IJPSR (2013), Vol. 4, Issue 4 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 30 December, 2012; received in revised form, 07 February, 2013; accepted, 25 March, 2013

OPTIMIZATION OF THE FORMULATION AND *IN-VITRO* EVALUATION OF CAPECITABINE NIOSOMES FOR THE TREATMENT OF COLON CANCER

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Keywords:
Niosomes, Capecitabine, Non-Ionic Surfactants, Colon targeting

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ABSTRACT: The goal of the present study was to investigate the feasibility of using non-ionic surfactant vesicles as carriers for the sustained delivery of water soluble anti-cancer drug Capecitabine, used in the treatment of colorectal cancer. The niosomal formulations were prepared using various non-ionic surfactants (span 40, span 60, tween 40 and tween 60) in the presence of cholesterol, by thin film hydration technique. The effect of process related variables like hydration time, sonication time, rotation speed of evaporation flask on the entrapment efficiency and *in vitro* drug release were evaluated. Formulation of Capecitabine niosomes was optimized by altering the proportions of Tween, Span and cholesterol. The formation, morphology and size of the drug loaded niosomes were determined by optical microscopy, transmission electron microscopy and particle size analyzer respectively. Results showed a substantial change in the release rate and in the % Entrapment of Capecitabine from niosomal formulations upon varying the type of surfactant and cholesterol content. *In-vitro* drug release results confirmed that the niosomal formulations have exhibited a higher retention of Capecitabine inside the vesicles such that the *in-vitro release* was slower compared to the drug solution. Highest drug entrapment (59.1±0.72%) and sustained release (67.95±0.65%) was obtained with vesicles formed using tween 60 and cholesterol in 4:1 ratio. The optimized niosomal formulation was subjected to stability studies at 4±2°C and 27±2°C for a period of three months.

INTRODUCTION: Capecitabine is an anticancer drug which is widely used in the treatment of colorectal cancer and breast cancer.

QUICK RESPONSE CODE

IJPSR:
ICV (2011)- 5.07

Article can be accessed online on:
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Colorectal cancer, commonly known as bowel cancer, is a cancer from uncontrolled cell growth in the colon, rectum or appendix. According to the American Cancer Society, colorectal cancer is one of the leading causes of cancer related deaths in the United States and is the fourth most common cancer worldwide.[1] Fluoropyrimidines like Capecitabine remain the cornerstone of chemotherapy regimens in the treatment of metastatic colorectal cancer (mCRC), even with the availability of newer cytotoxic and targeted biologic agents [2].

The half-life of Capecitabine is 45 minutes which results in frequent administration of the dosage form and increased side effects. To overcome these problems and to improve the patient compliance, a prolonged release and a site specific formulation is desirable. Niosomes are one of the drug carriers made from inexpensive and easily available food additives of non-ionic surfactants. These are bilayered structures, which can entrap both hydrophilic and lipophilic drugs. [3] Niosomes act as a controlled release formulation, which establishes and maintains the drug concentration at the target site for longer intervals of time.

MATERIALS AND METHODS: Capecitabine was obtained as a gift sample from Burgeon Pharmaceuticals. Span 40, Span 60, Tween 40 and Tween 60 were purchased from SD fine chemicals, Mumbai. Cholesterol was procured from Himedia, Mumbai. Chloroform was bought from Rankem laboratories. All other chemicals used were of Analytical grade.

Experimental Procedure:

1. **Drug Excipient Compatibility Studies** [4]: The possibilities of drug-excipient (cholesterol, nonionic surfactant) interactions were further investigated by XRD analysis and FT-IR spectrum study. The X-ray diffraction pattern (XRD) of Capecitabine, cholesterol and the formulations with different non-ionic surfactants were recorded using PANalytical Xpert pro X-ray diffractometer with Ni filtered Cu K_α radiation over the 2θ range of 10-90 and the peaks were indexed. The FT-IR spectrum of pure drug and combination of drug with different non-ionic surfactants were recorded by using a Perkin Elmer (Jasco V650, USA) FTIR spectrophotometer. The spectrum was recorded in the wavelength region of 4000 to 400cm^{-1} and the resolution was 4 cm^{-1}.

2. **Formulation of Capecitabine Niosomes** [5]**:** The niosomal formulations were prepared by thin film hydration technique. Accurately weighed quantities of drug, non-ionic surfactant (Tween or Span) and cholesterol were dissolved in 10ml of solvent mixture (Chloroform: Methanol 2:1 ratio) in a round bottom flask. The thin films formed under reduced pressure in a rotary flash evaporator were hydrated with 10ml of phosphate buffered saline pH 7.4 and the flask was kept rotating at 60ºC at various revolutions per minute (rpms). The empty niosomes were also prepared by the same method but without the drug.

3. **Optimization of Process-Related Variables:**

a. **Effect of Sonication Time** [6]: The niosomal formulations containing tween 60 at different ratios and a fixed amount of cholesterol (1:1, 2:1, 3:1, 4:1, 5:1) were subjected to ultrasonic vibration using Vibronic's Ultrasonicator. To study the effect of sonication time, the formulations were subjected to sonication for various time intervals (1min, 2mins, 3mins, 4mins and 5mins). The entrapment efficiency of the formulations was measured.

b. **Effect of Hydration Time** [6]: The niosomal formulations containing tween 60 at different ratios and a fixed amount of cholesterol (1:1, 2:1, 3:1, 4:1, 5:1) were hydrated with 10 ml of phosphate buffered saline pH 7.4 for 30 minutes, 45minutes, 60minutes, 75minutes and 90minutes. The entrapment efficiency of the formulations was calculated by ultracentrifugation method.

c. **Effect of Rotational Speed of Evaporator Flask** [6]**:** The thickness and uniformity of the film depends upon the rotational speed of the evaporator flask. The niosomal formulations were subjected to various speeds ie. 50 rpm, 100 rpm, and 150 rpm of evaporator flask. The appearances of the formulations were checked by visual observation. The entrapment efficiency of the formulations was calculated by ultracentrifugation method.

d. **Effect of Osmotic Shock** [6]**:** 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.

4. **Drug Content Analysis** [7]: The amount of drug in the formulation was determined by lysing the niosomes 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 240nm in the UV-Visible Spectrophotometer (Jasco V650, USA) using empty niosomes as a blank and the drug content was calculated.

5. **Estimation of Entrapment Efficiency** [7]: The entrapment efficiency of the formulations were determined by centrifuging 1 ml of the suspension at 14,000 rpm for 60 minutes maintaining a temperature of 4ºC, using a refrigerated centrifuge in order to separate niosomes from unentrapped drug. The free drug concentration in the supernatant was determined at 240nm using UV-Visible Spectrophotometer (Jasco V650, USA). The percentage of drug entrapment in niosomes was calculated by using the following formula,

$$\% \text{ Drug entrapment} = \frac{(\text{Total drug} - \text{Drug in supernatant liquid})}{\text{Total drug}} \times 100$$

6. ***In-vitro* Release Study** [8,9]: *In-vitro* drug release pattern of niosomal suspension is carried out by dialysis bag (Himedia dialysis membrane of molecular weight cut off 12,000-14,000). The niosomal preparation was placed in a dialysis bag with an effective length of 5 cm which acts as a donor compartment. Dialysis bag was placed in a beaker containing 100 ml of phosphate buffered saline of pH 7.4, which acts as a receptor compartment. The temperature of the receptor medium was maintained at 37±1ºC 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 pH 7.4. The sink condition was maintained throughout the experiment. The collected samples were analyzed spectrophotometrically at 240 nm using UV-Visible Spectrophotometer (Jasco V650, USA). Each study was performed in triplicate. The *in vitro* release studies were also carried out for the pure drug by the same method.

7. **Optimization of the Formulation** [6]: The molar ratio of non-ionic surfactant to cholesterol and other process related variables were optimized based on entrapment efficiency and drug content. Niosomal formulations using various surfactants (Tween 40, Span 40 and Span 60) were also prepared in the thin film hydration technique. The best formulations were chosen among them.

8. **Kinetics of Drug Release** [10, 11]**:** To study the kinetics and mechanism of drug release, the release data of the *in-vitro* dissolution study of niosomes were fitted in various kinetic models (Capecitabine encapsulated with cholesterol and four different non-ionic surfactants), such as Zero order kinetic (equation 2), First order kinetic (equation 3) and Higuchi kinetic (equation 4)

$$Q_t = K_0 t \quad \ldots\ldots(2)$$

$$\ln (Q_0 - Q_t) = \ln Q_0 - K_1 t \quad \ldots\ldots(3)$$

$$Q_t = K_h t^{1/2} \quad \ldots\ldots(4)$$

$$Q_0^{1/3} - Q_t^{1/3} = K_{HC} \text{ X } t \quad \ldots\ldots(5)$$

Wherever Q_t is the percentage of drug released at time t, Q_0 is the initial amount of drug present in the formulation and K_0, K_1 and K_h are the constants of the equations. The following plots are made; Q_t vs. t (Zero order kinetic model), ln (Q_0-Q_t) vs. t (first order kinetic model) and Q_t vs. $t^{1/2}$ (Higuchi kinetic) respectively from equation 2, equation 3 and equation 4. Further to confirm the mechanism of drug release, data is fitted with korsmeyer-peppas equation,

$$M_t/M_\alpha = K_p t^n \quad \ldots\ldots(6)$$

Where M_t/M_α is the fraction of the drug release at time t, K_p is the rate constant and "n" is the release exponent. The value of "n" is calculated from the korsmeyer-peppas equation. It is used to interpret different mechanisms of drug release. Hixson Crowell cube root kinetics (equation 5) used to understand the progressive dissolution of matrix as a function of time.

9. **Photomicroscopy and Transmission Electron Microscopy** [12]: Vesicle dispersions were characterized by photo and transmission electron microscopy for vesicle formation, vesicle size and morphology. Small amounts of the formed niosomes were spread on a glass slide and examined for the vesicles structure using (Leica light microscope) with varied magnification powers (10X and 40X) and the photomicrographs were taken. Samples were placed onto a carbon coated grid and they were negatively stained with 1% phosphotungstic acid. They were dried at room temperature and then examined by Transmission Electron Microscopy (FEI Tecnai Spirit G^2 transmission electron microscope operating at 120 kV).

10. **Particle Size Analysis** [13]: The particle size determination of the niosomal formulations was carried out using laser diffraction (Malvern Particle size Analyzer) and the mean of vesicular diameter was calculated.

11. **Stability Studies** [14]: Stability studies were carried out to investigate the leaching of drug from niosomes during storage. The ability of vesicles to retain the drug was assessed by keeping the selected niosomal suspension in sealed glass ampoules at 4 ± 2^0C, 25 ± 2^0C for 3 months. Samples were withdrawn periodically and analyzed for aggregation, drug entrapment and residual drug content.

RESULTS AND DISCUSSION

XRD Analysis: From the XRD analysis, the drug and the formulation with different non-ionic surfactants were performed and clearly shown in **Figure 1(A)**. Capecitabine had shown the characteristic peaks at 2θ of 20.56º, 25.82º, 28.63º, 36.34º and 40.35º because of its crystallinity. In the figure: 1 (B) cholesterol had shown characteristic peaks at 2θ of 10.59º, 12.91º, 14.41º, 15.72º, 17.38º, 18.39º, 21.86º and 42.60º because of its crystallinity. However, those peaks were not found in the formulations (C) (D) (E) (F) with different non ionic surfactants used in the niosomal formulations.

For the most part, XRD peaks depend only on the crystal size. Wherein, the present studies, formulations with different non-ionic surfactants, the characteristic peak of Capecitabine are encapsulated by the noise of layered cholesterol and different non-ionic surfactants. From this, it is distinguish that the XRD signals of encapsulated drug Capecitabine is very complicated to notice, which shows the drug is dispersed at a molecular level in the cholesterol and with different non-ionic surfactants. As a result, no crystals are found individually in the formulations, as drug encapsulated by the cholesterol and with different non-ionic surfactants as shown in Figure 1.

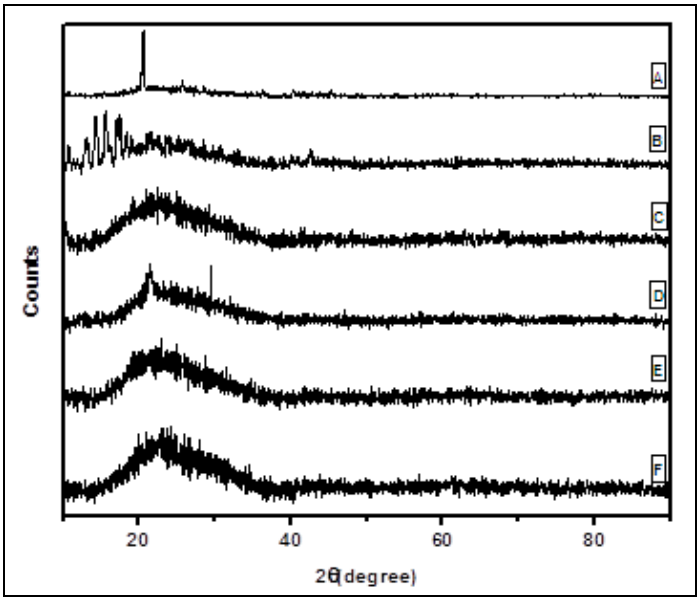


Figure 1: show A) XRD of pure drug Capecitabine B) XRD of Cholesterol C) XRD of Capecitabine + Span40 + Cholesterol D) XRD of Capecitabine + Span60 + Cholesterol E) XRD of Capecitabine + Tween40 + Cholesterol F) XRD of Capecitabine + Tween60 + Cholesterol

FT-IR Analysis: From the FTIR graph, the individual pure drug Capecitabine and Cholesterol shows their intensity peaks. Whereas, the characteristics peaks of Capecitabine shows the sharp peak at 3523cm^{-1} N-H stretching, a board peak at 3242cm^{-1} of O-H stretching, 2926cm^{-1} C-H stretching, 1775cm^{-1} C=O stretching, 1606cm^{-1} C=C stretching and 1115cm^{-1}C-O bending vibrations. The cholesterol shows the wave number 3392cm^{-1} O-H stretching, 2931cm^{-1} C-H stretching, 1458cm^{-1} C=C stretching, and 1070cm^{-1} C-O bending vibrations.

For the formulation drug encapsulated with span40 and cholesterol the peaks produce at the wave number 3397 cm^{-1} O-H stretching, 2932cm^{-1} C-H stretching, 1731cm^{-1} C=O stretching, 1463cm^{-1} C=C stretching and 1063cm^{-1} C=O bending vibrations. The formulation drug encapsulated with span60 and cholesterol show the peaks at the wave number 3385cm^{-1} O-H stretching, 2857cm^{-1} C-H stretching and 1730cm^{-1} C=O stretching vibrations.

Moreover the prepared formulations with other two different non-ionic surfactants Tween40 shows characteristics peaks at 3432cm^{-1} O-H stretching, 2932cm^{-1} C-H stretching, 1729cm^{-1} C=O stretching and 1104cm^{-1} C-O bending vibrations. The formulation drug with Tween60 show a board peak around 3426cm^{-1} O-H stretching, 2926cm^{-1} C-H stretching, 1649cm^{-1} C=O Stretching and 1109cm^{-1} C-O bending vibrations as shown in the Figure: 2.

Hence, the FTIR spectra of Capecitabine, cholesterol, non ionic surfactants and the niosomal formulation showed corresponding peaks which indicate that there is no interaction between the drug and the excipient.

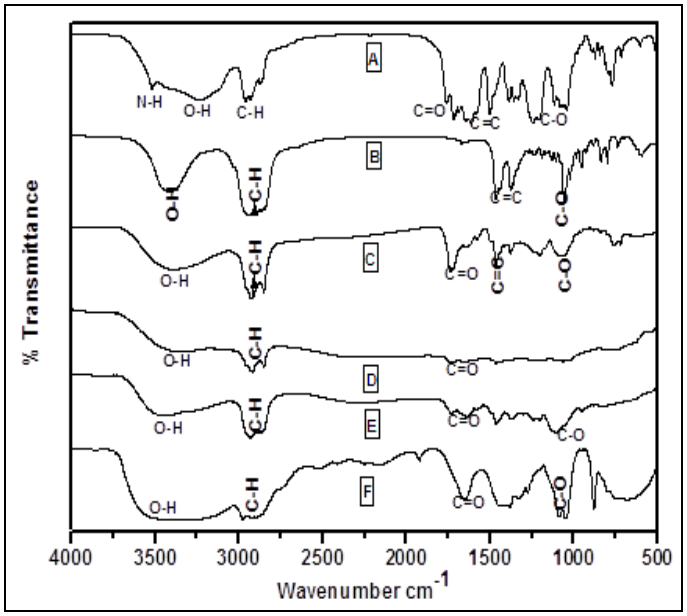


Figure 2: show A) FTIR graph of pure drug Capecitabine B) FTIR graph of Cholesterol C) Capecitabine + Span40 + Cholesterol D) FTIR graph of Capecitabine + Span60 + Cholesterol E) FTIR graph of Capecitabine+ Tween40 + Cholesterol F) FTIR graph of Capecitabine + Tween60 + Cholesterol.

Preformulation Studies: Pre-formulation studies were carried out with non ionic surfactant Tween 60 at 25, 50, 75, 100 and 125 µM with a fixed amount of Capecitabine (10 mg) and 25µM cholesterol. At concentrations 25, 50 and 75 µM, vesicles were not formed properly and they were not stable. At concentrations 100 and 125 µM of Tween 60, Spherical vesicles were formed. At 125 µM concentration, the vesicles were seen aggregated. So the formation of Niosomes using 100 µM of non-ionic surfactant was found to be superior to other molar ratios. The process related variables such as Sonication time, hydration time and speed of rotation of flask evaporator were investigated.

Optimization of Process Related Variables:

A) Effect of Sonication Time: Formulations were sonicated three times for 15 min (for each time) with an interval of 5 min. Spherical niosomal vesicles were not observed after 5 minutes of Sonication. The entrapment efficiency decreased when the sonication time was increased above 3 minutes. Exposure to ultrasound for 5 minutes and above damaged the vesicles. Three minutes of sonication resulted in uniform unilamellar vesicles (**Table 1**).

TABLE 1: EFFECT OF SONICATION TIME

S. No.	Sonication Time (minutes)	Entrapment Efficiency
1	0	49.23 ± 0.54
2	1	55.96 ± 0.41
3	2	58.45 ± 0.59
4	3	59.10 ± 0.72
5	4	58.28 ± 0.36
6	5	50.34 ± 0.81

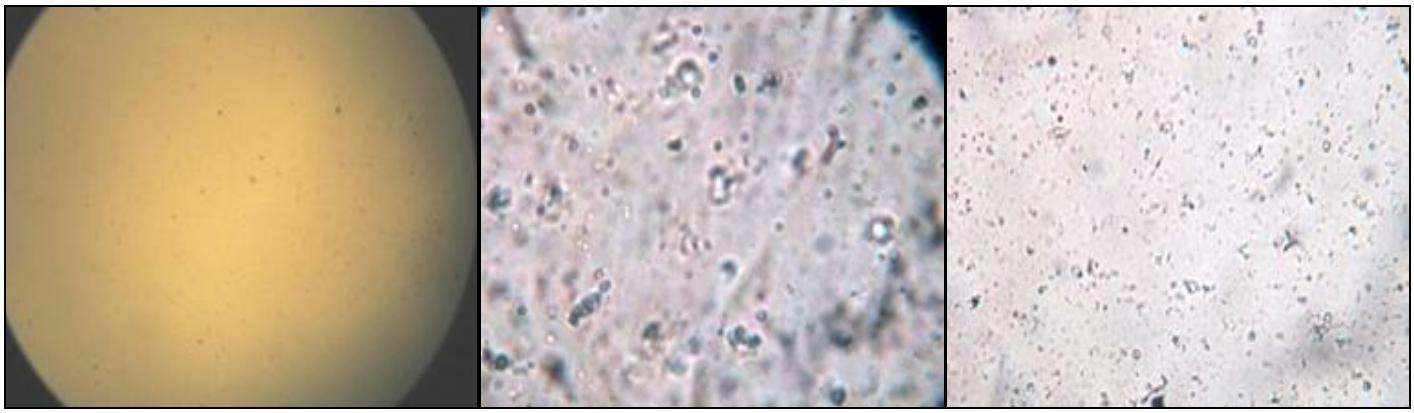
B) Effect of Hydration Time: The niosomal formulations were hydrated with 10 ml of phosphate buffer saline (pH 7.4), for 30, 45, 60, 75 and 90 minutes. The entrapment efficiency of the formulations is shown in **Table 2**. The results indicate that increase in the hydration time of the film from 30 to 45 minutes lead to higher entrapment efficiency but further increase in the hydration time did not produce any significant impact on it.

TABLE 2: EFFECT OF HYDRATION TIME

S. No.	Hydration Time (minutes)	Entrapment Efficiency
1	30	54.38 ± 0.61
2	45	59.10 ± 0.72
3	60	59.08 ± 0.36
4	75	59.14 ± 0.52
5	90	58.96 ± 0.29

C) Effect of Rotational Speed of Rotary flask evaporator: The thickness and uniformity of the film depended on the rotational speed of the flask. A speed of 100 rpm produced a uniformly thin lipid film, which upon hydration produced spherical vesicles. Lower rpm (50 rpm) resulted in non-uniform films and higher rpm (150 rpm) produced thick films which on hydration formed aggregates of vesicles.

D) Effect of Osmotic Shock: The niosomal formulations were treated with hypotonic (0.5% NaCl), hypertonic (1.6% NaCl) and isotonic saline (0.9% NaCl) solutions. The effect of osmotic shock was shown in **Figure 3.** 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 oral use.



A. Hypertonic (1.6% NaCl) **B. Hypotonic (0.5% NaCl)** **C. Isotonic Saline (0.9% NaCl)**

FIGURE 3: EFFECT OF OSMOTIC SHOCK

Formulation of Capecitabine Niosomes: Niosomal formulations were prepared with the ingredients as shown in Table 3 by thin film hydration technique using increasing molar ratios of non ionic surfactants (Span 40, Span 60, Tween 40, Tween 60) and constant molar ratio of cholesterol (25µmol). The formulation details are shown in **Table 3**.

TABLE 3: FORMULATIONS OF CAPECITABINE NIOSOMES

Formulation Code	Surfactant	Ratio of		Entrapment Efficiency*	Cumulative % Drug Release* at the end of 24th hour
		Surfactant	Cholesterol		
F1	SPAN 40	1	1	09.54±0.93	99.56±0.77
F2	SPAN 40	2	1	11.45±0.84	94.21±0.87
F3	SPAN 40	3	1	21.85±0.26	90.01±0.45
F4	SPAN 40	4	1	**32.22±0.35**	**85.43±0.19**
F5	SPAN 60	1	1	11.34±0.43	96.01±0.85
F6	SPAN 60	2	1	22.51±0.49	86.26±0.25
F7	SPAN 60	3	1	24.09±0.16	81.67±0.64
F8	SPAN 60	4	1	**32.84±0.26**	**74.01±0.51**
F9	TWEEN 40	1	1	29.43±0.26	84.58±0.26
F10	TWEEN 40	2	1	31.15±0.72	81.24±0.54
F11	TWEEN 40	3	1	39.27±0.85	78.47±0.92
F12	TWEEN 40	4	1	**46.47±0.17**	**74.69±0.25**
F13	TWEEN 60	1	1	36.72±0.21	85.80±0.16
F14	TWEEN 60	2	1	50.56±0.36	76.33±0.12
F15	TWEEN 60	3	1	54.79±0.42	71.29±0.31
F16	TWEEN 60	4	1	**59.10±0.72**	**67.95±0.65**

*Mean ±SD (n=3)

Entrapment Efficiency: The niosomes were prepared at four different micro molar ratios (1:1, 2:1, 3:1, 4:1) for each of the non-ionic surfactant keeping cholesterol and Capecitabine concentration (10 mg) as constant. The entrapment efficiency of the formulations was determined by centrifugation process. The entrapment efficiency of the formulations was observed to be between 9.54% and 59.10%. The entrapment efficiency was found to be 32.22, 32.84, 46.47 and 59.10% for Niosomes prepared with Span 40, Span 60, Tween 40 and Tween 60 respectively in 4:1 molar ratio (**Table 3 and Figure 4**).

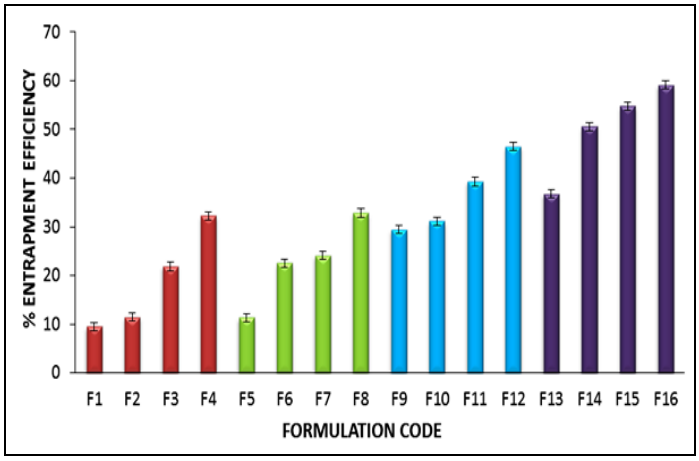


FIGURE 4: ENTRAPMENT EFFICIENCY OF DIFFERENT FORMULATIONS

Effect of Non-Ionic Surfactants on Entrapment Efficiency: It is clear that, the increase in surfactant concentration increases the entrapment efficiency of the formulation (Table 3). The entrapment efficiency of niosomes prepared with 4:1 molar ratios of non ionic surfactants are as follows,

32.22 %, 32.84 %, 46.47 % and 59.10%

for F4, F8, F12 and F16 respectively. From the results, it is observed that the formulation containing 100µmol concentration of non-ionic surfactant and 25 µmol concentration of cholesterol has higher entrapment efficiency than other formulations. Increasing the concentration of non ionic surfactants increases the entrapment efficiency.

F16 has higher entrapment efficiency compared to other formulations. Surfactants with longer alkyl chains generally give larger vesicles. This might be the reason for higher entrapment efficiency of vesicles prepared with stearyl chain surfactants.

The order of non ionic surfactants that resulted in better entrapment efficiency is as follows,

Tween 60 > Tween 40 > Span 60 >Span 40

***In- vitro* Release Studies:** The *In-vitro* drug release study of Capecitabine niosomes was done using dialysis bag diffusion technique in phosphate buffer saline of pH 7.4. The results are shown in Table 3.

The cumulative % drug release at 24 hours for the span 40 series is 99.56% for formulation F1 whereas it is 94.21 %, 90.01 % and 85.43 % for formulations F2, F3 and F4 respectively. The cumulative % drug release at 24 hours for the span 60 series is 96.01% for formulation F5 whereas it is 86.26 %, 81.67 % and 74.01 %, for formulations F6, F7 and F8, respectively. The cumulative % drug release at 24 hours for the tween 40 series is 84.69 % for formulation F9 whereas it is 81.67 %, 78.47 % and74.59 % for formulations F10, F11 and F12, respectively.

The cumulative % drug release at 24 hours for the tween 60 series is 85.80% for formulation F13 whereas it was 76.37 %, 71.29 % and 67.95 % for formulations F14, F15 and F16, respectively. Significant changes in release were observed upon changing the type of surfactant used in the bilayer of Capecitabine niosomes. The experimental studies showed that the rate of drug release is inversely proportional to the entrapment efficiency of the drug.

Effect of Surfactants on the Release Rate from Formulation: From the release studies, the formulation F16 shows slower and prolonged drug release than other formulations. This may be due to higher entrapment efficiency of the formulation.

Among the formulations prepared using span 40 (F1 to F4), F1 shows maximum drug release in 24 hours and the increasing order of percentage drug release was found to be,

F1 > F2> F3 > F4

Among the formulations prepared using span 60 (F5 to F8), F5 shows maximum drug release in 24 hours and the increasing order of percentage drug release was found to be,

F5 > F6> F7 > F8

Among the formulations prepared using Tween 40 (F9 to F12), F9 shows maximum drug release in 24 hours and the increasing order of percentage drug release was found to be,

F9 > F10> F11 > F12

Among the formulations prepared using Tween 60 (F13 to F16), F13 shows maximum drug release in 24 hours and the increasing order of percentage drug release was found to be,

F13 > F14> F15 > F16

From the above reports, it is concluded that increasing the surfactant concentration decreased the drug release.

Kinetics of Drug Release: The *in-vitro* release data was applied to various kinetic models to predict the mechanism of drug release of formulations F1 to F16. The release constant was calculated from the slope of appropriate plots, and the regression coefficient (r^2) was determined. From the results shown in **Table 4**.

TABLE 4: *IN- VITRO* RELEASE KINETICS OF CAPECITABINE NIOSOMES PREPARED USING DIFFERENT SURFACTANTS

BATCH CODE	ZERO ORDER		FIRST ORDER		HIGUCHI MODEL		KORSMEYER-PEPPAS		HIXSON-CROWELL	
	R^2	$KO^{(h-1)}$	R^2	$K1^{(h-1)}$	R^2	$KH^{h(-1/2)}$	R^2	n	R^2	$KHC^{(h-1/3)}$
F4	0.865	4.208	0.930	-0.046	0.934	20.81	0.835	0.693	0.915	-0.105
F8	0.864	3.754	0.917	-0.028	0.934	18.58	0.849	0.424	0.903	-0.083
F12	0.916	3.680	0.954	-0.027	0.916	17.52	0.895	0.270	0.874	-0.088
F16	0.923	3.348	0.946	-0.022	0.868	15.46	0.981	0.050	0.944	-0.069

All the formulations followed first-order kinetics and their r^2 value lay between 0.917 and 0.954, indicating the release to be dose dependent.

1) The drug release was proportional to the square root of time indicating that Capecitabine release from niosomes was diffusion controlled.

2) The n value for the Korsemeyer-Peppas model for Capecitabine niosomal formulation was found to be between 0.050 and 0.693 which confirms the Non-Fickian type diffusion or an anomalous diffusion mechanism with erosion.

3) The drug release pattern from Capecitabine loaded niosomes follows **Higuchi's model and first order of release.**

Optical Microscopy and Transmission Electron Microscopy: Optical microscopy and TEM images of optimized niosomal formulation (F16) were recorded. The particles are almost spherical and homogenous. The niosomes are in Nanometric size range of about 100 to 300 nm. The results showed that the Capecitabine loaded niosomes have a spherical shape with smooth surface and discrete without any aggregation or agglomeration (**Figure 5 and 6**).

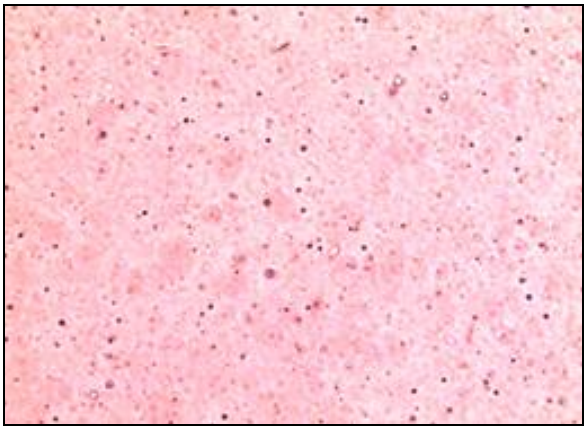


FIGURE 5: OPTICAL MICROGRAPH OF F16

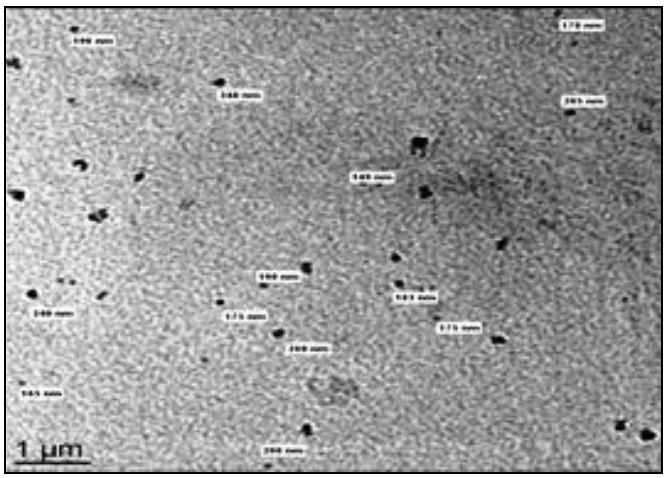


FIGURE 6: TEM IMAGE OF F16

Particle Size Analysis: The particle size determination of the niosomal formulations was carried out using laser diffraction (Malvern Particle Size Analyzer) and the mean of vesicular diameter was found to be 131.9nm as shown in the **Figure 7**.

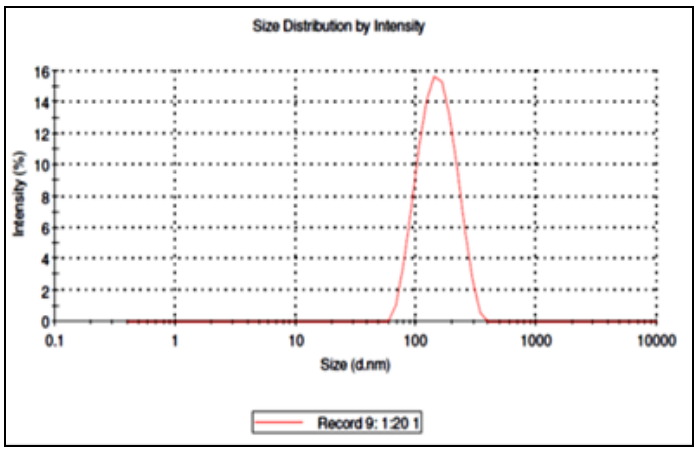


FIGURE: 7 PARTICLE SIZE DISTRIBUTION OF F16

Stability Studies of Capecitabine Niosomes: Stability studies of the selected formulations (F16) were carried out by storing at 4°C (refrigeration temperature) and 25°C ± 2°C for a period of three months as per ICH (International Conference on Harmonization) guidelines.

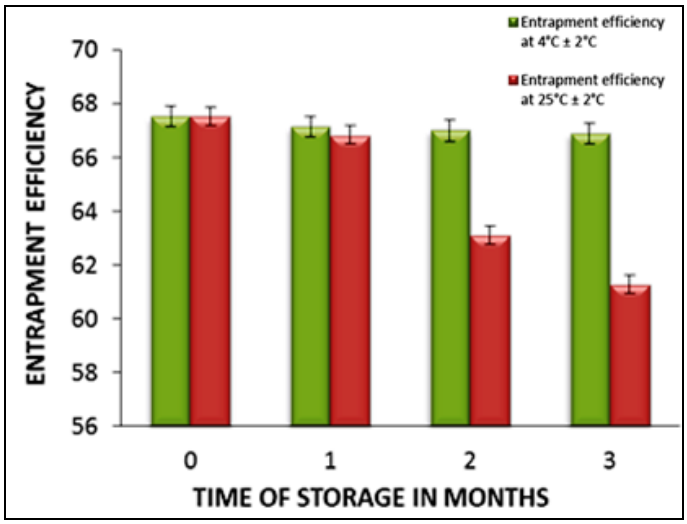


FIGURE 8: STABILITY GRAPH OF FORMULATION F16

The entrapment efficiency of the drug in the niosomal dispersion was estimated immediately after the preparation and after every month for three months (Figure 8). The drug leakage from the vesicles was least at 4°C. This may be attributed to phase transition of surfactant and lipid causing leakage of vesicles at higher temperatures during storage. Hence the Niosomes can be stored at 4°C.

CONCLUSION: It is concluded that, the thin film hydration technique is a useful method for the successful incorporation of hydrophilic drugs. The formulations were characterized with respect to size, entrapment efficiency, *in- vitro* drug release and stability under specific conditions. It has been concluded that the Niosomes prepared with **Tween 60 (F16)** has more entrapment efficiency and releases drug slowly **(67.95±0.65)** in a sustained manner as compared to other formulations. The drug release pattern from Capecitabine loaded Niosomes follows **Higuchi's model and first order release.**

The prolonged release of the drug from the niosomes suggests that the frequency of administration may be reduced. Further, as the particles are in nanometeric size range, the bioavailability may be increased and effective targeting may be achieved. Future investigations in animals, human volunteers, pharmacological and toxicological investigations in animals and human volunteers may help to exploit the niosomes as prosperous drug carriers for targeting drugs more efficiently. Hence, we can conclude that niosomes provide controlled release of drug and these systems are used as drug carriers for the delivery of cytotoxic drugs with fewer side effects.

ACKNOWLEDGEMENT: We gratefully acknowledge support for this work from SAIF, IIT Madras for FTIR Characterization. The work has been done in IIT Madras. One of the authors is thankful to Madras medical college of pharmacy support of this work.

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How to cite this article:
Anbarasan B, Rekha S, Elango K, Shriya B and Ramaprabhu S: Optimization of the formulation and *in-vitro* evaluation of Capecitabine Niosomes for the treatment of Colon Cancer. *Int J Pharm Sci Res* 2013; 4(4); 1504-1513.