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# FORMULATION AND EVALUATION OF NIOSOMES CO-LOADED WITH 5-FLUOROURACIL AND LEUCOVORIN: CHARACTERIZATION AND *IN VITRO* RELEASE STUDY

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## ABSTRACT

In the present study, niosomes co-loaded with 5-Fluorouracil and Leucovorin was prepared and evaluated for their characterization and in vitro drug release. Formulation of niosomes was optimized for highest percentage of drug entrapment. Microscopic observation confirmed that all particles were uniform in size and shape. The entrapment efficiency was optimized using different concentration of cholesterol and non-ionic surfactants. The in vitro release studies of drug from niosomes exhibited a prolonged drug release as observed over a period of 12 h. The negative values of zeta potential indicated that the 5-Fluorouracil and Leucovorin loaded niosomes were stabilized by electrostatic repulsive forces. Results from stability study have shown that the drug leakage from the vesicles was least at 4°C followed by 25°C and 37°C. The mechanism of release of 5-FU and LV was found Non-Fickian and Fickian diffusion respectively. Niosomes can be formulated by optimised process parameters to enhance 5-FU and LV entrapment efficiency and sustainability of release. These improvements in 5-Fluorouracil and Leucovorin niosomal formulation may be useful in developing a more effective combination for cancer therapy.

## KEYWORDS

Niosomes, Multiple drug loading, Leucovorin, 5-Flurouracil and Colon cancer.

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## INTRODUCTION

In recent years, niosomes as a drug carrier has been received much attention in pharmaceutical academia and industrial research. Generally niosomes have a bilayer structure and are formed by self-association of nonionic surfactants and cholesterol in an aqueous phase[1]. It has many advantages like biodegradable, biocompatible, and nonimmunogenic, long shelf life, exhibit high stability and achieves the delivery of drug at target

site in a controlled and/or sustained manner. Also niosomes may alleviate the disadvantages associated with liposomes such as chemical instability, variable purity of phospholipids and high cost[2]. Encapsulation of a large number of drugs with a wide range of solubility in niosomes using various types of nonionic surfactants has been extensively studied[3].

Combination therapy with drugs of different therapeutic effects shows an improving efficacy in the treatment of various diseases. Particularly, cancer treatment requires simultaneous administration of different combination of drugs due to the molecular complexity of cancer diseases[4,5]. Niosomes show potential in combination of drug delivery and targeting combine transdermal and tumor targeting ability in cancer therapy[6,7]. 5-Fluorouracil (5-FU) has been in clinical use as an anticancer drug for more than 30 years. Although 5-FU has a broad spectrum of anticancer activity including common solid tumors present in the gastrointestinal system. But only a minority of patients treated with 5-FU experience an objective response to therapy[8]. Leucovorin is an active metabolite of folic acid and an essential coenzyme for nucleic acid synthesis. Leucovorin may significantly increase both the clinical efficacy and the clinical toxicity of 5-FU in cancer patients. From literature we found that 5-Flurouracil in combination with leucovorin is well acknowledged in the treatment of cancer[9-11]. But only a few approaches consider the developing new drug delivery systems co-loaded with 5-FU and Leucovorin has been investigated.

The purpose of this study was to evaluate the process parameters that critically affect the formulation of niosomes with respect to entrapment and release of 5-FU and Leucovorin. There is little information in the literature on optimizing the different processing variables that are important in the formulation of 5-FU and Leucovorin niosomes for development of an improved drug delivery system. In this investigation, we optimized concentration of cholesterol and non ionic surfactants (Span 40, Span 60, Tween 40 and Tween 60) used in the niosomal formulations. The niosomes was characterized such as entrapment efficiency, particle size analysis, surface morphology (SEM and TEM) and zeta potential. Measurements of in vitro drug release of 5-FU and leucovorin were done to assess the effectiveness of the drug delivery system.

## MATERIAL AND METHODS

### Drugs and Chemicals

5-Fluorouracil and Leucovorin was procured from Sigma Aldrich, India. Cholesterol, polyoxyethylene sorbitan monopalmitate (Tween 40), polyoxyethylene sorbitan monostearate (Tween 60), sorbitan monopalmitate (Span 40), sorbitan monostearate (Span 60) and dicetyl phosphate (DCP) were procured from Merck, India. All materials used in the study were of analytical grade.

### FTIR Spectral analysis

FTIR spectral analysis of pure drug and excipients was carried out and observation was done to analyze any changes in the chemical nature of the drug after combining it with the excipients occurred. The drug samples were mixed with KBR and the pressure (600 $Kg/cm^2$) were applied to get pellets and scanned with the IR instrument (Shimadzu, 8400 Series, Tokyo, Japan) from 400-4000$cm^{-1}$.

### Differential Scanning Colorimetry (DSC)

In differential Scanning Colorimetry, the samples were heated from 25°C to 100°C at a constant temperature increment of 10° C/min and purged with nitrogen gas at 40 ml/min.

### Preparation of the niosomes

Niosomes were prepared by a thin film hydration technique using a mixture of surfactants encompassing (span 40, span 60, tween 60 and tween 40) and cholesterol, at different specified ratios as given in Table No.1. Surfactant and cholesterol was dissolved in 8 ml of diethyl ether and the drugs were dissolved in 2 ml of ethanol. The mixture was then transferred to a round bottom flask, and the solvent was evaporated under reduced pressure at a temperature 20-25º, using a rotary flash evaporator until the formation of a thin lipid film. The resultant film was made wet with 10 ml of phosphate buffer saline pH 7.4. The hydration was

continued for 1 h, while the flask was kept rotating at 55-65°. The hydrated niosomes were sonicated for 20 min using a bath sonicator to obtain niosomal dispersion.

**Entrapment efficiency for the Niosomes**

The niosomal dispersion is a homogeneous milky white suspension which is centrifuged at 12000 rpm for 15 min and entrapment efficiency of niosomes were evaluated by measuring the UV absorption of the supernatant. The corresponding calibration curves were made by testing the supernatant of blank niosomes. Each sample was measured in triplicate. 5-FU was measured at 266nm and elicited an intense characteristic peak whilst LV displayed a characteristic peak at 288 nm. The percentage of the entrapment of the drug can be obtained for the niosomal formulations.

**Particle Size Analysis (PSA)**

The size division of the niosomes was analyzed using the Beckman particle size analyzer (Beckman Coulter, Delsa nano C, Brea, USA) prepared with a dry accessory system. Samples were mixed with water and maintained at a temperature of 25°C.

**Zeta Potential Analysis**

The zeta potential was measured using a Beckman Coulter (Beckman Coulter Delsa Nano C, Brea, USA). The sample was diluted with double distilled water and transferred to cuvettes and maintained at a temperature of 25°C.

## MORPHOLOGICAL ANALYSIS OF NIOSOMES

**Optical microscopy**

Characterizations of vesicle dispersions were done by photo microscopy for vesicle formation and morphology. The size and shape of vesicles in niosomes formulations were observed by optical microscopy using a calibrated eyepiece micrometer, and images were captured at resolution of 400 X with a digital camera (Olympus, 8.1 megapixel, Japan).

**Transmission electron microscopy (TEM)**

Transmission electron microscopy (TEM) was used in the morphological analysis of niosomes. Samples were processed by spreading a drop of freshly prepared nano-suspension onto copper grid with carbon film support and excess solution was cleared using a filter paper. Samples were allowed to dry at room temperature and observed directly with TEM (JEM-100, JEOL, Tokyo, Japan) without further staining.

**Scanning Electron Microscopy (SEM)**

The SEM is used to identify the particle size and the surface morphology of the niosomes. For niosomes, the drop of the sample is placed on the covered glass slide and then dried by applying vacuum, later it was coated with gold to a thickness of 100A using VEGAS TESCAN Vacuum evaporator and the image was captured for the niosomal formulation.

***In-vitro* release studies for the niosomes**

In vitro release of 5-FU and leucovorin niosomes was conducted by a dialysis membrane having a pore size of 2.4 mm (LA-395-5Mt Himedia Pvt. Ltd, Mumbai, India) with 75 ml of pH 7.4 phosphate buffer at 37°C. Briefly in a 100 ml beaker 75ml of pH 7.4 phosphate buffer was taken. A 2 ml of formulation was taken into a dialysis bag and immersed into the buffer solution. The dialysis membrane was activated earlier using by soaking in 1% w/v NaOH over night. Then the flask was placed on a magnetic stirrer. The stirring was done at 250 rpm and the temperature of the buffer was maintained at 37°C. Sampling was done by withdrawing 1 ml of aliquots from a beaker. Immediately 1 ml of new buffer was added to keep the sink condition. Samples were analyzed after sufficiently diluting with buffer by using a UV-Visible Spectrophotometer (UV/VIS-Double beam Spectrophotometer, V-530, Jasco, Tokyo, Japan) at a wavelength of 266 nm for 5-FU and 288 nm for LV [15].

***In vitro* release kinetics study**

The release data obtained via the above procedure was derived using Ritger and Peppas model to devise its release mechanism. The initial 60% cumulative release data were used to estimate the diffusion exponent 'n' by using following equation.

$$M_t / M_\infty = Kt^n$$

Where $M_t$ is the amount of drug released at time t, $M\infty$ the nominal total amount of drug released, K the kinetic constant, and n the diffusion exponent

that is used to characterize the release mechanism. For spheres, a value of $n \leq 0.43$ indicates Fickian release and 'n' value between 0.43 and 0.85 is an indication of non-Fickian release (both diffusion-controlled and swelling-controlled drug release). An 'n' value ≥0.85 indicates case-II transport that involves polymer dissolution and polymeric chain enlargement or relaxation.

**Stability studies**

Stability studies for the optimized niosomes (F4) were carried out at refrigeration temperature, room temperature and elevated temperature (4 ± 2°C, 25 ± 2°C/ 60 % RH ± 5 % RH and 37 ± 2°C/ 65 % RH ± 5 % RH) for a period of three months. At definite time intervals, the samples from each batch were taken and evaluated using following parameters like appearance, size, assay, and dissolution.

## RESULTS AND DISCUSSION

**FT-IR spectral analysis**

The development of a successful formulation depends only on a suitable selection of excipients. Hence the physical state of the drug 5-Flurouracil and leucovorin and the combination of drug and chloestrol and surfactant used for niosomes preparation were studied by FTIR to study the drug and excipients compatibility. The physicochemical compatibility of the drugs and the excipients was obtained by FTIR studies with the interpretation values of the FTIR were mentioned in Figure No.1.

The spectra were recorded for pure drugs, surfactants, cholesterol and optimized formulations using FTIR spectrophotometer. The spectra of pure 5-FU, LV and niosomes loaded with 5-FU and LV was shown the intense characteristic peaks at 3160, 1727, 1662, 1426, 1247, 811.7 and 547cm$^{-1}$ are detected due to the vibration of imide stretch (amide II and amide III) and aromatic ring in the structure of 5-FU . In the case of the FTIR spectra of LV, some absorption bands are observed at 1609, 1324, 1190, and 763cm$^{-1}$ corresponding to the vibration of NH stretch, aromatic ring, C O, C–H groups. The characteristic peaks of 5-Fluorouracil and leucovorin appeared in spectra without any remarkable change position in that of prepared niosomes. The results revealed that no chemical interaction between drugs and excipients.

**Optimization of niosomes**

The optimization of the niosomes was made by altering the cholesterol ratio and evaluating the niosomes by using in vitro drug release. The uniformity of the particle size and the formation of the particles are considerably noted. Following which the sonication process was carried out. The larger the cholesterol content the formation of the flakes in the solution takes place and the formation of the thin film in the round bottom flask is increasing timely. Optimum amount is by taking the equal proportion of the cholesterol and the surfactant (Span 40, Span 60, Tween 40 and Tween 60) gives a better formulation.

From composition of all the formulations (Table No.1), the best formulation is obtained from the as we increase the cholesterol concentration and keeping the surfactant concentration constant the formation of the niosomes is uniform and the visibility of the cholesterol flakes in the formulation is nil. But the time taken for the organic solvents to get evaporated is increasing. The film which is formed on the round bottom flask is kept overnight for the complete removal and drying of the organic solvents from the film. The best formulation is taken from the further evaluation.

Large volume of organic solution of lipids is most easily dried in a rotary evaporator fitted with a cooling coil and a thermostatically controlled water bath. Rapid evaporation of solvent is carried out by gentle warming (20° - 40°C) under reduced pressure (400 - 700 mmHg). Rapid rotation of the solvent containing flask increases the surface area for evaporation. In case, where sufficient vacuum is not attainable or if the concentration of lipids is particularly high, it may be difficult to remove the last traces of chloroform from the lipid. Therefore, it is recommended as a matter of routine that after rotary evaporation some further means is employed to bring the residue to complete dryness. Attachment of the flask to the manifold of lyophilizer and overnight exposure to high vacuum is a good method.

Increasing the sonication time resulted into reduction in percent drug entrapment the decrease drug entrapment is due to leakage of the drug during sonication. Cholesterol provides endurance against mechanical strain during sonication and centrifugation. Sonication brings about size reduction by breaking large niosomes to smaller ones and in doing so, leakage of small quantities of drug from the niosomes occur. Hence the sonication time was optimized to 15 minutes, and further reduction in the size by increasing sonication time was not attempted.

**Particle Size Analysis**

The particle size of the niosomes was analyzed by using the particle size analyzer (Malvern) and it is shown in the Figure No.2. The particles of the drug loaded niosomes are in the size of 0.1 to 1 µ range. More number of the particles was in the 0.1µ to 0.8 µ approximately and also found some larger particle size distribution.

**Optical microscopy**

The formulated niosomes were viewed through optical microscope and image was shown in Figure No.3. The microscopic methods include the use of Bright field, phase contrast microscope and fluorescent microscope and are useful in evaluating the vesicle size of large vesicles (>1µm) particularly the upper end of the size distribution for miltilamellar vesicles. Vesicular dispersion appropriately diluted is wet mounted on a haemocytometer and photographed with a phase contrast microscope. The negatives then can be projected on a piece of calibrated paper using a photographic enlarger at X 1250 (Vyas and Katar, 1991), diameters of approximately 500 vesicles are measured and thus this method is tedious and coupled with the limitation of resolution, hence electron microscopic methods with greater resolutions are preferred.

**Morphological studies**

The TEM and SEM images of the 5-FU and LV loaded niosomes are shown in the Figure No. 4 and 5**.** The images are shown the discrete formation of the structure and the internal morphology. The mean size of the niosome increased with progressive increase in the HLB value because surface free energy decreases on increasing hydrophobicity of surfactant[12]. Surfactants with longer alkyl chains generally give larger vesicles. These result reasonable with that of higher entrapment efficiencies with span 60 niosomes.

**Differential scanning Colorimetry**

The drugs 5-Flurouracil, Leucovorin, cholesterol, span 40, span 60, span 80 and mixture of drug and excipients were subjected to DSC studies for testing the compatibility of the drug with the excipients used in the formulation. DSC thermo gram was shown in Figure 6. The results clearly indicates that there is no interaction between drug and excipients.

The surface charge of 5-Fluorouracil and leucovorin niosomes was measured using Zeta potentiometer. The zeta potential graph was shown in Figure No.7. Zeta value of niosomes was found to be – 10 mV. Niosomes has a Negative Zeta Potential which indicates that it has excellent stability due weak electrostatic repulsive force exists in the niosomal bilayer. The noisome formulations have more tendencies to be in suspended condition/ dispersed condition for longer duration[13]. Particles with zeta potential close to zero are less able to be phagocytosed than charged particles.

**Drug entrapment efficiency**

The quantity of the drug entrapped in the niosomes is very essential to know before studying the behaviours of this entrapped drug in physical or biological system. The process and formulation variables like cholesterol was altered and optimized to obtain the niosomes with maximum drug entrapment. 5-Fluorouracil and leucovorin entrapped niosomes were subjected to percentage drug entrapment. The entrapment efficiency of drug containing span 60 was found to be 72.21% which showed maximum percent drug entrapment where as those containing span 40, span 60, tween 40, tween 60 were found to encapsulate 66.35, 72.21, 50.44, and 52.24. This result indicates that span 60 is the more suitable surfactant along with higher concentration of cholesterol for enhancing maximum entrapment for the drugs 5-Fluorouracil and leucovorin.

With increase in the cholesterol concentration more number of niosomes per ml of the niosomal

dispersion was formed, though the optimum concentration resulting into an increased percent drug entrapment. The solvent mixture composition used was chloroform / methanol, is an attempt to enhance percent drug entrapment. The solvent mixture composition was optimized to 1:1 (5ml v/v). The presence of large volume of hydration medium helps in faster and efficient hydration of the niosomes. Therefore, the volume of the hydration medium was optimized to 10ml. The Entrapment Efficiency of the formulations was calculated and Tabulated in the Table No.2.

Incorporation of cholesterol into niosomes at ratios up to 1:2 increased the encapsulation efficiency of drugs. Inclusion of cholesterol increases the viscosity of the formulation indicating more rigidity of the bilayer membrane. Moreover, drug partitioning will occur more easily in highly ordered systems of surfactant and cholesterol. The ability of the lamellar surfactant phase to accommodate drug, depends upon the structure of the surfactant phase. The entrapment efficiency of Span 60 was higher than Span 40. The higher entrapment may be due to the solid nature, hydrophobicity, and high-phase transition temperature of the surfactant[12].

***In vitro* drug release**

The formulated niosomes were subjected to *in vitro* drug release. The amount of 5-Fluorouracil and leucovorin diffused was estimated spectrophotometrically at nm 266 and 288 nm respectively[14,15]. The *in vitro* release profile of drugs was shown in Table No.3 and 4. Niosomes containing 5-Fluorouracil prepared with span 60 showed 99.65 % of drug release up to 12 hours shown in Figure No.8. 5-Fluorouracil release from the formulation containing span 40, Tween 40 and Tween 60 was observed almost 100% drug release within 10 hours. These results showed that niosomal formulation containing 5-Fluorouracil prepared with span 60 has sustained release up to 12 hours. Leucovorin release profile from niosomal formulation was shown in Figure No.9. Niosomes containing leucovorin prepared with span 60 showed 99.91 % of drug release up to 12 hours. Leucovorin release from the formulation containing span 40, Tween 40 and Tween 60 was observed almost 100% drug release within 10 hours. These results showed that niosomal formulation containing leucovorin prepared with span 60 has sustained release up to 12 hours.

The slower release of 5-Fluorouracil and leucovorin from the lipid layer may be due to slow partitioning and diffusion of 5-Fluorouracil and leucovorin from the lipid layer to the surrounding aqueous layer. Increasing cholesterol markedly reduces the efflux of the drug. Inclusion of cholesterol fills the pores in vesicular bilayers and abolishes the gel-liquid phase transition of liposomal and niosomal systems resulting in niosomes that are less leaky. This confirms that cholesterol in the formulation acts as a membrane stabilizing agent that helps to sustain drug release. Differences in the *in vitro* release profiles may be due to vesicle size, lamellarity, and membrane fluidity as a function of chain length of surfactant and cholesterol content. *In vitro* release data of niosomal formulation containing 5-Fluorouracil and leucovorin prepared with span 60 was selected for kinetics study analysis.

***In vitro* release kinetics**

In vitro release kinetics data was sown in Table No.5. The results shown that formulation F4, Span 60 with cholesterol follows zero-order kinetics. Calculation of Higuchi's correlation coefficient confirms that drug release was proportional to the square root of time indicating that drug release from niosomes was diffusion controlled. The *n* value from the Korsmeyer-Peppas model for release 5-FU from niosomal formulation was 0.67 which confirms the Non Fickian type diffusion, whereas release LV from niosomal formulations follow an Fickian diffusion mechanism ($n > 0.47$).

**Stability study**

The stability study result indicates that stability studies for the optimized niosomes (F4) were carried out at refrigeration temperature, room temperature and elevated temperature (4 ± 2°C, 25 ± 2°C/ 60 % RH ± 5 % RH and 37 ± 2°C/ 65 % RH ± 5 % RH) for a period of three months. Niosomes were evaluated for physical appearance, size, assay and dissolution studies. 5 – FU and LV loaded niosomes have not shown any significant change in storage condition 4°C ± 2°C. But drug leakage was

found in conditions 25 ± 2°C/ 60 % RH ± 5 % RH and 37 ± 2°C/ 65 % RH ± 5 % RH. This may be attributed to phase transition of surfactant and lipid causing vesicles leakage at higher temperature during storage[13]. Hence, it is concluded from the obtained data that the optimum storage condition for niosomes was found to be 4°C.

**Table No.1: Formulation composition of Niosomes**

| S.No | Type of formulation | F1 | F2 | F3 | F4 | F5 | F6 | F7 | F8 |
|---|---|---|---|---|---|---|---|---|---|
| 1 | Drug | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| 2 | Cholesterol | 10 | 20 | 10 | 20 | 10 | 20 | 10 | 20 |
| 3 | Span 40 | 10 | 10 | - | - | - | - | - | - |
| 4 | Span 60 | - | - | 10 | 10 | - | - | - | - |
| 5 | Tween 40 | - | - | - | - | 10 | 10 | - | - |
| 6 | Tween 60 | - | - | - | - | - | - | 10 | 10 |
| 7 | Drug :cholesterol: SA | 1:1:1 | 1:2:1 | 1:1:1 | 1:2:1 | 1:1:1 | 1:2:1 | 1:1:1 | 1:2:1 |

**Table No.2: Entrapment Efficiency of the Niosomal formulations**

| S.No | Formulations Code | 5-FU (%) | LV (%) |
|---|---|---|---|
| 1 | F1 | 62.44 | 63.28 |
| 2 | F2 | 66.35 | 65.02 |
| 3 | F3 | 68.30 | 64.86 |
| 4 | F4 | 72.21 | 69.45 |
| 5 | F5 | 47.05 | 54.76 |
| 6 | F6 | 50.44 | 57.92 |
| 7 | F7 | 50.92 | 59.55 |
| 8 | F8 | 52.24 | 60.27 |

**Table No.3: *In-vitro* release study of the 5 – FU from Niosomes**

| S.No | Time(hr) | % Drug Released | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | F1 | F2 | F3 | F4 | F5 | F6 | F7 | F8 |
| 1 | 1 | 25.12 | 20.78 | 23.43 | 19.23 | 28.34 | 23.02 | 24.72 | 21.66 |
| 2 | 2 | 42.04 | 39.02 | 40.12 | 30.22 | 40.22 | 33.22 | 35.15 | 32.17 |
| 3 | 4 | 51.06 | 48.9 | 52.22 | 46.89 | 61.27 | 48.82 | 49.42 | 50.92 |
| 4 | 6 | 69.35 | 70.33 | 68.79 | 65.88 | 72.52 | 67.94 | 70.21 | 66.25 |
| 5 | 8 | 90.23 | 83.45 | 88.16 | 79.43 | 84.67 | 82.64 | 82.56 | 81.92 |
| 6 | 10 | 99.34 | 95.74 | 95.34 | 89.58 | 99.96 | 99.52 | 98.9 | 95.99 |
| 7 | 12 | 99.9 | 99.13 | 100.02 | 99.65 | 100 | 99.99 | 100 | 100 |

**Table No.4: *In-vitro* release study of the Leucovorin from Niosomes**

| S.No | Time (hr) | % Drug Released | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | F1 | F2 | F3 | F4 | F5 | F6 | F7 | F8 |
| 1 | 1 | 38.96 | 36.72 | 35.07 | 30.01 | 40.04 | 39.02 | 39.27 | 38.12 |
| 2 | 2 | 50.22 | 50.38 | 48.39 | 45.22 | 52.78 | 51.35 | 50.55 | 49.55 |
| 3 | 4 | 65.15 | 62.12 | 62.07 | 59.12 | 66.9 | 66.17 | 65.22 | 62.49 |
| 4 | 6 | 79.6 | 76.2 | 72.98 | 70.36 | 84.56 | 72.56 | 79.21 | 78.01 |
| 5 | 8 | 92.1 | 90.08 | 90.14 | 85.43 | 95.9 | 88.54 | 90.56 | 88.32 |
| 6 | 10 | 99.34 | 98.94 | 99 | 93.12 | 99 | 98.18 | 99.82 | 100 |
| 7 | 12 | 99.9 | 100 | 100 | 99.91 | 99.98 | 99 | 100 | 100 |

**Table No.5: *In vitro* release kinetics of 5-FU and LV from niosomes**

| S.No | Formulation code | Higuchi $R^2$ | Korsmeyer-Peppas | | Zero order | | First order | | Release mechanism |
|---|---|---|---|---|---|---|---|---|---|
| | | | $R^2$ | *N* | $R^2$ | $K_0$ (%mg/h) | $R^2$ | $K_1$ ($h^{-1}$) | |
| 1 | F4 (5- FU) | 0.996 | 0.998 | 0.67 | 0.969 | 8.01 | 0.770 | 0.161 | Non Fickian |
| 2 | F4 (LV) | 0.996 | 0.995 | 0.47 | 0.907 | 7.44 | 0.745 | 0.194 | Fickian |

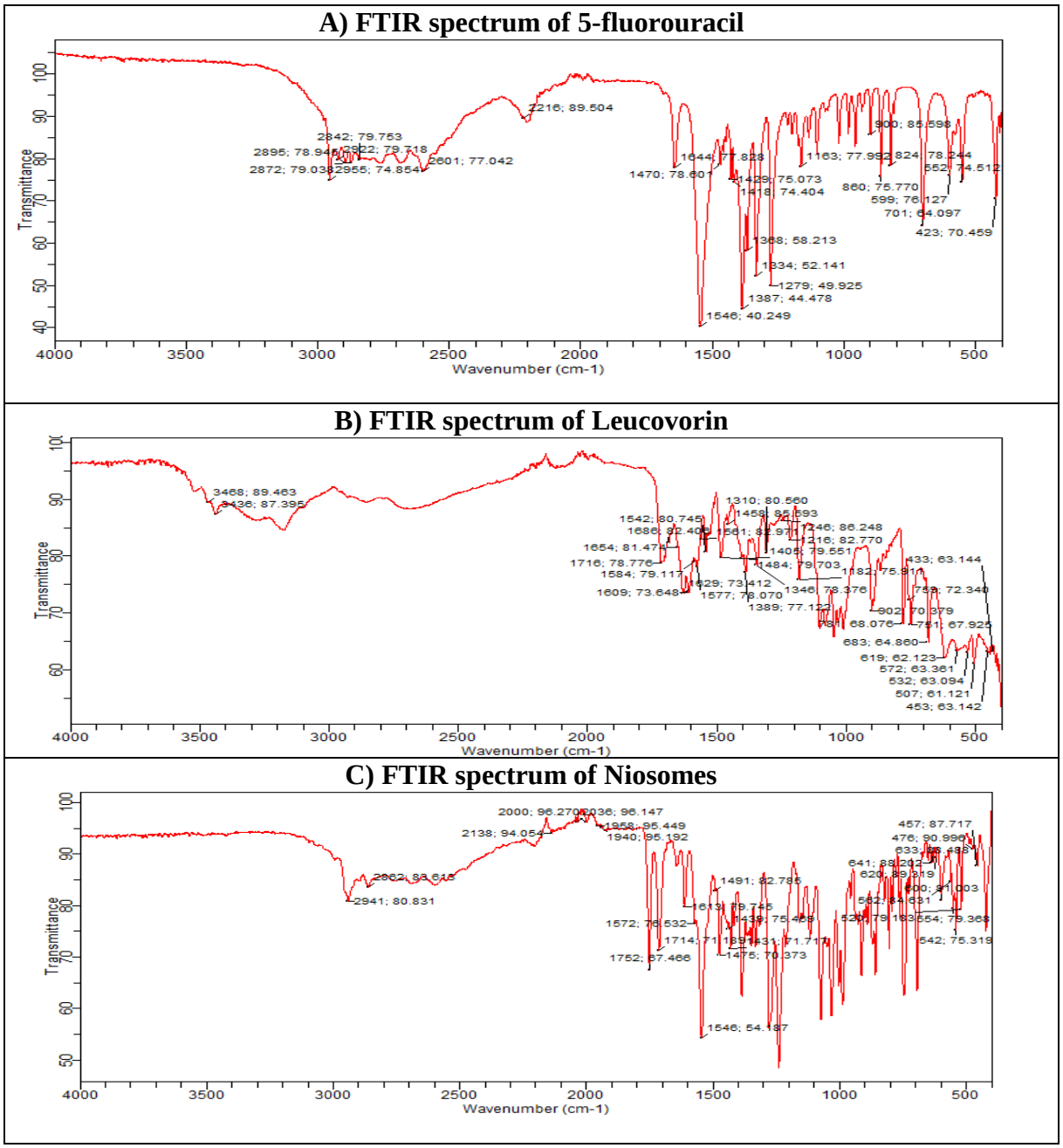


**Figure No.1: FTIR spectrum**

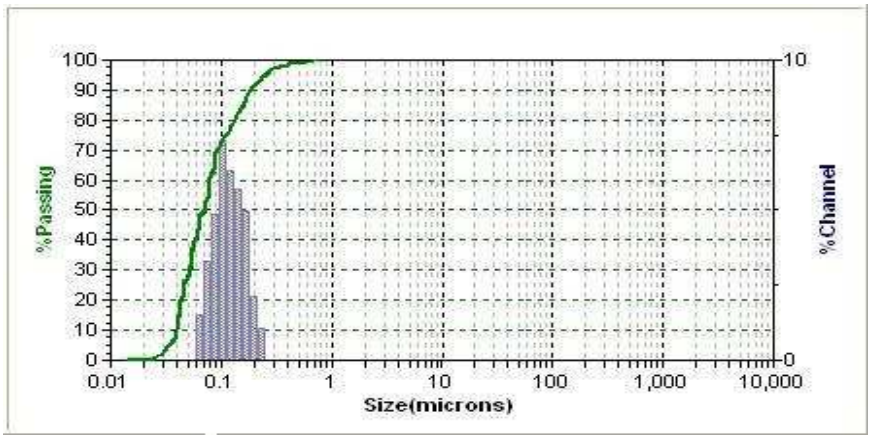


**Figure No.2: Particle size analysis of niosomes**

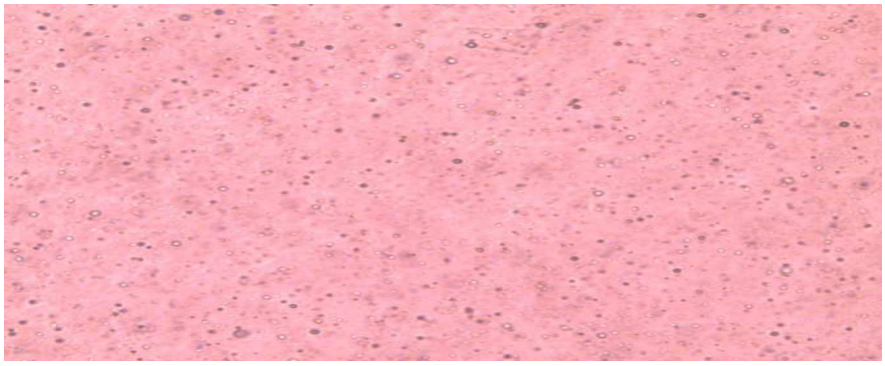

**Figure No.3: Optical microscopy images of Niosomes**

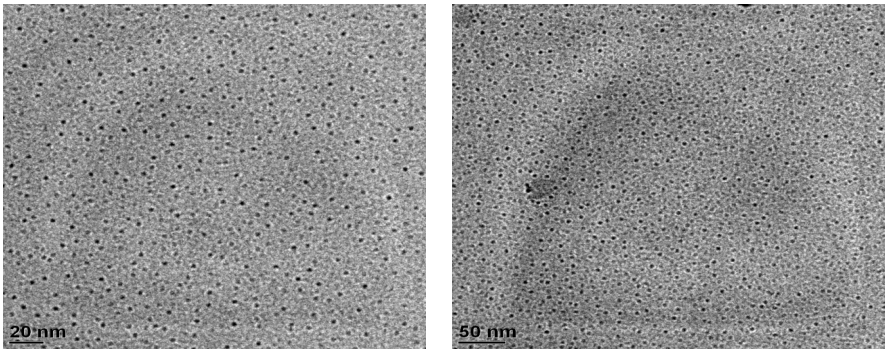


**Figure No.4: TEM image of Niosomes**

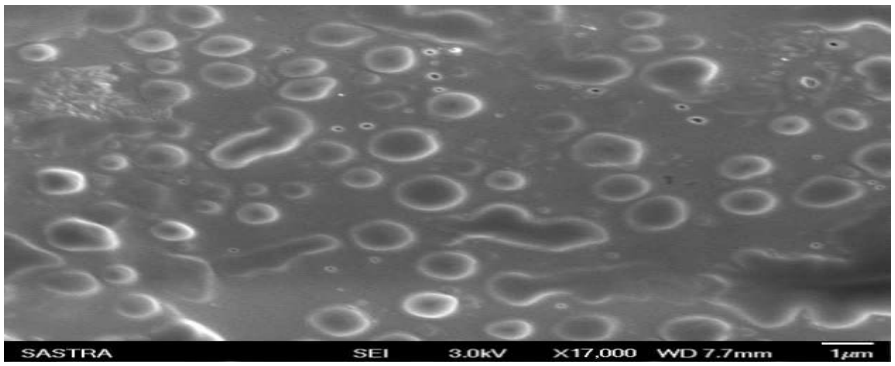


**Figure No.5: SEM image of Niosomes**

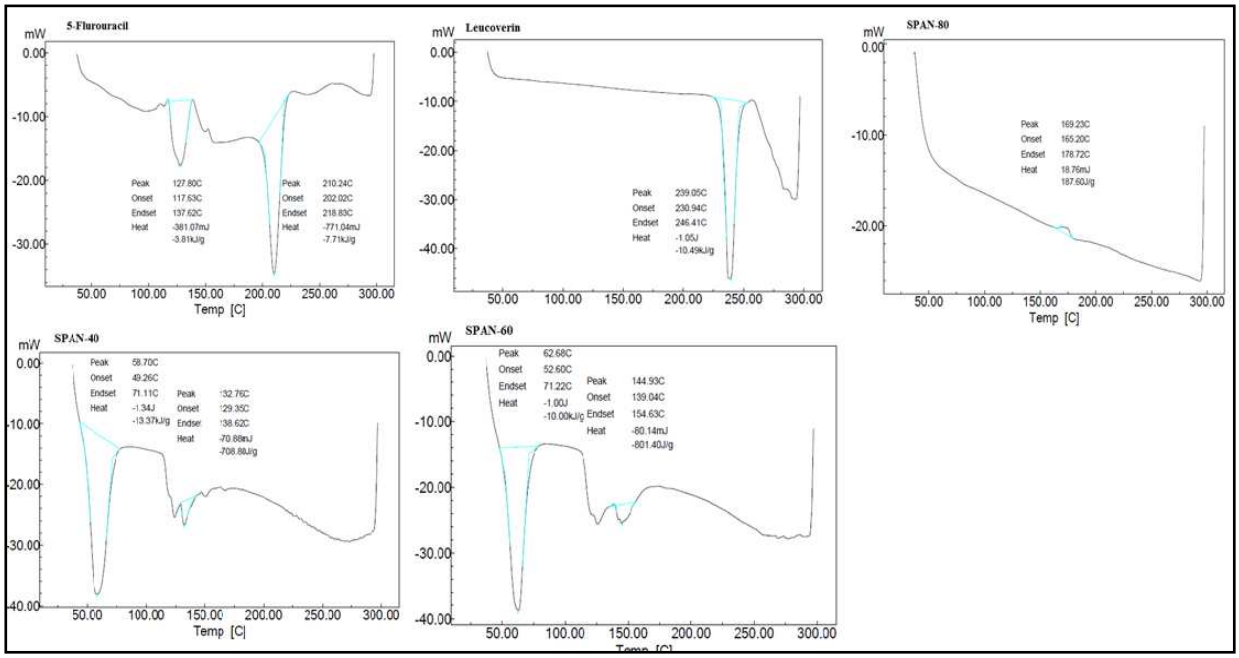


**Figure No.6: DSC Studies of niosomes Zeta potential**

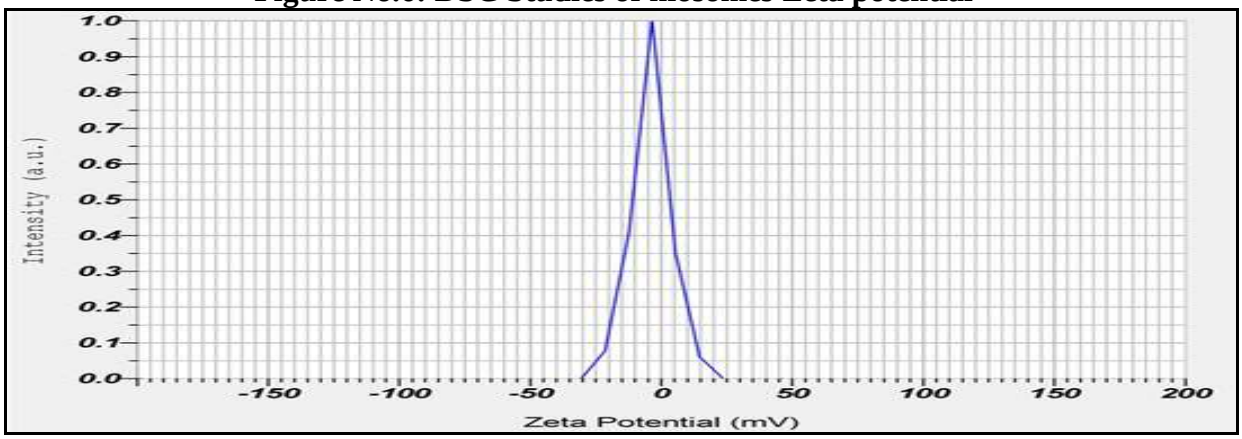


**Figure No.7: Zeta potential of Niosomes**

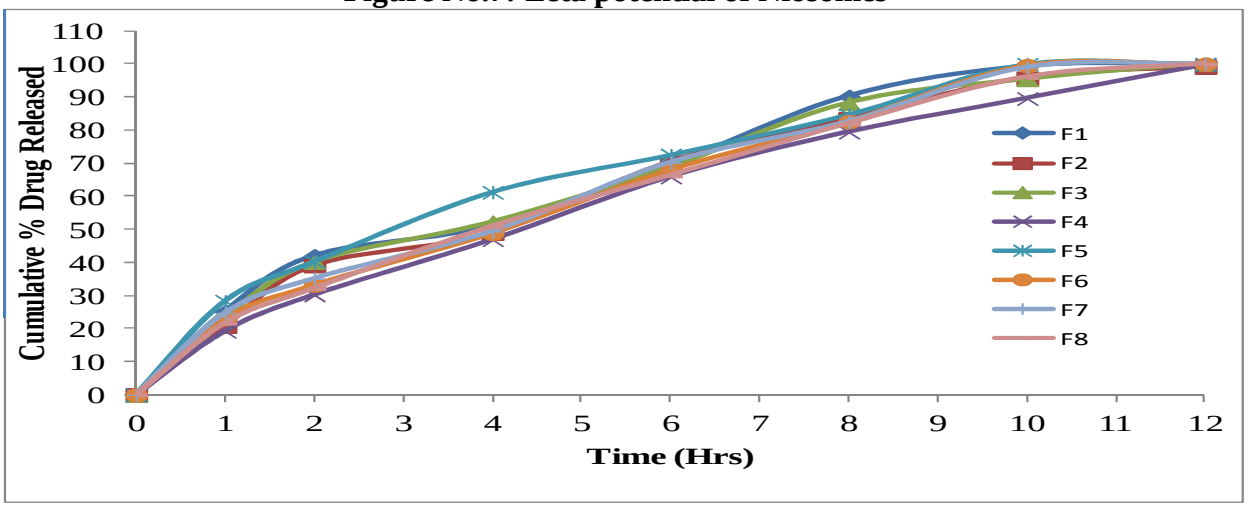


**Figure No.8: *In-vitro* release study of the 5 – FU from Niosomes**

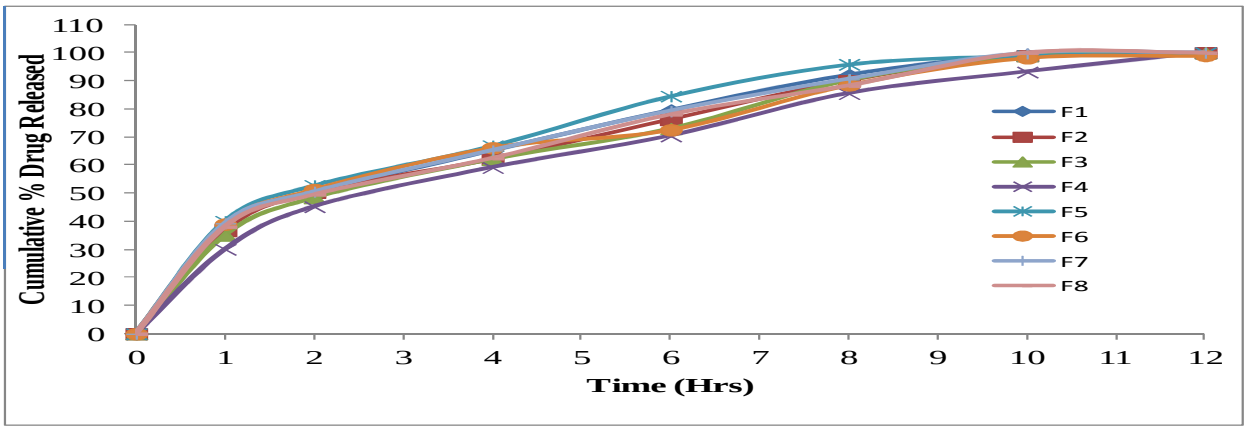


**Figure No.9: *In-vitro* release study of the Leucovorin from Niosomes**

## CONCLUSION

Niosomal formulations containing 5- Fluorouracil and leucovorin were successfully prepared with different surfactants like Span 40, Span 60, Tween 40, and Tween 60 by thin film hydration technique. The evaluation parameters revealed that 5-Fluorouracil and leucovorin niosomes shows reduced particle size distribution with better entrapment efficiency. Niosomes has negative surface charges which indicate excellent stability. In vitro release of 5-Flurouracil and Leucovorin from niosomes was carried out. Formulation F4 was shown better sustained release among other formulations. In vitro release kinetics study was done for formulation F4 to find out the release mechanism. The release of 5-FU from niosomal formulation follow Non Fickian type diffusion, whereas release LV from niosomal formulations follows Fickian diffusion mechanism. The result suggests that niosomal formulation can provide consistent and prolonged release of the entrapped drug molecules. Niosomal delivery system can provide sustained action of the entrapped drug and reduce the side effects associated with frequent administration of the drug and potentiate the therapeutic effects of the drug.

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## CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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