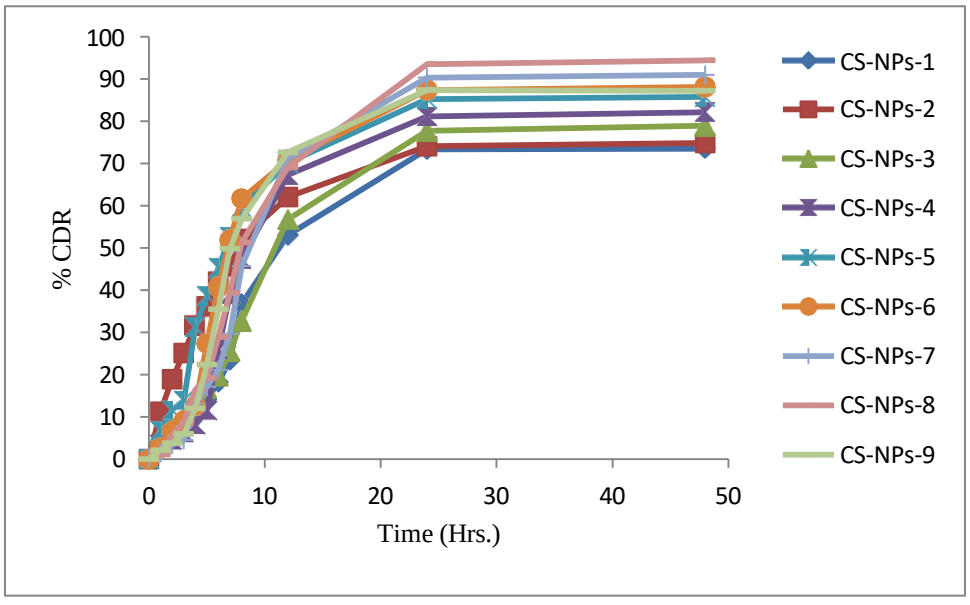

Fig 16 : % Drug release CS-NPs

Table 7: drug release kinetic model

Optimized Formulations (Batch no.)	Drug release kinetic model				
	Zero order R^2	First order R^2	Higuchi model R^2	Kors-peppas R^2	Hixson-Crowell R^2
CS-NPs-8	0.7331	0.8692	0.8743	0.8848	0.8374

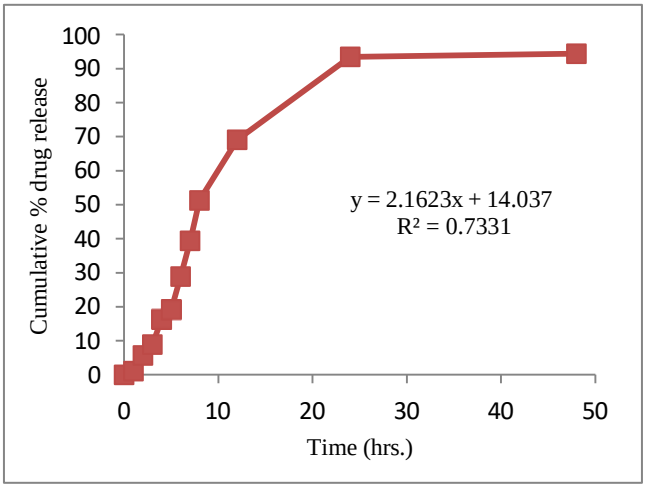

Fig 17: Zero order drug release kinetics of CS-NPs-8

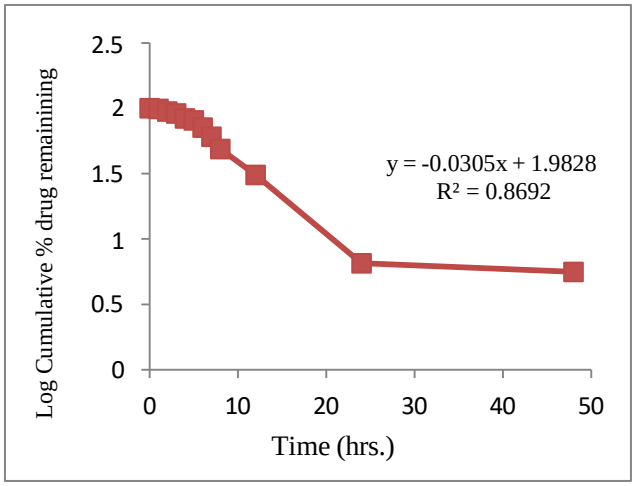

Fig 18: First order drug release kinetics of CS-NPs-8

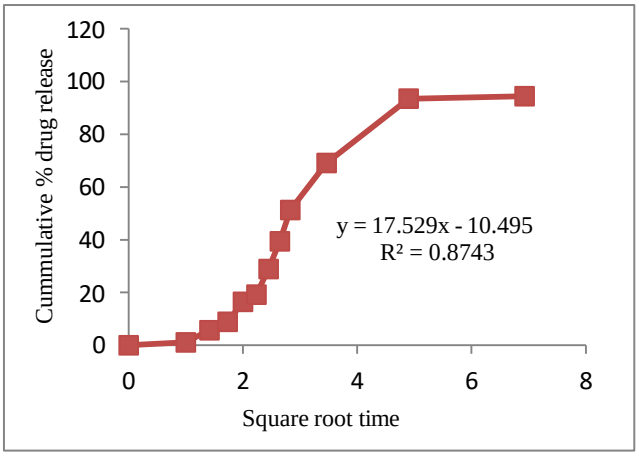

Fig 19: Higuchi order drug release kinetics of CS-NPs-8

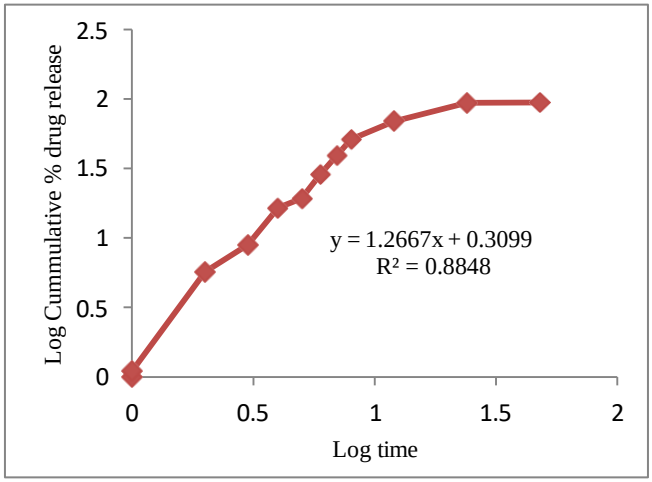

Fig 20: Kors-peppas order drug release kinetics CS-NPs-8

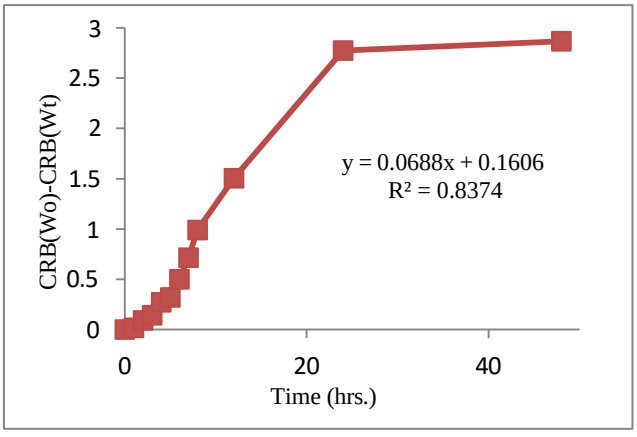

Fig 21 : : Hixson order drug release kinetics of

Table 8 : Stability studies of CS-NPs-8

Storage condition	Tested after time (days)	Physical appearance	% Drug content
25ºC ± 2ºC (60%± 5%RH)	0	No change	29.74±0.025
	15	No change	29.68±0.011
	30	No change	29.42±0.015
	45	No change	29.28±0.012
	60	No change	29.12±0.031
	75	No change	28.78±0.059
	90	No change	28.29±0.042
40ºC ± 2ºC (75%± 5%RH)	0	No change	29.74±0.025
	15	No change	29.32±0.022
	30	No change	29.09±0.061
	45	No change	28.68±0.052
	60	No change	28.32±0.042
	75	No change	28.11±0.034
	90	No change	27.79±0.037

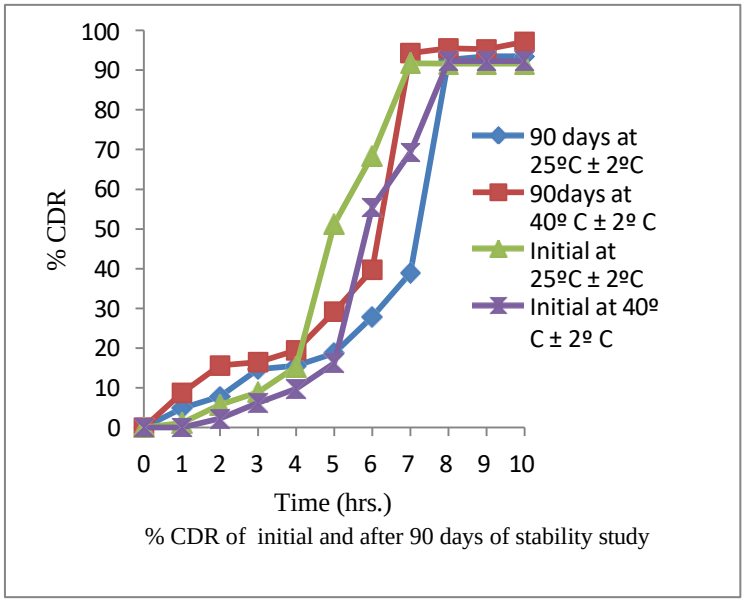

Fig 22 : Comparison of in-vitro drug release profile initial and after storage for 90 days, at 25ºC ± 2ºC (60%± 5%RH) and 40º C ± 2º C (75%± 5%RH)

Conclusion :

Sodium tripolyphosphate was used as the cross-linking agent in the ionotropic gelation process to effectively produce chitosan nanoparticles containing capecitabine. The method of preparation was found to be simple, non-toxic, organic solvent free, convenient and cheap. Capecitabine was compatible with chitosan and TPP was confirmed by FT-IR and DSC studies, performed to test the compatibility between drug and polymer. The mean particle size was observe 166.5 nm with polydispersity index was 0.307 , depending upon the concentration of the polymer. The zeta potential of the nanoparticles was 37.4mv . The Capecitabine loaded chitosan-tripolyphosphate nanoparticles had a maximum entrapment efficiency of 85.74±2. The optimized formulation was particularly CS-NPs-8, which was characterized further by FT-IR, DSC, SEM and TEM, particle size distribution and zeta potential. In vitro release study was done in simulated intestinal fluid (phosphate buffer pH 7.4). It was found that almost 55% of Capecitabine was released after 8 hours in simulated intestinal fluid (phosphate buffer pH 7.4) .Thereafter, the release became sustained upto a period of 48 hours. Kinetic modeling studies of the drug release behavior showed Fickian diffusion of the drug from the formulations. Results from the stability studies at 25ºC ± 2ºC (60%± 5%RH) and 40º C ± 2º C (75%± 5%RH) indicated good stability of the optimized formulation as there was no significant change in the observed physical parameters. Drug loading capacity was determined by UV spectrophotometer at 215nm. The results of the present study clearly confers promising potentials of chitosan nanoparticles for delivering an anti-cancer drug like Capecitabine to Colorectal cancer (disease originating from the epithelial cells) and also as a potential alternative to conventional dosage form as the sustained release will be effective for reducing the dosing frequency. As chitosan is biodegradable and biocompatible it is expected that the use of chitosan in formulation will not have any deleterious effect or toxic response in our body even if the nanoparticles are used for prolonged periods. Capecitabine is one of the choices for the treatment of colon cancer.

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