Research Article

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Development and characterization of combination of zidovudine and lamivudine liposomal drug delivery system

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ABSTRACT

Zidovudine is an antiretroviral drug with activity against Human Immunodeficiency Virus (HIV) Type 1. Lamivudine (Epivir-HBV) is used to treat hepatitis B infection. Lamivudine and Zidovudine are nucleoside reverse transcriptase inhibitors with activity against Human Immunodeficiency Virus Type 1 (HIV-1). Combination of zidovudine and liposomes were developed by thin film hydration method. Drug and excipient compatibility studies measured by using FTIR. These liposomes were evaluated for particle size, drug entrapment efficiency, SEM, in vitro drug release studies and stability studies. Percentage drug entrapment of liposomes was found to be within the range of 65.98-85.55%. In vitro dissolution was carried out for 8 hours and the percentage drug release for all the formulations was in the range of 83.56-93.72%. Optimized formulation follows zero order kinetics.

Keywords: Zidovudine, Lamivudine, FTIR studies, Thin film hydration technique, In vitro drug release studies.

INTRODUCTION

Liposomes are spherical microscopic vesicles composed of one or more lipid bilayers, separated by water or aqueous buffer compartments with a diameter ranging from 25nm to 1000µm. [1] 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. [2] Lamivudine (3TC) and zidovudine (AZT) are nucleoside reverse-transcriptase inhibitors commonly used in the treatment of AIDS in HIV-infected patients. However, only temporary and limited benefits are observed in HIV-infected patients treated with conventional therapy of a single drug or in combination. [3] The limited ability of these agents to reduce viral burden, the rapid development of resistance and toxic side effects have limited their long-term efficacy. In this study, the ethanol injection method was used to prepare a liposomal rapamycin-delivery system, and the formulation was optimized. [4, 5]

MATERIALS AND METHODS

Materials

Zidovudine and lamivudine obtained as gift sample from Aurobindo Laboratories Ltd, Phosphotidyl choline and cholesterol were

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purchased from S. D chemicals. All other ingredients used throughout the study were of analytical grade and were used as received.

Methodology [6, 7, 8]

Fourier Transform Infra-red Spectroscopy (FT-IR) Analysis

The Fourier transform infra-red analysis was conducted for the analysis of drug polymer interaction and stability of drug during microencapsulation process. Fourier transform infra-red spectrum of pure drug and microspheres were recorded.

Preparation of liposomes

Lamivudine and zidovudine-loaded stealth liposomes were prepared by thin film hydration technique. Required quantity of drug and phospholipids with variable concentration of cholesterol was dissolved in 10 ml solvent system of chloroform and methanol mixture in a 250 ml round bottom flask. The organic solvent system was removed using a rotary evaporator under reduced pressure to obtain a thin film on the wall of the flask. During the process, the conditions such as speed (100 rpm) and temperature (40°±5°C) were maintained constant. The flask was removed and left overnight in a desiccator under reduced pressure to remove the solvent residuals completely. Then, the lipid film was hydrated using phosphate buffer saline pH 7.4 at 60±2°C. The resultant suspension was vortexed for about 2 min and allowed to stand for 2-3 h to allow complete swelling of the lipid film. Then, the suspension was sonicating using probe sonicator for about 5 min and extruded through a polycarbonate membrane of 0.2 µg aperture size.

Table -1: composition of lipids for preparation of liposome

Ingredients	F1	F2	F3	F4	F5	F6	F7	F8
Phosphatidylcholine	100	150	200	250	300	350	400	450
Cholesterol	50	75	100	100	75	50	100	75
Solvent(Methanol)	10	10	10	10	10	10	10	10
Chloroform	10	10	10	10	10	10	10	10
Zidovudine	25	25	25	25	25	25	25	25
Lamivudine	25	25	25	25	25	25	25	25
Phosphate buffer pH 7.4	10	10	10	10	10	10	10	10

EVALUATIONS OF LIPOSOMES [9, 10, 11]

Drug entrapment efficiency

Entrapment efficiency of liposomes were measured by centrifugation technique. Aliquots (1 ml) of liposomal solution 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 drugs and absorbance recorded at 255nm and 270 nm. 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 255nm and 270 nm.

Amount of drugs in supernatant and sediment gave a total amount of Zidovudine and lamivudine in 1 ml dispersion.

Formula

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

Particle size analysis

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

In Vitro Drug release study

The drug release studies were carried out by Franz diffusion cell. 10ml ml Franz diffusion cell containing 10 ml Phosphate buffer. Phosphate buffer pH 7.4 (10 ml) was placed in a 10 ml beaker. The beaker was placed on a magnetic stirrer and the medium was calibrated at 37 ± 5^0C. Dialysis membrane was taken and one end of the membrane was sealed. After separation of non-entrapped combination of zidovudine and lamivudine liposomal dispersion was filled in the dialysis membrane and other end was closed. The dialysis membrane consists of the sample was suspended in the medium. 1ml of liquid were withdrawn at specific intervals, and these 1 ml liquid diluted with 10ml of 7.4 phosphate buffer and the apparatus was immediately replenished with same quantity of fresh buffer medium.

Drug release kinetics

Several theories and kinetic models describe the dissolution of drug from immediate release and modified release dosage forms. There are several models to represent the drug dissolution profiles where f (t) is a function of time related to the amount of drug dissolved from the pharmaceutical dosage form. Some analytical definitions of the Q(t) function are commonly used, such as zero order, first order, Higuchi, Korsmeyer-Peppas, Hixson-Crowell models. These models are used to characterize drug dissolution/release profiles.

Zero Order Kinetics

This model represents an ideal release profile in order to achieve the pharmacological prolonged action. Zero order release constitutes drug release from the dosage form that is independent of the amount of drug in the delivery system (that is, a constant release rate). The following equation is used to express the model:

Qt = Qo + Kot

Where,

Qt is the amount of drug dissolved in time t

Qo is the initial amount of drug in the solution

Ko is the zero order release constant

For practical purposes the equation is rearranged

Percent drug released = Kt

First Order Kinetics

First order release constitutes drug release in a way that is proportional to the amount of drug remaining in its interior; in such a way that amount of drug released by unit time diminish. The following equation is used to express the model:

log Qt = log Qo + Kt/2.303

Where,

Qt is the amount of drug dissolved in time t

Qo is the initial amount of drug in the solution

K is the first order release constant

For practical purposes the equation is rearranged:

Log % of drug unreleased = Kt/2.303

This model is applicable to dosage forms such as those containing watersoluble drugs in porous matrices.

Higuchi Model

Higuchi describes drug release as a diffusion process based in Fick's law, square root dependent. The following equation is used to express the model:

Qt = Kht1/2

Where, Qt is the amount of drug dissolved in time t

Kh is the first order release constant

For practical purposes the equation is rearranged:

Percent drug released = Kt1/2

This model is applicable to systems with drug dispersed in uniform swellable polymer matrix as in case of matrix tablets with water soluble drugs.

Peppas-Korsmeyer Model

This model is widely used when the release mechanism is not well known or when more than one type of release phenomenon could be involved The following equation is used to express the model

$Qt/Q\infty = Ktn$

Where, Qt is the amount of drug dissolved in time t

$Q\infty$ is the amount of drug dissolved in infinite time

n is the release exponent indicative of drug release mechanism

K is the kinetic constant

For practical purposes the equation is rearranged:

Log percent drug released = log k +n log t

Peppas used n value in order to characterize different release mechanism concluding for values of n = 0.5 for Fickian diffusion and values of n, between 0.5 to 1.0 for anomalous transport

(corresponds to diffusion, erosion and swelling mechanism or mixed order kinetics) and higher values of n, n=1 or n>1 for case-II transport (corresponds to erosion and relaxation of swollen polymer layer).

Stability studies[12]

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 combination of zidovudine and lamivudine 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 90 days.

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.

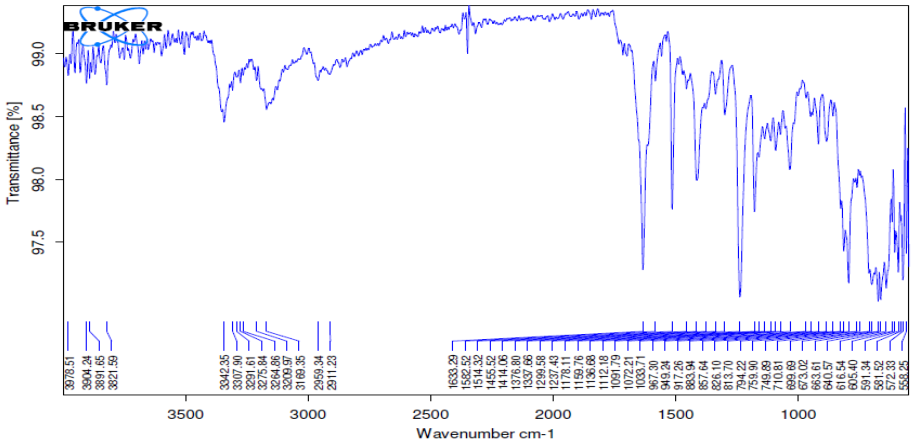

Fig-1: FTIR Studies of Pure Drug (Zidovudine)

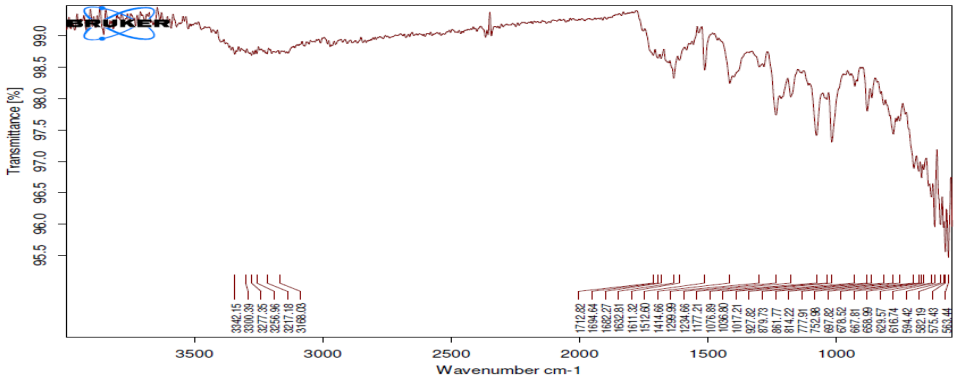

Fig- 2: FTIR Studies of Pure Drug (Lamivudine)

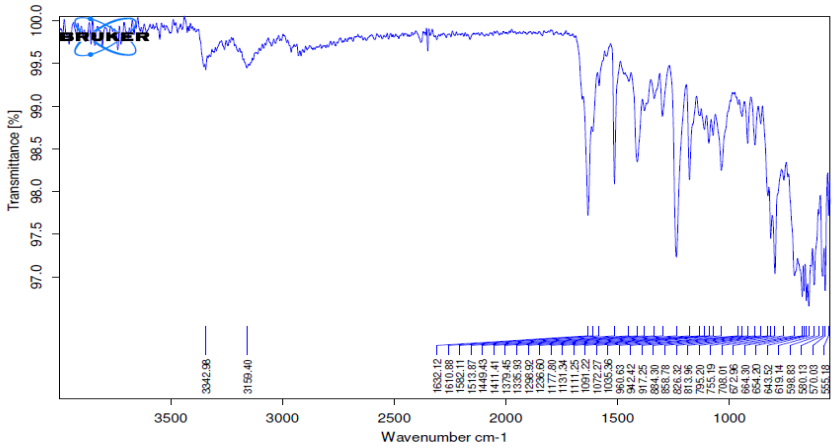

Fig-3: Optimized formulation of Liposomes

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

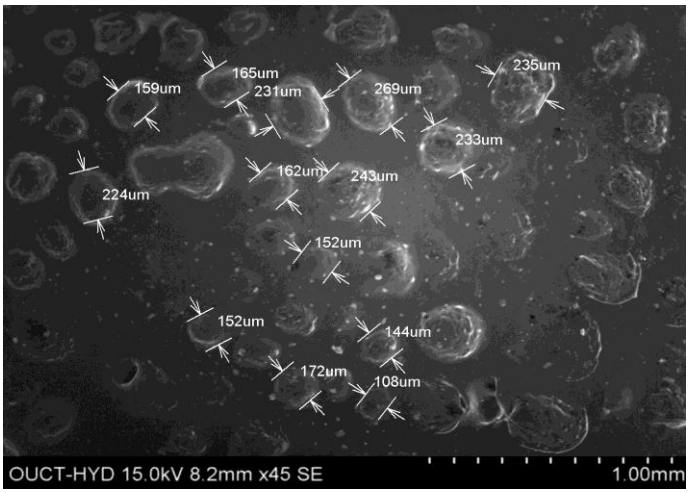

Fig-4: Particle size of optimization formulation

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

Sr. No	F. no	Particle size(µm)
1	F1	232
2	F2	219
3	F3	208
4	F4	229
5	F5	228

6	F6	218
7	F7	203
8	F8	234

Drug entrapment efficiency

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

Sr. No	F.No	PDE
1	F1	68.25
2	F2	73.90
3	F3	70.35
4	F4	65.98
5	F5	85.55
6	F6	82.75
7	F7	79.20
8	F8	78.85

Drug release studies

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

Time	Batch code							
	F1	F2	F3	F4	F5	F6	F7	F8
0	0	0	0	0	0	0	0	0
1	14.19	15.28	16.49	13.18	17.25	16.15	12.60	14.12
2	23.22	23.66	20.28	22.21	20.18	22.25	23.40	27.02
3	33.40	36.25	37.41	33.69	31.12	31.28	33.26	35.60
4	42.62	44.19	42.16	46.18	42.28	40.50	41.62	46.55
5	53.86	59.60	60.25	52.60	55.63	51.25	56.32	51.60
6	64.50	68.35	72.72	70.25	72.28	68.60	67.72	64.52
7	78.22	79.21	84.28	81.30	85.32	72.28	72.10	78.20
8	85.43	89.28	90.22	91.75	93.72	85.80	89.56	83.56

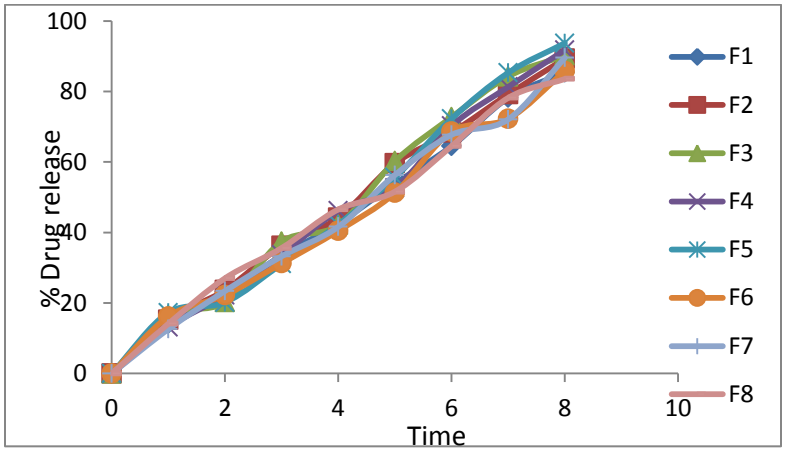

Fig-5: *In vitro* drug release of various formulations

All the three batches of formulation F5 were found to release the drug in 8 h. The cumulative percentage release was found to be 93.72 %.

Kinetic modelling of drug release

All the nine formulation of prepared combination of zidovudine and lamivudine liposomes 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)
1. Cumulative percent drug released vs. square root of time (Higuchi's
2. Classical Diffusion Equation)
3. Log of cumulative % release Vs log time (Peppas Exponential Equation)
4. (Percentage retained) $1^{/3}$ Vs time (Hixson – Crowell Erosion Equation)

Zero order kinetics

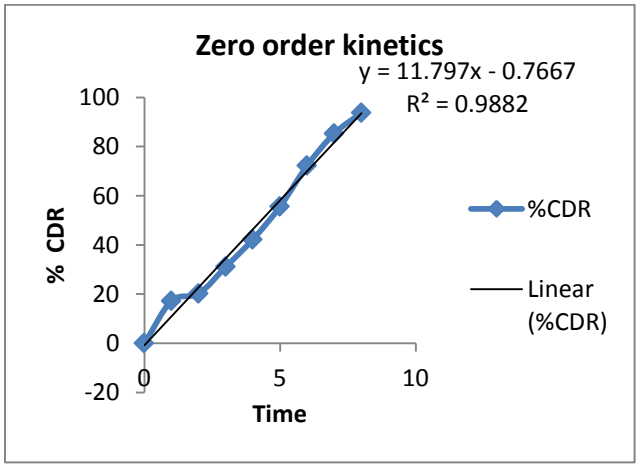

Fig-6: Zero order kinetics

First order kinetics

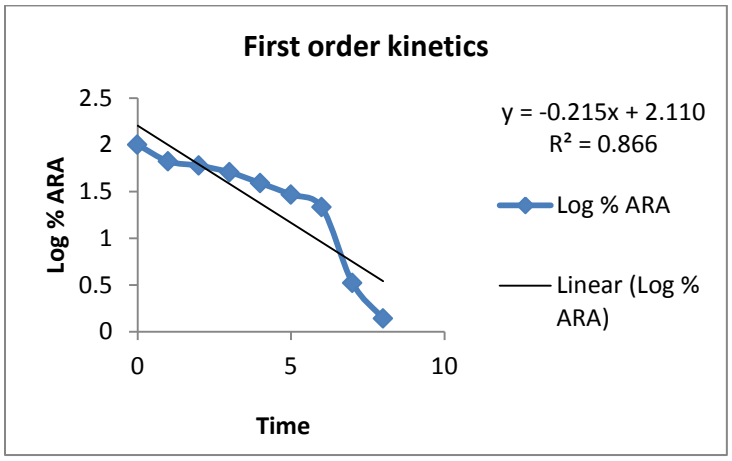

Fig-7: First order kinetics

Higuchi model

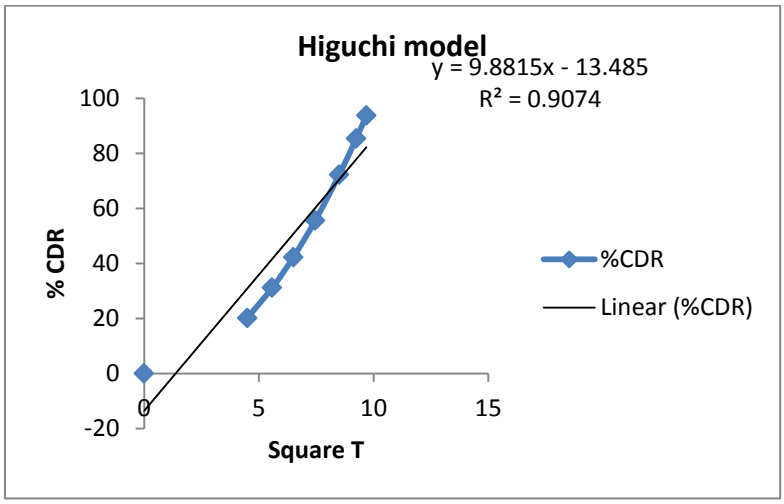

Fig-8: Higuchi model

Krosmayer peppas

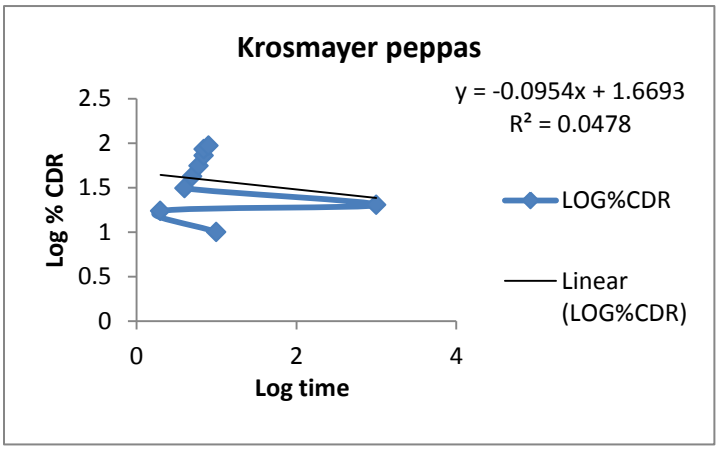

Fig-9: krossmayer peppas

The values of in vitro release were attempted to fit into various mathematical models. Plots of zero order, first order, Higuchi matrix and Pappas. Regression values are higher with Zero order release kinetics.

Table-5: Regression equations of optimaized formulation

F. No	In vitro release in phosphate buffer P^H 7.4 Regression values			
	Zero order	First order	Higuchi Plot	Kross mayerpeppas
F_5	0.988	0.866	0.907	0.047

The table indicates that r^2 values are higher for Higuchi's model compared for all the microspheres formulations. Hence Zidovudine and lamivudine release from all the microspheres followed diffusion rate controlled mechanism.

Stability studies

There was no significant change in physical and chemical properties of the tablets of formulation F-5 after 3 months. Parameters quantified at various time intervals were shown;

Table-6: Results of stability studies of optimized formulation F-5

Formulation Code	Parameters	Initial	1st Month	2nd Month	3rd Month	Limits as per Specifications
F-5	**25^0C/60%RH % Release**	93.72	93.70	93.62	93.50s	**Not less than 85 %**
F-5	**30^0C/75% RH % Release**	93.72	93.68	93.61	93.54	**Not less than 85 %**
F-5	**40^0C/75% RH % Release**	93.72	93.65	93.56	93.52	**Not less than 85 %**

CONCLUSION

From the performed work it was concluded that Zidovudine and Lamivudine possesses all requisite qualities required for liposomal drug delivery. Among the various formulation, the combination F5 was found to be most suitable because of high encapsulation efficiency with smaller particle size. The formulation F5 comprising Phosphatidylcholine, cholesterol, full fills the requirement of good liposomal formulation. In vitro drug release up to 8 h and more than 93.72% drug released. Follows Pappas model in release studies. It shows encapsulation efficiency of 85.55% and particle size of 228 µm.

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