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Formulation and evaluation of liposomal drug delivery system of 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 short biological half-life. This study aimed at formulating and evaluating liposomal formulation of Decitabine in order to enhance 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. F1 emerged as the most satisfactory formulation in so far as its properties were concerned. Further, release of the drug from the most satisfactory formulation (F1) was evaluated through dialysis membrane to get the idea of drug release. The mechanism of dug release was governed by Peppas model.

Keywords: Liposomes, Decitabine, Bioavailability.

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. Liposomes composed of one or more amphiphilic phospholipids bilayer membranes that can entrap both hydrophilic and hydrophobic drugs. Spherical vesicle with a membrane composed of phospholipid bilayer used to deliver drug in to a cell. Decitabine a treatment of myelodysplastic syndrome (MDS). Decitabine half life is 30 minutes. Decitabine is soluble in water, slighty soluble in methanol and ethanol. Decitabine liposomes are prepared by using thin film hydration technique by using rotary evaporator. Liposomes were prepared using Phosphotidyl choline, cholesterol and prepared liposomes were evaluated by particle size, drug entrapment, drug release studies and stability studies.

MATERIAL AND METHODS
MATERIAL

Decitabine was collected as a gift sample from aurbindho, Hyderabad and various excipients like Phosphotidyl choline, cholesterol and chloroform were purchased from AR chemicals, Hyderabed.

METHODODOLOGY

Method of preparation

Thin film hydration technique

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 No 1 : Optimized formula for liposome preparation

Sr. No.	Constituents	Quantity
1	Phosphatidylcholine	250 mg
2	Cholesterol	25 mg
3	Solvent(Chloroform)	5 ml
4	Drug	200 mg
5	Phosphate buffer pH 7.4	10 ml

RESULTS AND DISCUSSION

Drug and 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.

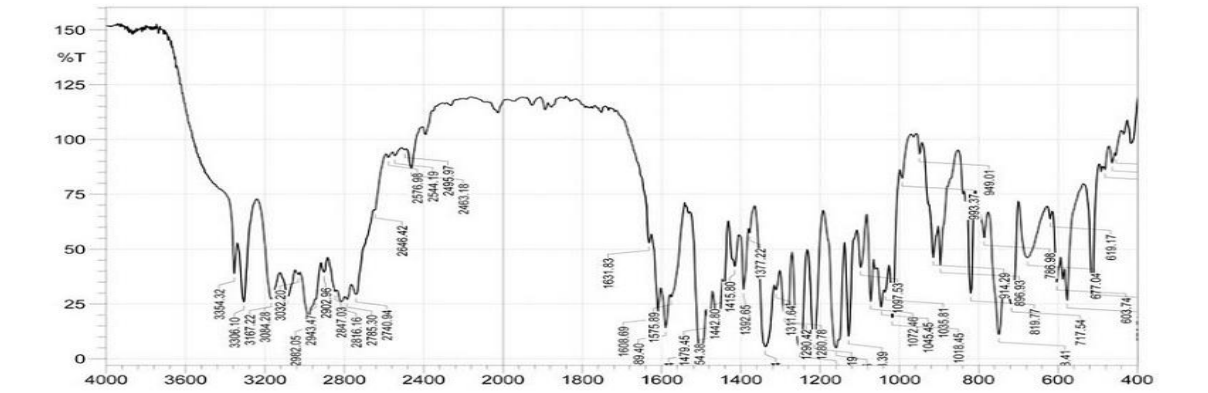


Figure No 1: FTIR Spectra of Glipizide pure drug

Table No 2: FTIR spectra data for pure Decitabine

S. no	Functional groups	FTIR absorption band of pure Decitabine
1	C-N	1215
2	CH(Alkane)	2847
3	N-H(Bending)	1630
4	OCH_3	1159
5	C=C	3306

EVALUATION OF LIPOSOMES

Drug entrapment efficiency of liposomes

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

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

Table No 3: Results of entrapment efficiency of liposomes of formulations

Observations	Batch code		
	F1	F2	F3
1	48.23	47.62	47.47
2	47.38	48.70	49.15
3	49.73	46.80	47.63
Mean	48.33	47.71	48.08
Mean ± S.D.	48.33±1.000	47.70±0.566	48.09±1.545

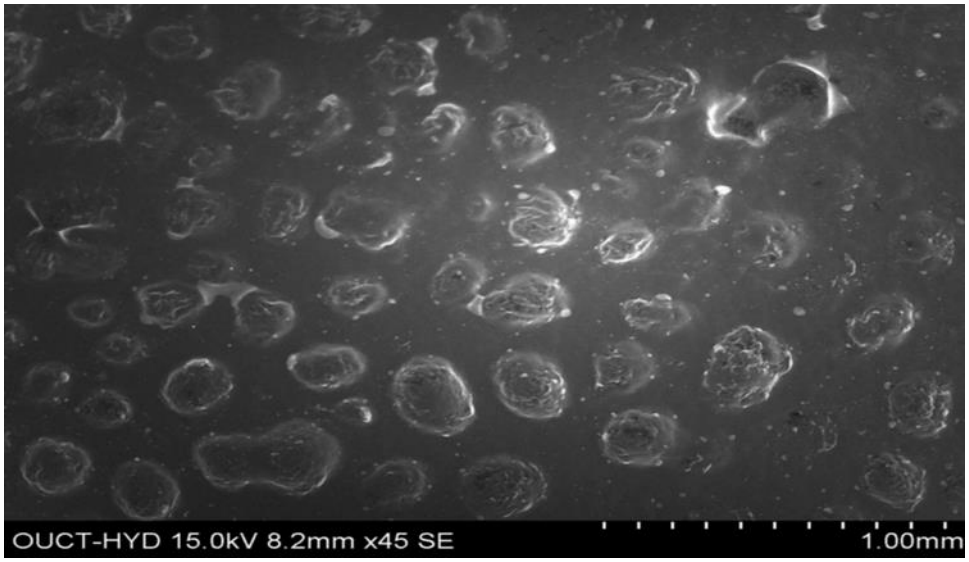
Now, let H_0 be the hypothesis that there is no significant difference between the batches.

Particle size analysis

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.

Vesicle shape

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



Vesicle size

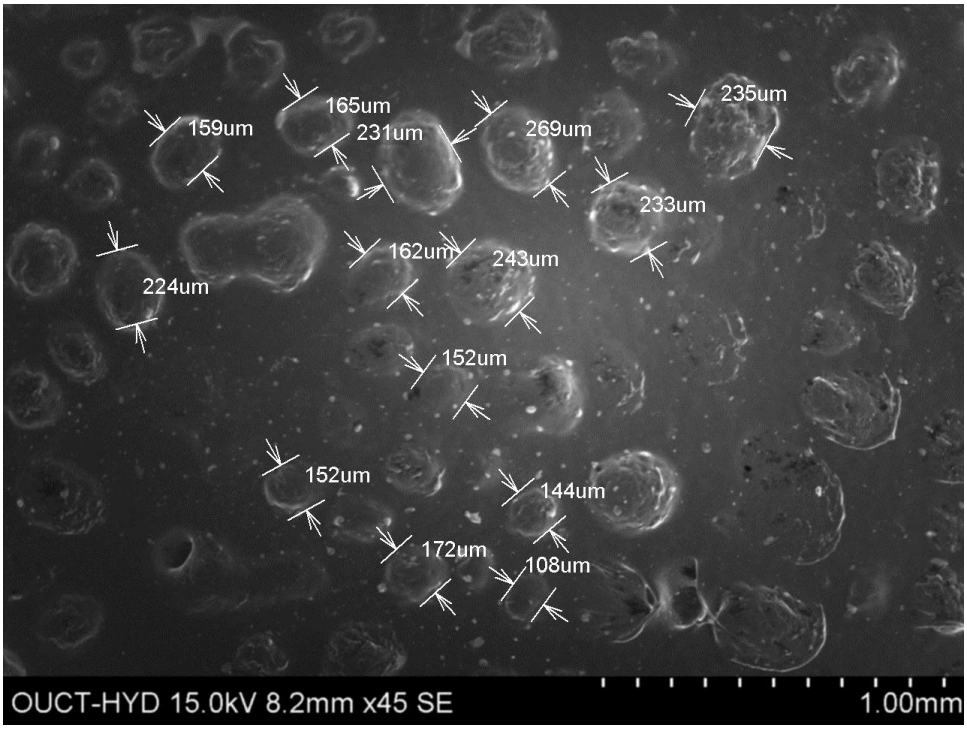


Figure No 3: Particle size of Decitabine liposomes

Table: Vesicle size

Formulation	Size (µm)
F1	126
F2	120
F3	214

Table No 4: Results of particle size of liposomes

Observations	Batch code		
	F1	F2	F3
1	6.43	7.22	6.08
2	7.15	6.74	7.20
3	6.62	7.43	6.70
Mean	6.72	7.22	6.5
Mean ± S.D.	6.76±0.097	7.24±0.050	6.7±0.062

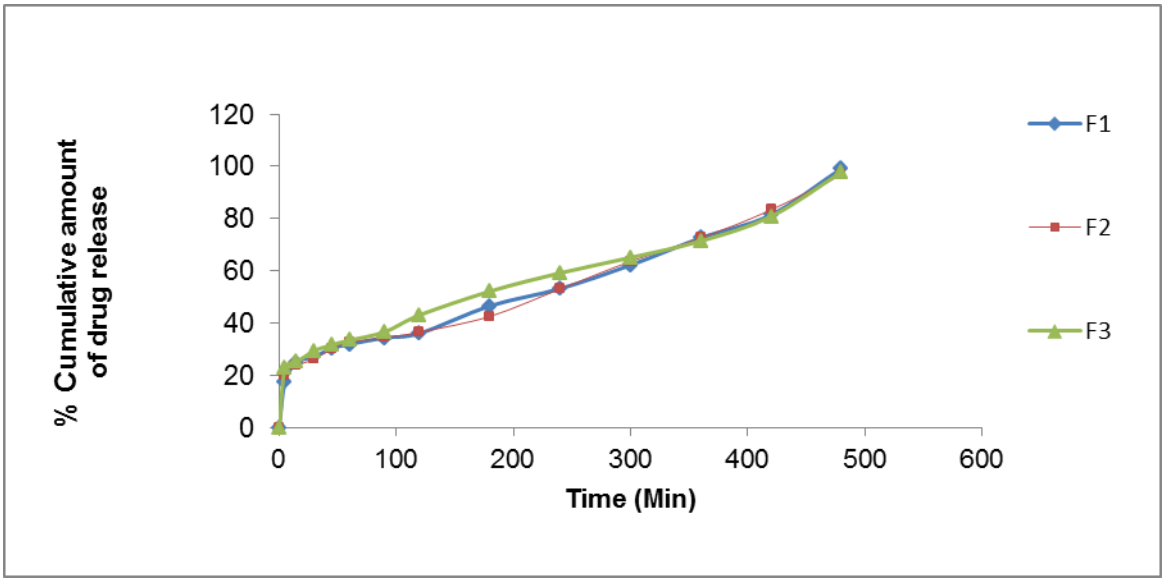
Now, let H_0 be the hypothesis that there is no significant difference between the batches.

In Vitro Drug release study

The release studies were carried out in 250 ml beaker containing 100 ml Phosphate buffer. Phosphate buffer pH 7.4 (100 ml) was placed in a 250 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. 5ml of aliquots were withdrawn at specific intervals, filtered after withdrawal and the apparatus was immediately replenished with same quantity of fresh buffer medium.

Table 5: Cumulative percentage drug release from verious formulation of liposomes

Time (Min)	Batch code		
	F1	**F2**	**F3**
0	0	0	0
5	17.38±1.05	19.86±1.17	22.80±1.60
15	24.07±2.26	23.63±3.53	25.18±0.52
30	27.14±0.79	26.10±0.24	28.32±2.26
45	30.32±1.20	30.35±0.11	31.72±1.62
60	31.85±1.09	32.62±0.42	32.35±1.21
90	34.41±1.03	34.44±0.23	36.54±1.01
120	36.14±0.87	36.53±4.67	43.09±0.74
180	45.52±1.45	42.51±0.57	52.12±0.89
240	53.21±0.88	53.10±4.00	59.26±.89
300	62.20±1.19	62.61±2.59	65.10±1.51
360	72.65±0.81	72.58±9.49	71.39±1.37
420	81.48±1.7	83.38±7.92	80.88 ±0.8
480	99.22±0.35	96.80±2.51	97.69±1.09



(Mean ± S.D., n=3)

Figure 4: *in vitro* drug release of various formulations

All the three batches of formulation F1 were found to release the drug in 8 h. The cumulative percentage release was found to be 99.22%.

Study of drug release kinetics

Drug release kinetics for formulations F1-F3 were shown in table . Figure and shows Higuchi's and Peppas Korsmeyer's plot respectively.

Table No 6: Drug release kinetics for the various formulations of liposomes

Formulation	Correlation coefficient (r) values		
	Zero order	First order	Peppas model
F1	0.8350	0.9156	0.9420
F2	0.8623	0.8632	0.9217
F3	0.7965	0.8722	0.9422

The correlation coefficient (r) values showed that formulations follow peppas model drug release.

Stability studies

Stability studies were carried out for a period of two month at 4±2^{0}C, 25±2^{0}C and 37±2^{0}C. The entrapment efficiency was estimated at an interval of 15 days. The results of stability studies are shown in table 7.

Table No 7: Stability studies for the formulation F1

Sampling Intervals (Days)	% drug entrapped at		
	4 ± 2^{0}C	25 ± 2^{0}C	37 ± 2^{0}C
0	48.82	48.82	48.82
15	48.63	47.46	46.29
30	48.42	44.44	42.13
45	47.06	40.62	37.95
60	47.80	39.12	35.10

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 F1 was found to be most suitable because of high encapsulation efficiency with smaller particle size. The formulation F1 comprising phosphatidylcholine, cholesterol 9:1 ratio, fulfills the requirement of good liposomal formulation. *In vitro* drug release upto 8 h and more than 99.22% drug released. Follows peppas model in release studies. It shows encapsulation efficiency of 48.92% and particle size of 6.24 µm.

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