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PREPARATION AND EVALUATION OF DECITABINE LIPOSMOES

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Abstract:
The aim of this study was to Formulation and In-vitro evaluation of liposomal drug delivery system of Decitabine. Decitabine is an anticancer medication is indicated for treatment of patients with myelodysplastic syndrome (MDS). Decitabine Liposomes are prepared by the thin film hydration method using the soya lecithin as the phospholipid. This study mainly explains about the effect of concentration of soya lecithin, cholesterol and Tween 80. The prepared liposomes were characterized by scanning electron microscopic method respectively. The In-vitro release studies were performed and the drug release kinetics was evaluated using linear regression method. The objective of the present study was to develop liposome containing Decitabine and the prepared liposomes were evaluated for size, shape, drug entrapment efficiency, In-vitro drug release and stability. Decetabine loaded liposomes formulation had good ability to encapsulate drug and elicted favorable physicochemical characteristics. The intestinal absorption and antitumor capacity of Decitabine was significantly enhanced by using liposomes. These results suggest that liposomes could be a promising perioral carrier for Decitabine.

Key Words: Liposomes, (MLV) multi lamellar vesicles, Thin film hydration method, Decitabine, Lecithin, Cholesterol, Tween 80.

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INTRODUCTION:

Rational research in drug delivery began in 1950's with the advent of polyclonal antitumor antibodies developed for tumour targeting of cytotoxic drugs to experimental tumors. This had triggered a series of concerted efforts evolved with the emergence of a plethora of deliver systems [1-8]. Liposomes were discovered in the early 1960's by British hematologist Dr Alec D Bangham (published 1965) and subsequently became the most extensively explored drug delivery system. However, it took several years from the early to the late 60's before the system was realized as a potential drug carrier. At first they were used to study in vivo simulated biomembrane behavior. Subsequent to that liposome has become an essential therapeutic tool most notably in drug delivery and targeting. Not surprisingly, liposomes have covered predominantly medical, albeit some non-medical areas like bioreactors, catalysts, cosmetics and ecology. However, their predominance in drug delivery and targeting has enabled them to be used as therapeutic tool in fields like tumour targeting, gene and antisense therapy, genetic vaccination, immune modulation, lung therapeutics, fungal infections, and skin care and topical cosmetic products [8,9]. The name liposome s derived from two Greek words: 'Lipos' meaning fat and 'Soma' meaning body. [9-12]. Liposomes are spherical microscopic vesicles composed of one or more concentric lipid bilayers, separated by water or aqueous buffer compartments with a diameter ranging from 25 nm to 10000 nm. They are commonly composed of one or more amphiphilic phospholipids bilayer membranes (and thus also called as phospholipid vesicles) that can entrap both hydrophilic and hydrophobic drugs[13-18]. A liposome is a spherical vesicle with a membrane composed of a phospholipid bilayer used to deliver drug or genetic material into a cell. Liposomes can be composed of naturally-derived phospholipids with mixed lipid chain like egg phosphatidylethonalimine or of pure components like DOPE (dioleolylphosphatidylethanolamine).

Decitabine is indicated for treatment of patients with myelodysplastic syndrome (MDS). Half life is 30 mins decitabine is slightly soluble in ethanol\water methanol\water sparingly soluble in water soluble in dimethylsulfoxide. Decitabine liposome's were prepared using soya lecithin, cholesterol, Tween80, and chloroform as solvent by thin film hydration method using rotary evaporator. The prepared Liposomes were evaluated by drug entrapment study, particle size analysis. In vitro drug release study an mechanism of release kinetics using Higuchi's plot and korsemeyer Peppas plot and stability studies [19-20].

MATERIALS AND METHOD:

Decitabine was obtained from Aurobindo laboratories Ltd, Soyabean lecithin was purchesed from Hi-Media laboratories Pvt. Ltd, Mumbai, Tween 80, methanol &Potassium dihydrogen phosphate obtained from Merck specialities Pvt, Ltd, Mumbai, Cholestrol ,Chloroform and Sodium chloride, Potassium cholride, Di-sodium hydrogen ortho phosphate purchesed from S.D. Fine chemicals Pvt, Ltd, Mumbai.

Preparation:

The preparation of liposomes with Soybean lecithin was prepared by dried thin film hydration technique using a rotary evaporator (Aditya scientific). Drug, Soya lecithin, cholesterol, tween 80 and were dissolved in 10 mL chloroform in 250mL round bottom (RB) flask. The chloroform was evaporated under vacuum using rotary flash evaporator 65-70rom, which allows soya lecithin to form a thin dry film on the walls of the flask. This system was maintained at vacuum and 40°C for an additional 10min, after complete removal of organic solvent as indicated by visual observations. Vesicles were prepared by hydrating the lipid film in the presence of 10mL phosphate buffer pH 7.4. Liposomes formed were sonicated for 30 min. to reduce the size of the vesicles. [8]

The composition and ratios of lecithin, cholesterol and Tween 80 for different types of Liposomes were mentioned in Table No. 3

Drug-Excipient Compatibility Studies:

Infrared (IR) spectroscopy was conducted using a FTIR Spectrophotometer (Bruker) and the spectrum was recorded in the wavelength region of 4000 to 400 cm−1. The procedure consisted of dispersing a sample (drug alone or mixture of drug and excipients) in KBr and compressing into discs by applying a pressure of 5 tons for 5 min in a hydraulic press. The pellet was placed in the light path and the spectrum was obtained.

Drug Entrapment Efficiency or Drug Content:

Entrapment efficiency of Liposomes was determined by centrifugation method. Aliquots (1 ml) of liposomal dispersion were subjected to centrifugation on a laboratory centrifuge (REMI CM-12 PLUS) at 3500 rpm for a period of 90 min. 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 231 nm. Amount of Decitabine in supernatant and sediment gave a total amount of Decitabine in 1 ml dispersion.

The amount of drug loaded was determined by the formula:

Drug loading = Total of drug amount of drug in solution – amount drug present in supernatant

% of drug content = (amount loaded / Total drug) x 100

Particle Size Analysis:

Particle size of the formulations was observed under a scanning electron microscope (Hitachi), one drop of Liposomes suspension were mounted on the stab covered with clean glass and coated with gold and were observed under the scanning electron microscope at an accelerating voltage of 15KV and photomicrographs of suitable magnification was obtained. The SEM of the formulation given in Figure No. 4

Zeta Potential Analysis:

The significance of zeta potential is that its value can be related to the stability of colloidal dispersions. So, colloids with high zeta potential (negative or positive) are electrically stabilized while colloids with low zeta potentials tend to coagulate or flocculate. A value of 25mV (positive or negative) can be taken as the arbitrary value that separates low-charged surfaces from high-charged surfaces. The zeta potential was analyzed by MALVERN ZETASIZER

***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±50C. Dialysis membrane was taken and one end of the membrane was sealed. After separation of non-entrapped Decitabin, liposome dispersion was filled in the dialysis membrane and other end was closed. The dialysis membrane containing the sample was suspended in the medium. Aliquots were withdrawn (5 ml) at specific intervals, filtered and the apparatus was immediately replenished with same quantity of fresh buffer medium.

Release Kinetic Model:

The diffusion data obtained from diffusion profile was fitted by to various kinetic models (Zero order, First order Higuchi, Koresmeyer peppas, Hixon Crowell) and the best fit model was obtained by regression analysis.

- Zero - order kinetic model – Cumulative % drug released versus time.
- First – order kinetic model – Log cumulative percent drug remaining versus time.
- Higuchi's model – Cumulative percent drug released versus square root of time.
- Korsmeyer equation / Peppa's model – Log cumulative percent drug released versus log time.
- Hixon crowell -Time vs cube root % drug remaining.

The release mechanism is determined by finding out **n** value from Koresmeyer peppas model.

Short Term Stability Studies:

Stability studies were performed to inspect the leakage of the drug from the liposome during storage. Liposomal suspensions of Decitabine of optimized formulations were sealed in 20 mL glass vials and stored at refrigeration temperature (2–8 °C) and room temperature (25 ± 2 °C / 60 ± 5 % R.H) for a period of 2 months. Samples from each liposomal formulation which are kept for examination were withdrawn at definite time intervals. The withdrawn samples were In-vitro drug release studies at 231 nm.

RESULTS AND DISCUSSION:

Table 1: Interpretations of FTIR Spectra for Pure Drug Decitabine

S.No.	Functional Groups	Range of Groups Wave Number cm^{-1}	Assesment of Peak Wavenumber cm^{-1}
1.	N-H stretching	3400-3500	3489.23,3442.94,3064.89
2.	C-H stretching(alkane)	2960-2850	2939.52,2899.01,2823.79,2044.54
3.	C=O stretching(aldehyde)	1720-1740	1722.43
4.	N-H bending	1500-1650	1625.99,1602.85,1585.49,1566.20
5.	C=C stretching(aromatic)	1450-1600	1496.76,1452.40
6.	C-N vibration	1000-1400	1390.68,1369.46,1315.45, 1269.16,1246.02,1168.86, 1101.35,1074.35,1026.13
7.	C-H bendig(aromatic)	750-850	850.61,827.48,804.32, 777.31,748.38,705.95

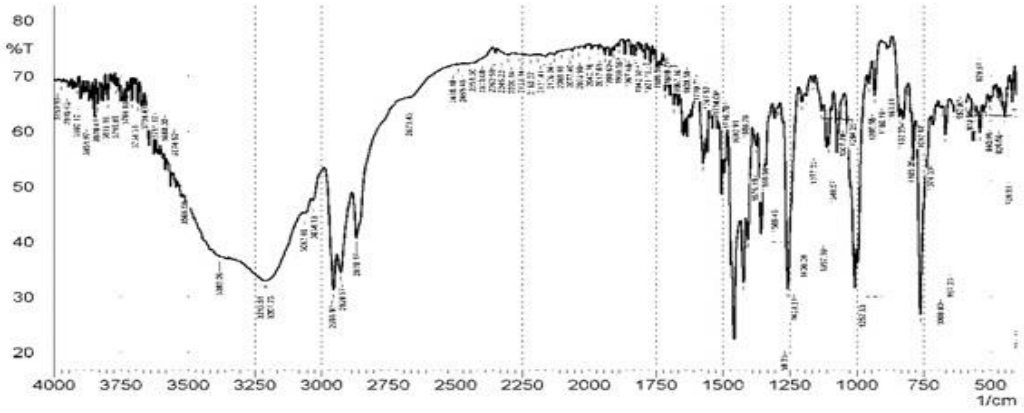

Fig 1: FTIR of Decitabine Spectrum

Table 2: Interpretation of FTIR Spectra of Optimized Formulation

S.No	Functional groups	Range of groups Wavenumber cm^{-1}	Assesment of peak Wavenumber cm^{-1}
1.	N-H stretching	3400-3500	3417.86
2.	C-H stretching(alkane)	2960-2850	2931.80
3.	C=O stretching(aldehyde)	1720-1740	1720.50
4.	N-H bending	1500-1650	1627.92,1620.21,1602.85, 1585.49
5.	C=C stretching(aromatic)	1450-1600	1492.90,1454.33
6.	C-N vibration	1000-1400	1369.46,1315.45,1269.16, 1246.02,1170.79,1141.86, 1101.35,1056.99,1024.20
7.	C-H bendig(aromatic)	750-850	846.75,827.46,802.39, 777.31,744.52,705.95

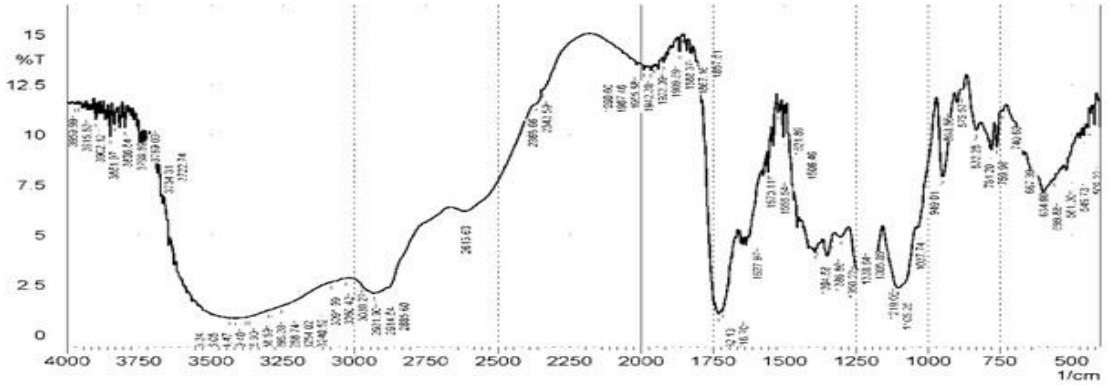

Fig 2: FTIR of Optimized Formulation

Table 3: Qualitative and Quantitative Lipid Compositions of Different Formulations

Ingredients	F1	F2	F3	F4	F5	F6
Drug(mg/ml)	20	**20**	20	20	20	20
Soya lecithin (mg)	240	**270**	210	180	210	180
Cholesterol(ml)	60	**30**	90	120	90	120
Chloroform(ml)	5	**5**	5	5	5	5
Tween 80(ml)	…..	**0.5**	…..	…..	0.5	0.5
PBS 7.4(ml)	10	**10**	10	10	10	10
Hydration time	20	**35**	30	20	30	30

Table 4: Drug Entrapment Efficiency of Decitabine

S.NO	Formulation code	%Entrapped Drug
1	F1	79.75
2	F2	82.96
3	F3	82.62
4	F4	78.39
5	F5	78.86
6	F6	78.81

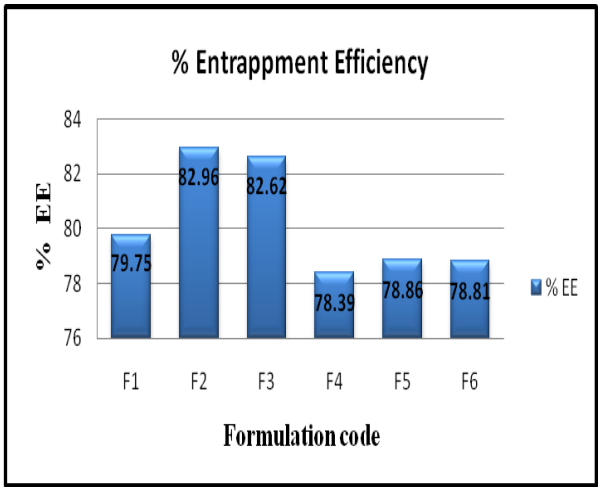

Fig 3: Percentage of Drug Entrapment Efficiency of Plot for F1 to F6 Formulations

Inference : The percentage entrapment was maximum F2 is 82.96% and minimum for F4 is 78.39 % .The data suggests that concentrations with respect to the formulation represent the critical value up to which the entrapment increased and beyond that its start decreasing.

Particle Size Analysis:

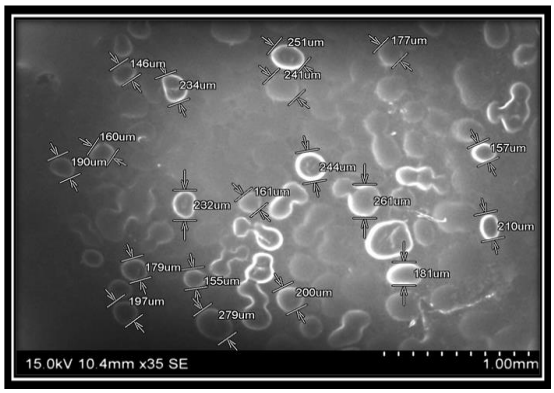

Fig 4: SEM Photography of Liposomal Solution for F2 Formulation.

Inference: The shape and morphology of the liposome droplet was determined by SEM show the round shape, smooth surface and nano size range of vesicle. Demonstrating Multi lamellar vesicles structure under electron microscopic study confirming the vesicle characteristics.

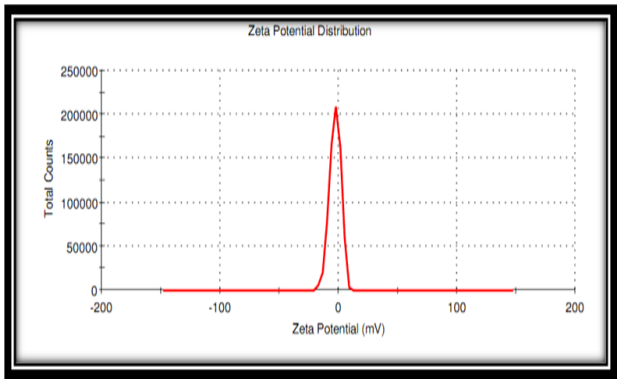

Fig 5: Zeta Potential for Decitabine Liposomal Solution for F2 Formulation.

Inference: The zeta potential of optimized formulation (F2) which is selected based on entrapment efficiency. The value was -0.271 mV which indicates that the surface of liposomes is dominated by the anions and proved that prepared liposome have sufficient charge to avoid aggregation of vesicles.

***In Vitro* Dissolution Data:**

Inference: The in vitro dissolution profile prepared formulations was determined by membrane diffusion method. The dissolution was carried out for a period of 24 hrs in 7.4 pH phosphate buffer.

The cumulative percent release of F1 to F6 formulations at various time intervals was calculated and tabulated in Table No 5. The cumulative percent drug release in all formulations was plotted against time in Figure No 6.The Maximum percent of drug release was found in F2 formulation which contains maximum drug entrapment.

Release Kinetics:

The release kinetics of F1 F2, F3, F4, F6 formulations was studied. All formulations follow Zero order release kinetics and follow case II transport when it applied to the Korsmeyer-Peppa's Model for mechanism of drug release. F2 formulation has better kinetic results when compared to F1 to F6 formulations. The results are shown in Table no 7 to 11 and Figure no 8 to 12

Table 5: *In Vitro* Cumulative % Drug Release Profile of Decitabine Liposomal Formulations

Time(Hr)	F1	F2	F3	F4	F5	F6
0	0	0	0	0	0	0
1	8.29	5.68	10.65	12.74	13.11	15.13
2	15.36	12.21	20.21	25.31	26.05	28.06
4	33.53	28.77	39.85	40.5	42.92	45.01
6	58.21	51.65	63.56	68.9	67.53	68.62
10	78.85	74.25	81.56	83.78	84.86	85.07
12	95.85	89.46	92.59	94.84	95.25	93.67
24	100	96.31	100	100	100	99.86

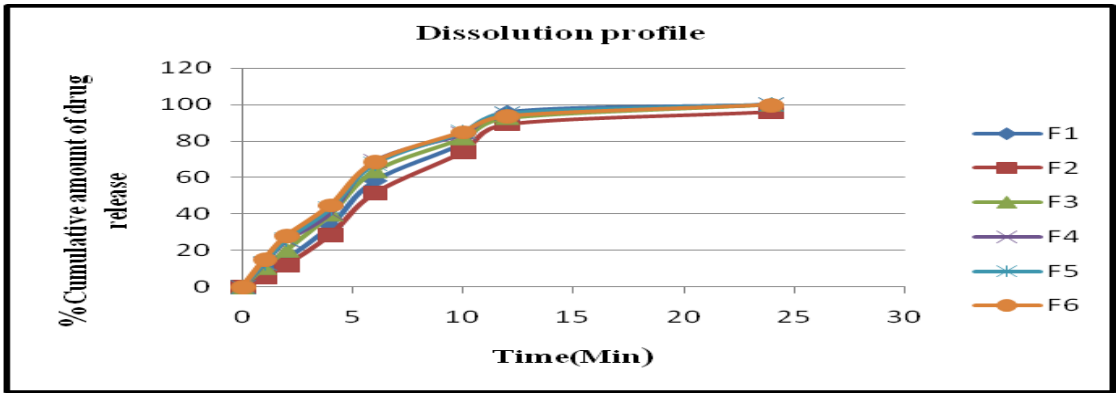

Fig 6: In Vitro Drug Release Study of F1 to F6

Table 6: Zero Order Release Model of Decitabine Liposomal Optimized F2 Formulation

S.No.	Time(Hrs)	%Cumulative drug release
0	0	0
1	1	5.68
2	2	12.21
3	4	28.77
4	6	51.65
5	10	74.25
6	12	89.46
7	24	96.31

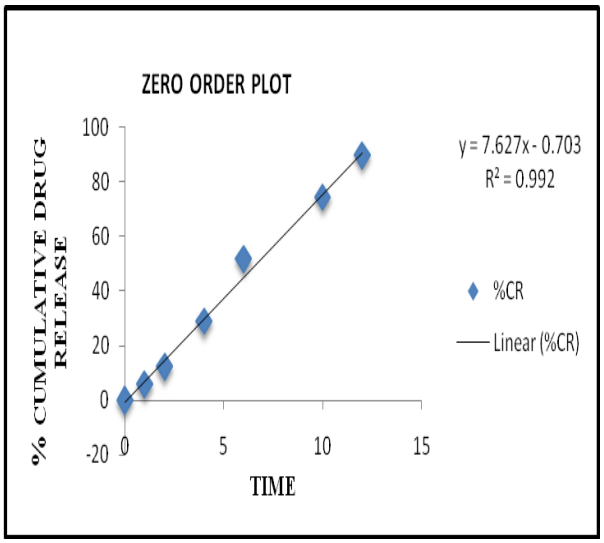

Fig7: Zero Order Plot for Optimized F2 Formulation

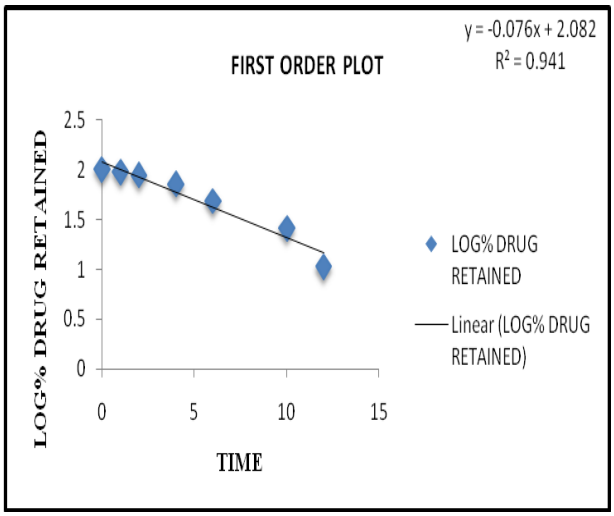

Fig 8: First Order Plot for Optimized F2 Formulation

Table 7: First Order Release Model of Decitabine Liposomal Optimized F2 Formulation

S.No.	Time(Hrs)	Log % Drug Retained
0	0	2
1	1	1.974604
2	2	1.943445
3	4	1.852663
4	6	1.684396
5	10	1.410777
6	12	1.022841
7	24	0.567026

Table 8: Higuchi Release Model of Decitabine Liposomal Optimized F2 Formulations

S.No.	Square root of Time	%Cumulative Drug Release
0	0	0
1	1	5.68
2	1.414214	12.21
3	2	28.77
4	2.44949	51.65
5	3.162278	74.25
6	3.464102	89.46
7	4.898979	96.31

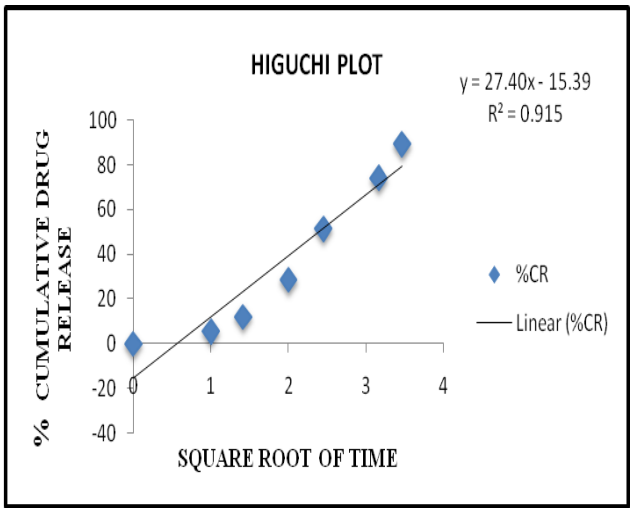

Fig 9: Higuchi Plot for Optimized F2 Formulation

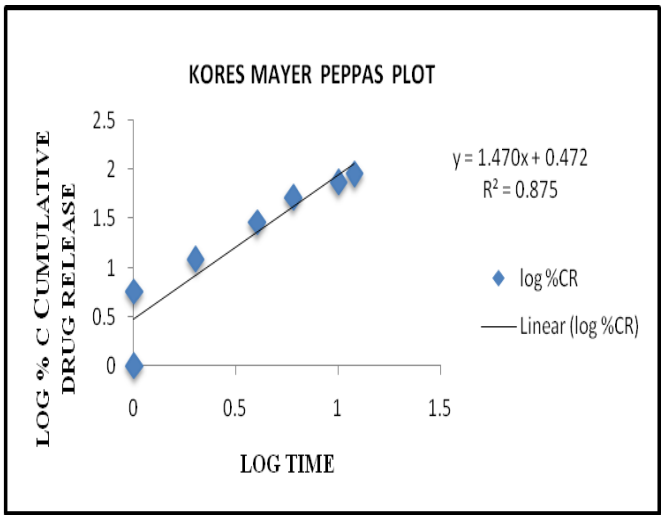

Figure 10: Koresmayer Peppas Plot for Optimized F2 Formulation

Table 9: Korsmeyer-Peppas Model for F2 Mechanism of Drug Release

S.No.	log TIME	log % Cumulative Drug Release
0	∞	0
1	0	0.754348
2	0.30103	1.086716
3	0.60206	1.45894
4	0.778151	1.71307
5	1	1.870696
6	1.079181	1.951629
7	1.380211	1.983671

Table10: Hixon Crowell Release Model of Decitabine Optimization F2 Formulation

S.No.	Time	Cube root of %drug remaining
0	0	4.641589
1	1	4.55199
2	2	4.444419
3	4	4.145284
4	6	3.643053
5	10	2.95297
6	12	2.192537
7	24	1.545286

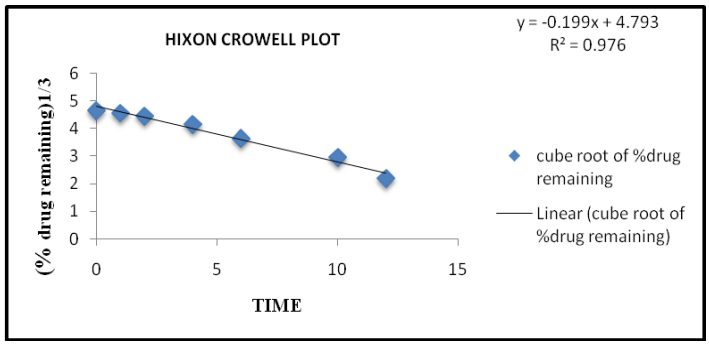

Fig 11: Hixon Crowell Plot For Optimized F2 Formulation

Table 11: Curve Fitting Data of Release Rate Profile of Formulations F1 to F6

Formulation code	Zero order(r^2)	First order(r^2)	Higuchi (r^2)	Hixon crowel(r^2)	kosermeyer-peppas (r^2)	kosermeyer-peppas Slope (n)
F1	0.988	0.885	0.929	0.957	0.821	1.414
F2	0.992	0.941	0.915	0.976	0.875	1.470
F3	0.971	0.966	0.956	0.991	0.763	1.353
F4	0.959	0.953	0.961	0.985	0.721	1.316
F5	0.963	0.954	0.968	0.990	0.714	1.310
F6	0.952	0.978	0.976	0.994	0.683	1.276

Table 12: Stability Dissolution Results of Optimized Formulation-F2

Formulation Code	Parameters	Initial drug release after 24 hrs	After 1st Month	After 2nd Month
F2	**25^{0}C/60%RH % Release**	96.31	96.3	96.29
F2	**30^{0}C/75% RH % Release**	96.31	96.29	96.28
F2	**40^{0}C/75% RH % Release**	96.31	96.28	96.28

Stability Studys: The liposomal formulation was tested for a period of 8 weeks at different temperatures of 25ºc and 40ºc with 60% RH and 75%RH for their drug dissolution.

DISCUSSION:

The entrapment efficiency of decitabine liposomes (Table 4) indicates that as the concentration of phosphatidyl choline decreases, drug entrapment efficiency of liposomes decreases which was due to the saturation of lipid bilayer with reference to the drug where low phosphatidyl choline content provides limited entrapment capacity. The cumulative drug release maximum percent of drug release was found in F2 formulation which contains maximum drug entrapment compared to F1, F3, F4, and F5, F6. The encapsulation efficiency of liposomes is governed by the ability of formulation to retain drug molecules in the aqueous core or in the bilayer membrane of the vesicles. Results of particle size analysis showed that, as the concentration of cholesterol increases particle size increases which was may be due to formation of rigid bilayer structure but this was up to a specific concentration as there was also decrease in size of formulation F2. The release kinetics mechanism of drug release F2 formulation has better kinetic results when compared to F1 to F6 formulations.

The kinetic treatment of the drug release data of the prepared formulations followed zero order drug release; the prepared formulations followed Higuchi profile, as the plot showed high linearity (R^2 = 0.992) indicating diffusion as one mechanism of drug release. F2 showed high linearity in Hixon plot (R^2 = 0.976) and Korsmeyer-Peppas plot slope value "n" was 1.470 the relative complexity of this formulation and its components may indicate super case-II transport i.e. that the drug release is controlled by more than one process. and The stability of the Decitabine liposomes was tested for a period of 8 weeks at different temperatures of 25ºc and 40ºc with 60% RH and 75%RH. The liposomes stored at 25ºc and 40ºc were found to be stable for duration of two months.

CONCLUSION:

The Decitabine loaded liposome formulation had good ability to encapsulate drug and elicited favorable physicochemical characteristics. The intestinal absorption and antitumor capacity of Decitabine was significantly enhanced by using liposomes. These results suggest that liposomes could be a promising perioral carrier for Decitabine. From the executed experimental results, it could be

concluded that the lipids like Soya lecithin, Cholesterol and Tween80 were suitable carrier for the preparation of Decitabine Liposomes. Though the preliminary data based on *in-vitro* dissolution profile, release kinetics and stability studies proved that the suitability of such formulations, Still a thorough experiment will be required based on the animal studies. There after we can find the actual mode of action of this kind of dosage form. Therefore, a future work will be carried out as follows,

- Long term stability studies
- *In-vitro* Cytotoxicity studies
- *In-vivo* Pharmacological work on animals.
- *In-vivo* pharmacokinetic studies on animals.

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