FORMULATION AND EVALUATION OF LIPOSOMES CONTAINING ANTI-CANCER DRUG DECITABINE

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ABSTRACT: ***The drug release from Liposomes depends on many factors including the composition of Liposomes, the type of drug encapsulated and nature of the cell. Once it is released a drug that normally crosses the membrane of a cell will enter the cell, other drugs will not enter. Decitabine is a drug with narrow therapeutic index and short biological half-life. This study aimed at developing and optimizing liposomal formulation of Decitabine in order to improve its bioavailability. In evaluation study the effect of the varying composition of lipids on the properties such as encapsulation efficiency, particle size and drug release were studied. Phase transition study was carried out to confirm the complete interaction of Decitabine with bilayer structure of liposome. Moreover, the release of the drug was also modified and extended over a period of 8 h in all formulations. F3 emerged as the most satisfactory formulation in so far as its properties were concerned. Further, release of the drug from the most satisfactory formulation (F3) was evaluated through dialysis membrane to get the idea of drug release.***

Keywords: Liposomes, Decitabine, bioavailability, thin film hydration technique, in vitro drug release studies.

I. INTRODUCTION

Liposomes are spherical microscopic visicles composed of one or more lipid bilayers, separated by water or aqueous buffer compartments with a diameter ranging from 25nm to 1000µm.[1] Depending on the nature of the drugs, liposomes have one or more phospholipid bilayers, to transport drug materials[2]. Decitabine liposomes are prepared by using thin film hydration technique by using rotary evaporator. Decitabine is a cytidine deoxynucleoside analog, which acts by inhibiting DNA methyltransferase, inducing DNA hypomethylation7,8. It is used for the treatment of acute myeloid leukemia (AML) in patients aged ≥65 years. However, it can only be administered intravenously due to very low oral bioavailability and a large distribution volume. Decitabine is a hydrophilic drug (log P=−2.2), with a short half-life (25 minutes), and is sensitive to harsh conditions[3] Multilamellar vesicles (MLVs) were prepared using thin lipid film hydration method. Various factors such as phosphatidylcholine and cholesterol ratio, lipid and drug ratio, incorporation of charged species and pH etc. were studied which may affect the size, shape and incorporation efficiency of liposomes.[4] Liposomes were evaluated by optical microscope. Analysis of drug content was carried on UV spectrophotometer. *In vitro* release rate studies were conducted on specially designed. Thus the present study was to refine the formulation of elastic liposomes of Decitabine in order to enhance its bioavailability.[5]

II. MATERIALS AND METHODS

2.1. Materials

Decitabine was obtained from Hetero labs private limited (HYD, India). Phosphotidyl choline and cholesterol were procured from Vijaya chemicals, Hyderabad and other chemicals and reagents used were of analytical grade.

2.2 Methods

Drug and excipient compatibility studies[6]

Drug excipients compatibility studies were performed to know the compatibility of excipient with drug at accelerated conditions. The study was conducted by preparing homogenous mixture of excipients with drug and filled in HDPE bags and LDPE bags. Glass vials were exposed to 600 C and 400C/75 %RH for 4 weeks and LDPE bags were exposed to 400C±75 %RH for 4 weeks. Samples were observed periodically for any physical change.

Preparation of liposomes [7,8]

Method

Liposomes were prepared by physical dispersion method using different ratio of lipids.

In this method the lipids were dissolved in chloroform. This solution of lipids in chloroform was spread over flat bottom conical flask. The solution was then evaporated at room temperature without disturbing the solution. The

hydration of lipid film form was carried out with aqueous medium phosphate buffer (pH 7.4). For this the flask was inclined to one side and aqueous medium containing drug to be entrapped was introduced down the side of flask and flask was slowly returned to upright orientation. The fluid was allowed to run gently over lipid layer and flask was allowed to stand for 2 h at 37^0C for complete swelling. After swelling, vesicles are harvested by swirling the contents of flask to yield milky white suspension. Then formulations were subjected to centrifugation. Different batches of liposomes were prepared in order to select an optimum formula. All batches of liposomes were prepared as per the general method described above.

Table - 1: composition of lipids for preparation of liposome

Ingredients	F1	F2	F3	F4	F5	F6
Phosphatidylcholine	200	250	300	350	400	450
Cholesterol	50	75	100	100	75	50
Solvent(Chloroform)	10	10	10	10	10	10
Decitabine	10	10	10	10	10	10
Phosphate buffer pH 7.4	10	10	10	10	10	10

Evaluations of liposomes

Particle size analysis[9]

All the prepared batches of liposomes were viewed under microscope to study their size. Size of liposomal vesicles from each batch was measured at different location on slide by taking a small drop of liposomal dispersion on it and average size of liposomal vesicles were determined.

Drug entrapment efficiency of liposomes[10]

Entrapment efficiency of liposomes were determined by centrifugation method. Aliquots (1 ml) of liposomal dispersion were subjected to centrifugation on a laboratory centrifuge (Remi R4C) at 3500 rpm for a period of 90 min. The clear supernatants were removed carefully to separate non entrapped Decitabine and absorbance recorded at 245nm. The sediment in the centrifugation tube was diluted to 100 ml with phosphate buffer pH 7.4 and the absorbance of this solution was recorded at 245 nm.

Amount of Decitabine in supernatant and sediment gave a total amount of Decitabine in 1 ml dispersion.

% entrapment of drug was calculated by the following formula

$$\% \text{ Drug Entrapped (PDE)} = \frac{\text{Amount of drug in sediment}}{\text{Total amount of drug}} \times 100$$

***In Vitro* Drug release study**[11]

The release studies were carried out in 10 ml Franz diffusion cell containing 10 ml Phosphate buffer. Phosphate buffer pH 7.4 (10ml) was placed in a 10 ml beaker. The beaker was assembled on a magnetic stirrer and the medium was equilibrated at 37 ± 5^0C. Dialysis membrane was taken and one end of the membrane was sealed. After separation of non entrapped Decitabine liposomal dispersion was filled in the dialysis membrane and other end was closed. The dialysis membrane containing the sample was suspended in the medium. 1sml of aliquots were withdrawn at specific intervals, filtered after withdrawal and the apparatus was immediately replenished with same quantity of fresh buffer medium.

Mechanism of drug [12]

The models used were zero order (equation 1) First order (equation 2) and Higuchi model (equation 3) and Koresmeyer Peppas model (equation 4).

i) zero order kinetics:

$$R = K_o t \quad \text{-- (1)}$$

R=cumulative percent drug

Ko=zero order rate constant

ii) First order kinetics

$$\log C = \log Co - K_1 t / 2.303 \quad \text{-- (2)}$$

where C = cumulative percent drug

K_1 = first order rate constant

iii) Higuchi model

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

Where R = cumulative percent drug

K_H = higuchi model rate constant

iv) korsermeyer peppas model:

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

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

where K_k = korsermeyer peppas rate constant

'M t / M α' is the fractional drug , n = diffusional exponent, which characterizes the mechanism of drug (Simon Benita, 2007).

Stability studies[13]

The success of an effective formulation can be evaluated only through stability studies. The purpose of stability testing is to obtain a stable product which assures its safety and efficacy up to the end of shelf life at defined storage conditions and peak profile.

The prepared Decitabine liposomes were placed on plastic tubes containing desiccant and stored at ambient conditions, such as at room temperature, 40±2°c and refrigerator 2-8°c for a period of 30 days.

III. RESULTS & DISCUSSION

Drug - excipient compatibility studies (FT-IR)

The compatibility between the drug and the selected lipid and other excipients was evaluated using FTIR peak matching method. There was no appearance or disappearance of peaks in the drug-lipid mixture, which confirmed the absence of any chemical interaction between the drug, lipid and other chemicals.

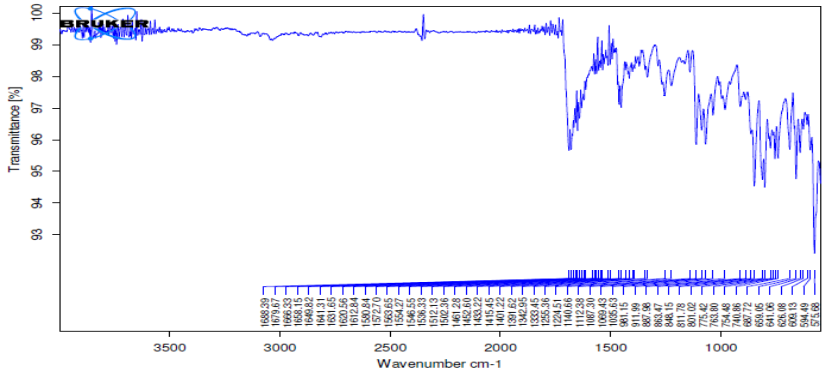

Fig.1. FTIR Studies of Pure Drug (Decitabine)

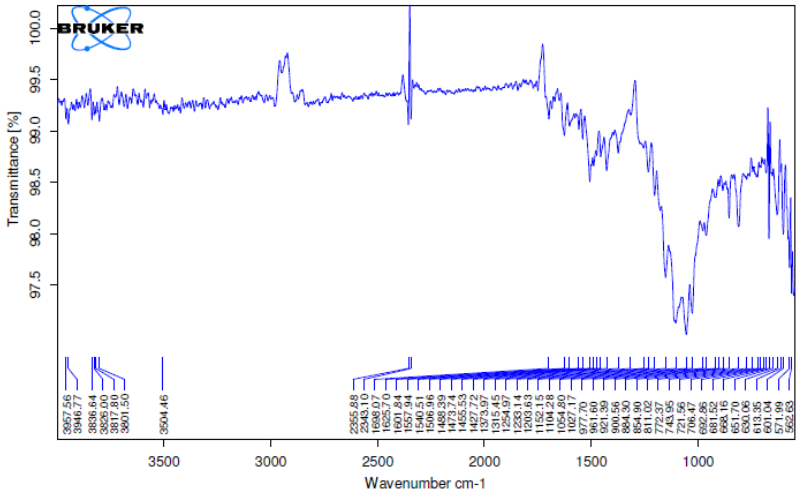

Fig.2. FTIR Studies of Decitabine and phosphotidylcholine

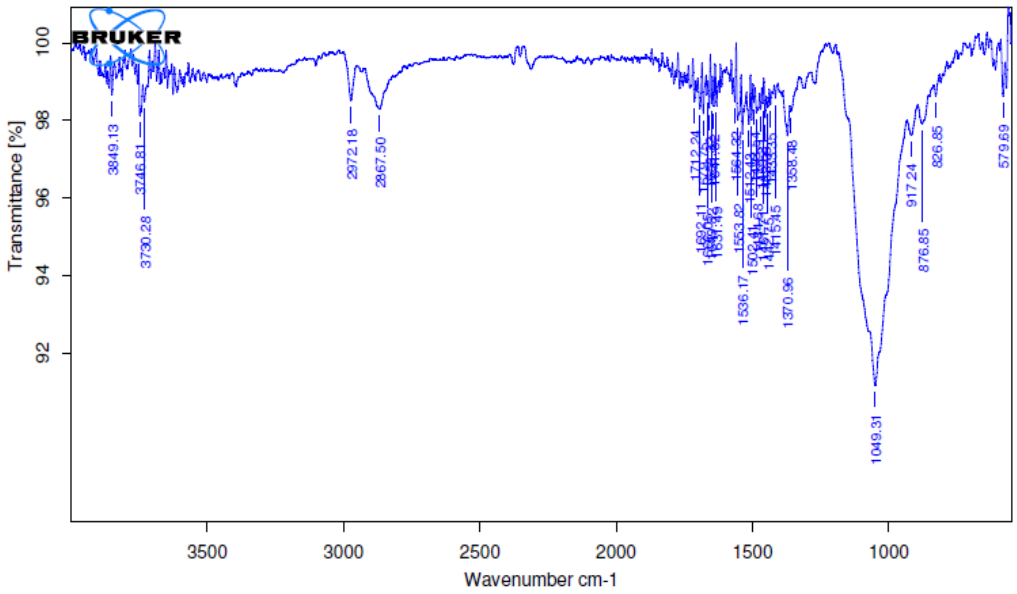

Fig.3. FTIR Studies of Decitabine and cholesterol

Particle size

Vesicle shape: Vesicle shape of the prepared formulation was found to be spherical from the SEM(scanning electron microscope) analysis at 15.00kV

2. Vesicle size:

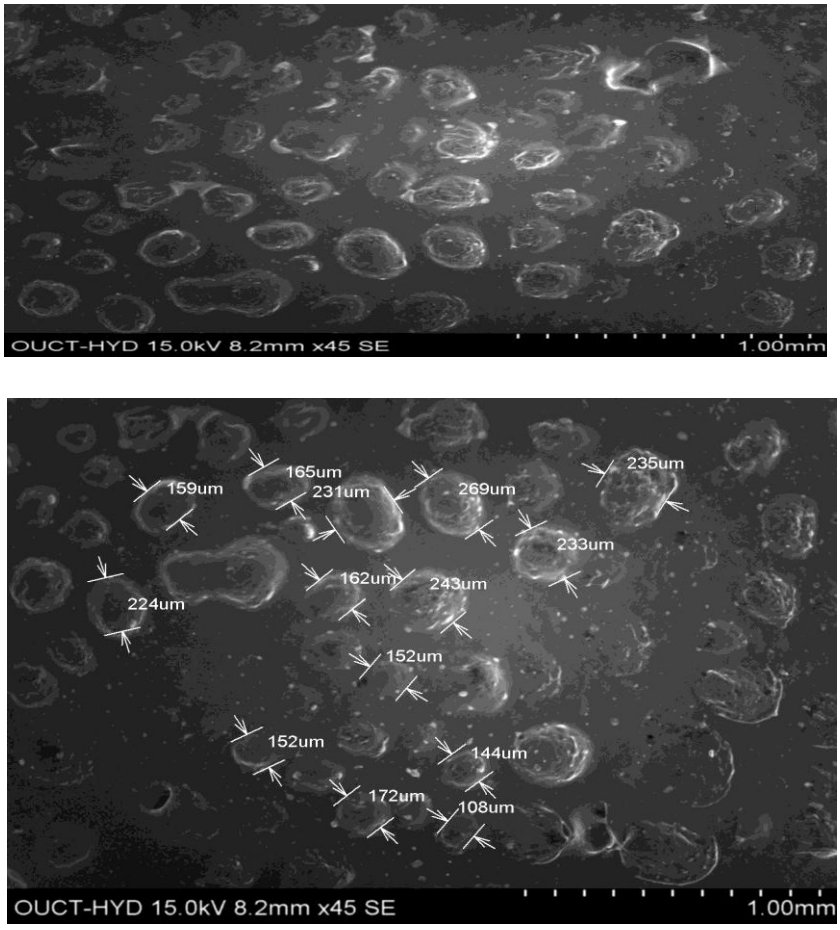

Fig.4. particle size of Decitabine liposomes

Table-2: Mean particle size (mps) of different formulation of liposomes

Sr. No	Formulation No.	MPS µm± SD
1	F1	7.10± 0.04
2	F2	8.09 ± 0.85
3	F3	11.81± 0.57
4	F4	6.98± 0.42
5	F5	9.85± 0.12
6	F6	10.25± 0.36

Drug entrapment efficiency

Table-3 : Different batches of liposome made by using different ratio of lipids

Sr. No	Formulation no.	PDE
1	F1	53.21± 0.79
2	F2	65.33 ± 0.90
3	F3	80.69 ± 0.64
4	F4	63.89± 0.79
5	F5	76.33 ± 0.54
6	F6	74.69 ± 0.43

Drug release studies

Table-4: Cumulative percentage drug release from various formulation of liposomes

Time (Min)	Batch code					
	F1	F2	F3	F4	F5	F6
0	0	0	0	0	0	0
1	17.35	19.85	22.60	18.35	20.80	23.12
2	23.08	23.63	34.18	27.09	33.12	34.18
3	35.15	36.10	47.30	35.13	46.10	40.30
4	40.39	49.32	51.35	46.38	50.32	53.39
5	51.81	61.52	63.24	54.51	61.55	62.24
6	62.47	74.25	75.54	64.40	72.13	76.52
7	76.16	85.15	83.08	72.16	80.32	81.08
8	85.50	92.43	97.10	88.74	93.69	94.59

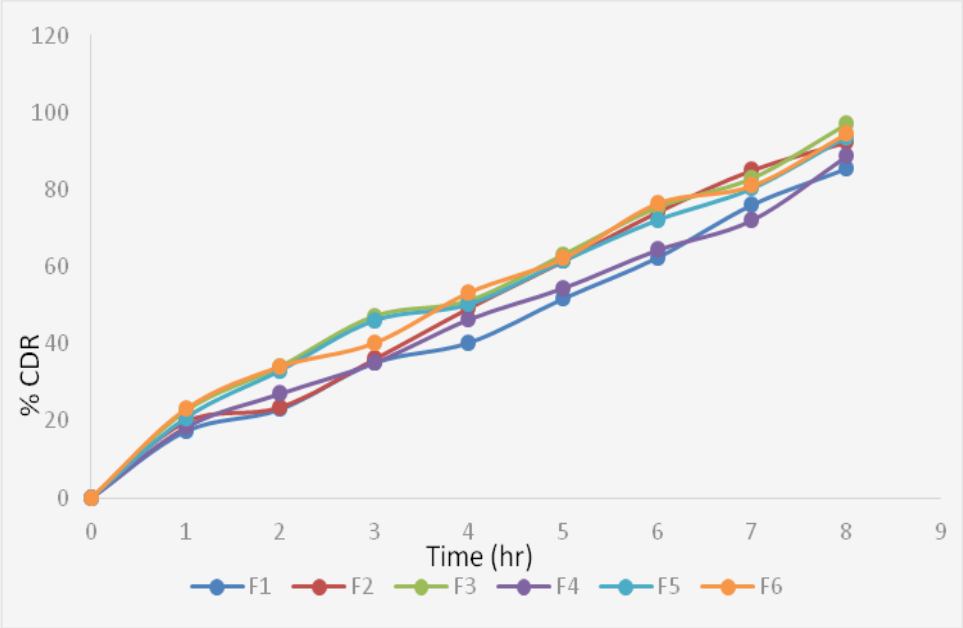

Fig.5. *In vitro* drug release of various formulations

All the three batches of formulation F3 were found to release the drug in 6 h. The cumulative percentage release was found to be 97.10%.

Kinetic modeling of drug release

All the six formulation of prepared liposomes of Decitabine were subjected to in vitro release studies these studies were carried out using dissolution apparatus.

The results obtaining in vitro release studies were plotted in different model of data treatment as follows:

1. Cumulative percent drug released vs. time (zero order rate kinetics)
2. Log cumulative percent drug retained vs. time (First Order rate Kinetics)
3. Cumulative percent drug released vs. square root of time (Higuchi's Classical Diffusion Equation)
4. Log of cumulative % release Vs log time (Peppas Exponential Equation)

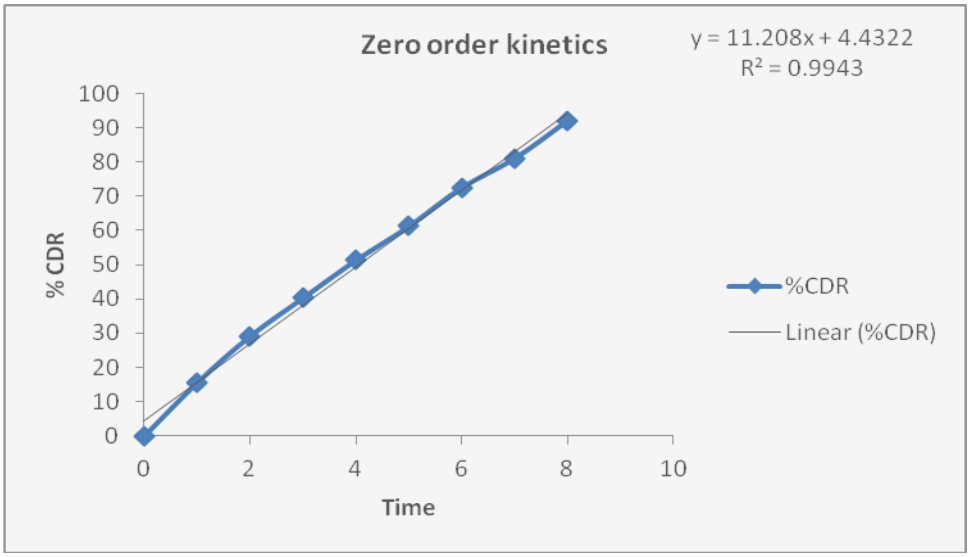

Fig.6. Zero order kinetics

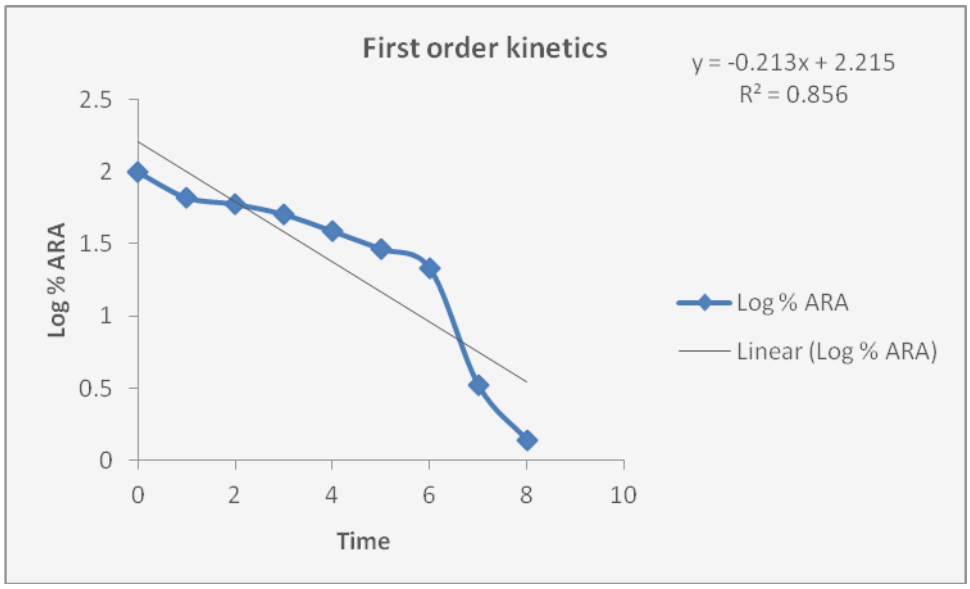

Fig.7. First order kinetics

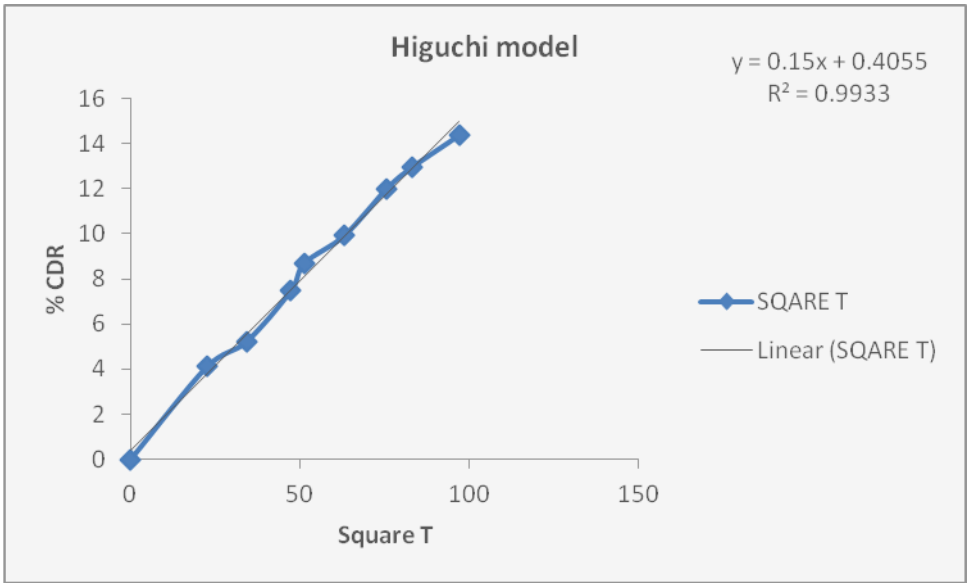

Fig.8. Higuchi model

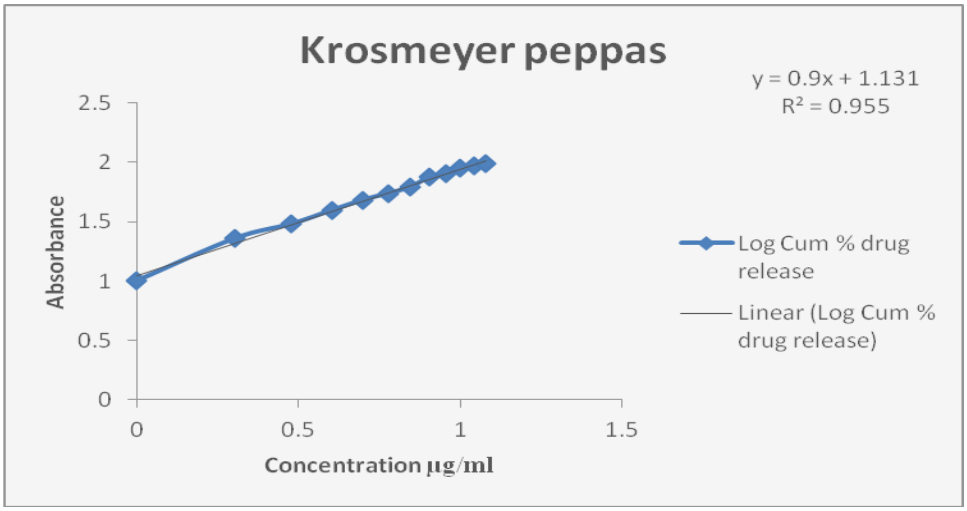

Fig.9. krossmayer peppas

The values of in vitro release were attempted to fit into various mathematical models. Plots of zero order, first order, Higuchi and peppas.

Regression values are higher with Zero order release kinetics. Therefore all the Decitabine liposomes follows Zero order release kinetics.

The table indicates that r^2 values are higher for Higuchi's model compared for all the liposomes. Hence Decitabine release from all the liposomes followed diffusion rate controlled mechanism.

Stability studies

There was no significant change in physical and chemical properties of the liposomes of formulation F-3 after 3 months. Parameters quantified at various time intervals were shown

Table-5 : Results of stability studies of optimized formulation F-3

Formulation Code	Parameters	Initial	1st Month	2nd Month	3rd Month	Limits as per Specifications
F-3	25^0C/60%RH % Release	97.10	97.11	97.12	97.14	Not less than 85 %
F-3	30^0C/75% RH % Release	97.10	97.12	97.15	97.16	Not less than 85 %
F-3	40^0C/75% RH % Release	97.10	97.13	97.14	97.15	Not less than 85

IV. CONCLUSION

From the performed work it was concluded that: Decitabine possesses all requisite qualities required for liposomal drug delivery. Among the various formulation, the combination F3 was found to be most suitable because of high encapsulation efficiency with smaller particle size.The formulation F3 comprising phosphatidylcholine, cholesterol, fulfills the requirement of good liposomal formulation. *In vitro* drug release up to 8 h and more than 97.10 % drug released. Follows Peppas model in release studies. It shows encapsulation efficiency of 80.69% and particle size of 11.81 µm.

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