International Journal of Multidisciplinary Research and Growth Evaluation.

Design and evaluation of liposomal formulation of zidovudine

Spandhana P [1*], Govindu Supraja [2], Dongiri Shirisha [3], Sabavat Usha Rani [4], Juveria Firdouse [5], Beemani Sree Veena [6]
[1] Department of Pharmaceutics, Bojjam Narasimhulu Pharmacy College for Women, Hyderabad, Telangana, India
[2-6] Bojjam Narasimhulu Pharmacy College for Women, Hyderabad, Telangana, India

* Corresponding Author: **Spandhana P**

Article Info

ISSN (online): 2582-7138
Volume: 04
Issue: 01
January-February 2023
Received: 04-01-2023;
Accepted: 21-01-2023
Page No: 386-389
DOI: https://doi.org/10.54660/.IJMRGE.2023.4.1.386-389

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. Zidovudineis a drug with narrow therapeutic index and short biological half-life. This study aimed at developing and optimizing liposomal formulation of Zidovudinein 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.F3emerged 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

1. Introduction

Liposomes

Amphiphilic molecules such as phospholipids can be used to form hydrophobic and hydrophilic compartments with in an aqueous environment. Phospholipids have a pair of long hydrocarbon chain(s) covalently bonded to a polar, zwitter ionic and/or ionic head group. Upon dispersion in aqueous medium, they can assume three possible forms as shown in Figure 1 [2].

2. Materials and Methods

2.1 Materials

Zidovudine as gift sample from Aurobindo Laboratories Ltd, Cholesterol, Chloroform, Phosphatidylcholine, Methanol, Di-sodium hydrogen ortho phosphate and Potassium dihydrogen phosphate were purchased from AR chemicals, Hyderabad.

2.2 Methodology

Preparation of liposomes

Method

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.

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

Evaluations of liposomes

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.

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 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

In Vitro Drug release study

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^{0}C. 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

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^{o}c and refrigerator 2-8^{o}c for a period of 90 days.

3. Results and 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 linteraction between the drug, lipid, and other chemicals.

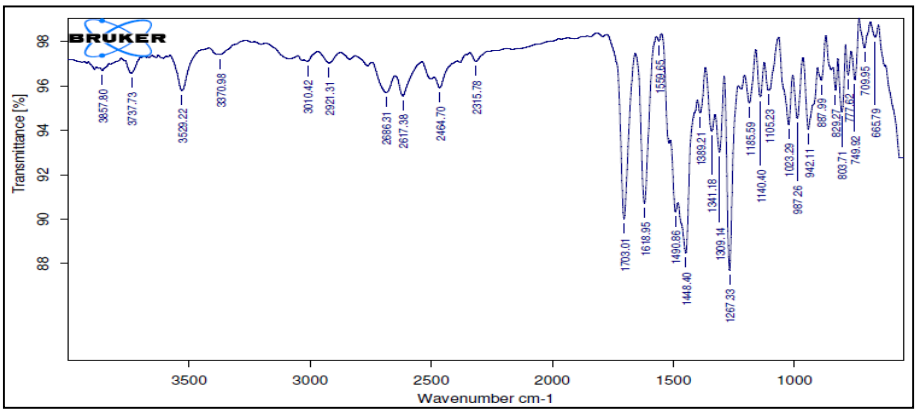

Fig 1: FT-IR Sample for Zidovudine

Table 2: Characteristic Peaks and frequency of Zidovudine

S.No.	Characteristic Peaks	Frequency range (cm-1)	Frequency (cm-1)
1	OH stretching	3887-3737	3500
2	OH Bending	2342-2421	2100
3	C-H stretching	2464-2315	2500
4	C-N stretching	1750-1653	1650

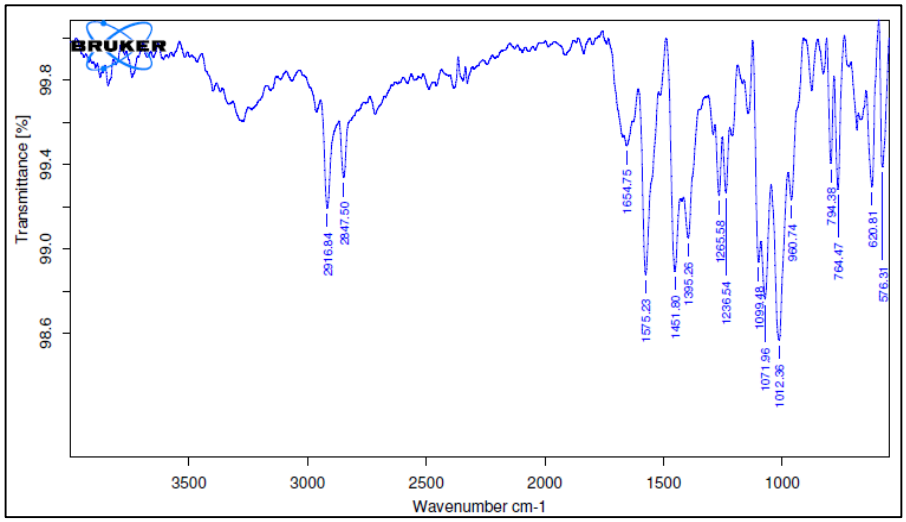

Fig 2: FT-IR Sample for Optimized Formulation

Table 3: Characteristic Peaks and frequency of Mixture

S. No.	Characteristic Peaks	Frequency range (cm-1)	Frequency (cm-1)
1	OH stretching	2916-2847	3000
2	OH Bending	1575-1421	1500
3	C-N stretching	1395-1236	1600

Entrapment efficiency

The percentage of entrapment efficiency of different liposomal batches design was found to be between ranges 75.74% to 85.45%. The maximum entrapment was observed in batch F3i.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 4: Results of entrapment efficiency of Zidovudine liposomes

S. no	Drug entrapment efficiency
F1	81.46
F2	78.26
F3	85.45
F4	75.74

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 5: Results of particle size of Zidovudine liposomes

F. no	Particle size(nm)
F1	96
F2	98
F3	87
F4	76

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 they are uniformly distributed.

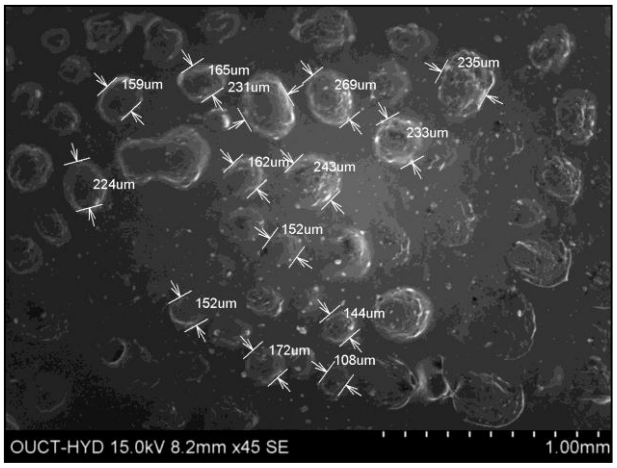

Fig 3: SEM analysis of Zidovudine liposome

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 6: Cumulative percentage drug release from various formulation of liposome

Time (hrs)	F1	F2	F3	F4
0	0	0	0	0
1	16.55	15.23	17.45	16.52
2	24.15	22.54	28.08	26.32
3	29.31	26.53	30.13	29.53
4	34.78	37.46	45.67	35.54
5	45.25	43.72	64.74	47.83
6	58.67	60.65	73.62	64.92
7	74.82	70.15	86.25	72.75
8	85.32	82.22	92.89	84.98

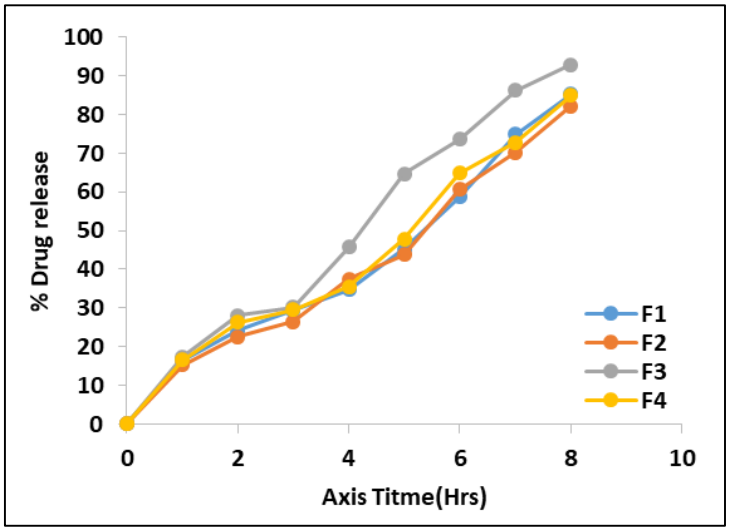

Fig 4

All the four batches of formulation F3 were found to release the drug in 8hrs. The cumulative percentage release was found to be 92.89%.

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 7: 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	92.89	92.89	92.89
15	91.89	91.68	91.50
30	91.09	90.38	90.25

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.

4. Conclusion

From the performed work it was concluded that

1. Zidovudinepossesses all requisite qualities required for liposomal drug delivery.
2. Among the various formulation (F1-F4), the formulation F3 was found to be most suitable because of high entrapment efficiency with smaller particle size.
3. The formulation F3comprising of cholesterol: lecithin is1:2ratio, fulfills the requirement of good liposomal formulation. *In vitro* drug release was studied upto 8hrs and the drug release was found to be91.72% with entrapment efficiency of 90.82% and particle size of 89 nm.
4. 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.

5. References

1. Benita S. Microencapsulation Method and Industrial Application, Drug and The Pharmaceutical sciences. 2002; 73:213.
2. Fuji G. Vesicles, Realization of the Drug Delivery Concept. 1996; 62:491-495.
3. Bangham AD, Standish MM. Diffusion of univalent ions across the lamella of swollen Phospholipids. J Mol. Biol. 1965; 13:238-252.
4. Vyas SP, Khar RK. Targeted and controlled Drug Delivery, Novel Carrier system, CBS Publishers and distributors, 2002, 249.
5. Gregoriadis G. Liposome technology, Liposome preparation and related techniques, 3rd edition. 2007; l:21.
6. Singh S, Gulati M. Advances in liposomal therapeutics, Targeting of drugs to lipid bilayer in liposomes: an approach for the success of liposomes as delivery systems. CBS Publishers and distributors. New Delhi. 2001, 4-34.
7. Scherphof G, Morselt H, Regts J. The involvement of the lipid phase transition in the plasma-induced dissolution of multi lamellarp hosphatidyl choline vesicles. Bio-chem. Biophys. Acta. 1979; 556:196–207.
8. Jain NK. Controlled and novel drug Delivery, CBS Publishers and distributors. 2002, 305,326,330.
9. Bangham AD. Techniques in life Science, lipid membrane biochemistry. Elsevier scientific publications. Ireland, 1982, 1-25.
10. Drummond DC, Meyer O, Hong K. Pharmacokinetic study of liposome encapsulated drug. Pharmacological reviews. 1999; 51(4):691-743.11.
11. Lasic DD. Stealth liposomes, Microencapsulation Method and Industrial Applications. 1996; 73:297-311.
12. Crommelin DJ, Schreier H. Liposomes, Colloidal Drug Delivery Systems, 1996, 73-157.
13. Liu R, Cannon JB, Li Y. Liposomes in Solubilization. Water Insoluble Drug Formulation CRC Press, New York, 2000, 355-358.
14. Patel HM, Ryman BR. Systemic and Oral administration of liposomes. Physical Structure to therapeutic applications. 1981, 409-439.
15. Jaggi M, Khar RK. Liposomes as Drug Carriers. Indian Drugs. 1989; 27(8):404.
16. Krensky AM, Strom TB. Immunomodulators: Immunosuppressive agents, Tolerogens and Immunostimulants. Goodman and Gilman's The Pharmacological Basis of Therapeutics Limbird LE (Ed.) 10th Ed., 2001, 1466-1480.