FOMULATION AND EVALUATION OF ZIDOVUDINE LIPOSOMAL DRUG DELIVERY SYSTEM

Bhaskar Vishlavath, P. Nithin, R. Supraja, N. Uha, P. Mounika*
Department of Pharmaceutics, Thalla padmavathi Pharmacy College, Orus gutta, Kareemabad, Warangal, Telangana, India.

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. Zidovudine is a drug with narrow therapeutic index and short biological half-life. This study aimed at developing and optimizing liposomal formulation of Zidovudine 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 Zidovudine 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, Zidovudine, bioavailability, thin film hydration technique, in vitro drug release studies.

I.INTRODUCTION

The Novel drug delivery system includes sustained release and controlled release drug delivery systems. Vesicular drug delivery system plays a major role in modeling biological membranes, and in the transport and targeting of active agents. Liposomes are multilamellar or unilamellar vesicles wherein an aqueous solution of solute(s) is enclosed in highly ordered bilayer made up of cholesterol and phosphotidyl choline. [1] Liposome has become an essential therapeutic tool most notably in drug delivery and targeting. Structurally, liposomes are concentric bilayered vesicles in which an aqueous volume is enclosed by a membranous lipid bilayer mainly composed of natural or synthetic phospholipids. [2] Phospholipids such as phosphatidylcholine (PC) and cholesterol were selected for the formation of liposomes into which the drug was incorporated. Phospholipids are amphipathic molecules as they have a hydrophobic tail and a hydrophilic or polar head. Cholesterol acts as a 'fluidity buffer' since below the phase transition it tends to make membrane less ordered while above transition it tends to make membrane more ordered thus suppressing the tilts and shifts in membrane structure specifically at the phase transition[3] Zidovudine (AZT) is the first anti-retroviral compound approved for clinical use but the main limitation to its therapeutic effectiveness is its dose-dependent hematological toxicity, low therapeutic index, short biological half-life, and poor bioavailability**.** The biological half life of AZT is 4 hours, thus necessiating frequent administration(3 to 4 times a day) to maintain constant therapeutic drug levels.[4,5]

II.MATERIALS AND METHODS

Zidovudine was collected as a gift sample from Aurobindo Laboratories Ltd, Hyderabad and various cholesterol, phosphotidylcholine and other excipients were purchased from AR chemicals, Hyderabad.

2.1 Methodology

Drug 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 high density polyethylene bags and low density poly ethylene bags. Glass vials were exposed to 60^0C and 40^0C/75 % relative humidity for 4 weeks and low density polyethylene bags were exposed to 40^0C±75 % relative humidity for 4 weeks. Samples were observed periodically for any physical change.

Preparation of liposomes

Table-1: Formulation Table

Formulation no	Zidovudine	Cholesterol	Phosphatidylcholine
F1	100	50	50
F2	100	75	150
F3	100	100	200
F4	100	125	250

Method[7,8]

Liposomes were prepared by thin film hydration technique by using rotary evaporator and 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.

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 Zidovudine and absorbance recorded at 230 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 250 nm.

Amount of Zidovudine in supernatant and sediment gave a total amount of Zidovudine 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 Zidovudine 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.

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 Zidovudine 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.

III.RESULTS & DISCUSSION

Drug-excipient compatibility studies (FT-IR)

The compatibility between the drug and the selected lipid and other excipients was evaluated using Fourier transform infrared spectroscopic 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.

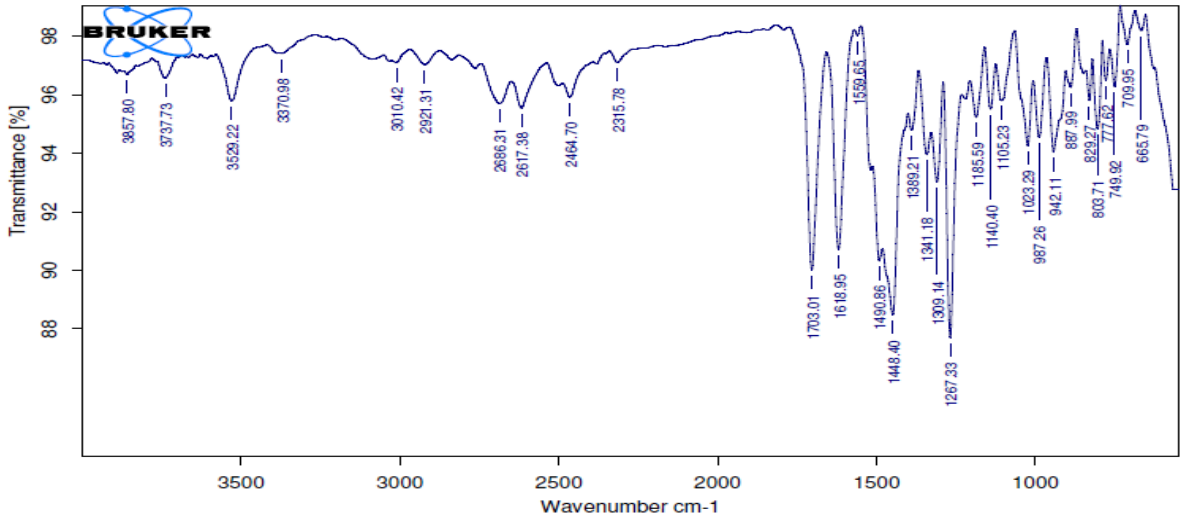

Fig-1: FT-IR Sample for Zidovudine

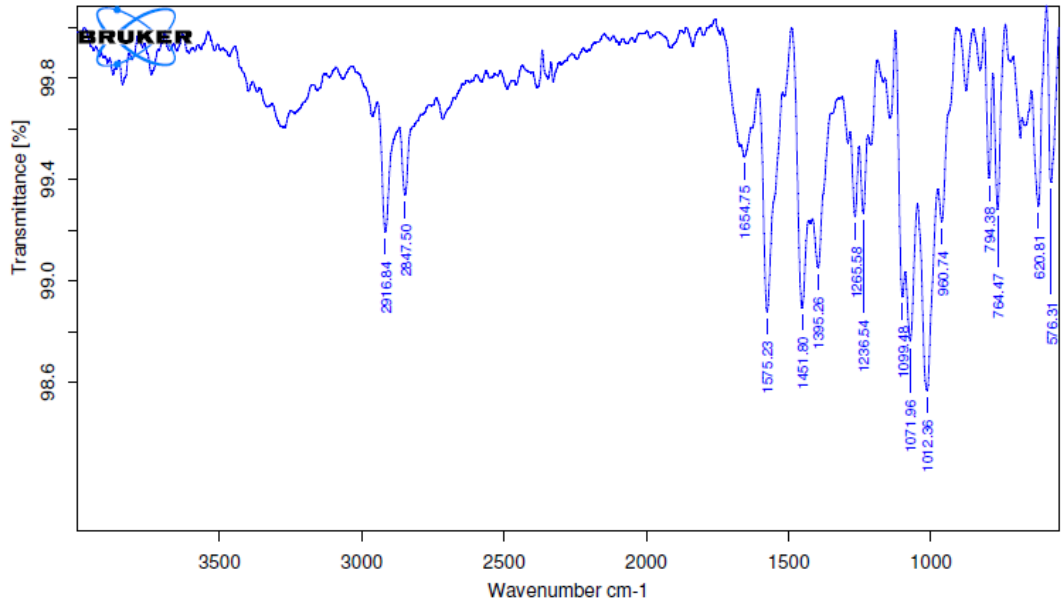

Fig-2: FT-IR Sample for Optimized Formulation

Entrapment efficiency

The percentage of entrapment efficiency of different liposomal batches design was found to be between ranges 75.70% to 85.85%. The maximum entrapment was observed in batch F3 i.e. 85.85%. It can be concluded that the formulation component variables i.e. drug : lecithin : cholesterol ratio, volume of organic phase and volume of aqueous phase and formulation process variables i.e. speed of rotation, vacuum, temperature and hydration time affect the entrapment efficiency of drug.

Table-2: Results of entrapment efficiency of Zidovudine liposomes

F. no	Drug entrapment efficiency
F1	83.16
F2	78.18
F3	85.85
F4	75.70

Particle size:

Liposomal formulations were subjected to particle size analysis and the particle size of the respective formulations was found and the optimized formulation has the particle size of 85 nm and the shape was found to be spherical from the Scanning electron microscopic analysis at 15.00k

Table-3: Results of particle size of Zidovudine liposomes

F. no	Particle size(nm)
F1	102
F2	98
F3	85
F4	77

Scanning electron microscopy:

Scanning electron microscopy micrograph of optimized drug loaded liposomes showed that the colloidal particles have uniform loose aggregates in spherical shape with a smooth surface and theyare uniformly distributed.

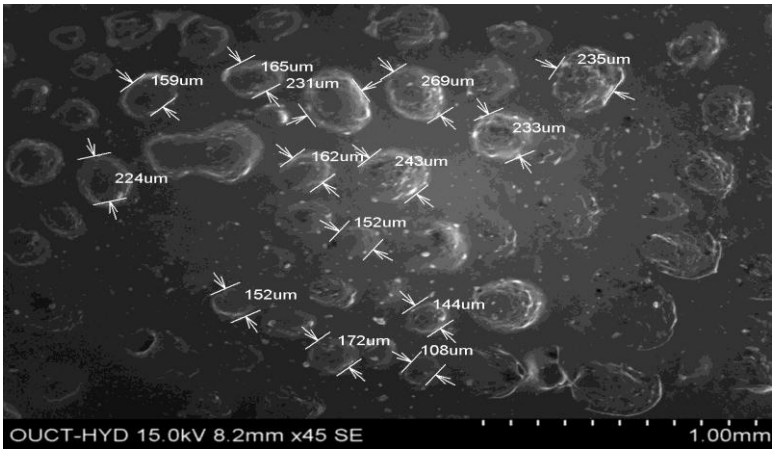

Fig-3: SEM analysis of Zidovudineliposome

Drug release studies

The release studies were carried out by Franz diffusion cell. The phosphate buffer along with liposomal solution was taken in diffusion cell and kept on magnetic stirrer and the samples were withdrawn at regular intervals and drug was determined spectrophotometrically.

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

Time (hrs)	F1	F2	F3	F4
0	0	0	0	0
1	15.20	16.52	17.40	16.55
2	22.50	26.32	28.08	24.15
3	26.53	29.53	30.10	29.31
4	37.40	35.50	45.63	34.70
5	43.70	47.80	51.70	45.20
6	60.65	54.92	63.62	58.67
7	70.15	72.75	76.25	64.82
8	77.20	83.30	88.89	77.23

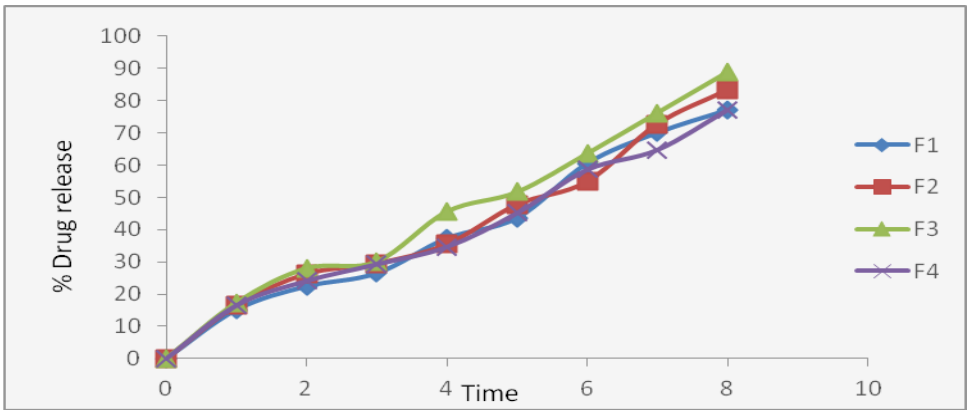

Fig-4: Drug release of all formulations

All the three batches of formulation F3 were found to release the drug in 8 hrs. The cumulative percentage release was found to be 88.89%.

Stability studies:

Stability studies were carried out for a period of 30 days at 4±2^{0}C, 25±2^{0}C and 37±2^{0}C. The entrapment efficiency was estimated at an interval of 30 days.

Table-5: Stability studies for the formulation F3

Sampling Intervals (Days)	% Drug releases at		
	4 ± 2^{0}C	25 ± 2^{0}C	37 ± 2^{0}C
0	88.89	88.89	88.89
15	88.75	88.68	88.50
30	88.45	88.38	88.25

Stability studies for the liposomal formulations were carried out for 30days at 3 different temperatures and was found that there was no incompatibilities between the drug and excipients.

IV. CONCLUSION

From the performed work it was concluded thatZidovudine possesses all requisite qualities required for liposomal drug delivery. Among the various formulation (F1-F4), the formulation F3 was found to be most suitable because of high entrapment efficiency with smaller particle size. The formulation F3 comprising of cholesterol: lecithin = 1: 2 ratio, fulfills the requirement of good liposomal formulation. *In vitro* drug release was studied up to 8 hrs and the drug release was found to be 88.89% with entrapment efficiency of 85.85% and particle size of 85 nm. Formulation F3 was subjected to stability studies of 1month and was found that there was no incompatibility between drug and excipients and no significant changes in the stability of the drug.

REFERENCES

1. Toshimitsu Yoshioka, Brigitte Sternberg, Alexander T Florence. Preparation and properties of vesicles (niosomes) of sorbitan mono esters (span 20, 40, 60 and 80) and a sorbitan tri ester (span 85). Int J Pharm. 1994;105:1-6.
2. Vyas SP, Khar RK. Targetted and controlled drug delivery. 1st Ed. CBS Publishers; 2002.
3. Rukholm G, Mugabe C, Azghani AO, Omri A. Antibacterial activity of liposomal gentamicin against Pseudomonas aeruginosa: a time-kill study. Int J Antimicrob Agents 2006;27:247-52.
4. Kieburtz KD, Seidllin M, Lambert JS, Dollis R, Reichman R, Valentine T. Extended follow-up of neuropathy in patients with AIDS and AIDS related complex treated with dideoxyinosine. J Acquir Immuno Defic Syndrom. 1992; 5: 60-64.
5. Kuksal A, Tiwary AK, Jain NK, Jain S. Formulation and in vitro, in vivo evaluation of extended- release matrix tablet of zidovudine: Influence of combination of hydrophilic and hydrophobic matrix formers. AAPS PharmSciTech. 2006; 7(1): Article 1. DOI. 10.1208/ pt070101.
6. Scherphof G, Morselt H, Regts J. "The involvement of the lipid phase transition in the plasma-induced dissolution of multilamellarphosphatidylcholine vesicles". Biochem. Biophys. Acta.1979, p.no.556:196–207.
7. Jain NK. "Controlled and novel drug Delivery", "CBS Publishers and distributors".2002.,p.no. 305,326,330.
8. Bangham AD. "Techniques in life Science", "lipid membrane biochemistry". "Elsevier scientific publications". Ireland. 1982, p.no.1-25.
9. Drummond DC, Meyer O, Hong K. "Pharmacokinetic study of liposome encapsulated drug". Pharmacological reviews 1999, p.no. 51(4): 691-743.11.
10. Lasic DD**. "**Stealth liposomes,Microencapsulation Method and Industrial Applications". Vol. 73, 1996, p.no. 297-311.
11. Crommelin DJ, Schreier H. „Liposomes, Colloidal Drug Delivery Systems". 1996, p.no.73-157.
12. Liu R, Cannon JB, Li Y. "Liposomes in Solubilization". "Water Insoluble Drug FormulationCRC Press", New York. 2000, p.no. 355-358.