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FORMULATION AND *INVITRO* EVALUATION OF LAMIVUDINE NIOSOMES

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Abstract:

The aim of present study is an attempt to formulate and evaluate controlled release niosomal formulations by using Lamivudine drug for potentially treating HIV and AIDS related condition. Lamivudine is an antiretroviral drug for the treatment of acquired immune deficiency syndrome (AIDS) & Hepatitis. The present study involves the preparation and characterization of Lamivudine entrapped niosomes and finding the drug carrier qualities of the niosomes. The formulation L1-L6 which were prepared by varying the concentration surfactant (Tween 20 & span 20) by ether injection method. The optimized formulation of lamivudine is prepared by ether injection method was subjected to characterization studies for different evaluation parameters such as vesicle size, % entrapment efficiency, drug content, in vitro release and the stability studies was carried out at different temperature. The present study demonstrates the controlled drug release after encapsulation of Lamivudine into niosomal preparation.

Key Words: *Lamivudine, Niosomes, controlled release, anti-retroviral, Immunodeficiency syndrome, Hepatitis, Tween 20, Span 20.*

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INTRODUCTION:

In the past few decades, considerable attention has been focused on the development of new drug delivery system (NDDS). The NDDS should ideally fulfill two prerequisites. Firstly, it should deliver the drug at a rate directed by the needs of the body, over the period of treatment. Secondly, it should channel the active entity to the site of action. Conventional dosage forms including prolonged release dosage forms are unable to meet none of these. At present, no available drug delivery system behaves ideally, but sincere attempts have been made to achieve them through various novel approaches in drug deliver [1]. Approaches are being adapted to achieve this goal, by paying considerable attention either to control the distribution of drug by incorporating it in a carrier system, or by altering the structure of the drug at the molecular level, or to control the input of the drug into the bio-environment to ensure an appropriate profile of distribution.

Novel drug delivery system aims at providing some control, whether this is of temporal or spatial nature, or both, of drug release in the body. Novel drug delivery attempts to either sustain drug action at a predetermined rate or by maintaining a relatively constant, effective drug level in the body with concomitant minimization of undesirable side effects. It can also localize drug action by spatial placement of controlled release systems adjacent to, or in the diseased tissue or organ or target drug action by using carriers or chemical derivatization to deliver drug to target cell type.

Different types of pharmaceutical carriers are present. They are particulate polymeric, macromolecular, and cellular carrier. Particulate type carrier also known as a colloidal carrier system, includes lipid particles (low- and high-density lipoprotein LDL and HDL, respectively), microspheres, nanoparticles, polymeric micelles and vesicular like liposomes, niosomes, pharmacosomes, virosomes, etc [2-4]. The vesicular systems are highly ordered assemblies of one or several concentric lipid bilayers formed, when certain amphiphilic building blocks are confronted with water. Vesicles can be formed from a diverse range of amphiphilic building blocks. The terms such as synthetic bilayers allude to the nonbiological origin of such vesiculogenes. Biologic origin of these vesicles was first reported in 1965 by Bingham and was given the name Bingham bodies [5].

The oral route remains the most promising route of drug delivery because of the low cost of therapy and high levels of patient compliance. An important requisite for the successful performance of oral SRDDS is that the drug should have good absorption throughout the gastrointestinal tract (GIT), preferably by passive diffusion, to ensure continuous absorption of the released drug. A major constraint in oral controlled drug delivery is that not all drug candidates are absorbed uniformly throughout the GIT. Such drugs are said to have an absorption window, which identifies the drug's primary region of absorption in the GIT. This limitation could be overcome, for various judiciously selected drugs, by prolonging the gastric residence time of the pharmaceutical dosage form.

In the present investigation lamivudine was employed in the controlled drug delivery system for extending the drug release for a prolonged period. Lamivudine comes under the class - Nucleoside Reverse Transcriptase Inhibitors (NRTIs). It is a nucleoside analogue, which was originally licensed for the treatment of HIV. For the treatment of AIDS, the dosage of conventional oral formulations of Lamivudine is 300mg per day (i.e. 150 mg twice daily, multiple times a day). Lamivudine is rapidly absorbed after oral administration and mean elimination half-life (t½) is 5 to 7 hours, thus necessitating frequent administration for a extended period (lifelong in AIDS and for one year in hepatitis patients) to maintain constant therapeutic drug levels.

Based on the biopharmaceutical properties the drug lamivudine is selected as a drug candidate for the formulation of niosomes. The present work was aimed to formulate the controlled release niosomes of Lamivudine by ether injection technique for extending the drug release from the dosage form for a prolonged period by non –ionic surfactants like tween 20 and Span 20 respectively.

MATERIAL AND METHODS:

Materials

Lamivudine was obtained as a gift sample from Apotex pharma Ltd, Bangalore. Tween 20, Span 20 Commercially procured from Yarrow chem. Products, Mumbai and cholesterol, commercially procured from Colorcon chemicals Asia pvt., Ltd., Mumbai.

Preparation of Lamivudine Loaded Niosomes by Ether Injection Method

This method provided a means of making niosomes by slowly introducing nonionic surfactant and cholesterol dissolved in diethyl ether mixed with 2 ml methanol previously containing weighed quantity of drug. The resulting solution was slowly injected using micro syringe into 10 ml hydrating phosphate buffer at a rate

of 1 ml/min on magnetic stirrer, and the temperature maintained at 60-65°C. Then the lipid solution was injected slowly into aqueous phase. Differences in temperature between the phases caused rapid vapourization of ether and resulted in the formation of niosomal vesicles [7].

EVALUATION OF LAMIVUDINE NIOSOMES

Morphological Characterization

Optical microscopy

The vesicle formation was confirmed by optical microscopy in ×45 resolution. The niosome suspension placed over a glass slide and fixed over by drying at room temperature, the dry thin film of niosome suspension observed for the formation of vesicles [8].

Drug content

Niosomal suspension equivalent to 5 mg was taken in a 10 ml volumetric flask, and the volume was made with 7.4 pH buffer to disrupt the vesicle by sonication for 40 min. 1mL of the solution was pipetted out in 50 ml volumetric flask; the volume was made with buffer and kept for sonication. Then the concentration of drug was analyzed by UV spectrophotometer at 235 nm [9,10].

Entrapment efficiency

After preparing niosomal dispersion, unentrapped drug was separated by centrifugation using pH 7.4 phosphate buffer for 45 min at 17,000 rpm. The resulting solution was analyzed by UV spectrophotometer at 235 nm for the total amount of entrapped drug [11,12].

Entrapment efficiency = Amount of drug taken – amount of drug in supernatant × 100
amount of drug

In Vitro Dissolution Studies

In-vitro drug release studies of niosomes were carried out by Franz diffusion cell. 1 ml of niosomal formulation was placed on cellophane membrane between the donor compartment and the receptor compartment containing 15 ml of pH 6.8 buffer. The samples were withdrawn using micro syringe at regular interval of time with replacing fresh buffer to maintain the sink condition. The absorbance of each sample was analyzed by UV spectrophotometer at 235 nm, and the *in-vitro* drug release was calculated [13,14].

Evaluation of Various Dissolution Parameters:

Various dissolution parameters such as zero order rate constant, first order rate constant, Higuchi constant and Peppa's constant were calculated from the dissolution data obtained from various formulations.

Characterization

Based on the dissolution studies performed on all the formulations, some of the optimized formulations were selected for further investigations such as FTIR, Analysis.

Infra- Red Spectroscopy

I.R Spectral studies were carried out on some selected Lamivudine by using BRUKER FTIR.

FTIR spectrophotometer was used for recording spectra in the region of 4000 – 400 cm^{-1} or in some cases down to 200 cm^{-1}. Triturate 1-2 mg of substance to be examined with 200 mg of finely powdered and dried KBr. These quantities were usually sufficient to give a disc of 10-15 mm diameter and a spectrum of suitable intensity [15,16].

RESULTS AND DISCUSSION:

Preparation of Lamivudine Loaded Niosomes by Ether Injection Method

This method provided a means of making niosomes by slowly introducing nonionic surfactant and cholesterol dissolved in diethyl ether mixed with 2 ml methanol previously containing weighed quantity of drug. The resulting solution was slowly injected using micro syringe into 10 ml hydrating phosphate buffer at a rate of 1 ml/min on magnetic stirrer, and the temperature maintained at 60-65 °C. Then the lipid solution was injected slowly into aqueous phase. Differences in temperature between the phases caused rapid vapourization of ether and resulted in the formation of niosomal vesicles [6]. The composition of lamivudine niosomes was given in Table1.

Table 1: Composition of Lamivudine Niosomes Formulations

Formulation	Drug (mg)	Tween 20(mg)	Span 20(mg)	Cholesterol(mg)	Diethyl Ether (ml)
L1	100	100	-	100	10
L2	100	200	-	100	10
L3	100	300	-	100	10
L4	100	-	100	100	10

L5	100	-	200	100	10
L6	100	-	300	100	10

Estimation of Lamivudine:
The spectrophotometric method used for the estimation of lamivudine in the dissolution medium was found to be linear and reproducible. The standard calibration curve yields a straight line, which shows that the drug follows Beer's law in the concentration range of 2-10 µg/ml. Reproducibility of the method was tested by analysing 6 separately weighed samples of drug Lamivudine. Thus, the method was found to be suitable for the estimation of lamivudine in dissolution media (6.8 p^H phosphate buffer). Calibration curve was shown in Figure1.

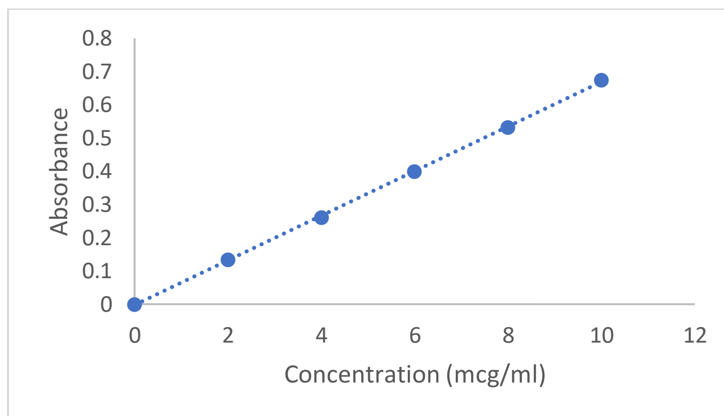

Figure 1: Calibration Curve for the Estimation of Lamivudine

Evaluation of physical parameters for Lamivudine Niosomes
The niosomal formulations are evaluated for physical parameters such as drug, entrapment efficiency and particle shape [17]. The drug content of all niosomal formulations were found in the range of 88± 0.50, 95± 0.12%. The entrapment efficiency of all niosomal formulations were found in the range of 84-94%. The particle shape of all niosomal formulations were spherical in shape. The results were given in Table 2.

Table 2: Evaluation of Lamivudine Niosomes Formulations

Formulation	Drug content (%)	Entrapment Efficiency (%)	Particle shape
L1	88± 0.50	84	Spherical
L2	89±0.25	86	Spherical
L3	90±0.02	88	Spherical
L4	90±0.32	89	Spherical
L5	94±0.54	90	Spherical
L6	95±0.12	94	Spherical

***In Vitro* Dissolution Studies:**
Diffusion studies were performed on all the niosomal formulations by using Franz Diffusion cell. By using 6.8 pH phosphate buffer. The drug release from the niosomal Formulations were failed to extend the drug up to 12hrs in the formulations L1 and L2 containing tween20 and release 84.93-86.92%. The drug release from the niosomal formulations were extended the drug release up to a period of 12hrs in the formulation L3 containing tween20 and release 92.66%. The drug release from the niosomal formulations (L4-L6) was extended up to 12hrs and release 90.55-94.87% from the formulations L4, containing span20. The results were shown in Figure 3.

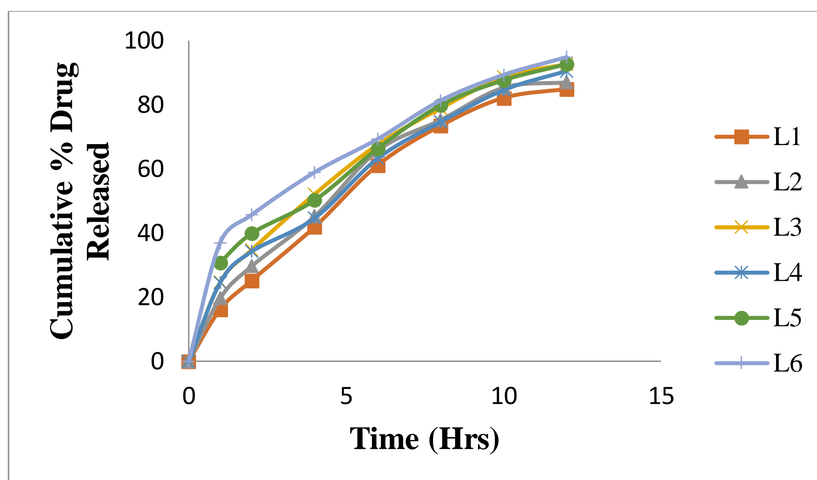

Figure 3: Drug Release Profiles of Lamivudine Niosomes

Evaluation of Various Dissolution Parameters:
The zero order plots for all the niosomal formulations were not linear. All the niosomal formulations were found to be linear with first order release rate with R^2 values in the range of 0.902 - 0.979. Thus the rate of drug release from all then niosomal formulations were concentration dependent and were linear with first order release rate constant (K_1). The Higuchi constants for all the niosomal formulations were in the range of 27-46 mg indicating the controlled drug release from the dosage form [18-20]. The amount of drug released *Vs* square root time plots were found to be linear with R^2 values in the range of 0.922 – 0.975. Thus, the drug release from the niosomal formulations was by diffusion process. The results were shown in figure 16-18. The release exponent (n values) for all the niosomal formulations were in the range of 0.6-0.5, indicated that the drug release was by non-Fickian diffusion. The log amount drug released v*s* log time plots were found to be linear with R^2 values in the range of 0.902 – 0.977. The results were given in Table3.

Table 3: Dissolution parameters of Lamivudine Niosomes

Formulation	Zero Order		First Order		Higuchi		Peppas	
	K_0 (mg/hr)	R^2	K_1 (h^{-1})	R^2	K_H ($mg/h^{1/2}$)	R^2	n	R^2
L1	6.856	0.666	0.274	0.902	23.186	0.922	0.634	0.902
L2	7.012	0.672	0.203	0.923	32.588	0.933	0.552	0.912
L3	7.660	0.861	0.214	0.933	33.634	0.945	0.587	0.923
L4	7.231	0.688	0.425	0.922	31.574	0.951	0.425	0.953
L5	7.054	0.691	0.549	0.946	27.017	0.967	0.484	0.961
L6	7.944	0.884	0.4005	0.979	37.888	0.975	0.550	0.977

CHARACTERIZATION
FTIR Studies:
FTIR studies of lamivudine and optimized formulations were carried out to study the interaction between the drug and excipients used. C=O stretching, O-H stretching, N-H stretching, Asymmetrical C-O-C stretching and Symmetrical C-O-C stretching of pure lamivudine and the optimized formulations were almost in the same region of wave number ranging from 3326.95cm^{-1} to 1160.39cm^{-1}. It showed that IR spectrum of lamivudine and optimized

formulations were having similar fundamental peaks and pattern. This indicated that there were no drug excipient interactions in the formulations. The results were shown in Figure 4, 5.

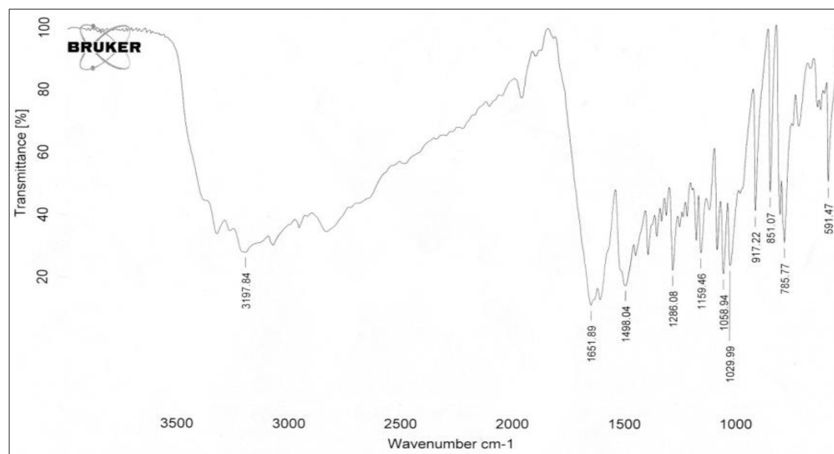

Figure 4: FTIR spectrum of Pure Drug of Lamivudine

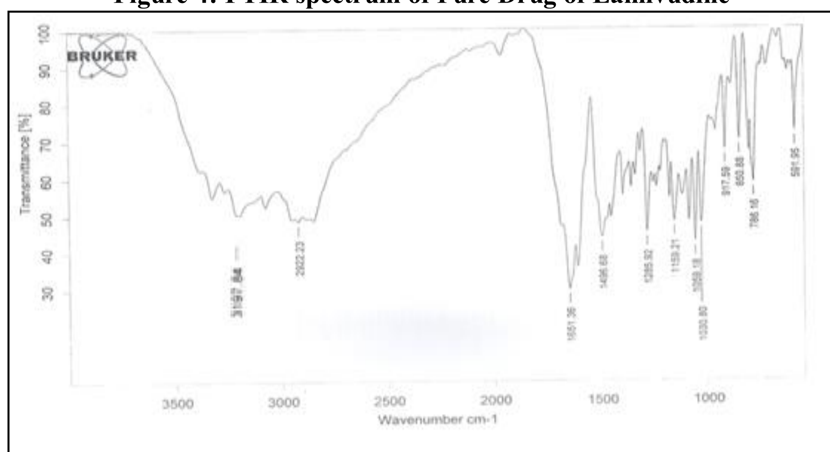

Figure 5: FTIR Spectrum of Optimized Formulation (L6)

Accelerated Stability Studies:

The formulations which showed good *in vitro* performance were subjected to accelerated stability studies. These studies were carried out by investigating the effect of temperature on the physical properties of the tablets and on drug release from the niosomal formulations L6 containing lamivudine. The results of these studies were given shown in Figure 6.

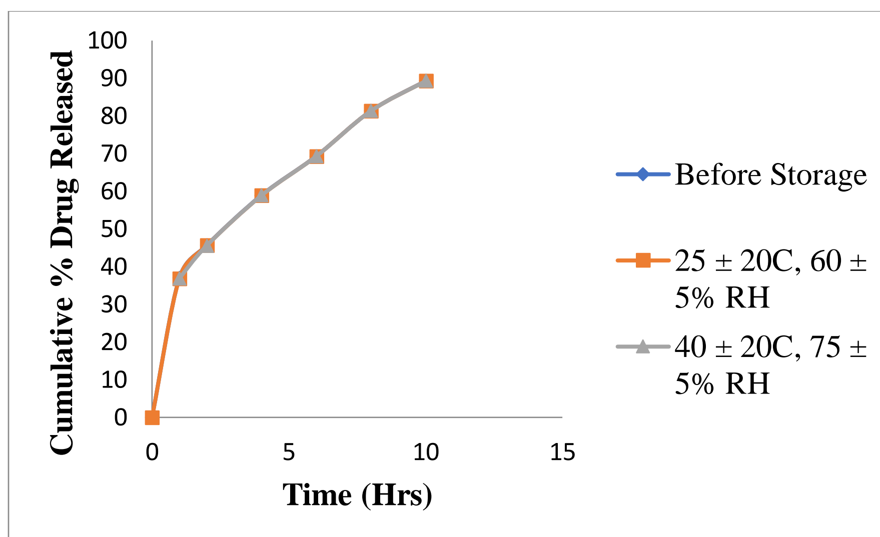

Figure 6: Drug Release profiles of Lamivudine Niosomes Before and After Storage at Different Conditions

CONCLUSION:

Lamivudine niosomes prepared by ether injection method showed good flexibility and physicochemical properties with good bioavailability. The drug release from the niosomal formulations (L4-L6) was extended up to 12hrs. In the formulations L4, L6 containing span 20. All the designed formulations of lamivudine displayed first order release kinetics. The optimized formulation L6 prepared by utilizing Span 20 showed the average drug release of 94.86% which was desirable for extending the drug release. The drug content on average was found to be 88± 0.50 to 95±0.12, and showed good entrapment efficiency and the FTIR studies indicated that there was no interaction between drug and polymer. Accelerated stability studies were carried out for optimized niosomal formulation. Drug release from the niosomal formulations after storage at different conditions remained unaltered and quite stable. Lamivudine niosomes prepared by ether injection technique were found to be suitable for the treatment of HIV. *Invitro* studies showed that plasma concentrations of drug were extending to more than 12hrs.

Future Prospects

Lamivudine niosomal formulations prepared by ether injection technique using tween 20 and span 20 showed drug release upto 12 hrs. Future studies can be focused on designing several Lamivudine-controlled release niosomal formulations by using different newer non-ionic surfactants. The *in-vivo* pharmacokinetic and pharmacodynamic studies can be performed on a suitable animal model.

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