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(RESEARCH ARTICLE)

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Development and characterization of Decitabine Niosomes

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Abstract

The present study was focused on formulating and evaluating Decitabine containing niosomes formulation for *In Vitro* studies. Niosomal formulations were prepared by using different ratio of surfactant (Tween 80 and Tween 20) and cholesterol by thin film hydration method and were evaluated for *In Vitro* characteristics, stability studies. Span 20 containing niosomal formulation displayed highest entrapment efficiency with desired particle size. SEM analyses showed that niosomal formulation was spherical in shape. Niosomes containing Tween 20 displayed higher percentage of drug release after 8 h as compared to other formulations. F-7 formulation was found to be stable at the end of the study on storage condition. The present study suggested that niosomal formulations provide sustained and prolonged delivery of drug with enhance bioavailability.

Keywords: Niosomes; Decitabine; Bioavailability; Thin Film Hydration Technique; *In Vitro* Drug Release Studies

1. Introduction

Niosomes are formations of vesicles by hydrating mixture of cholesterol and non-ionic surfactants. These vesicles are called niosomes [1]. These are formed by self-assembly of non-ionic surfactants in aqueous media as spherical, unilamellar, multilamellar system and polyhedral structures in addition to inverse structures which appear only in non-aqueous solvent [2]. Niosomes and liposomes are active in drug delivery potential and both increase drug efficacy as compared with that of free drug [3]. Niosomes preparation is affected by processes variables, nature of surfactants, and presence of membrane additives and nature of drug to be encapsulated. Niosomal drug delivery system perhaps is a useful strategy towards targeted drug delivery in cancer chemotherapy [4]. Decitabine is a hypomethylating agent. It hypomethylates DNA by inhibiting DNA methyltransferase. Decitabine is indicated for the treatment of myelodysplastic syndromes [5].

2. Material

Decitabine was obtained from Alkem Pvt Mumbai, Cholesterol and Tween 20 and 80 procured from SD fine chemicals Mumbai. Other chemicals and the reagents used were of analytical grade.

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3. Methodology

3.1. Fourier Transform Infrared Spectroscopy (FTIR) Study

FTIR is a useful technique to check and confirm any interaction that may occur between excipients and drug. The FTIR spectra of drug, excipients, briefly, solid sample (1 mg) along with 100 mg dried potassium bromide were compressed into a disc. For liquid sample, few drops of the sample were dripped onto NaCl or KBr aperture plate and sandwiched it under another aperture plate, such that no gas bubbles were trapped. The sample allowed formation of a thin liquid membrane between the two aperture plates. Thereafter, sample was scanned for absorbance over the range from 4000 to 400 (cm-1) wave numbers. The obtained spectrum was then compared with standard group frequencies of Decitabine [6].

3.2. Preparation of Niosomes

- **Niosome Preparation:** Decitabine niosomes were prepared using thin film-hydration method. Accurately weighed quantities of the surfactant (Tween 20 and Tween 80) and cholesterol in different Ratios in around-bottom flask. Afterwards, Decitabine dissolved in 5 ml of chloroform: methanol mixture (2:1) was added to the lipid solution. The organic solvents were removed under vacuum in a rotary evaporator at 40 ◦ C for 20 min to form a thin film on the wall of the flask, and kept in a desiccator under vacuum for 2 h to ensure total removal of trace solvents. After removal of the last trace of organic solvents, hydration of the surfactant film was carried out using 10 mL of distilled water at 55 ◦ C. The resulting niosomal suspension was mechanically shaken for 1 h using a horizontal mechanical shaking water bath at 55 ◦ C. Then, the vesicle suspension was sonicated in 3 cycles' of1min "on" and 1 min "off" leading to the formation of multi lamellar niosomes. The niosomal suspension was left to mature overnight at 4 ◦ C and stored at refrigerator temperature for further studies [7].

Table 1 Composition of Niosomal Decitabine (F1 to F8)

S.No.	Ingredients (mg)	F1	F2	F3	F4	F5	F6	F7	F8
1	Decitabine	20	20	20	20	20	20	20	20
2	Cholesterol	100	100	100	100	100	100	100	100
3	Tween 20	50	100	150	200	-		-	-
4	Tween 80	-		-	-	50	100	150	200
5	Methanol	5	5	5	5	5	5	5	5
6	Chloroform	5	5	5	5	5	5	5	5

3.3. Evaluation of Niosomes

3.3.1. Zeta-Potential

The sample was diluted with distilled water (1:100 (V/V)) and zeta potential was determined using Malvern Zetasizer (Nano ZS, Malvern Instruments, and United Kingdom). Measurement was based on the electrophoretic mobility of the particles, which was converted to the zeta potential by inbuilt software based on the Helmholtz-Smoluchowski equation [8].

3.3.2. Size and Size Distribution

Size and size distribution studies were done for niosomes prepared from Niosomes hydration. The Niosomes (100 mg) was hydrated in a small glass test tube using 10 ml of pH 7.4 phosphate buffer solution. The dispersion was observed under optical microscope at 40X magnification. Size and size distribution of 200–300 niosomes were noted using calibrated stage and ocular micrometers (Elico Instruments, Hyderabad). Similarly, size was noted for niosomes formed spontaneously from Niosomes after hydration without agitation in a cavity slide [9].

3.3.3. Entrapment Efficiency

To 0.2 g of Niosomes, weighed in a glass tube, 10 ml phosphate buffer pH 7.4 was added. The aqueous suspension was then sonicated. Niosomes containing Decitabine were separated from unentrapped drug by centrifugation at 9000 rpm

for 45 min at 4 ◦ C. The supernatant was recovered and assayed spectrophotometrically using UV spectrophotometer (UV-1800 Shimadzu, Japan), at 295 nm [10].

The encapsulation percentage of drug (EP) was calculated by the following equation

$$\mathbf{EP = [(C_t - C_r)/ C_t] * 100}$$

Where,

Ct, concentration of total Decitabine, Cr, concentration of free Decitabine.

3.3.4. Vesicle Physical Analysis

The shape, surface characteristics, and size of the niosomes were observed by scanning electron microscopy. Once again, 0.2 g of the Niosomes in a glass tube was diluted with 10 ml of pH 7.4 phosphate buffer. The niosomes were mounted on an aluminium stub using double-sided adhesive carbon tape. Then the vesicles were sputter-coated with gold palladium (Au/Pd) using a vacuum evaporator (Edwards) and examined using a scanning electron microscope (Hitachi 3700N, Germany) equipped with a digital camera, at 10 kV accelerating voltage [11].

3.3.5. In Vitro Drug Release Study

In Vitro release studies were carried out using unjacketed vertical Franz diffusion cells with a diffusional surface area of 6.154 cm^2 and 20 mL of receptor cell volume. Prior to the study, the dialysis membrane was soaked in phosphate buffer pH 7.4 Formulation equivalent to 5 mg of Decitabine was placed in the donor compartment. The receptor compartment consisting of PB pH 7.4 (containing 0.02 % w/v of ethanol to retard microbial growth) was maintained at 37±2 °C under constant stirring up to 24 hrs. The donor chamber and the sampling port were covered with lid to prevent evaporation during the study. Aliquots of 5 mL were withdrawn periodically at different time intervals and replaced with equal volume to maintain constant receptor phase volume. At the end of the study, the samples were suitably diluted and the amount of drug was determined spectrophotometrically at 295 nm [12].

3.3.6. Drug Release Kinetics [13]

The models used were zero order (equation 1) *First order* (equation 2) and *Higuchi model* (equation 3) and *Kores Meyer Pappas* model (equation 4).

Zero Order Kinetics:

$$R = Ko\ t \quad \ldots\ldots\ldots\ldots\ldots\ldots (1)$$

R=cumulative percent drug

Ko=zero order rate constant

First Order Kinetics

$$\log C = \log Co - K_1\ t / 2.303 \quad \ldots\ldots\ldots\ldots\ldots\ldots (2)$$

Where

C = cumulative percent drug

K_1 = first order rate constant

Higuchi Model

$$R = K_H\ t^{0.5} \quad \ldots\ldots\ldots\ldots\ldots\ldots (3)$$

Where

R = cumulative percent drug

K_H = higuchi model rate constant

Korsmeyer Peppas Model:

$$M t / M \alpha = K_k t^n$$

$$\log M t / M \alpha = \log K_{k+} n \log t \text{ -- } (4)$$

Where

$K_{k=}$ Korsmeyer Peppas rate constant

'M t / M α' is the fractional drug, n = diffusional exponent, which characterizes the mechanism of drug.

The obtained regression co-efficient (which neared 0.999) was used to understand the pattern of the drug from the Niosomes.

3.4. Stability Studies [14]

The formulations stored in glass vials covered with aluminium foil were kept at room temperature and in refrigerator (4 ◦ C) for a period of 90 days. At definite time intervals (10, 20, and 30 days), samples were withdrawn and hydrated with phosphate-buffered saline (pH 7.4) and observed for any sign of drug crystallization under optical microscope. Furthermore, the samples were also evaluated for particle size and percent retention of Decitabine.

4. Results and discussion

4.1. Drug - Excipient Compatibility Studies (FT-IR):

The IR spectrum of Decitabine and Excipients mixture was shown in respectively. In the present study, it has been observed that there is no chemical interaction between drug and the polymers used. From the figures it was observed that there were no changes in these main peaks in IR spectra of mixture of drug and polymers, which show there were no physical interactions because of some bond formation between drug and polymers. This further confirms the integrity of pure drug and compatibility of them with excipients.

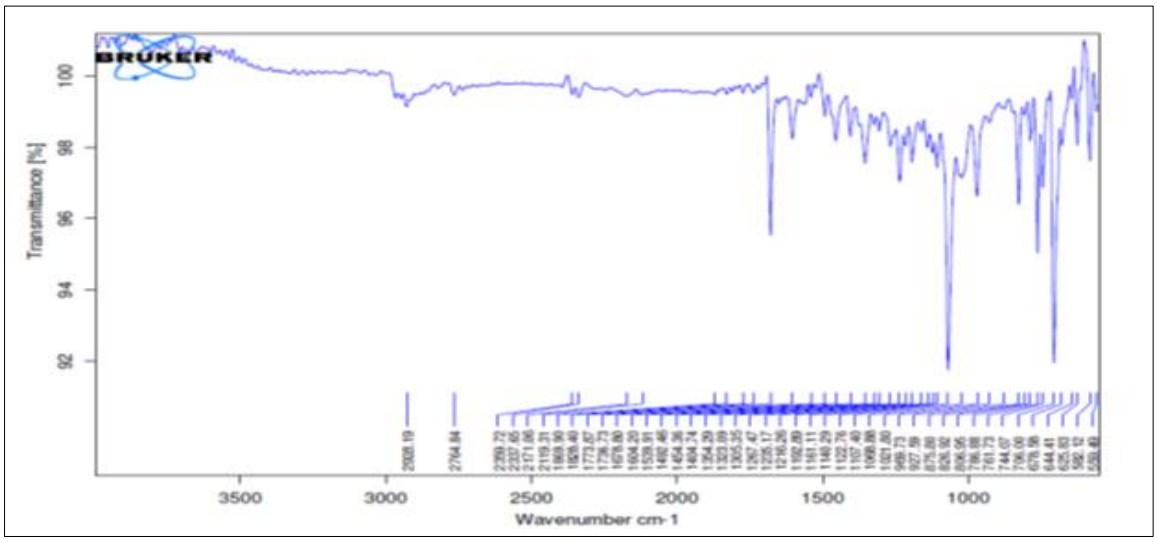

Figure 1 FTIR of Pure Drug

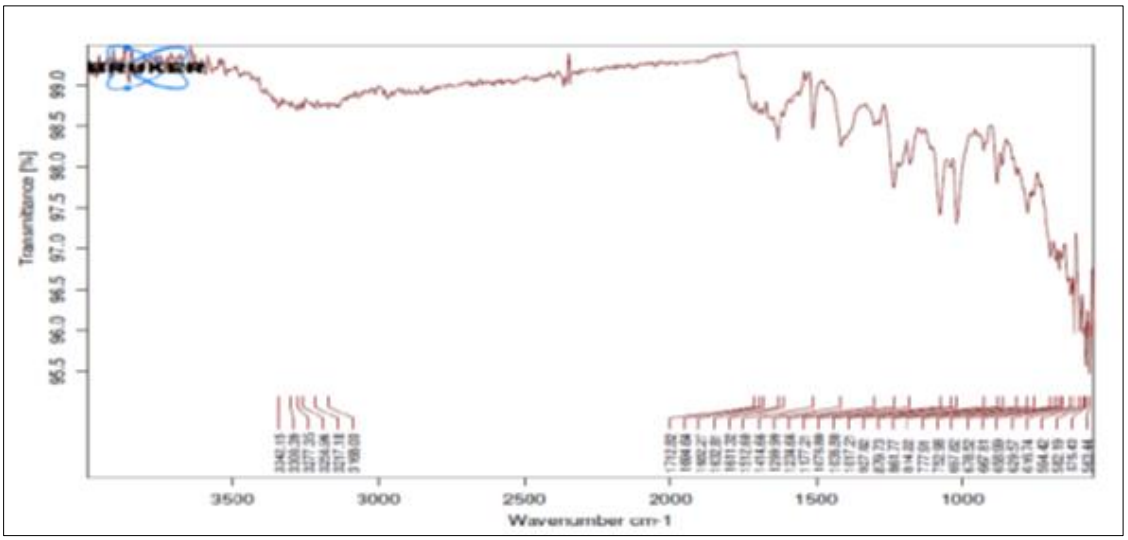

Figure 2 FTIR of Optimized Formulation

4.2. Evaluation parameters

4.2.1. Entrapment Efficiency

Separation of unentrapped drug from niosomal suspension was done by exhaustive dialysis method. A measured quantity of niosomal suspension was placed in a dialysis tube to which osmotic cellulose membrane was attached securely on one side and the dialysis tube was suspended in 100 ml of phosphate buffer pH 7.4 which was stirred continuously using magnetic stirrer. Through the osmotic cellulose membrane, the unentrapped drug was separated into the medium. For every one hour the whole medium was replaced with same quantity of fresh medium and the absorbance of collected medium reaches a constant reading indicating complete separation of unentrapped drug. The niosomal suspension in the dialysis tube was further lysed with propane–1–ol and the entrapped drug was estimated with the help of double beam UV spectrophotometer at 305 nm. The *entrapment efficiency* was measured in % with the help of following equation,

$$\%\ Entrapment\ efficiency = \frac{Amount\ of\ drug\ entrapped}{Total\ amount\ of\ drug\ added} \times 100$$

Table 2 Drug Entrapment Efficiency of All Formulation

F. No.	Drug Entrapment Efficiency
F1	80.26
F2	75.89
F3	79.63
F4	78.50
F5	77.51
F6	79.68
F7	77.50
F8	74.25

4.2.2. Determination of Vesicle Morphology and Size

The morphological characteristics of formulated niosomes were carried by using Scanning electron microscopy (SEM). A small drop of niosomal suspension was placed between two rivets fixed on a gold plated copper sample holder. The whole system was slushed under vacuum in liquid nitrogen. The sample was heated to -85 ^{0}C for 30 min to sublime the

surface moisture. Finally the sample was coated with gold and allowed the SEM to capture the images at a temperature of -120 ^{0}c and voltage of 5 kV.

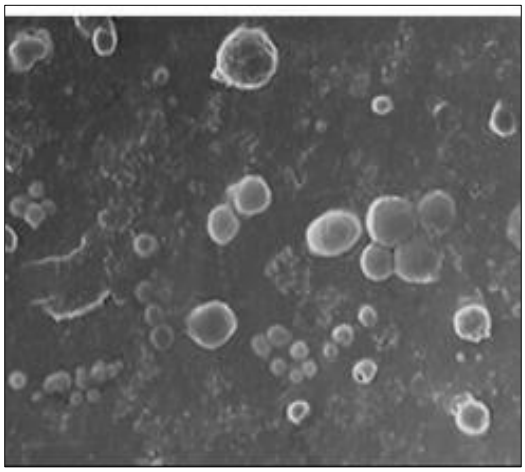

Figure 3 SEM Analysis of Optimized Niosomes

4.3. Zeta Potential

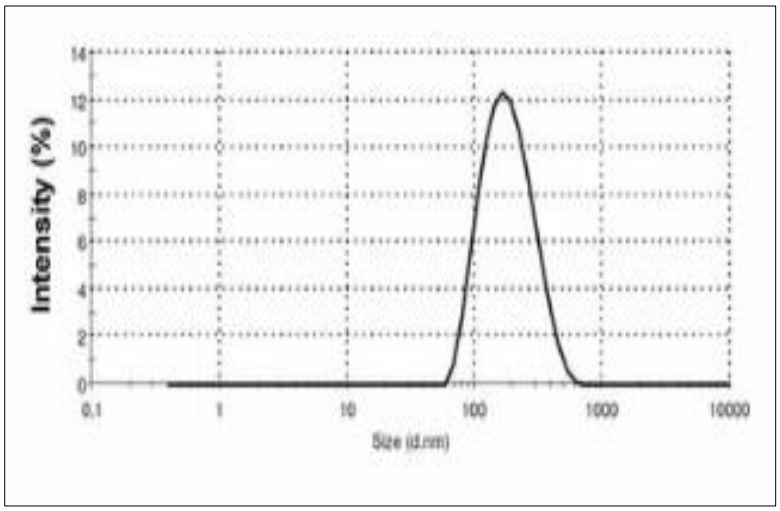

Figure 4 Particle Size Analysis of Optimized Niosomes

Table 3 Evaluation Studies of Particle Size and Zeta Potential Niosomes

F. No.	Particle Size (nm)	Zeta Potential
F1	185.63	-32
F2	178.20	-40
F3	184.93	-35
F4	182.35	-41
F5	179.80	-30
F6	181.22	-42
F7	175.86	-38
F8	184.58	-37

4.4. *In Vitro* Drug Release Studies

The release of drug from niosomes was investigated using dialysis tubing method. All the formulations were separately placed in a dialysis membrane of 5 cm length with closed ends which was washed and soaked in phosphate buffer pH 7.4 for about 15 min. The membrane was suspended in a beaker containing 500 ml of phosphate buffer pH 7.4 as diffusion medium maintained at a temperature of 37 ± 0.5 ^{0} C and stirred continuously by means of magnetic stirrer at a constant speed. At a regular time, interval of one-hour 5 ml of diffusion medium was withdrawn periodically for about 8 hrs and immediately replaced with same amount of fresh diffusion medium to maintain sink condition. The collected samples were measured spectrophotometrically at 305 nm.

Table 4 *In Vitro* Drug Release Profiles of Decitabine Niosomes (F1-F8)

Time (hrs)	% Cumulative Drug Released							
	F1	F2	F3	F4	F5	F6	F7	F8
0	0	0	0	0	0	0	0	0
1	19.68	15.82	16.89	15.34	14.58	17.82	13.65	14.96
2	28.96	27.41	25.94	27.96	25.96	22.25	29.85	27.56
3	36.85	34.68	32.52	30.25	35.68	34.96	36.52	35.89
4	49.68	45.35	44.21	43.58	46.93	45.98	44.85	46.58
5	53.82	51.68	50.92	52.34	53.25	50.25	52.14	55.58
6	69.86	65.98	64.58	67.92	64.19	65.58	67.98	65.55
7	77.86	75.64	74.96	73.21	76.98	72.17	75.17	74.10
8	93.35	91.25	90.25	90.21	92.21	93.02	92.55	90.22

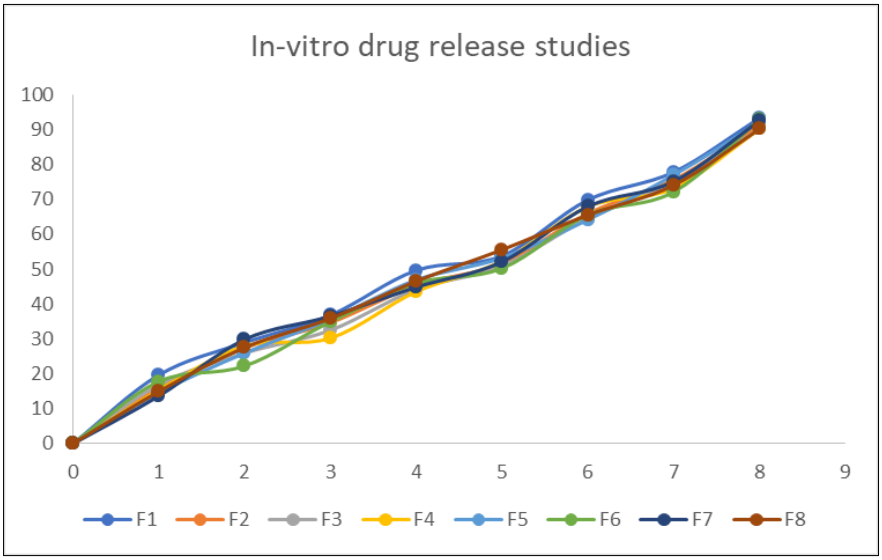

Figure 5 Drug Release Formulations

4.5. Release Order Kinetics

Table 5 Drug Release Kinetics of Formulation F1

TIME	%CDR	SQARE T	LOG T	LOG%CDR	ARA	LOG%ARA
0	0	0	0	0	0	0
1	19.68	1	0	1.29402509	80.32	1.9048237
2	28.96	1.41421356	0.30103	1.46179856	71.04	1.85150295
3	36.85	1.73205081	0.47712	1.56643749	63.15	1.80037335
4	49.68	2	0.60206	1.69618159	50.32	1.70174063
5	53.82	2.23606798	0.69897	1.73094369	46.18	1.66445393
6	69.86	2.44948974	0.77815	1.84422858	30.14	1.47914325
7	77.86	2.64575131	0.8451	1.8913144	22.14	1.34517762
8	93.35	2.82842712	0.90309	1.97011432	6.65	0.82282165

4.6. Zero Order Kinetics

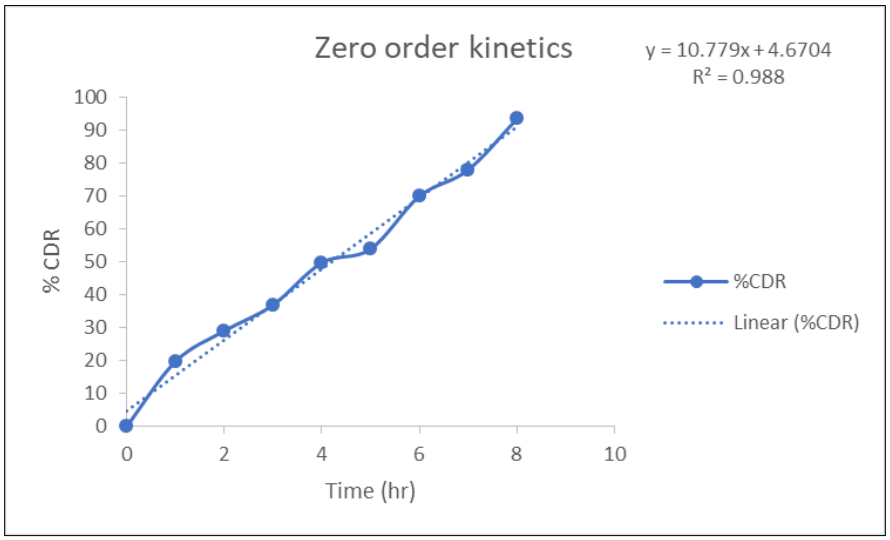

Figure 6 Zero Order Kinetics Optimized Formulation

4.7. First Order Kinetics

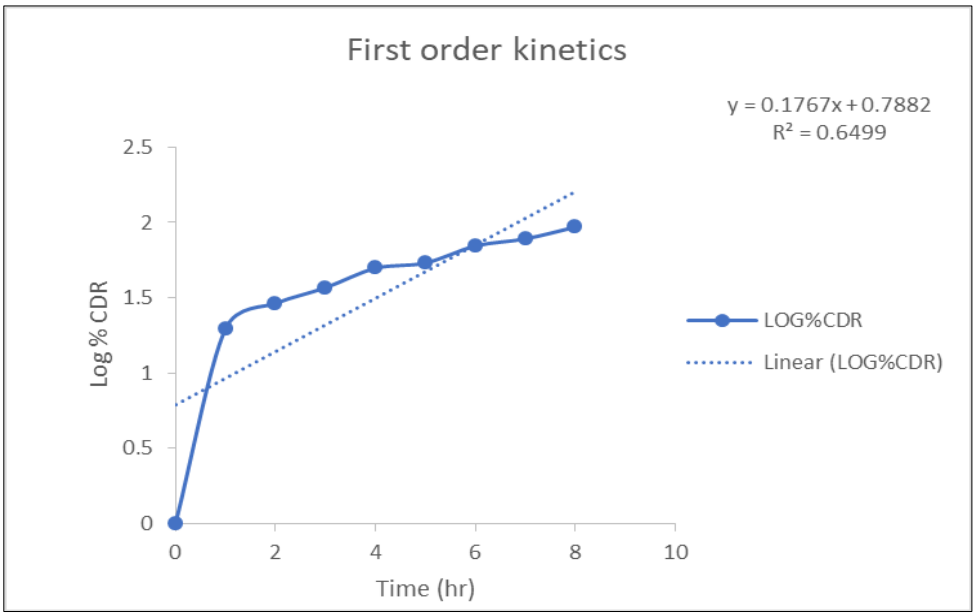

Figure 7 First Order Kinetics Optimized Formulation

4.8. Higuchi Model

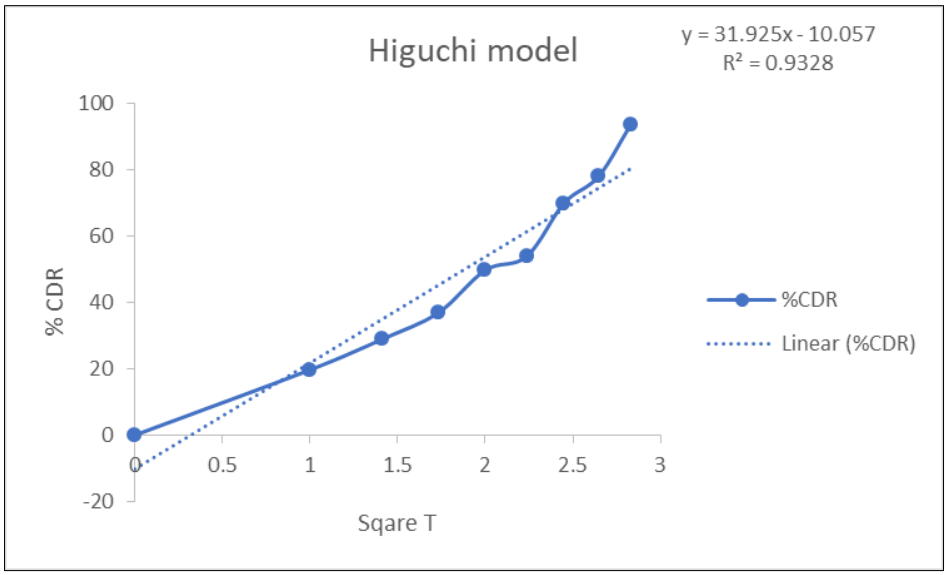

Figure 8 Higuchi Model of Optimized Formulation

4.9. Korsmeyer Peppas Model

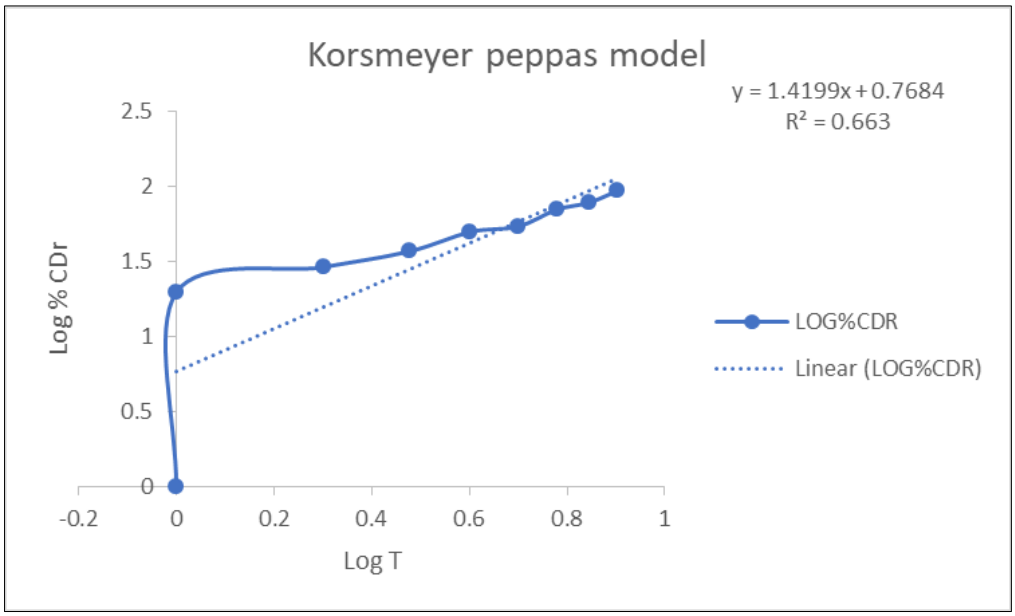

Figure 9 Korsmeyer Peppas Optimized Formulation

The values of *In Vitro* release were attempted to fit into various mathematical models. Plots of zero order, first order, Higuchi matrix, Peppas were respective.

4.10. Stability Studies

Optimized formulations F1 was selected for accelerated stability studies as per ICH guidelines. The patches were observed for color, appearance and flexibility for a period of three months. The folding endurance, weight, drug content, % cumulative drug release of the formulation was found to be decreasing. This decrease may be attributed to the harsh environment (40 ^{0}C) maintained during the studies.

Table 6 Stability Studies of Optimized Formulations at 40 ± 2 ^{0}C and 75 ± 5% RH for 3 Months

Formulation Code	Initial	1st Month	2nd Month	3rd Month	Limits as per Specifications
F-1	93.35	92.36	91.96	90.36	Not less than 85 %
F-1	93.35	92.95	91.85	90.58	Not less than 85 %
F-1	93.35	92.10	91.80	90.37	Not less than 85 %

5. Conclusion

The goal of the current formulation study on Decitabine is to create a niosomal drug delivery system and assess how well it functions *in vitro*. Different proportions of cholesterol and surfactant were used to create the formulations. High entrapment efficiency is regarded as the ideal or best Niosome formulation. This study discovered that the ratio of cholesterol to surfactant affected *entrapment efficiency*. Formulations were discovered to guarantee the drug's good oral bioavailability. The niosomes were seen to be smooth-surfaced, spherical vesicles. The highest entrapment efficiency was demonstrated by Formulation F1. These facts lead to the conclusion that niosomes may be a promising method for increasing Decitabine bioavailability.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

References

[1] Kausalya A, Borawake PD, Shinde JV, Chavan RS. Niosomes: A Novel Carrier Drug Delivery System. JDDT [Internet]. 15Jan.2021 [cited 13Jul.2024]; 11(1):162-70

[2] Ge X, Wei M, He S, Yuan WE. Advances of Non-Ionic Surfactant Vesicles (Niosomes) and Their Application in Drug Delivery. Pharmaceutics. 2019 Jan 29; 11(2):55.

[3] G. Parthasarathi SEM imaging predicts quality of niosomes from maltodextrin- based Proniosomes. Pharm. Res. 2001; 18: 656-661.

[4] Kazi KM, Mandal AS, Biswas N, Guha A, Chatterjee S, Behera M, Kuotsu K. Niosome: A future of targeted drug delivery systems. J Adv Pharm Technol Res. 2010 Oct; 1(4): 374-80.

[5] Parthasarathi G, Udupa N, Umadevi P, Pillai GK. Niosome encapsulated of vincristine sulfate: Improved anticancer activity with reduced toxicity in mice. J Drug Target. 1994; 2: 173–82.

[6] Deventra, Stermberg B, Florence AT. Preparation and properties of vesicles (niosomes) of Sorbitan monoesters (Span 20, 40, 60, and 80) and a sorbitantriester (Span 85) Int J Pharm.

[7] 1994; 105: 1–6.

[8] Piyush, editor. New York: Alan and Unwin and Soloman Press; 1985. The Physical Chemistry of Membranes; pp. 209–30.

[9] Hamid, Novel Niosomal Combination of Selenium Coupled with Glucantime against Leishmania tropica. Korean J Parasitol. 2019 Feb; 57(1):1-8.

[10] Chaudhari, p. D., & Desai, U. S. (2019). Formulation and evaluation of niosomal in situ gel of prednisolone sodium phosphate for ocular drug delivery. International Journal of Applied Pharmaceutics, 11(2), 97–116.

[11] Tulsi Bisht, Formulation Evaluation and Optimization of Niosomal Gel of Piroxicam, IOSR Journal Of Pharmacy Volume 9, Issue 5 Series. II (May 2019), PP. 01-07

[12] Namrata Mishra, Journal of Scientific and Innovative Research , Journal of Scientific and Innovative Research , JSIR 2014; 3(3): 337-341

[13] Rai A. Niosomes: An approach to current drug delivery-A Review. International Journal of Advances in Pharmaceutics. 2017; 06: 41-48.

[14] Srivastav A, Das P. To Study the Formulation of Niosome of Ofloxacin and Its Evaluation for Efficacy of Anti-Microbial. International Journal of Innovative Research in Science, Engineering and Technology. 2014; 3: 17958-17965.

[15] Bayindir Z, Yuksel N. Characterization of niosomes prepared with various non-ionic surfactants for paclitaxel oral delivery. Journal of Pharmaceutical Sciences. 2010; 99: 2049-2060.