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IN-VITRO AND *IN-VIVO* EVALAUATION OF NIOSOMAL FORMULATION FOR CONTROLLED DELIVERY OF CAPECITABINE

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ABSTRACT

The present study was focused on formulating and evaluating Capecitabine containing niosomal formulation for *in vitro* and *in vivo* pharmacokinetic behavior. Niosomal formulations (drug loaded) were prepared by using different ratio of surfactant span 60, pluronic P85 and cholesterol (1:1:1, 1:2:1 and 1:3:1) by thin film hydration method. Span 60, pluronic P85 and cholesterol ratio 1: 1:1 containing niosomal formulation CF1 displayed highest entrapment efficiency with desired particle size of 240nm. SEM analyses showed that niosomal formulation was spherical in shape. Formulation CF1 displayed higher percentage of drug release after 24 h as compared to other formulations. CF1 formulation was found to be stable at the end of the study on storage condition. Various pharmacokinetic parameters, namely t max, t1/2, AUC, AUMC, and MRT of niosomal formulation, were found to be 2-fold, 2.5-fold, 1-fold, 5-fold and 2.5-fold marketed drug, respectively. The C_{max} of the marketed formulation and developed niosomes were found to be and respectively 65.66 and 68.48ng/ml, respectively. The present study suggested that niosomal formulations provide controlled and prolonged delivery of drug with enhance bioavailability.

KEYWORDS

Niosomes, Breast cancer, Capecitabine, Pluronic P85, Controlled release and Target delivery.

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INTRODUCTON

Among the cancer related disease, the most prevalent would be breast cancer and statistically it is the common cancer diagnosed in women[1,2]. The treatment includes surgery, chemotherapy, radiation, hormone targeted therapy, and combination therapies. Chemotherapy, with which both natural and synthetic drugs could be used, is considered to be the choice of therapy. The limitations with conventional chemotherapeutic

approaches are their low therapeutic index, severe side effects, poor pharmacokinetic and pharmacodynamics performance. Hence, novel therapeutics is in need to be developed so that all types of breast cancers and colon rectal cancer could be treated. Niosomes drug delivery systems (NDDS) may be the effective way of delivering the drug for breast cancer for better cure[3-6].

In the present study, a site specific delivery of the anticancer drug, Capecitabine using PLGA polymers was prepared. Capecitabine has largely replaced 5-fluorouracil in several indications, including gastric cancer. Capecitabine is an antineoplastic and antimetabolite prodrug that is enzymatically converted to 5-fluorouracil in the tumor. It inhibits DNA synthesis and slows growth of tumor tissue. Pluronic P85 is an effective coploymeric nonionic surfactant for prolonging the circulation time of hydrophobicniosomes. RES uptake of drug is reduced by Pluronic P85and such particles have a better chance for accumulation at the target site[7,8].

In most cases, capecitabine produce side effects like myocardial infarction, angina, hand-foot syndrome, diarrhea, nausea, stomatitis, anemia, thrombocytopenia, hyperbilirubinemia in conventional dosage form. This can be overcome by delivering capecitabine asniosomes. Niosomes can deliver the drug in controlled manner throughout the breast by using a much reduced dosing schedule to increase the therapeutic efficiency[9,10].

MATERIAL AND METHODS

Chemicals

Capecitabine was a gift sample from East West Pharma, Uttarakhand. Laboratories Pvt. Ltd., India. Pluronic P85 was purchased from Pratham Chemical Company, Mumbai, Cholesterol and Span60 were purchased from Loba chemicals, India. Dialysis membrane, 12,000-14,000 Da was purchased from Himedia Laboratories Pvt. Ltd., LA, Mumbai, India. All other chemicals used were of analytical grade.

Preparation of niosomes by conventional thin film hydration method

Drug-loaded niosomal formulations were prepared by thin film hydration technique by using span60, copolymeric surfactants (pluronic P85), and cholesterol at various ratios is, 1:1:1, 1:2:1 and 1:3:1 Table No.1. Accurately weighted quantities of surfactants, copoymeric surfactants and cholesterol were taken to give the desired ratio and were dissolved in 10mL chloroform in a round bottom flask and DCP was added to the above mixture. Then, accurately weighed amount of drug (capecitabine-150mg) was added to the solvent. The solvent was evaporated in a rotary flash evaporator at a temperature of 60°C at 120 rpm until a smooth, dry lipid film was obtained followed by introducing under high vacuum through vacuum pump for at least three hours for removal of residual content of chloroform. Further flask was kept in vacuum desiccators overnight for complete removal of chloroform. Then film was hydrated with 10mL of PBS pH 7.4. The obtained noisome dispersions were stored in a refrigerator at 5±3°C[10].

Characterization of niosomes

FT-IR Spectrophotometric Analysis

FTIR study was done as a part of pre-formulation study for the selection of Excipients and to check the compatibility of drug with other Excipients. The discs were scanned over a wave number range of 400 to 4000 cm^{-1} in FTIR instrument (schimadzu FTIR - 8400S, Europe)[11,12].

Differential scanning calorimetric (DSC) Analysis

Differential scanning calorimetric (DSC) analysis is a fast and reliable method to understand the polymeric transitions, to screen drug excipients compatibility and provide maximum information about possible interactions. The DSC heating curves capecitabine, cholesterol, and non-ionic surfactants and Physical mixture of capecitabine was recorded using differential scanning calorimeter (DSC Q20V24.11 Build 124, USA)[11,12].

Estimation of entrapment efficiency

Entrapment efficiency of the capecitabine niosomes derived niosomal dispersions were be done by separating the unentrapped drug by dialysis method

and the drug remained entrapped in niosomes was determined by complete vesicle disruption using 0.1% Triton X-100 and analyzed UV spectro photometrically for the drug content after suitable dilution with pH7.4 phosphate buffer and filtered through what mann filter paper. The percentage of drug encapsulation (EE (%)) was calculated by the following equation:

EE %=[(Ct-Cr/Ct)] ×100%

Where Ct is the concentration of total drug, Cr is the concentration of free drug[13].

Estimation of percentage of drug content

The percentages of drug content of the formulations were determined by lysing method, using 50% n-propanol. 1ml of the niosomal preparations were pipetted out, sufficient quantity of 50% n-propanol was added and shaken well for the complete lysis of the vesicles. After suitable dilution with the phosphate buffered saline of pH 7.4, the absorbance of the solutions were measured at 290nm in the UV-Visible Spectrophotometer using plain niosomes as a blank and the percentage of drug content was calculated. The drug content is calculated following formula, %Drug content = Sample Absorbance/ Standard Absorbance× 100[13].

Vesicle size distribution measurements and surface charge

The vesicle size and surface charge of the niosome is determined by measuring the electrophoretic mobility of the niosomes using a Malvern zeta sizer (Malvern Instruments ltd, UK) Polydispersity index was determined as measures of homogeneity[14].

Effect of Osmotic shock

The effect of osmotic shock on niosomal formulations was investigated by monitoring the change in vesicle diameter after incubation of niosomal suspension in media of different tonicity i.e., 1.6% NaCl (hypertonic), 0.9% NaCl (isotonic) and 0.5% NaCl (hypotonic). Suspensions were incubated in these media for 3 hours and the change in vesicle size was measured by optical microscopy with a calibrated eyepiece micrometer[14].

Scanning electron microscope observation of niosomes (SEM)

The scanning electron microscopy (SEM) is one of the most important instruments used for analysis of surface morphology (roundness, smoothness, and formation of aggregates). The particle size of niosomes is a very important characteristic. The surface morphology such as roundness, smoothness, and formation of aggregates and the size distribution of niosomes were studied by Scanning Electron Microscopy (Hitachi, Japan). Niosomes were sprinkled onto the double- sided tape that was affixed on aluminum stubs. The aluminum stub was placed in the vacuum chamber of a scanning electron microscope[14].

***In-Vitro* release studies**

In vitro release rate of niosomes was carried out and the drug in pH 7.4 phosphate buffer saline was used as a control. Then niosomes was placed inside the pretreated dialysis membrane (mw-12,000-14,000Da, Hi media, LA 387, Mumbai.) with an effective length of 5 cm tied at both the ends. It was then transferred to a beaker containing 100ml of phosphate buffer saline pH 7.4. The temperature was maintained at 37±0.5ºC and the medium was agitated at a speed of 50 rpm using a magnetic stirrer. 5 ml of the samples were collected at a predetermined time and replenished immediately with the same volume of fresh buffer. The sink condition was maintained throughout the experiment. The collected samples were analyzed spectrophotometrically at 240 nm using UV-Visible Spectrophotometer. The *in- vitro* release studies were also carried out for the control drug by the same method[14].

Optimized chromatographic conditions

Based on the above studies, the following chromatographic conditions were selected for the estimation of selected drug in plasma samples.

Preparation of standard and sample solutions[15]

Standard stock solution of capecitabine

100mg of capecitabine working standard was accurately weighed and transferred into a 100ml volumetric flask and dissolved in acetonitrile and made upto the volume with the same solvent to produce a 1mg/ml of capecitabine. The stock solution was stored in refrigerator at -20±2^{0}C until analysis. The stock solution was diluted to suitable concentration for spiking plasma to obtain

calibration curve (CC) standards and quality control (QC) samples.

Calibration curve standards and quality control samples of capecitabine

Working solutions for calibration and control were prepared from stock solution by an adequate dilution using water. Calibration standards for control plasma were prepared by spiking this stock solution to obtain the concentration levels of 1, 3, 7.5, 15, 30, 60, 90 and 120ng /ml in rat plasma. Quality control plasma were prepared as bulk, at a concentration 10.0ng /ml (LQC), 50ng /ml (MQC) and 500ng /ml (HQC). These samples were below -50^0C until use.

c) Standard stock solution of Gemicitabine (Internal standard)

100mg of internal standard was accurately weighed and transferred into a 100ml volumetric flask, dissolved in acetonitrile-water mixture (1:1) and made up to volume with the same solvent to produce a 1mg/ml of internal standard. The stock solution was stored in refrigerator at 20 ±2^0C until analysis. The stock solution was diluted to suitable concentration with HPLC grade water.

Plasma samples

Calibration standards, validation QC samples and plasma samples were prepared by adding 0.2ml plasma to 2ml centrifuge tube and added 50ng /ml internal standard and 0.4ml of precipitating agent (10%v/v percholic acid) vortexed for 2 min. The resulting solution was centrifuged at 4000rpm for 7 min. the supernatant layer was separated and estimated by HPLC.

Validation of HPLC methods

Validation of methods was carried out after the development of HPLC methods.

Linearity

Linearity and range of methods were analyzed by preparing calibration curves using different concentration of the standard solution containing the internal standard. The calibration curve was plotted using response factor and concentration of the standard solutions.

***In- vivo* pharmacokinetic study in animal model**[15]

Female albino Wistar rats (weighing 200-300gm) were used for the bioavailability study. Animals were housed in the standardized conditions at the animal house of the Vinayaka mission's college of pharmacy, Tamilnadu. Protocol no.P.COL/16/2018IAEC.VMCP) All animals were acclimatized and kept under constant temperature (25°C + 2°C). All animal procedures were performed in accordance to the approved protocol for use of experimental animals set by the standing committee on animal care of the Vinayaka Mission's College of Pharmacy. Eighteen female albino rats were divided into three groups, each group containing six animals. The animals were fasted overnight for 12hrs.The study was designed as a single oral dose on the study day; the first group was fed with PBS of pH orally. Group 1 control group, Group 2 treated with marketed formulation of capecitabine equivalent to 150mg/kg[16-18]. Group 3 received optimized formulation (CF1) equivalent to 150mg/kg, respectively by oral route

Blood samples (about 0.5 ml) were withdrawn from the retro orbital plexus of eye at 0.5, 1, 2, 3, 4, 6, 8, 12 and 24 after dosing, in eppendroff tubes containing 1-2 drops of sodium citrate as anticoagulant. The blood samples were centrifuged by cooling centrifuged immediately at 4000 rpm for 10 minutes to separate plasma. The plasma was transferred in to air tight containers and stored at deep freeze condition (-70^0c) until analyzed by HPLC.

Pharmacokinetic analysis

Maximum plasma drug concentration (C_{max}), time to peack (T_{max}), area under the plasma drug concentration-time profile (AUC0-t and AUC0-∞), the elimination half-life (t½) and elimination rate constant(K_{el}) were calculated using WinNonlin standard edition version 5.1 for individual drug treatments from the observed plasma concentration-time data.

The measured plasma concentration was used to calculate the area under the plasma concentration-time profile time zero to the last concentration time

point (AUC $_{(0-t)}$). It was determined by the trapezoidal method. AUC $_{(0-\infty)}$ was determined by the following equation:

$$AUC(0-\infty) = \frac{AUC(0-t) + C(t)}{kel}$$

K_{el} was estimated by fitting the logarithm of the concentration verus time to a straight line over the observed exponential decline. The Wagner-Nelson method was used to calculate the percentage of the dose absorbed.

$$F_{(t)} = C_{(t)} + k_{el}\, AUC_{(0-t)}$$

Where $F_{(t)}$ is the amount absorbed.

The percentage absorbed is determined by dividing the amount absorbed at any time by the plateau value, k_e AUC $_{0-\infty}$ and multiplying this ratio by 100

$$\%\ \text{dose absorbed} = \frac{C(t) + (kel\ AUC(0-t)}{kel\ AUC(0-\infty)} X100$$

RESULTS AND DISCUSSION

FTIR compatibility Study

The drug Capecitabine showed the sharp peak at 3519cm^{-1} N-H stretching, a board peak at 3222cm^{-1} of O-H stretching, 2931cm^{-1} C-H stretching, 1718cm^{-1} C=O stretching, 1614cm^{-1} C=C stretching and 1116cm^{-1} C-O bending vibrations and the sharp characteristics peaks. The FTIR spectra of the pure drug and other formulations indicated that no interactions between the drug and other components of the formulation. Shown in Figure No.1A and B.

Differential scanning calorimetric (DSC) Analysis

In DSC measurements, Capecitabine pure drug showed the melting endotherm at 115.67^{o}C it was compared with the thermogram obtained from the physical mixture. An endothermic peak revealed clearly that there was no interaction between the pure drug and the physical mixtures were shown in the Figure No.2A and 2B along with its corresponding melting point.

Evaluation parameters of developed niosomal formulation

Estimation of entrapment efficiency of capecitabine niosomal formulations CF1-CF3

The entrapment efficiency (%EE) of capecitabine niosomes in CF1-CF3 formulation varied between 70.90±0.80% and 90.03±0.31% (Table No.5). From the results, it was observed that entrapment efficiency of drug loaded niosomal formulation was found to be increased on equal ratio of the surfactant and co-polymeric surfactants (1:1), which has lower molecular weight and lowest PEO/PPO ratio and own longer hydrophobic PPO chain than PEO chain, increases the hydrophobicity level, thus contributing to increasing entrapment efficiency, whereas entrapment efficiency decreases on further increase in co-polymeric surfactant ratio from 1 : 2 and 1: 3 This may be due to delicate wall of the vesicles that might have undergone some leakage of the drug with time. This can explain the decrease of entrapment efficiency. The observed results were shown in Table No.2.

Estimation of percentage of drug content of formulations CF1-CF3

The percentage of drug content of Capecitabine loaded niosomes in CF1-CF8formulation was found to be in the range of 98.12±0.53% to 99.01±0.60%. The results were indicated that the uniform distribution of drug in prepared niosomal formulations. The observed results were shown in Table No.2.

Particle size and size distribution measurements of formulation CF1to CF3

The vesicle size and surface charge of the niosome was done by using a Malvern zeta sizer. The capecitabine niosomes size was varied between 240.5±2.13 nm and 290.4±3.92nm. Results shown that as the amount of copolymer increased from 1:1:1 to 1:3:1, the vesicle size also increased by the same ratio. This can be explained that at higher copolymer concentration was increased the viscosity of polymer solution, thereby producing bigger vesicle size, which were later hardened due to the evaporation of chloroform. Formulation CF1 containing equal ratio of span60 and pluronic P85

produced optimum size of noisomes (240.5±2.13 nm).

The quality and uniformity of the dispersed systems is expressed with the polydispersity index values. The values less than 0.7 are considered as suitable measurements. In the niosome formulations, PDI values ranged between 0.23±0.03 and 0.27±0.05. The low PDI values demonstrated the narrow size distribution and uniformity of the niosomal formulation. The observed results were shown in Table No.3 and Figure No.3.

Surface charge of pluronic modified capecitabine niosomes

One of the parameters for interpreting the stability of colloidal systems is their zeta potential. As the zeta potential increases, the charged particles repel one another, and this stabilizes the system against agglomeration and prevents faster settling. Systems with the zeta potential value of >-30 mV or >+30mV are considered to be stable. The zeta potential of the niosomes was changed within a range of-32.5±0.4mV and-42.50±0.5 mV in this respect all the formulations have adequate stability due to higher repulsion, high electrostatic stabilization as a result of this precipitation retarded for all the formulations. So evenly distributed niosomes were obtained all the three formulations. The observed results were shown in Table No.3 and Figure No.4.

Scanning electron microscope observation of formulation CF1

The SEM micrographs of niosomes are given in Figure No.5A and B. The SEM images confirmed the formation of niosomes. The shapes of the vesicles were spherical, and they were similar with the typical niosome micrographs obtained in prior studies. The size of the niosomes was around the average particle size (240nm) measured by Zetasizer. The particle size distribution histogram revealed the bimodal size distribution of optimized CF7 formulation.

Osmotic Shock of capecitabine niosomal formulation

The niosomal formulations CF1-CF3 were treated with hypotonic (0.5% NaCl), hypertonic (1.6% NaCl) and isotonic saline (0.9% NaCl) solutions. Increase in the vesicle size was observed in formulation incubated with hypotonic solution. In hypertonic solution, the formulation shrunk uniformly. Formulations incubated with saline showed a slight increase in vesicle size. This demonstrates that Capecitabine niosomes could be diluted with normal saline for parental use as shown in Table No.4.

***In-Vitro* release studies of niosomal formulations CF1 to CF3**

The release profile of drug from niosomes clearly indicates that the concentration of polymers slows the release of capecitabine from niosomes. At the end of 24hrs, *in vitro* drug relased from formulations CF1 to CF3 was found to be 92.11±0.57% to 99.85±1.04%.in phosphate saline pH7.4, as shown in the Figure No.7. The formulation containing both surfactant and pluronic P85 ratio of 1:1, 1:2 and 1:3 showed maximum *in vitro* release of 99.85±1.04%, 94.47±0.54 and 92.11±0.57% for 24hrs. The cumulative percentage release at the end of 24 hrs was below 100% for all the dosage forms, this may be due to the relatively slow erosion of the niosomes based on the copolymeric concentration. Among various formulations, CF1 was found to have a good release pattern and controlles release upto 24 hrs it could be suggested that the developed pluronic P85 modified niosomes could act as controlled released niosomal carrier it was selected as the optimized formulation and used for the further studies.

Cumulative Drug Release of the optimized formulation CF1 was compared with Control Formulation of Conventional tablets. It was found that more than 98% of control tablet was released within 4 hours. The formulation CF1 showed better drug release throughout the time intervals in a controlled release manner when compared with control tablet (CT).Comparative *in vitro* drug release profile is shown in Figure No.7.

Optimization of chromatographic conditions

Optimizations of the chromatographic conditions are intended to take into account the various goals of method development. The requirement of HPLC methods being used for estimation of drugs in

biological fluids. Reverse phase HPLC method was chosen for capecitabine.

From the UV spectra, the detection wavelength selected was 240nm for capecitabine. The wavelength selected gave good peak response.

Validation of HPLC methods

Estimation of the drugs in plasma samples were carried out using optimized chromatographic conditions.

Linearity

It was observed that the optimized were linear within a specific concentration and range of the drug. The calibration was plotted between the response factor and concentration of standard solution. The linearity range for capecitabine was found to 1 to 120ng /ml. The results indicate no significant variability of slopes over the optimized concentration range as shown in Figure No.8.

Estimation of capecitabine niosomes in plasma samples

The solution of calibration samples and quality control tablet and capecitabine plasma samples were injected with the optimized and validated chromatographic conditions and the chromatograms were recorded. The retention times of internal standard and the capecitabine were found to 3.02 and 7.38 min, respectively are shown in Figure No.9.

***In vivo* data analysis**

Pharmacokinetic parameters such as peak plasma concentration (C_{max}), time to peack (T_{max}), area under the plasma drug concentration-time profile (AUC0-t and AUC0-∞), AUMC0-∞, the elimination half life (t½), elimination rate constant (K_{el}) and MRT were calculated separately and the blood levels data of selected formulation was compared and are presesented in the Table No.5.

***In- vivo* pharmacokinetic study in animal model**

It was found that the release of capecitabine loaded niosomes was extended over a period of 24hrs when compared to marketed formulation 10hrs as shown in Table No.5. The C_{max} for after administration of the marketed formulation and developed niosomes were found to be and respectively 65.66 and 68.51ng/ml, respectively. The t_{max} for capecitabine after administration of the marketed formulation and developed niosomes was 4hrs and 8hrs, respectively. AUC_{0-t} for capecitabine after administration of the marketed formulation and developed niosomes were found to be 192.47 and 300.78ng/ml, respectively and ($AUC_{0-\infty}$) 200.13 and 396.59ng/ml respectively. The elimination rate constant (k_{el}) for capecitabine after administration of the marketed formulation and developed niosomes was 0.38 h^{-1} and 0.17h^{-1}, respectively. The elimination half-life ($t_{1/2}$) for capecitabine after administration of the marketed formulation and developed niosomes was 1.83 h and 4.20 h, respectively. The C_{max} of prepared capecitabine niosomes was higher than the marketed formulation. However the formulated niosomes required more time to reach t_{max} is (8hr) as compared with marketed formulation t_{max} (4hr). Value of C_{max} and t_{max} clearly indicated the drug release was extended over a period of 24hrs after oral administration in rats. The decrease in clearance establishs the efficacy of niosomes sustaining the action of the drug from the absorption site, provide therapeutic and prolonged plasma concentration of drug in the body. The smooth and extended absorption phase coupled with maintenance of plasma concentration for longer duration after administration in niosomes suggests reduced chance of dose-dependent side effects of capecitabine.

Table No.1: Composition of Capecitabine loaded niosomes

S.No	Ingredients	CF1	CF2	CF3
1	Capecitabine(mg)	150	150	150
2	Span60(mg)	50	50	50
3	Pluronic P85 (mg)	50	100	150
4	cholesterol(mg)	50	50	50
5	Dicetyl phosphate(mg)	5	5	5
6	Chloroform(ml)	10	10	10

Table No.2: % of entrapment efficiency and % of drug content of pluronic modified capecitabine loaded niosomes

S.No	Formulation code	Span 60: Coploymeric surfactant ratio	% of Entrapment efficieny*	% of Drug Content*
1	CF1	1:1	90.03±0.31	99.01±0.60
2	CF2	1:2	78.90±0.80	98.12±0.53
3	CF3	1:3	70.54 ±0.43	98.51±0.36

*Mean ± SD, (n=3)

Table No.3: Vesicle size, Zeta potential analysis of niosomal formulations CF1-CF3

S.No	Formulation code	Vesicle Size (nm)	PDI	Zeta Potential(mV)
1	CF1	240.5±2.13	0.24±0.02	-42.01±0.5
2	CF2	265.0±1.94	0.27±0.05	-39.2±0.8
3	CF3	290.4±3.92	0.23±0.03	-32.5±0.4

*Mean ± SD, (n=3)

Table No.4: Effect of osmotic shock on niosomal formulations CF1 to CF3

		Average vesicle size (nm) after incubation with			
S.No	Formulation code	PBS pH7.4	Hypertonic 0.5% Nacl	Normal 0.9%Nacl	Hypertonic 1.6%Nacl
1	CF1	209.0±1.94	213.0±0.41	210.8±1.05	Shrunk
2	CF2	240.5±2.13	243.8±0.32	241.0±1.28	Shrunk
3	CF3	290.4±3.92	293.6±1.21	291.7±1.15	Shrunk

*Mean ± SD, (n=3)

Table No.5: Pharmacokinetic parameters of the developed capecitabine loaded niosomes and marketed formulation in rats

S.No	Pharmacokinetic parameters	Marketed formulation	Developed capecitabine niosomes (CF1)
1	$C_{max\ (ng.ml^{-1})}$	65.66±4.21	68.51±9.37
2	$T_{max\ (h)}$	4.0±0.54	8.0±1.03
3	$AUC_{0\text{-}t\ (ng.h.ml^{-1})}$	192.47±24.90	300.78±25.40
4	$AUC_{0\text{-}\infty\ (ng.h.ml^{-1})}$	200.14±32.74	396.60±33.16
5	$AUMC_{0\text{-}\infty\ (ng.h.ml^{-1})}$	1173.367±86.02	5873.083±97.88
6	$K_{el\ (h^{-1})}$	0.378±0.010	0.165±0.047
7	$T_{1/2\ (h)}$	1.83±0.61	4.20±1.25
8	MRT(h)	5.87±0.57	14.80±0.31

The data were reported as an average of 6 measurements (mean ± S.D.)

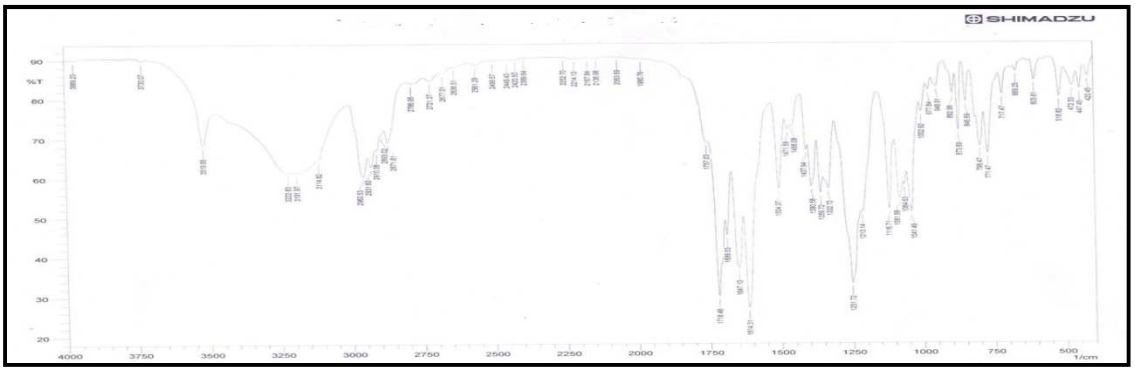

Figure No.1A: FTIR graph of pure capecitabine drug

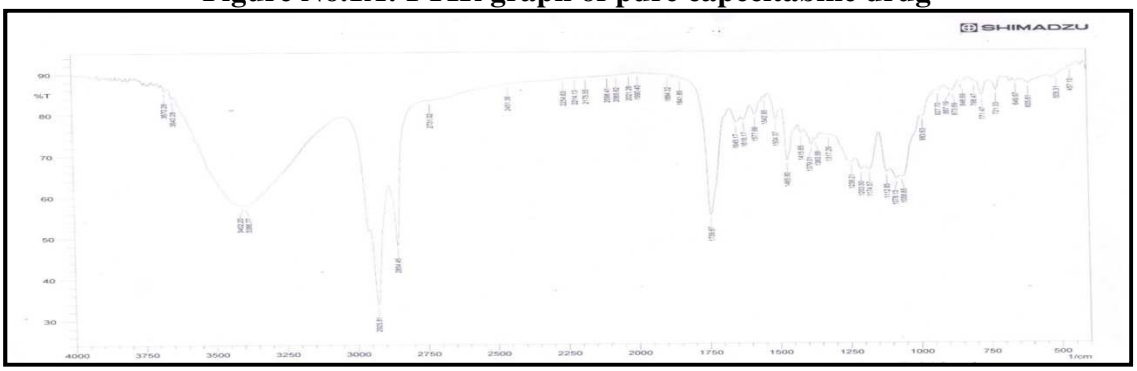

Figure No.1B: FTIR graph for optimized Capecitabine niosomal formulation CF1

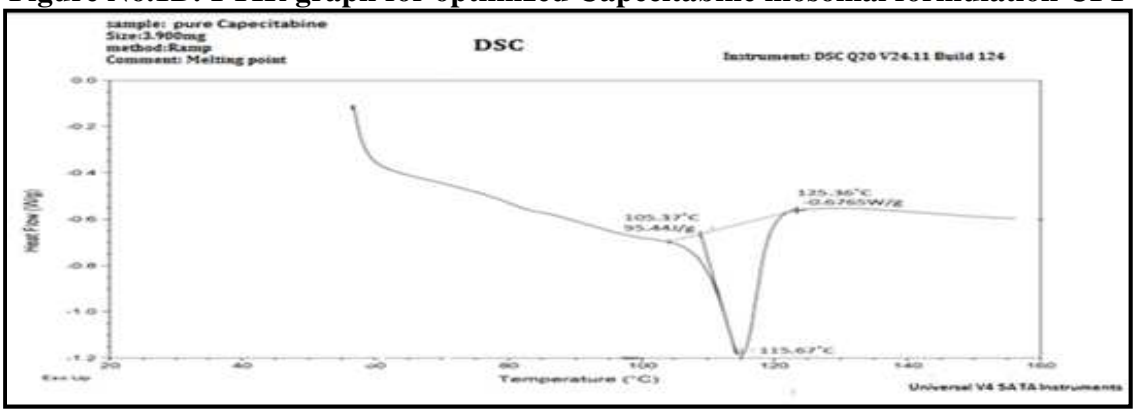

Figure No.2A: DSC thermogram of pure Capecitabine drug

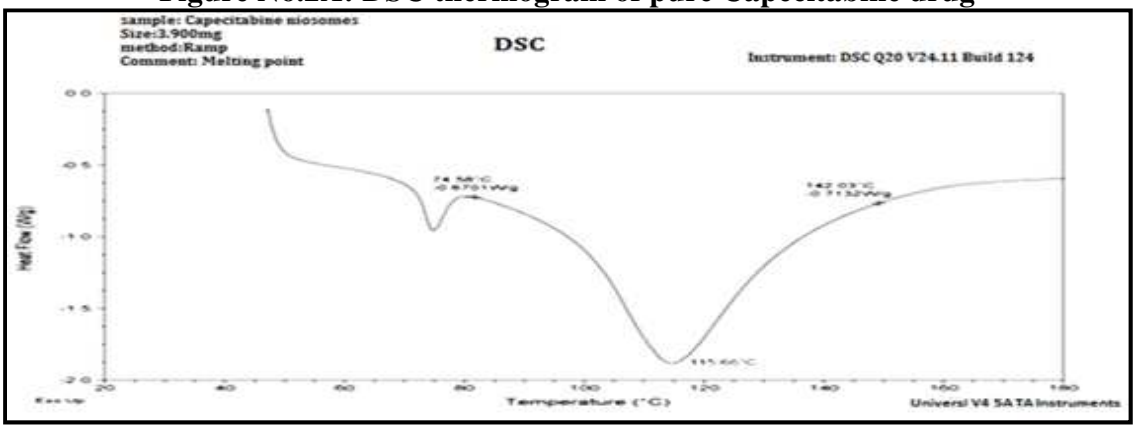

Figure No.2B: DSC thermogram of optimized Capecitabine niosomal formulation (CF1)

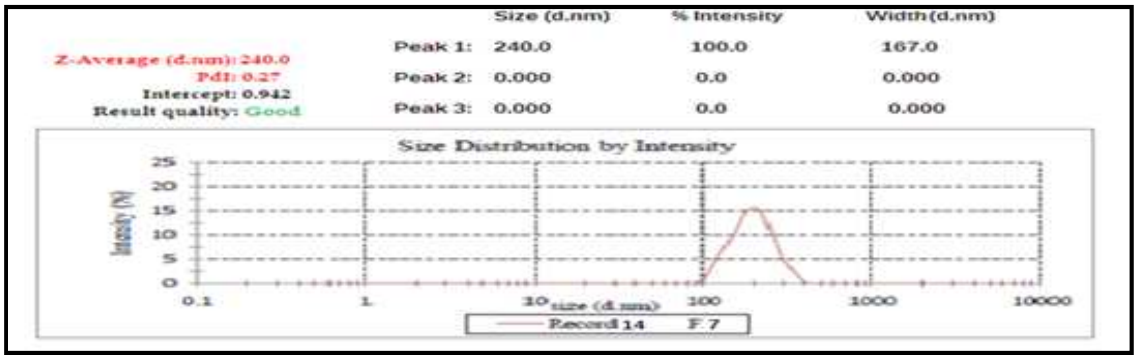

Figure No.3: Size distribution by intensity of optimized Capecitabine niosomal formulation (CF1)

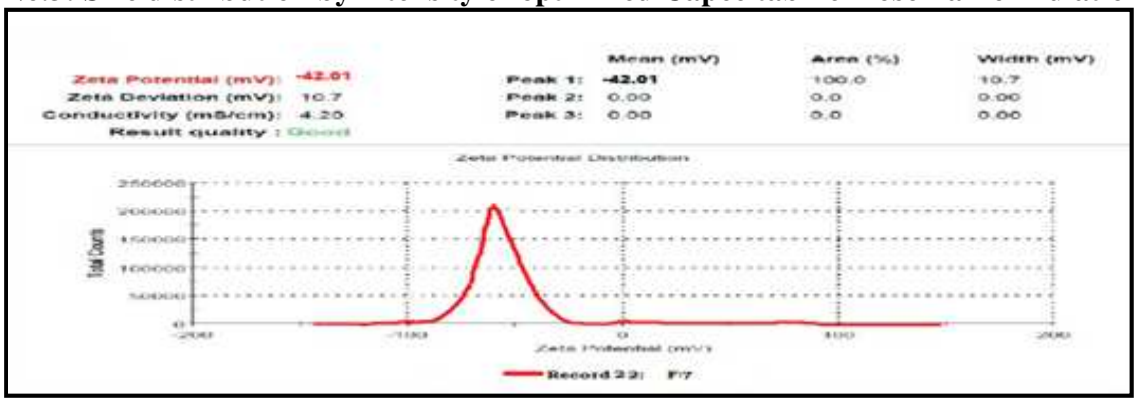

Figure No.4: Zeta potential of optimized Capecitabine niosomal formulation (CF1)

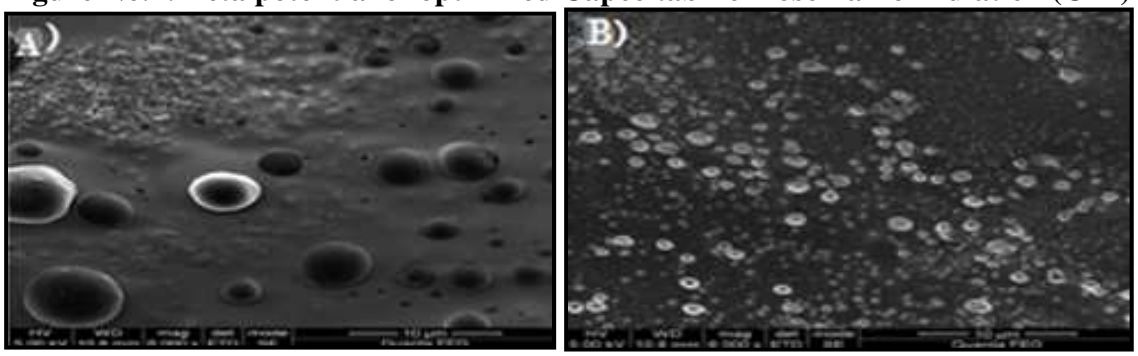

Figure No.5A and B: Scanning electron microscopic image of optimized niosomal formulation CF1 (500X magnification) and (200X magnification)

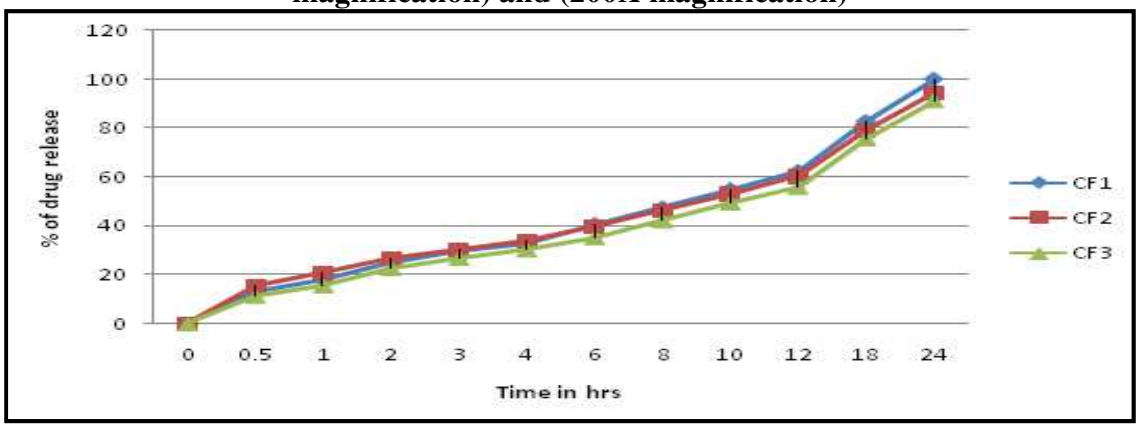

Figure No.6: Percentage of *in-vitro* drug release profile of formulations CF1 to CF3

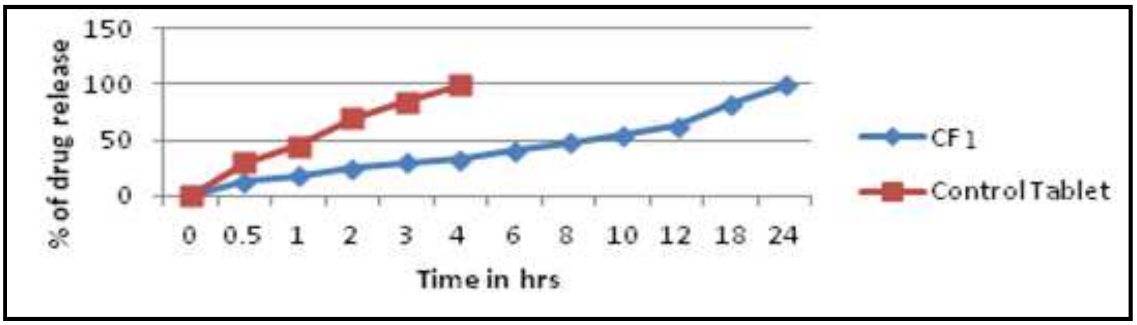

Figure No.7: Comparison of optimized formulation CF1 with Control tablet

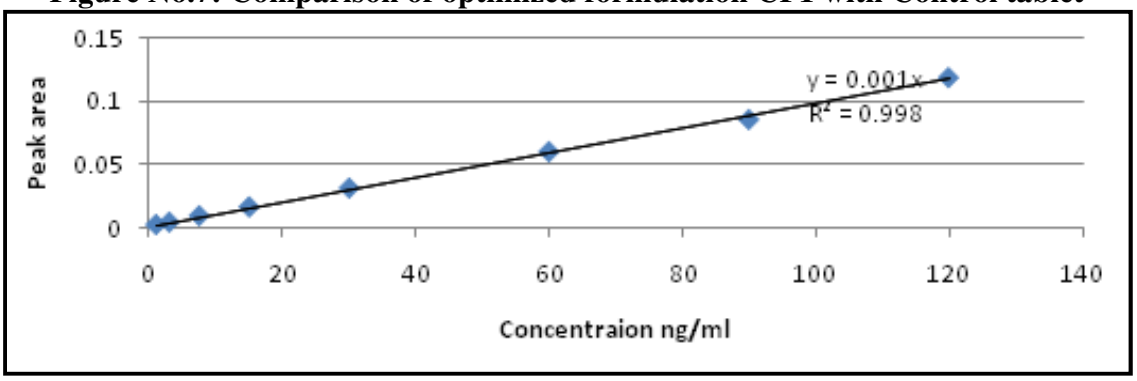

Figure No.8: HPLC Calibration curves of Capecitabine

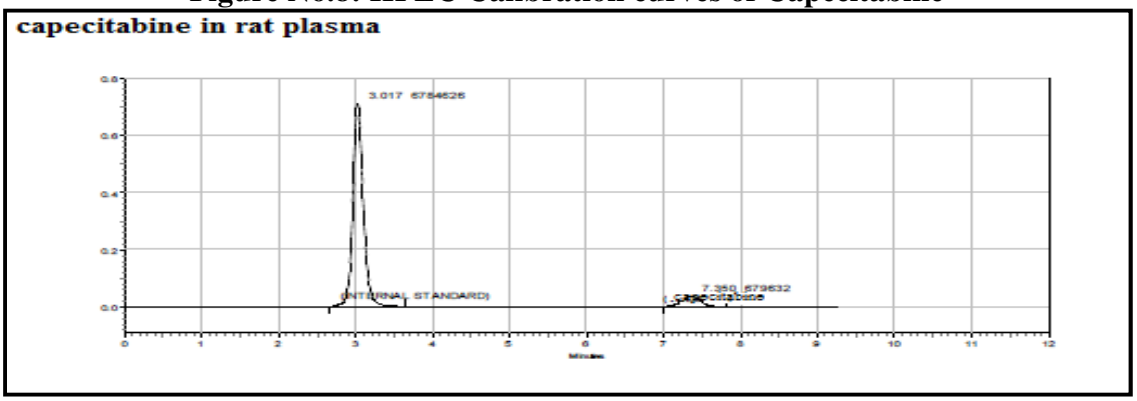

Figure No.9: Typical chromatogram of rat plasma of capecitabine niosomes

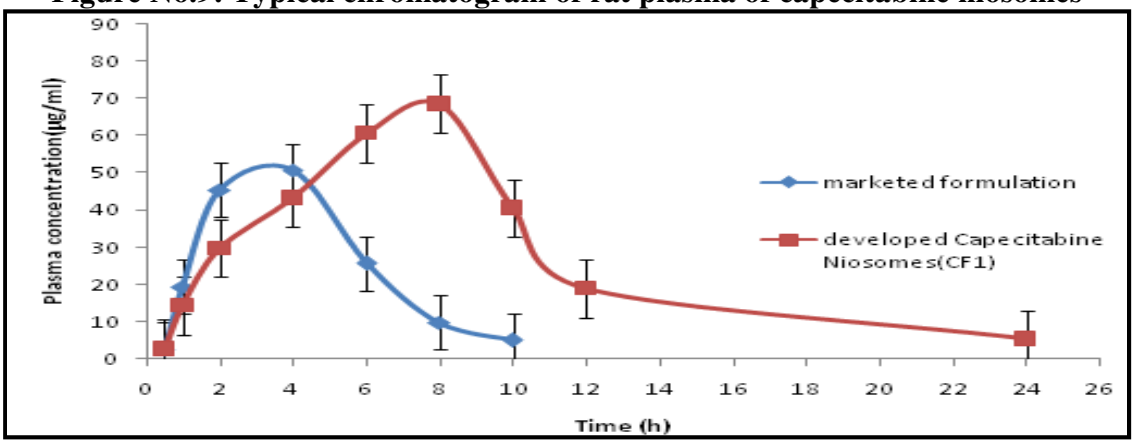

Figure No.10: Plasma concentration-time profile of developed capecitabine loaded niosomes (CF1) release and marketed formulation

CONCLUSION

In this study, Capecitabine loaded niosomes were successfully prepared using different combination of nonionic surfactant, copolymeric surfactant and cholesterol. The physicochemical properties and in-vitro release of capecitabine from niosomes were affected by the span 60, pluronic P85 and cholesterol molar ratio. The developed niosomes improved the pharmacokinetic parameters of capecitabine and boosted its bioavailability through prolonging its duration of action inside the body as well as decreasing its elimination rate constant compared to the marketed formulation showing the potential of niosomes as promising nanoparticulate carriers for cancer target delivery of capecitabine.

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CONFLICT OF INTEREST

The author declares that there is no conflict of interests regarding the publication of this paper.

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