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EFFECT OF SPAN-80 IN THE FORMULATION LAMIVUDINE NIOSOMAL GEL

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ABSTRACT

The present investigation was to develop controlled release formulation of Lamivudine loaded niosomal gel formulation and they were prepared by thin film hydration method using span 80 of different ratios and cholesterol. The formulation were optimized with above method with respect to compatibility studies, vesicle size, particle size, entrapment efficiency, drug content, *in vitro* release and release kinetics. The FT-IR and DSC investigation shows the drug and excipients were compatible. The *In vitro* release and release kinetics studies indicates that all the formulation exhibits retarded release for 24 hrs and it follows non- fickian diffusion mechanism with higuchi order release.

KEYWORDS

Niosome, Span 80, Cholesterol, Thin film hydration and FT-IR.

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INTRODUCTON

Controlled drug delivery systems, which release the drug in continuous manner by both dissolution controlled as well as diffusion controlled mechanisms. To control the release of the drugs, which are having different solubility properties, the drug is dispersed in swell able hydrophilic substances, an insoluble matrix of rigid non swell able hydrophobic materials or plastic materials[1].

Niosomes are a novel drug delivery system, in which the medication is encapsulated in a vesicle. Niosomes offer remarkable advantages over conventional drug delivery system. Biodegradability, biocompatibility,

chemical stability, low cost, easy handling and less toxicity[2,3].

Transdermal drug delivery permits controlled release of the drug into the patient, it enables a steady blood level profile which causes reduced systemic side effects and improved efficacy over other dosage forms. The main aim of transdermal drug delivery system is to administer drugs into systemic circulation through skin at predetermined rate. In recent years, owing to advantages offered by transdermal drug delivery, a number of antiretroviral agents have been studied by authors. Lamivudine is an antiretroviral agent and an it a non-nucleotide reverse transcriptase enzyme inhibitor which is selected as a drug candidate. Human immunodeficiency virus (HIV) is a retrovirus that causes irreversible destruction of the immune system. 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)[4].

The use of lipid vesicles as drug delivery systems for skin treatment has attracted increasing attention in recent years. 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[4,5].

In literature, there are no reports on transdermal delivery of niosomal vesicular system for Lamivudine.

MATERIALS AND METHOD

Lamivudine was obtained as a gift sample from Strides Arco labs ltd, Bangalore. Span 60 span 80, cholesterol, chloroform and methanol were purchased from SD fine chemicals ltd, (Mumbai, India). Phosphate Buffer Saline pH 7.4 (PBS pH 7.4) and Phosphate Buffer Saline pH 6.8 (PBS pH 6.8) were prepared as described in the Indian Pharmacopoeia (1996). The animal studied conducted for this research work has certified by the IAEC with a approval no. (BCP/IAEC/PCEU/05/15), Bharathi college of pharmacy, Bharthi nagara, Mandya (dist), Karnataka.

Method

Formulation and evaluation of niosomal gel

Niosomes were prepared by thin film hydration technique by using surfactant and cholesterol. The type of surfactant was optimized, keeping drug: cholesterol: surfactant molar ration at 1:0.5:1, 1:1:1, 1:1.5:1 and 1:2:1. (Table No.1). Accurately weighed quantities of surfactant (span 80) and CHOL were dissolved in 10 ml chloroform using a 100 ml round bottom flask. The weighed quantity of drug is added to the solvent mixture. The solvent mixture was removed from liquid phase by flash evaporation at 60°C to obtain a thin film on the wall of the flask at a rotation speed of 150 rpm. The complete removal of residual solvent can be ensured by applying vacuum. The dry lipid film was hydrated with 10 ml phosphate buffer saline of pH: 7.4 at a temperature of 60 ± 2°C for a period of 1hour until the formation of niosomes.

Formulation of Niosomal Gel

Based upon the results of formulated drug loaded niosomes, the batches with good entrapment efficiency were selected for further formulation of niosomal gel. For the formulation of niosomal gel, the gel base was prepared by dispersing 1% w/w carbopol 940 in a mixture of water and glycerol (7:3), the dispersion is then neutralized and made viscous by addition of sufficient amount of triethanolamine by cold mechanical method.

CHARACTRIZATION OF NIOSOMES

Preliminary studies

FT-IR studies

The compatibility of the drug and polymer were studied by the FTIR spectrometer using Shimadzu 8400-S, Japan. Two percent (w/w) of the sample with respect to potassium Bromide disc was mixed with dry KBr. The mixture was grind into a fine powder using a pestle and mortar and then compressed into a KBr disc in a hydraulic press at a pressure of 1000 psi. The characteristic peaks were recorded. FT-IR spectrum of Lamivudine was compared with FT-IR spectra of drug and polymers (Figure No.1 and 2).

Differential scanning calorimetry

Niosomal pellets were lyophilized. Differential scanning calorimetric (DSC) thermo grams for individual components, Span 80, Cholesterol, as well as the drug powder, were investigated. A heating rate of 5°C/min was employed over a temperature range (30–250 °C) (Figure No.3 and 4).

Vesicle Shape and Size analysis of niosomes[6]

The size and shape of the vesicles were determined by optical microscopy and SEM (Figure No.5).

Particle size distribution and Zeta potential[7]

Zeta potential of the optimized formulation was measured by instrument zetasizer nano ZS using DTS software (Malvern Instrument Limited, UK) using M3-PALS Technology.

Entrapment efficiency[7]

Entrapment efficiencies of niosomal formulations were carried out in triplicate by centrifugation method. The niosomal suspension was centrifuged at 8000 rpm for 10 min at 3°C. Then the solid mass was separated from the supernatant and then suitable dilutions were prepared with PBS (pH: 7.4). The drug concentration was assayed by UV-visible spectrophotometer method at 270.6 nm. The percentage of drug entrapment was calculated.

$$\text{Entrapment efficiency (\%)} = \frac{\text{Total amount of drug} - \text{free amount of drug} \times 100}{\text{Total amount of drug}}$$

Drug content[8]

Lamivudine content in niosomes was assayed by an UV spectrophotometry method. Niosomes containing equivalent to 10 mg of drug were dissolved in a 10 ml of methanol. After suitable dilution absorbance was measured by UV spectrophotometer against blank at λmax 270.6 nm and drug content was calculated.

***In vitro* release study**[9]

In vitro release pattern of niosomal suspension was carried out in dialysis bag method. Lamivudine niosomal suspension equivalent to 10 mg was taken in dialysis bag and the bag was placed in a beaker containing 100 ml of pH: 7.4 Phosphate buffer. The beaker was placed over magnetic stirrer having stirring speed of 100 rpm and the temperature was maintained at 37±0.5°C. 1 ml sample were withdrawn periodically and were replaced by fresh buffer. The samples were assayed by UV Spectrophotometer at 270.6 nm using phosphate buffer pH 7.4 as blank and cumulative % of drug released was calculated and plotted against time.

EVALUATION OF NIOSOMAL GEL[10]

Visual Appearance and pH

The prepared gels were examined for clarity, colour, homogeneity, presence of foreign particles and fibres.

PH

2.5 gm of gel was dispersed in 25 ml distilled water and the pH was examined using digital pH meter.

Drug content

Drug content uniformity of niosomal gel was determined by analyzing the drug concentration in the sample taken from four different points. The gel samples were dissolved in 50 ml PBS (pH: 6.8) and stirred at 100 rpm to facilitate rupture of the vesicles. Drug (Lamivudine) content was determined using UV spectrophotometer at 270.6 nm.

***In-vitro* drug release studies**

In- vitro release studies were performed using diffusion cells. Phosphate buffer pH: 7.4 were used as receptor fluid. Nylon membrane (0.22 μm) was soaked in phosphate buffer pH: 7.4 for 1 hrs before carrying out the study. 500 mg of niosomal gel (F4) containing 10 mg drug was placed onto the donor compartment. Samples were collected at 1, 2, 3, 4, 5, 6, 7, 12 and 24 hrs intervals and analyzed by U.V.

Release kinetics[11]

In order to describe the kinetics of the release process of drug in all formulations, various Equations were used, such as Zero order rate equation, first order equation, Higuchi's model, Peppas model (Table No.3).

Skin irritation studies[12]

Three young rats of white strain will be taken for skin irritation studies. The test will be carried out using drug free polymeric gel as control and niosomal gel containing drug for the observation of Erythema.

Stability studies

The optimized niosomal gel was placed in vials and sealed with aluminium foil for a short term accelerated stability study at 25º±2ºC/ 60±5% RH and 5º±3ºC as per modified International Conference on Harmonization guidelines. Samples were analyzed every 30 days for appearance, gelling studies and drug content.

RESULTS AND DISCUSSION

Vesicle shape and size of niosomes

SEM images and microscopic evaluation showed that most of the vesicles were spherical in shape and the optimized formulation F4 was studied SEM analysis in which the niosomal particles were appeared as discrete

and round in shape with irregular surface due to the presence of unentrapped drug and observed mean particle size range as 10μm. The result of SEM was sown in Figure No.7.

The particle size analysis also done by using Malvern particle size analyzer for the optimized formulation of F4 he average particle size was found to be 708.1 nm (Figure No.6).

Zeta potential

Zeta potential is a key factor for evaluation of the stability of colloidal dispersion. The zeta potential was measured for the optimized Formulations F4 was found to be -68.1 mV shown in Figure No.8.

Entrapment efficiency

The percentage entrapment efficiency of all the formulation was shown in Table No.2. The formulation F4 was found to be 91.4 %. The high drug entrapment may be observed due to increase in the surfactant ratio (Table No.2).

Drug content

Drug content for all formulation was shown in Table No.2. The formulation F4 was showing more drug content due to increased entrapment efficiency and it was found to be 90.02 % (Table No.2).

***In vitro* drug release**

The cumulative percentage of drug release from various niosomal formulations were shown in Figure No.8-12. The experimental studies showed that the rate of drug release depends on the percentage of drug entrapment efficiency. Cumulative % drug release was plotted against time (t). The percent drug release from F1-F4 was observed as follows F1- 93.10%, F2- 85.60%, F3- 81.70%, F4- 80.00% after 24 hrs at the end of 24 hrs. Formulation F4 showed higher drug release than other formulations. Hence it was choosen to formulate the niosomal gel (Figure No.10)

EVALUATION OF NIOSOMAL OF NIOSOMAL GEL

Visual appearance

The Visual appearance of the all Lamivudine niosomal gel formulations was checked and showed off white colour, opaque, odourless with smooth appearance.

pH measurement

All the prepared Lamivudine niosomal gel was checked for their pH. All the formulations were showing pH in the range of 6.02-6.18. The optimized formulation F4 was showing pH: 6.70.

Drug content (%)

All the prepared Lamivudine niosomal gel were subjected to drug content uniformity and represented in Table No.4. The Lamivudine in optimized niosomal gel formulation F4 shows 87.31% which indicated the drug uniformly dispersed throughout the formulation.

***In vitro* drug release and Release kinetics**

The result of *in-vitro* release of Lamivudine from the gel formulation is given in Table No.5. However, the results clearly show that the gels have ability to retain the drug for prolonged periods. The % CDR of niosomal gel formulation F4 was found to be 75.02 % which follows Higuchi model The 'n' values for all the formulation were found to be more than 0.5. This indicates that the release approximates Non- Fickian diffusion mechanism.

Skin irritation study

The skin irritation study of niosomal gel formulations F4 was performed and tabulated in Table No.6. The Average primary irritation index of formulations F4 was found to be 0.16 and it shows that the niosomal gel formulation did not show any irritation and Erythema after 7 days.

Stability studies

The accelerated stability study for optimized niosomal gel formulation F4 was performed for 6 month according to ICH guide lines. Drug content, pH: % CDR were fixed as physical parameters for stability testing and stability studies of selected formulation F4 shows that negligible changes in Drug content, pH: % CDR. This revealed that the formulation stable on storage at 40 ± 2°C and 75 ± 5% and the results were given in the Table No.7.

Table No.1: Formulation design of Lamivudine niosomes

S.No	Formulation code	F1	F2	F3	F4
1	Lamivudine	100	100	100	100
2	Span 80	50	100	150	200
3	Cholesterol	100	100	100	100
4	Phosphate buffer (pH 7.4) ml	10	10	10	10

Table No.2: Drug content entrapment efficiency

S.No	Formulation code	% Entrapment efficiency	% Drug content
1	F1	84.2	76.72
2	F2	86.6	81.07
3	F3	90.0	87.52
4	F4	91.4	90.02

Table No.3: Datas for different kinetic models

S.No	Formulation code	Zero order	First order	Higuchi plot	Peppas plot	'n' values for peppas
1	F1	0.873	0.958	0.987	0.991	0.634
2	F2	0.901	0.993	0.980	0.86	0.741
3	F3	0.880	0.984	0.977	0.98	0.656
4	F4	0.951	0.967	0.949	0.989	0.812

Table No.4: Appearance, pH, Viscosity (cps) and Drug content of niosomal gel

S.No	Formulation code	Appearance	pH	Viscosity	% Drug content
1	F4	Off white, opaque, odourless with smooth appearance	6.7	11,550	87.31%

Table No.5: Comparative *in vitro* release profile of Lamivudine niosomal gel

S.No	Formulation code	Zero order	First order	Higuchi plot	Peppas plot	
					r^2	'n'
1	F8-G1	0.91	0.981	0.986	0.724	0.926

Table No.6: Reading after Skin irritation study of niosomal gel F4

S.No	Skin responses	Days	F4-G1 formulation	
			Score	
			Rat 1	Rat 1
1	Erythema and scar formation	1	0	0
		3	0	1
		7	1	0
2	Edema formation	1	0	0
		3	0	0
		7	0	0
3	Primary irritation index	---	0.16	0.16

Table No.7: Accelerated stability studies the optimized formulation F4-G1 at 40 ± 2°C and 75 ± 5%

S.No	Parameters	Duration in Months			
		0	1	3	6
1	Drug content (%)	91.62	90.02	87.24	81.20
2	pH	6.40	6.45	6.55	6.78
3	% CDR	55.61	53.26	51.04	49.41

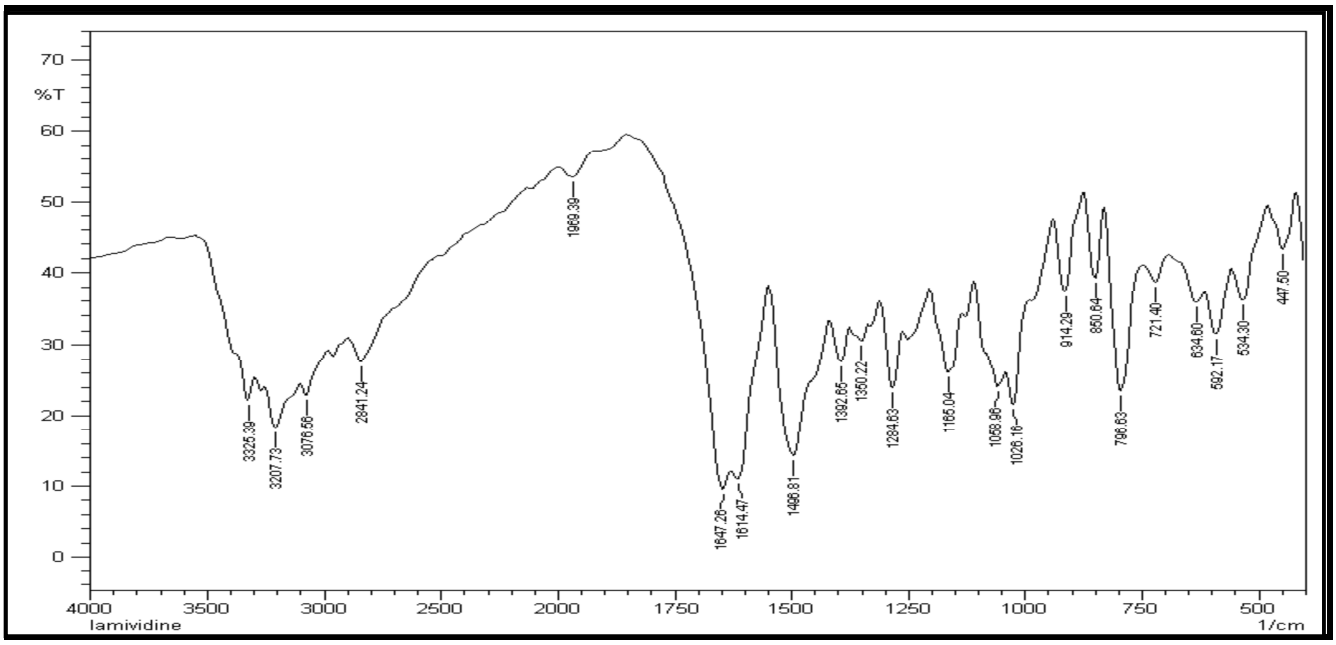

Figure No.1: FTIR spectra of pure drug Lamivudine

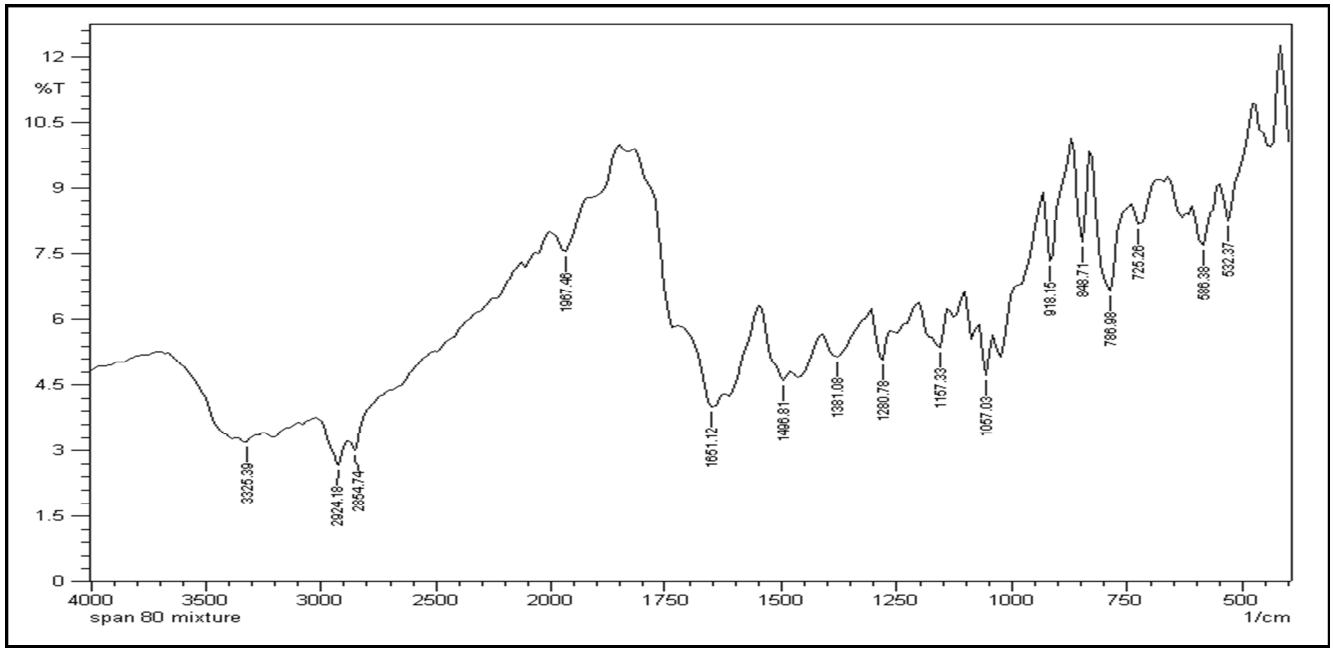

Figure No.2: FTIR spectra of physical mixture of Lamivudine, with Excipients (span 80, cholesterol)

DSC Investigation

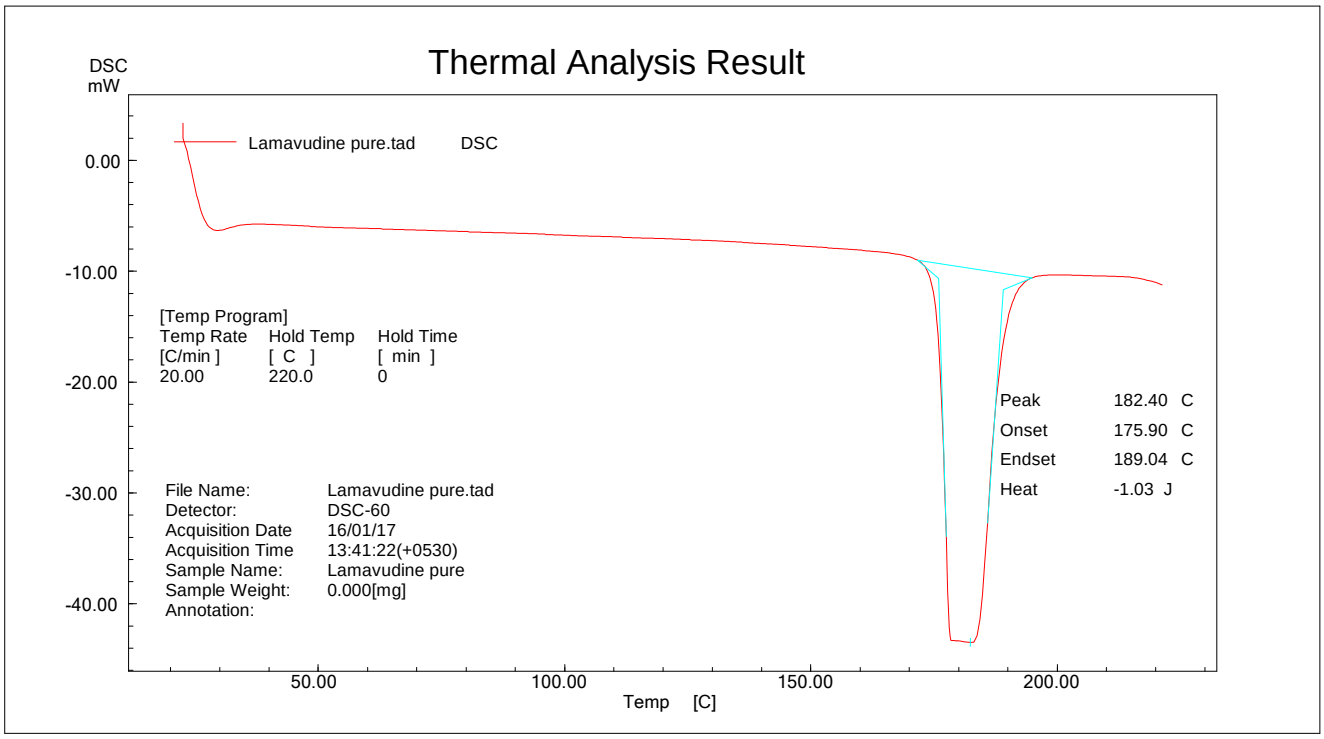

Figure No.3: DSC Thermograph of pure drug Lamivudine

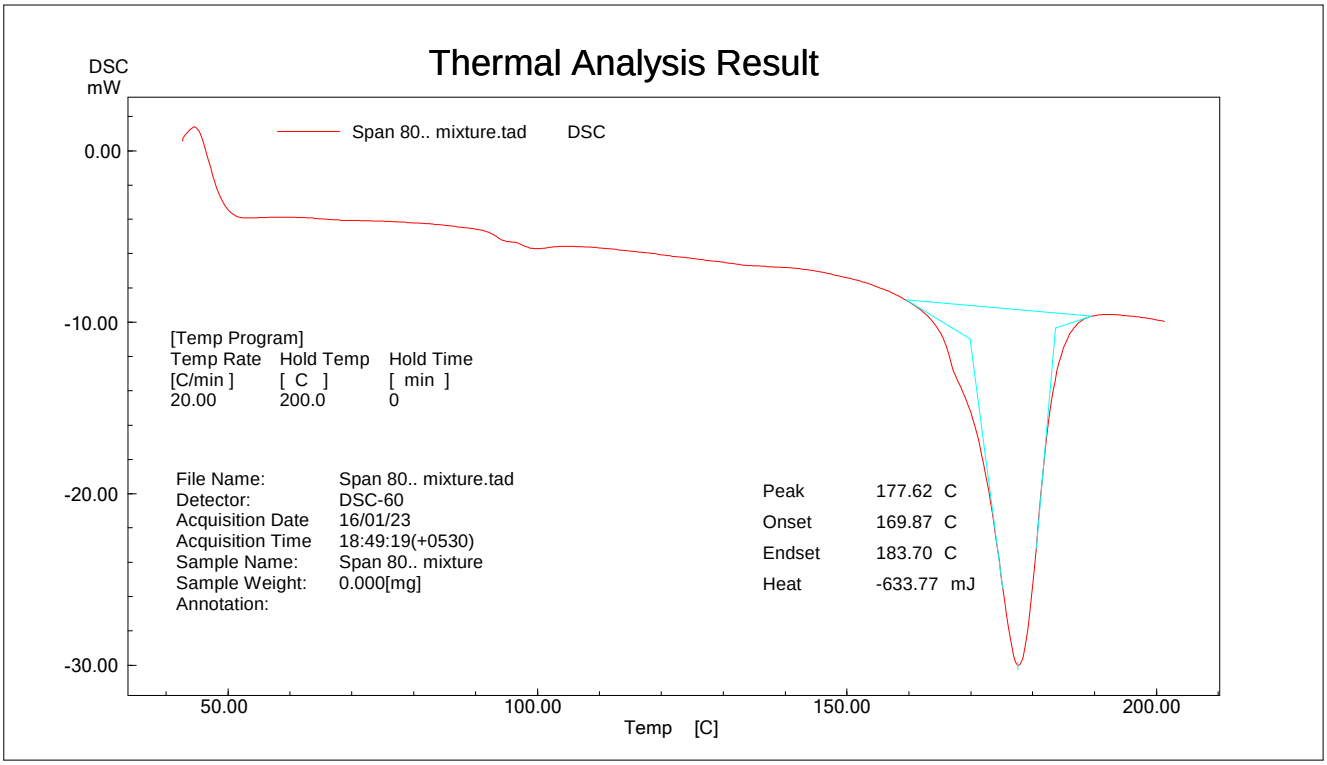

Figure No.4: DSC thermo graph of pure drug with excipients (Span-80 and Cholesterol)

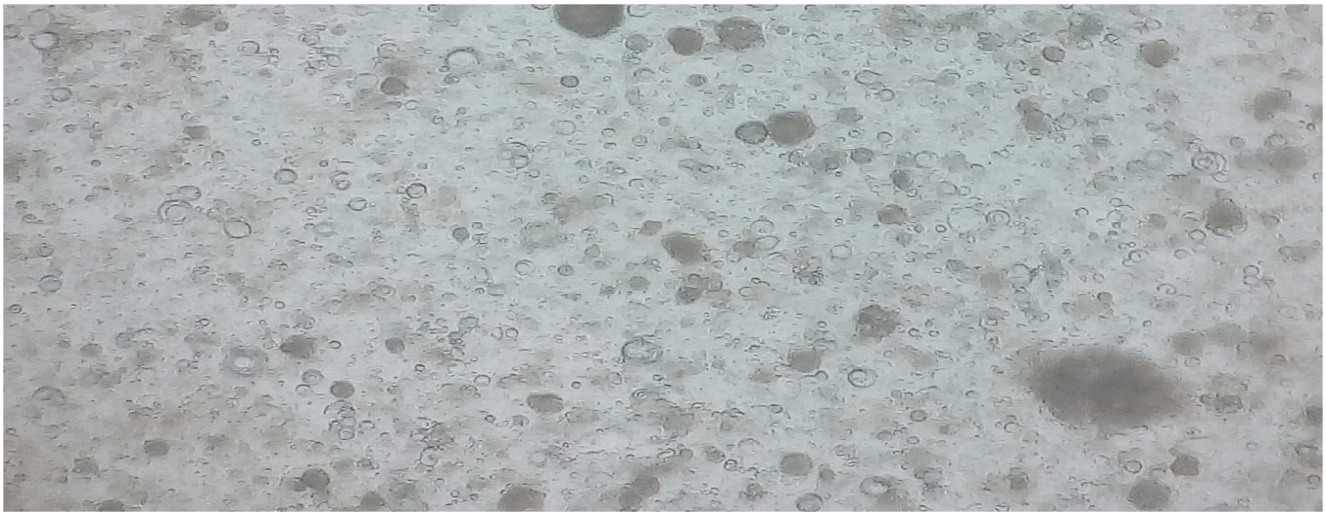

Figure No.5: Photomicrographs of optimized formulation F4

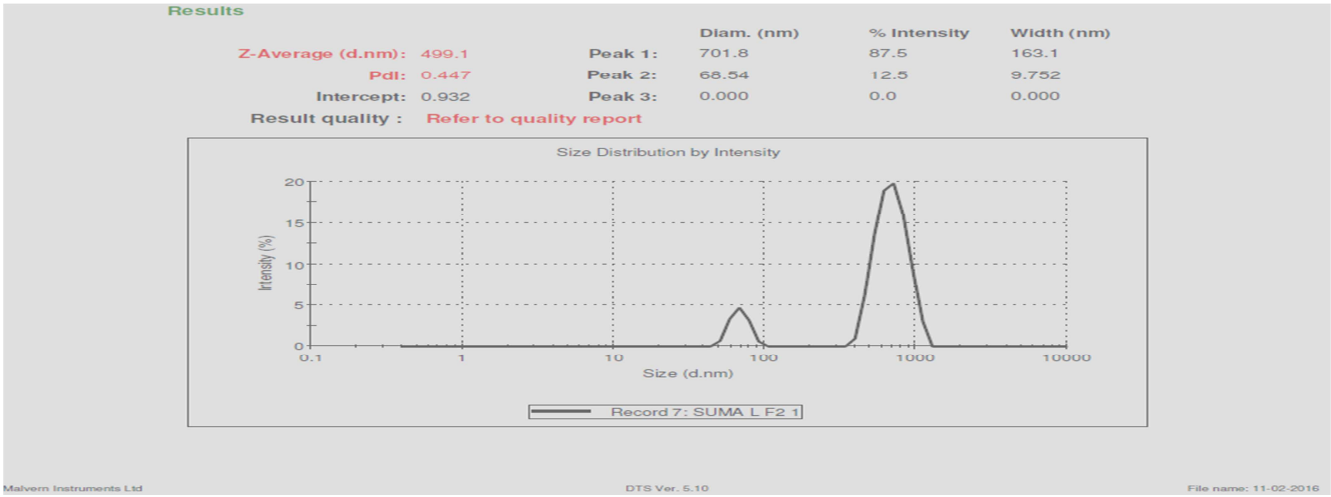

Figure No.6: Particle size distribution analysis of formulation F4

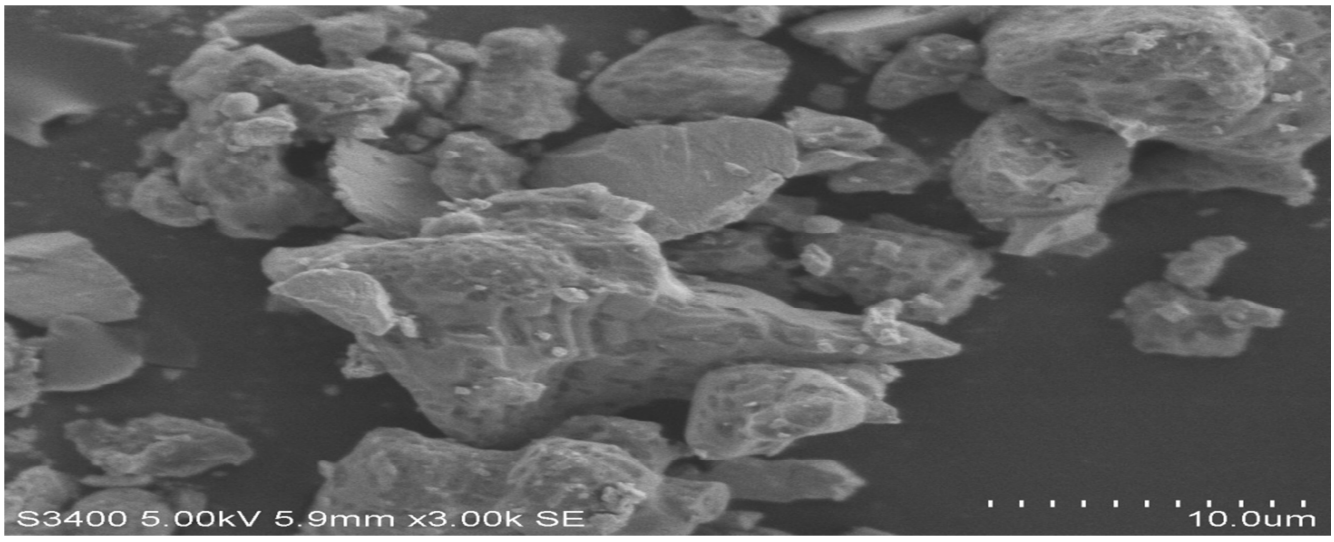

Figure No.7: SEM of the optimized formulation F4

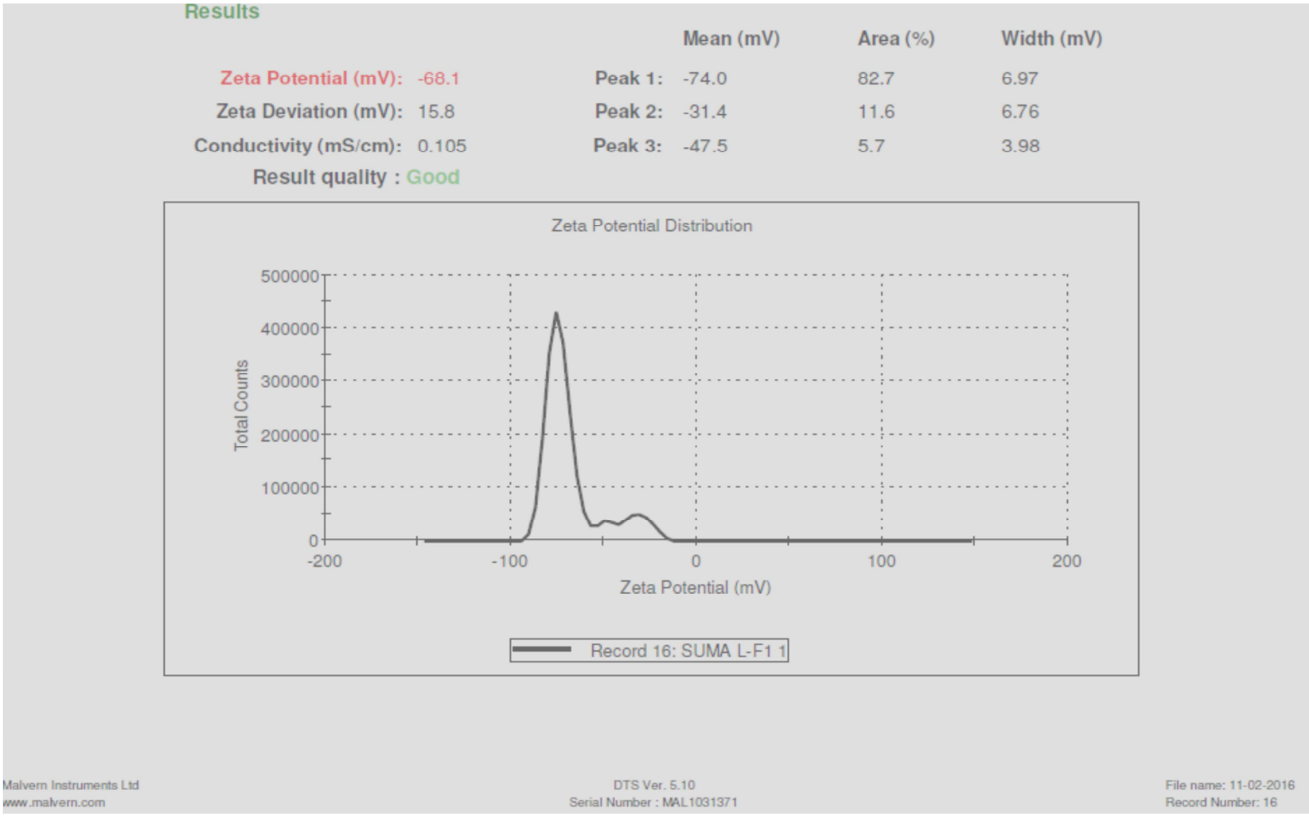

Figure No.8: Zeta potential analysis of F4

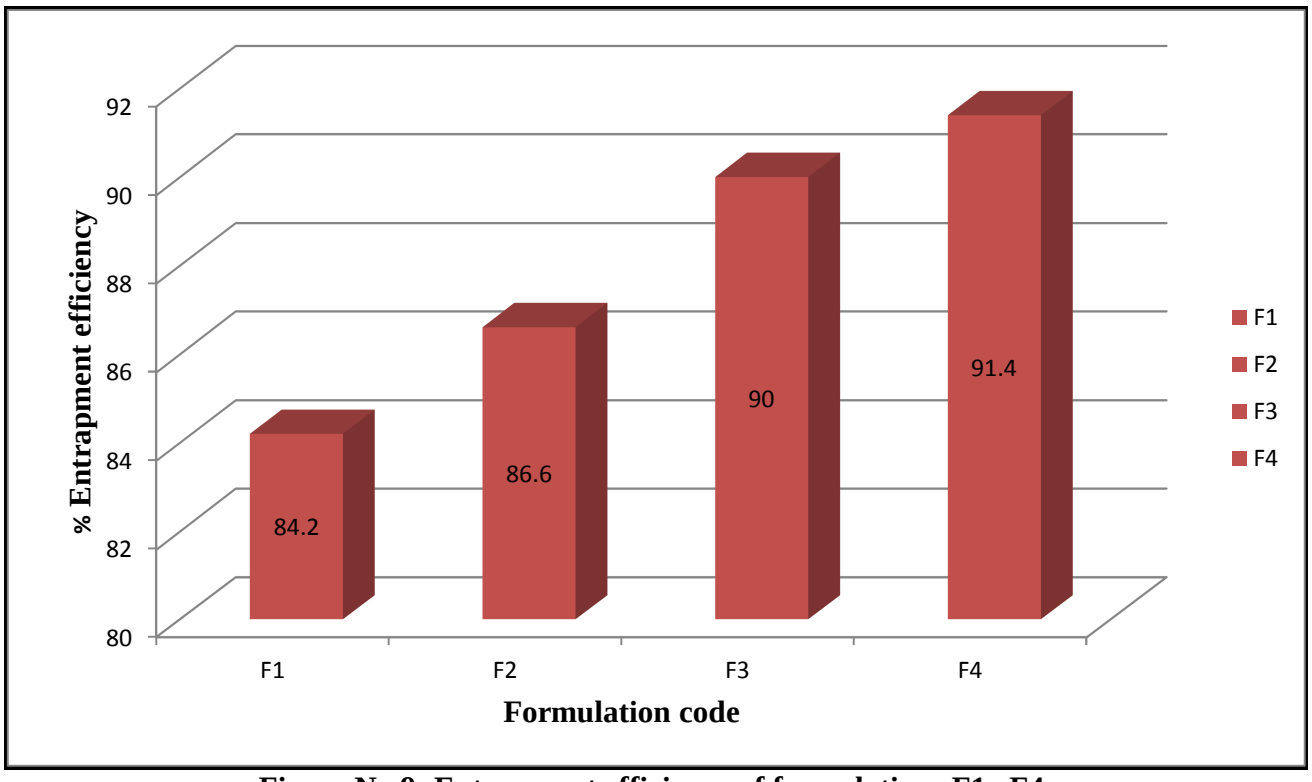

Figure No.9: Entrapment efficiency of formulations F1– F4

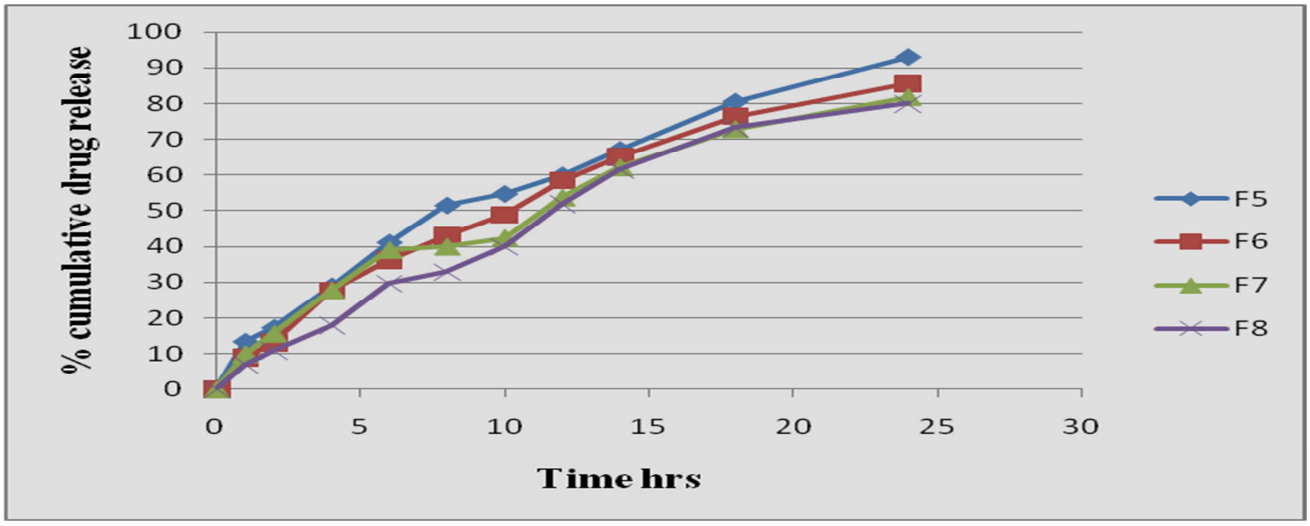

Figure No.10*: In-vitro* release profile of F1-F4

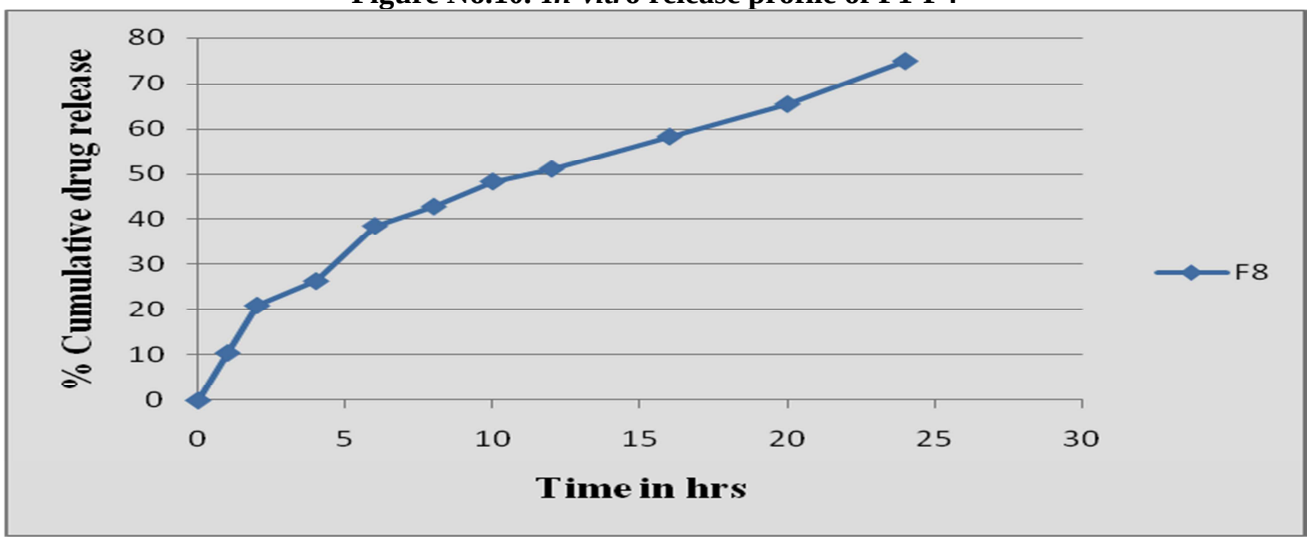

Figure No.11: % CDR of niosomal gel formulation F4

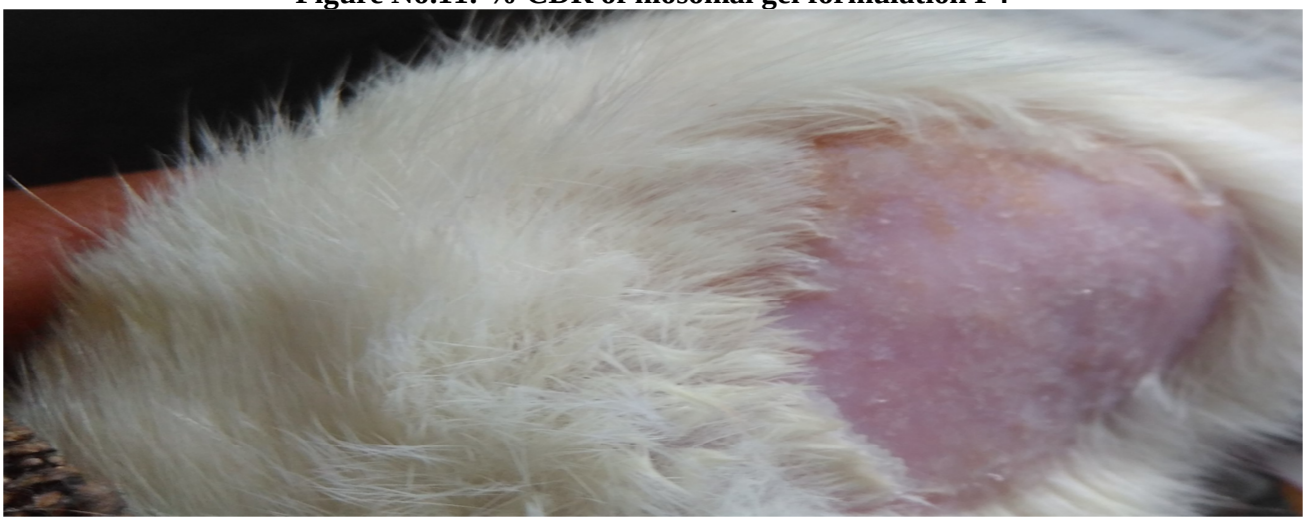

Figure No.12: Rat skin for observation of Erythema and edema (After 7 days) for gel

CONCLUSION

Thin film hydration technique used for the preparation Lamivudine niosomes using cholesterol and surfactant span 80 was found to be good techinique to encapsulate hydrophobic drug in non-ionic surfactants. It has shows the reasonable entrapment efficiency, suitable particle size and good Invitro release on increasing surfactant ratios. Further the gel formulation prepared with the span 80 niosomes found that no skin irritation to animal which can be used for further studies, when studied on the albino rat skin. From the stability studies of niosomal gel formulation F4-G1 showed negligible changes in pH, drug content and % CDR which revealed that the formulation are stable on storage.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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