Formulation and evaluation of Lamivudine ethosomes for the treatment of AIDS disease

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Abstract

The main objective of the present work was to develop transdermal delivery of lamivudine for the treatment of AIDS, from ethosomes. Ethosome become an area of research interest in formulation because of it's enhance skin permeation and improve entrapment efficiency. Lamivudine and Stavudine loaded ethosomal carriers were prepared, optimized and characterized for microscopy, vesicular size, entrapment efficiency, stability and in-vitro release study. The entrapment efficiency of optimized formulation containing lamivudine ethanome F7 was found to be highest (74.12%) while F4 formulation showed least entrapment efficiency (58.81 %). Transmission Electron Microscope (TEM) indicated that ethosome have a discrete spherical structure without aggregation. It has been observed the formulation containing phospholipid (3 gm) with 40 ml ethanol with Tween 80 and SLS has maximum entrapment efficiency. Percentage drug release of Lamivudine drug from ethosome formulation F7 was observed to be 78.49 % while for stavudine drug it observed to be 76.91% at 120 min. It was observed that ethosome formulation F7 showed maximum drug release as compared to other formulation.

Keywords: Lamivudine, ethosomal, entrapment efficiency, Transmission Electron Microscope.

INTRODUCTION

Human immunodeficiency virus (HIV) is a retrovirus that causes irreversible destruction of the immune system. During the last decade, even though attempts were being made to eradicate HIV but it was found that eradication of HIV is highly unlikely, and effective antiretroviral therapy is required on a long-term basis to maintain viral suppression and reduce disease progression. Lamivudine is a commonly used hydrophilic antiviral drug for treatment of acquired immunodeficiency syndrome (AIDS and hepatitis. Lamivudine has a short biological half-life (4-6 hour) and requires frequent administration for a prolonged period of time (lifelong in AIDS and for one year in hepatitis patients).[1,2] Stavudine is used in the treatment of HIV-1 infection, but is not a cure. It is not normally recommended as initial treatment.Stavudine can also reduce the risk of developing HIV-1 infection after coming into contact with the virus either at work (e.g., needlestick) or through exposure to infected blood or other bodily fluids. It is always used in combination with other HIV medications for the better control of the infection and a reduction in HIV complications.

Transdermal route is, therefore, a better alternative to achieve constant plasma levels for prolonged periods of time, which additionally could be advantageous because of less frequent dosing regimens.

The major advances in vesicle research was the finding that some modified vesicles possessed properties that allowed them to successfully deliver drugs in deeper layers of skin. Transdermal delivery is important because it is a non-invasive procedure for drug delivery. Further, problem of drug degradation by digestive enzymes after oral administration, gastric irritation and discomfort associated with parenteral drug administration can be avoided. Flexible liposomes are common vectors in transdermal drug-delivery systems, with relatively good liquidity and deformability. In recent years, ethosomes have become new liposome carriers with high deformability; high entrapment efficiency and a good transdermal permeation rate in the drug delivery system, and are suitable for transdermal administration. Compared with other liposomes, the physical and chemical properties of ethosomes make these more effective for drug delivery through the stratum corneum into the blood circulation, which is very important in the design of a transdermal drug delivery system.

Ethosomes

Ethosomes are novel drug delivery system that enable drugs to reach the deep skin layers and systemic circulation .These are soft, malleable non-invasive vesicles which encapsulate active agent for its enhanced delivery.Drug delivery from such vesicles results in the formation of a drug reservoir in the horny layer of the skin and is generally characterized by a lack of penetration into the deeper layers of the skin. This behavior is useful both for local treatment of skin disorders and for cosmetic formulations. Specific drug accumulation at the site of action and decreased systemic drug absorption can impart increased efficiency as well as decreased side effects for a compound applied topically. More recently, Touitou's group studied the delivery of dyphylline incorporated in unilamellar liposomes from polyethylene glycol (PEG), carbopol gel, a PEG enhancer base and water. Conversely, when caffeine delivered from small unilamellar liposomes, was found mostly localized into the skin. By using quantitative autoradiography, it was also found that the concentration of the drug was greatest in the epidermis, lowest in the dermis, and relatively high in the appendages. As discussed above, conventional liposomal systems were demonstrated to be effective at delivering active agents to the upper layers of the skin and novel lipid carriers that composed of ethanol, phospholipid, and water termed as Ethosomes.

All components of the Ethosomal systems are considered as being safe for pharmaceutical and cosmetic use. Ethosomal systems were found to be significantly superior at delivering drugs through the skin in terms of both quantity and depth when compared to liposomes and too many commercial transdermal and dermal delivery systems. Ethosomes are sophisticated vesicular delivery carriers that are capable of delivering various chemical applications. Visualization by dynamic light scattering showed that

Ethosomes could be unilamellar or multilamellar through to the core. These novel delivery systems contain soft phospholipid vesicles in the presence of high concentrations of ethanol. Ethosomal systems are sophisticated conceptually, but characterized by simplicity in their preparation, safety and efficiency - a rare combination that can expand their applications.[6, 7]

The enhanced delivery of actives using ethosomes over liposomes can be ascribed to an interaction between ethosomes and skin. It is thought that the first part of the mechanism is due to the' ethanol effect', whereby intercalation of the ethanol into intercellular lipids increasing lipid fluidity and decreases the density of the lipid multilayer. This is followed by the 'ethosomes effect', which includes inter lipid penetration and permeation by the opening of new pathways due to the malleability and fusion of ethosomes with skin lipids, resulting in the release of the drug in deep layers of the skin [7,8]

The ethosomes are vesicular carrier comprise of hydroalcoholic or hydro/alcoholic/glycolic phospholipid in which the concentration of alcohols or their combination is relatively high. Typically, ethosomes may contain phospholipids with various chemical structures like phosphatidylcholine (PC), hydrogenated PC, phosphatidic acid (PA), phosphatidylserine (PS), phosphatidylethanolamine (PE), phosphatidylglycerol (PPG), phosphatidylinositol (PI), hydrogenated PC, alcohol (ethanol or isopropyl alcohol), water and propylene glycol (or other glycols). Such a composition enables delivery of high concentration of active ingredients through skin. Drug delivery can be modulated by altering alcohol: water or alcohol-polyol: water ratio.

Some preferred phospholipids are soya phospholipids such as Phospholipon 90 (PL-90). It is usually employed in a range of 0.5-10% w/w. The concentration of alcohol in the final product may range from 20 to 50%. The concentration of the non-aqueous phase (alcohol and glycol combination) may range 22 to 70 % .[9-11]

Material & Methods

Materials

Lamivudine was received as a gift sample from Dr. Reddy's Laboratories Ltd, Hyderabad, India. High purity soyaphosphatidyl choline (99%, PC) and others chemical were purchased from Sigma Chemicals.

Preparation of Standard Curve [7]

Stock solution: Accurately weighted 100 mg of Lamivudine and Stavudine was dissolved separately in 10 ml of methanol in 100 ml of volumetric flasks and volume was made up to 100ml with pH 7.4 phosphate buffer to get a solution 1000µg/ml concentration.

Standard solution: From primary stock solution of 10 ml was pipette out in a 100 ml of volumetric flask and volume was made up to the mark with pH 7.4 buffer to get a concentration of 100 µg/ml. Aliquot of standard drug solution ranging from 1ml to 8ml were transferred in to 10ml volumetric flask and were diluted up to the mark with pH 7.4 phosphate buffer. Thus the final concentration ranges from 10-60 µg/ml. Absorbance of each solution was measured at 270 nm and 263 nm against pH 7.4 phosphate buffer as a blank. A plot of concentrations of drug versus absorbance was plotted.

FT–IR spectral analysis

The development of a successful formulation depends only on a suitable selection of excipients. Hence the physical state of the drug Lamivudine , Stavudine and the excipients used in ethosome formulation individually and the combination of drug and excipients used for ethosomes preparation are studied by FTIR (Fourier transform infrared spectroscopy) to know the drug–polymer compatibility. The physicochemical compatibility of the drugs and the polymer was obtained by FTIR studies (Fig 4.1 to 4.6). The interpretation values of the FTIR are mentioned in the Table

Preparation of Ethosomes suspension:

Ethosomes were prepared by solvent dispersion method: Soya phospotidylcholine up to (2-3%),drug taken and dissolved in (30-40%) of 90% ethanol by use of magnetic stirrer (Remi Motors Mumbai), to this solution fine stream of distilled water was added with help of syringe, then whole system was stirred for 30 minutes at 700 rpm in a close vessel. SLS and Tween 80 were added for increasing solubility. [11,12]

Evaluation of Ethosome suspension[13,14]:

Fourier transform-infrared ray spectroscopy (FT-IR) Studies

The interaction studies between drug, phospholipid and formulations (F7) were studied using FT-IR spectroscopy.

Image analysis of ethosomes by optical microscope

Visualization done by image analysis compound microscope .The compound microscope is attached with the digital camera: Nikol, coolpix, L20, through which image analysis was done, photographs were captured.

Vesicular shape and surface morphology

Transmission Electron Microscope (TEM) was used as a visualizing aid for ethosomal vesicles. Samples were dried on carbon-coated grid and negatively stained with aqueous solution of phosphotungstic acid. After drying the specimen was viewed under the microscope.

Determination of Entrapment efficiency of ethosomes suspension:

Aliquots of ethosomal suspension (10 ml) were subjected to centrifugation using cooling ultracentrifuge (Remi) at 12000 rpm for 90 minutes. The clear supernatant was siphoned off carefully and the

absorbance was recorded at λmax 255 nm using UV/Vis spectrophotometer (Shimadzu UV 1700). The percent entrapment was calculated using the formula.

$$\% EE = [Qt-Qs/Qt] X100$$

Where, EE is the entrapment efficiency, Qt is amount of drug added, Qs is amount detected in the supernatant.

Table 1: Preparation of Ethosomes suspension containing Lamivudine drug

Ingredient	F1	F2	F3	F4	F5	F6	F7	F8
Lamivudine (gm)	1	1	1	1	1	1	1	1
Stavudine(mg)	200	200	200	200	200	200	200	200
Phospholipid (gm)	2	2	2	2	3	3	3	3
Ethanol (ml)	40	30	40	30	40	30	40	30
Tween 80(ml)	-	-	2	2	-	-	2	2
SLS (mg)	-	-	500	500	-	-	500	500
Distilled Water(ml)	q.s. to 100ml	q.s. to 100ml	q.s. to 100ml	q.s. to 100ml	q.s. to 100ml	q.s. to 100ml	q.s. to 100ml	q.s. to 100ml

In-vitro drug release study

Franz diffusion cell (fabricated in our Lab.) with a diameter 3.7 cm was used in in-vitro release studies. A glass tube with both end open, 10 cm height and 3.7 cm outer diameter was used as a permeation cell. A one gram sample was accurately weighed and placed on a semipermeable cellophane membrane to occupy a circle of 3.7 cm diameter. The loaded membrane was stretched over the lower open end of a glass tube of 3.7 cm diameter and made water tight by rubber band. The tube (donor compartment) was immersed in a beaker containing 100 ml of phosphate buffer pH 6.8 (receptor compartment) .The cell was immersed to a depth of 1 cm below the surface of buffer. The system temperature was maintained at 37°±1° and speed was maintained at 30 rpm throughout the experiment by magnetic stirrer (Fig.1). Samples 3 ml were withdrawn at intervals of 15, 30, 45, 60, 90 and 120 min, the volume of each sample was replaced by the same volume of fresh buffer to maintain constant volume. Samples were analyzed without dilution or filtration for drug content spectrophotometrically at 270 nm and 263 nm.

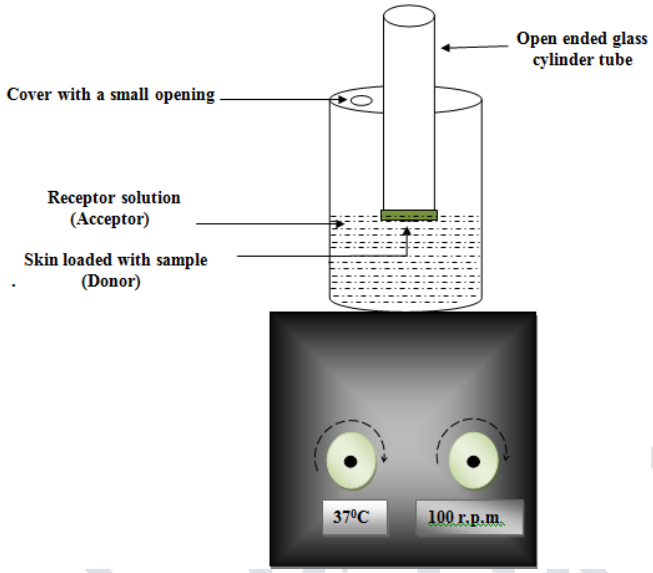


Figure 1: Fabricated diffusion cell for drug release study

RESULT AND DISCUSSION

Standard curve of Lamivudine

Table no.2 and Fig-2 shows the standard curve for Lamivudine in phosphate buffer pH 7.4. The method obeyed Beer's law limit in the concentration range of 2-12 mcg/ml at 270 nm with a regression value of 0.996

Table no2: Standard curve of Lamivudine.

S.No	Concentration (Mcg/ml)	Absorbance at 270 nm
0	0	0
1	2	0.055
2	4	0.096
3	6	0.145
4	8	0.182
5	10	0.218
6	12	0.263

Standard curve of Stavudine

Table no.3 and Fig-3 shows the standard curve for Stavudine in phosphate buffer pH 7.4. The method obeyed Beer's law limit in the concentration range of 2-12 mcg/ml at 263 nm with a regression value of 0.997

Table no3: Standard curve of Stavudine.

S.No	Concentration (Mcg/ml)	Absorbance at 263 nm
0	0	0
1	2	0.018
2	4	0.035
3	6	0.049
4	8	0.062
5	10	0.079
6	12	0.093

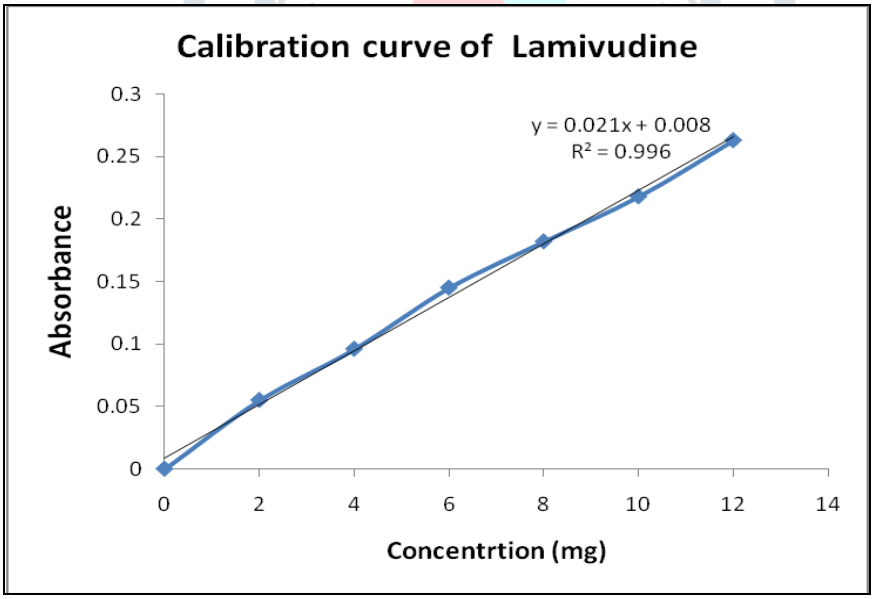


Fig: 2 Standard curve of Lamivudine using Phosphate buffer 7.4 pH at 270 nm

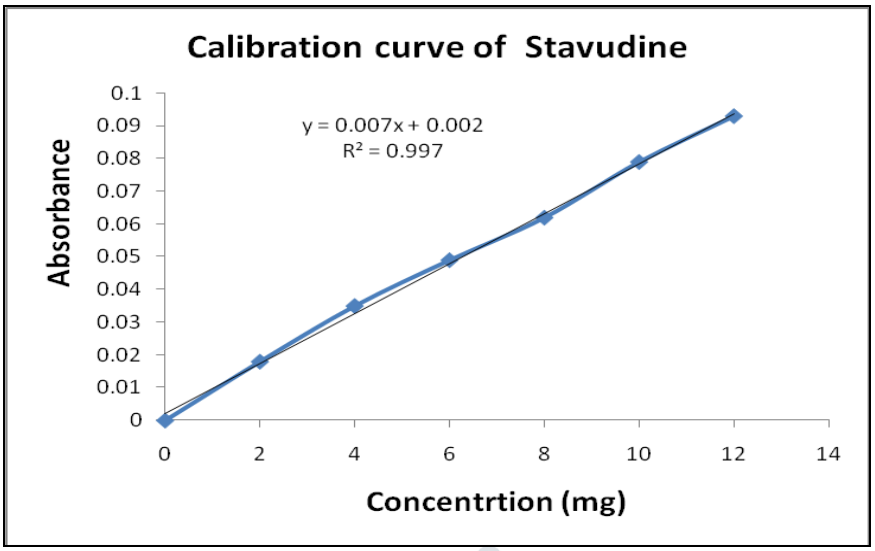


Fig: 3 Standard curve of Stavudien using Phosphate buffer 7.4 pH at 263 nm.

Fourier Transform-Infrared Ray Spectroscopy Studies (FT-IR) Studies:

Drug polymer compatibility studies were carried out using FT-IR spectroscopy to establish any possible interaction of Lamivudine and stavudine drug with the excipients used in the formulation. The FT-IR spectra results indicated that mixture of pure drug and excipients has no major change in the position of peaks. This shows that there is no possible interaction between drug and excipients.(fig.4-7)

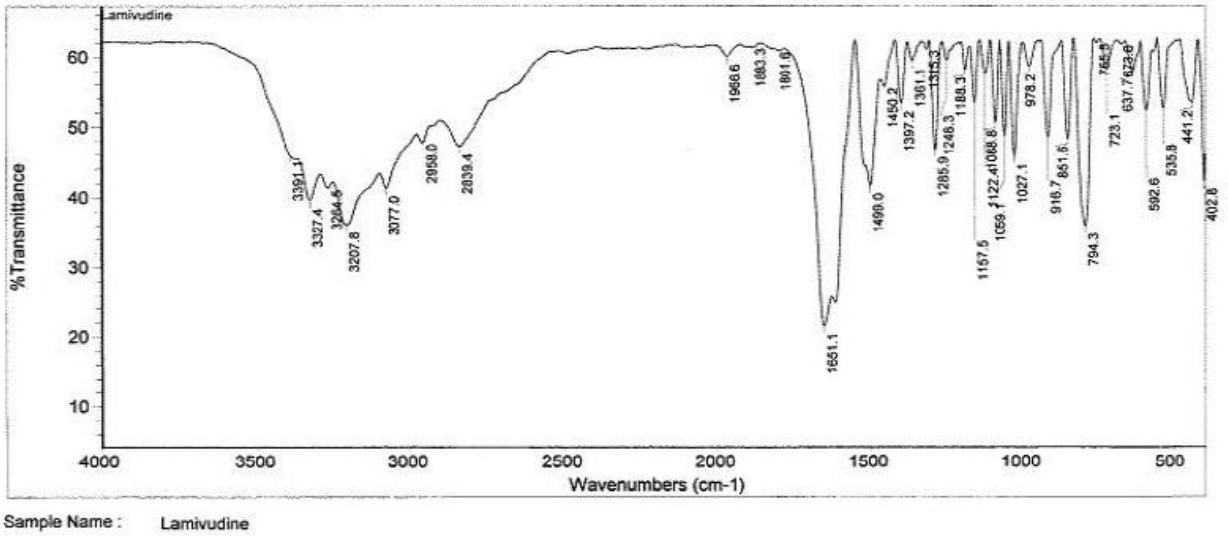


Fig 4: FTIR of Lamivudine

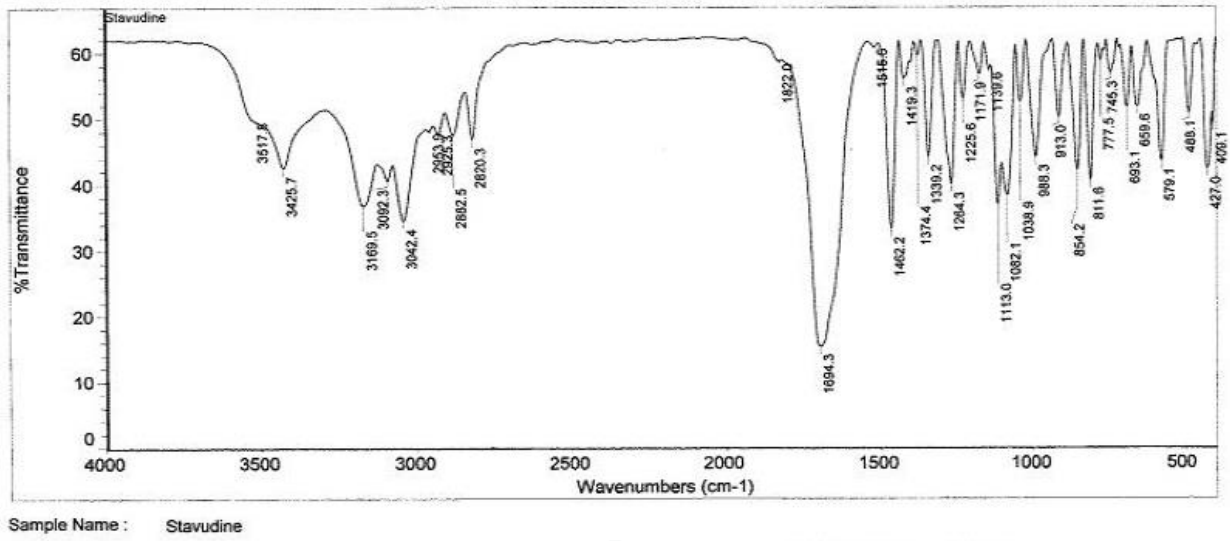


Fig 5: FTIR of Stavudine

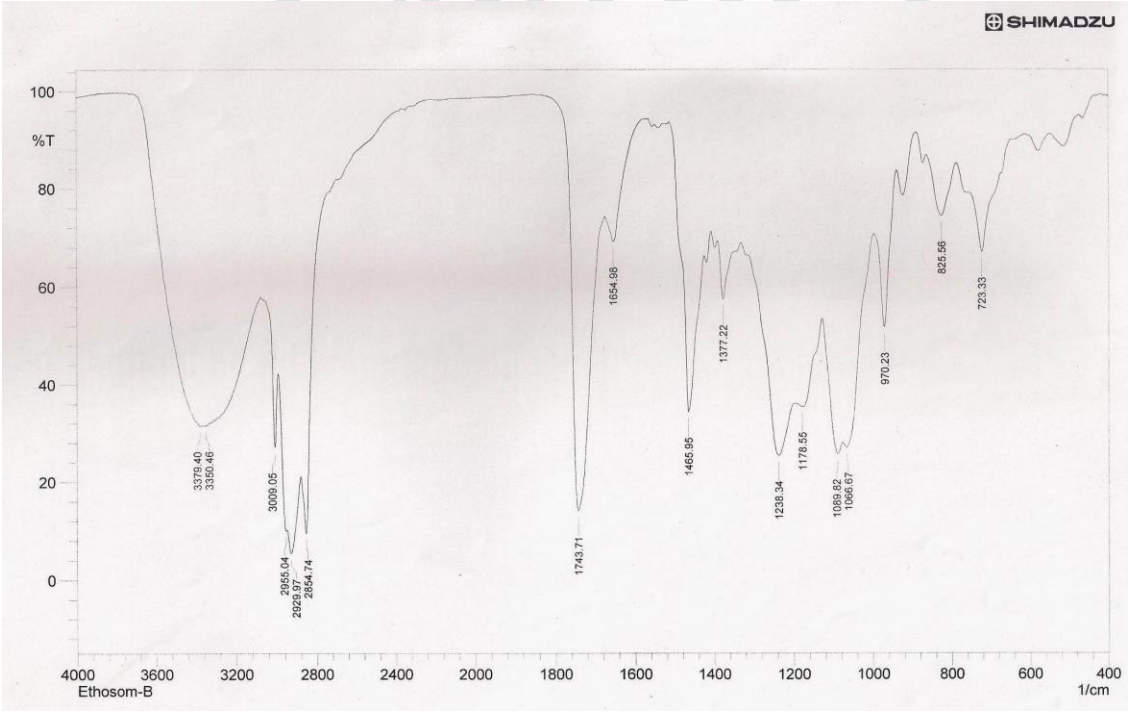


Figure 6 : FTIR spectra of Ethosomal gel base

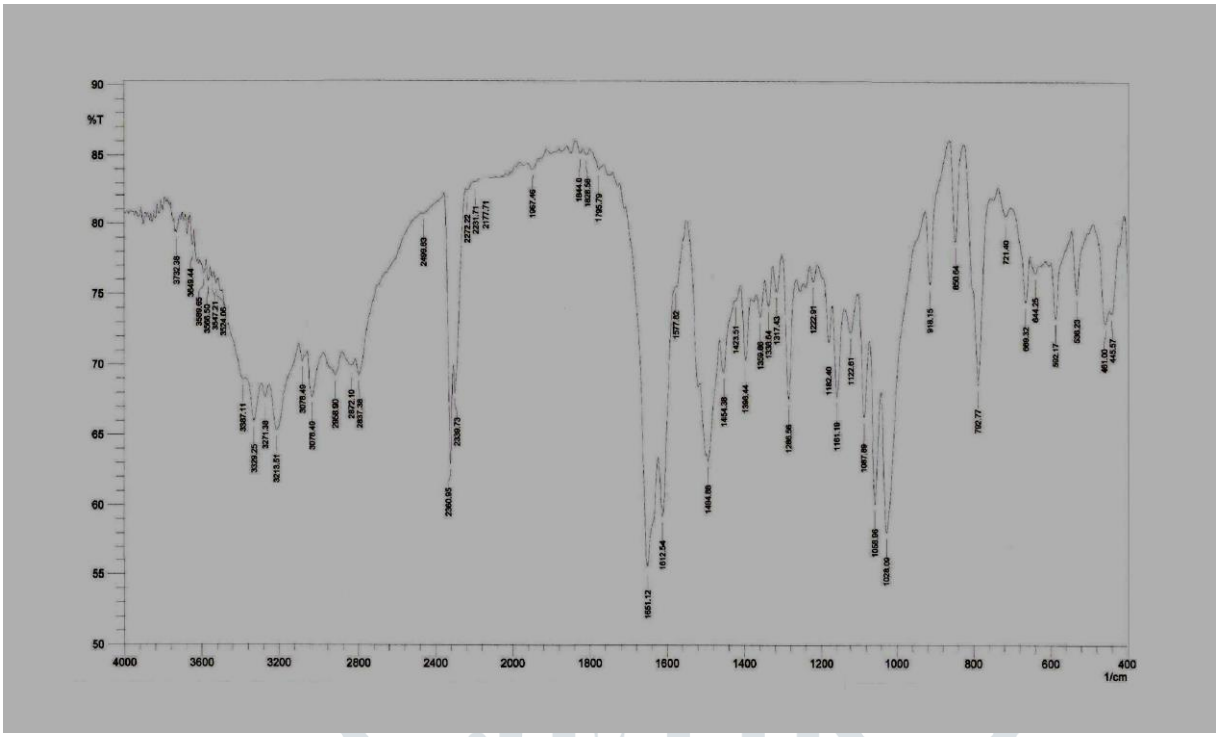


Fig 7 : FTIR of Lamivudine and Stavudine Loaded ethosome

Image analysis of ethosomes by optical microscope

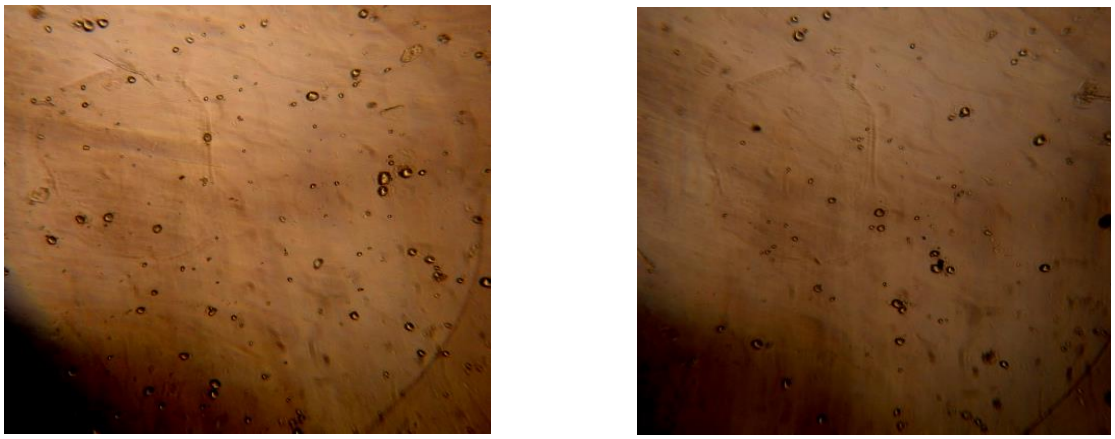
For the initial vesicle characterization of ethosome suspension were examined by compound microscope. The result revealed that all formulation shown spherical shaped vesicles like structure without aggregation process.(Fig.8)

Vesicular shape and surface morphology by TEM

The vesicular shape and surface morphology of ethosomes formulation **(**F7) examined by Transmission Electron Microscope (TEM). The TEM image showed that ethosomes were spherical shaped. (Fig.9)

Determination of Entrapment efficiency of ethosomes suspension:

The entrapment efficiency of various ethosomes formulations are presented in Table.2. The entrapment efficiency of formulation containing lamivudine and Stavudine ethasome F7 was found to be highest (74.12%) while F4 formulation showed least entrapment efficiency (58.81%). It has been observed the formulation containing phospholipid (3 gm) with 40 ml ethanol has maximum entrapment efficiency.



(A) F8 Ethosome formulation 10*2.0 (B) F7 Ethosome formulation 10*2.0

Fig :8. Ethosomes Image by optical microscope

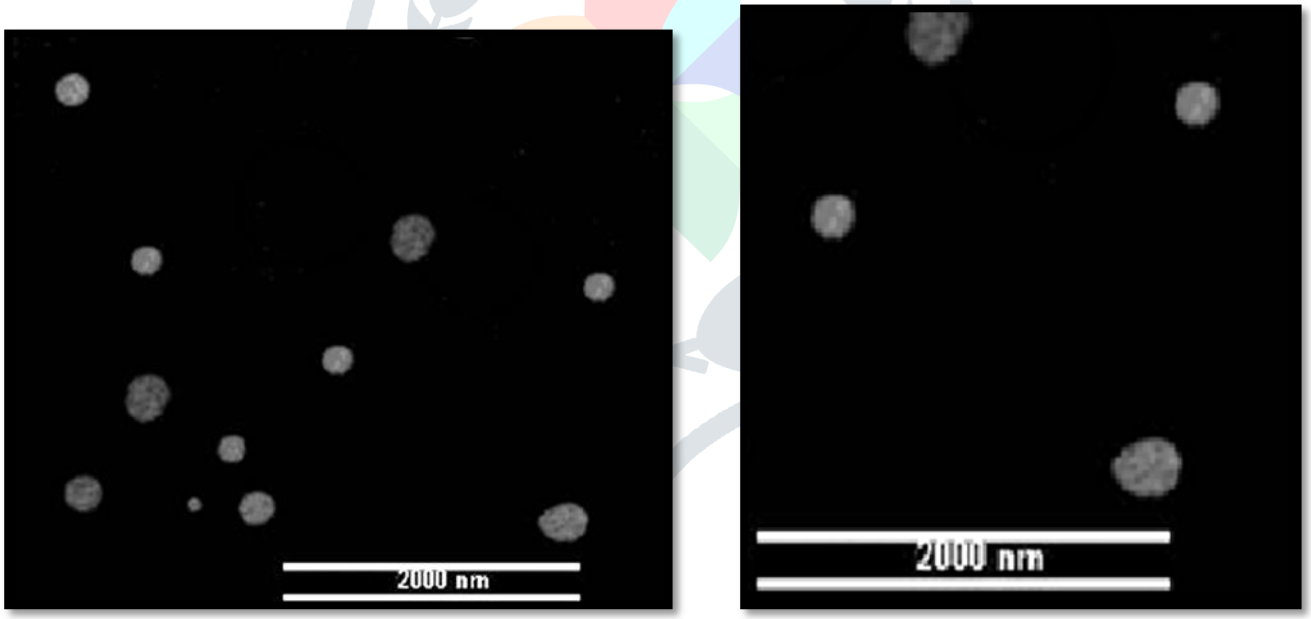


Fig:9 Transmission electron microphotograph Visualization of ethosomes formulation (F7) by transmission electron microscopy (×8400)

Table 2: Entrapment efficiency of ethosomes suspension

S/No.	Ethosomes	Qt	Qs	%EE
1.	F3	1	0.0550	61.19
2.	F4	1	0.0440	58.81
3.	F7	1	0.1296	74.12
4.	F8	1	0.1060	71.32

In-vitro drug release study

Percentage drug release of Lamivudine drug from ethosome formulation F7 and F8 was observed to be 27.33 % and 26.43% (at 30 min.) and 78.49 % & 72.92% (at 120 min.) respectively while Percentage drug release of stavudine drug from ethosome formulation F7 and F8 was observed to be 26.12 % and 25.68% (at 30 min.) and 76.91% & 70.12% (at 120 min.) respectively. It was observed that ethosome formulation F7 showed maximum drug release as compared to other formulation. (Table.3,4 Fig.10,11)

Table 3: Percentage Drug release of Formulated Ethosomal Gel at 270 nm

Time Interval (Min)	% Drug release of Formulation	
	F7	F8
15	18.31	16.93
30	27.33	26.43
45	35.91	34.56
60	46.78	45.21
90	61.43	60.13
120	78.49	72.92

Table 4 Percentage Drug release of Formulated Ethosomal Gel at 263 nm

Time Interval (Min)	% Drug release of Formulation	
	F7	F8
15	17.11	15.78
30	26.12	25.68
45	33.21	32.27
60	45.34	43.87
90	59.68	57.24
120	76.91	70.12

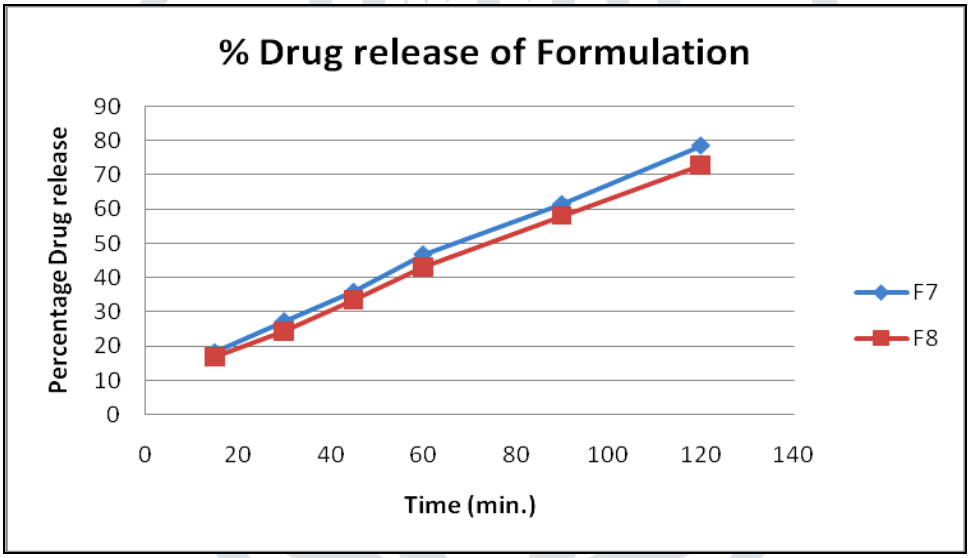


Fig.10: Percentage Drug release of Formulated Ethosomal at 270 nm

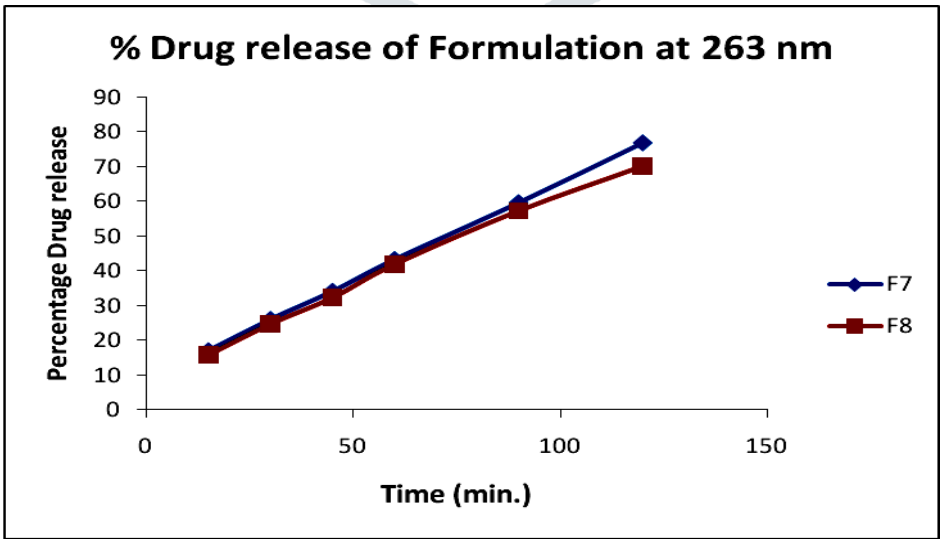


Fig.11: Percentage Drug release of Formulated Ethosomal at 263 nm

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