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Formulation and evaluation of lamivudine encapsulated niosomes

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ABSTRACT

The aim of this present work is to formulate Lamivudine niosomes by two different methods in various ratios of surfactant span 80. Lamivudine niosomes prepared by two methods those are hand shaking and ether injection. By by using span 80 and cholesterol from which the best formulation was selected and characterize in terms of vesicles size distribution, entrapment efficiency and invitro release studies. Interaction of drug and the different ingredients in the niosomes were studied by FTIR spectra and found to be compatible. Results: The niosomes size range of prepared formulation 0.5-5µ and 0.5-2.5µ by hand shaking method and ether injection method respectively. The entrapment efficiency of lamivudine niosomes was determined by separating the entrapped drug by dialyzing method. The entrapment efficiency of the drug was found to be more than 82% in case of niosomes prepared by hand shaking process. The in vitro release profile of drug from niosomes was studied in 0.1 N HCL using dialyzing method and result indicated 73.84% drug release for the formulation prepared by hand shaking method and It took an extended period of 24 hours of drug release. In vitro release of lamivudine from niosomes was very slow when compared to the release from pure lamivudine solution.

Keywords: Lamivudine, Diethyl ether, Ether injection method (ES), Hand shaking method (HS), Span 80, Cholesterol.

INTRODUCTION

One of the approaches for target specific drug delivery system is niosomal drug delivery system which approaches by having localized drug action with low size and low permeability through site specific. Localization of drug action results in enhancement of efficacy or potency of the drug and at the same time reduces its systemic toxic effects. The niosomes are targeted to reticuloendothelial system and to other organs, other than

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reticuloendothelial system in the body by use of antibodies, immunoglobulins. Many cells possess the intrinsic ability to recognize and bind to the drug carrier system. Niosomes can provide relatively constant and sustained blood stream levels of drug concentration. Niosomes can be injected into circulation and thus can serve as an intravascular drug depot. Sustained release action of niosomes could be applied to drugs with low therapeutic index and low water solubility, since these could be maintained in the circulation via niosomal encapsulation.

Lamivudine which is a reverse transcriptase inhibitor or antiviral drug, used as a model drug to be encapsulated niosomal drug delivery system. Lamivudine has a short biological half life, which necessitates multiple daily dosing and hence a novel delivery system such as niosomes, can be used to encapsulate the drug so that it maintain a therapeutic plasma concentration for a longer period of time, thereby increasing the bioavailability of the drug. Hence this niosomal delivery may reduce the frequency of dosing intervals and may improve patient compliance. More over Lamivudine is used in the treatment of HIV and Hepatitis B for a long period, which may induce toxic side effects. Therefore it is desirable to deliver them to target tissue in the right manner at the right time, by encapsulating in niosomes, we can minimize the drug dose, which in turn can reduce the toxic side effects and a sustained and controlled release rate of Lamivudine can be achieved. The prepared niosomes are to be characterized of their size, shape, entrapment efficiency, leakage studies, osmotic shock and invitro drug release. The best formulation is to be selected on the basis of evaluation characteristics.

PREPARATION OF LAMIVUDINE NIOSOMES BY HAND SHAKING METHOD

Cholesterol and span 80 were taken in specified ratios of (1:1, 1:2&1:3) and transferred in to a clean round bottom flask. Then the lipid mixture was dissolved in 10 ml of diethyl ether. The flask was continuously vortexed to form a thin film along the sides of the flask. An appropriate amount of Lamivudine was dissolved in phosphate buffer saline (PBS) pH.7.4. This was poured into added to the thin film and vortexed continuously for a period of 30 min at room temperature.

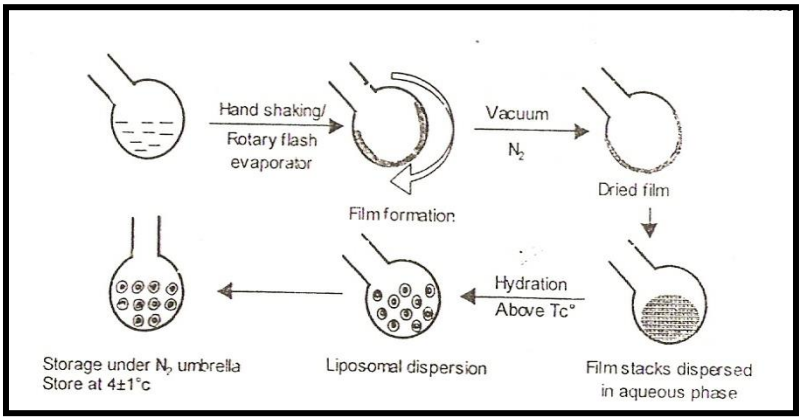

Fig 1 Preparation of Lamivudine Niosomes By Hand Shaking Method.

Table 1 Composition of the Lamivudine Niosomal formulations

Formulation Code	Drug	Span 80	Cholesterol	Diethyl ether
HS 1	300 mg	50 mg	50 mg	10 ml
HS 2	300 mg	100 mg	50 mg	10 ml
HS 3	300 mg	150 mg	50 mg	10 ml

PREPARATION OF LAMIVUDINE NIOSOMES BY ETHER INJECTION METHOD

Cholesterol and span 80 were taken in prescribed ratio (1:1, 1:2 &1:3) in a 50 ml beaker. The mixture was dissolved in diethyl ether and the solution was slowly injected into a beaker containing Lamivudine in phosphate buffer saline (PBS) pH 7.4. The temperature maintained during the injection was 40-60^{o}c. The differences in temperature between phases cause rapid vaporization of ether resulting in spontaneous vesiculation.

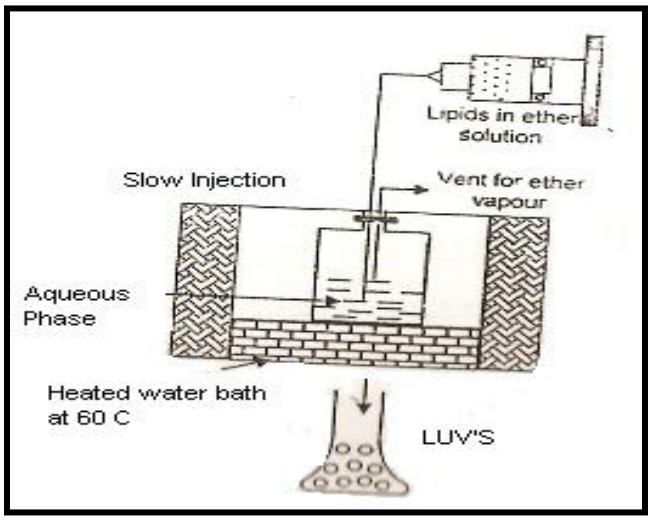

Fig 2 Preparation of Lamivudine Niosomes by Ether Injection Method.

Table 2 Composition of the Lamivudine Niosomes Formulations

Formulation Code	Drug	Span 80	Cholesterol	Diethyl ether
ES 1	300 mg	50 mg	50 mg	10 ml
ES 2	300mg	100 mg	50 mg	10 ml
ES 3	300 mg	150 mg	50 mg	10 ml

EVALUATION OF DIFFERENT BATCHES OF PREPARED LAMIVUDINE NIOSOMES FORMULATION

Morphology

The prepared niosomal vesicles were mostly round in shape and some vesicles are slightly elongated. The vesicles are slightly yellow in colour. Individual vesicles could be seen as shown in the photograph (fig-13-16), more over these vesicles were viscous and it can visualize under microscope after dilution with water.

Vesicles size determination

Calibration of eyepiece micrometer using stage micrometer

1 m.m of stage micro meter =1000 microns
1 m.m of stage micro meter = 100 equal divisions
Therefore 100 division =1000 microns.
Hence one division of stage micrometer =1000/100
= 10 micron.
First division of stage micrometer coincides with fifth division of eye piece micrometer in 45 X.
1/5 X 10 =2 microns.

After calibration stage micrometer was replaced with a glass slide containing the niosomal

preparation. Then the vesicles were counted using the eye piece micrometer.

Observation made were

- Type of vesicle
- Average mean diameter of vesicles
- Size range where maximum number of vesicles occurs
- Size range of the vesicles

By optical microscopy 12, 13, 17

Taking one drop of prepared niosomes (diluted with water if needed) in a clean glass slide. Then it is placed on the stage of the optical microscope. First it was viewed under low power then changed to high power and the vesicles were counted up to 100 numbers.

By scanning Electron microscopy (SEM) 15

The size of the vesicles was measured by scanning electron microscopy (HITACHI S – 150). Small amount of sample of niosomes suspension was taken in cover slip on the specimen stub. It was then coated with carbon and then with gold vapor using Hitachi vacuum evaporator, model HITACHI S 5 GB. The samples were viewed under scanning electron microscope, which was operated at 15 kilovolts and then photographed.

Percentage Encapsulation of drug

The total amount of drug will not be encapsulated into the vesicle (niosomal vesicle) they may be present in free form (unencapsulated). So for the3determination of percentage drug entrapment in the vesicle, it is necessary to separate unentrapped drug in formulation.

The separation of unentrapped drug can be done by the following techniques

1. Exhaustive Dialysis.
2. Separation by gel filtration.
3. Centrifugation (7000 X G for 30 min.)
4. Ultra Centrifugation (150000 X G for 1.5 H).

Lamivudine encapsulated niosomes were separated from unentrapped drug by dialysis method [10] for 24 hrs. The formulation were transferred into a standard flask, then lysed with1ml of 2.5% SLS solution, then incubated at $37^0 \pm 1^0$ C for 2 hrs. Then it is filtered through whatman filter paper. Filtrate of 1 ml was diluted to 10 ml with PBS and absorbance of the resulting solution was measured spectrophotometrically at 308 nm [55].

Determination of percentage of drug encapsulated in the niosomes:

This was carried out to find out the percentage of drugs encapsulated in the niosomes by using the following formula,

$$\text{Percentage of Drug Encapsulated} = \frac{\text{Amount of encapsulated drug in niosomes (mgs)}}{\text{Amount of drug used (mgs)}} \text{X } 100$$

Osmotic shock

Effect of osmotic shock was determined by measuring the change in vesicles size followed by incubating the vesicular suspension in media of different tonicity such as a hypertonic media with 1.5% w/v NaCl solution, normal saline with 0.9% w/v NaCl, hypotonic media with 0.5%w/v NaCl and PBS. The vesicular suspension was incubated in these media for 3 h following which the change in vesicle size was measured.

Drug leakage studies from vesicles

Vesicle stability with respect to drug leakage and drug degradation upon storage was studied at refrigeration (4^0c), room temperature (25^0c) and high temperature (37^0c) for a period of one month on niosomes samples containing a known amount of Lamivudine, contained in light resistant containers. Samples were withdrawn at weekly intervals, and entrapment efficiency was determined.

In Vitro Release Pattern of Lamivudine Niosomal Formulations

The niosomal preparation was taken in a dialysis tube, which acts as a donor compartment. Dialysis tube was placed in a beaker containing 250 ml of phosphate buffer saline of pH 7.4, which acts

as a receptor compartment. The temperature of receptor medium maintained at $37^0 \pm 1^0$ c and medium agitated at a moderate speed using magnetic stirrer. 1 ml aliquots of sample was taken and made up to 10 ml with PBS. 1 ml of diffusion medium was replaced after every withdrawal so that the volume of the diffusion medium was maintained. The collected samples were analysed at 308 nm.

RESULTS AND DISCUSSION

Niosomes of Lamivudine were prepared by hand shaking method and ether injection method using cholesterol and various ratios of Span 80. Niosomes were evaluated for morphology, vesicle size determination, and percentage of drug encapsulation, drug leakage studies from vesicles, osmotic shock and in vitro release profile.

Compatibility studies

The IR Spectrum of pure Lamivudine drug was compared with the IR spectrum of physical mixture of drug and excipients. (Fig.6.1.1)

There is no appearance or disappearance of any characteristics peaks. This shows that there is no interaction between the drug and excipients used in the vesicles preparation.

Procedure

Weighed amount of drug (3mg) was mixed with 100mg of potassium bromide (dried at 40-50^{o}c). The mixture was taken and compressed under 10-ton pressure in a hydraulic press to form a transparent pellet. The pellet was scanned in IR spectrophotometer.

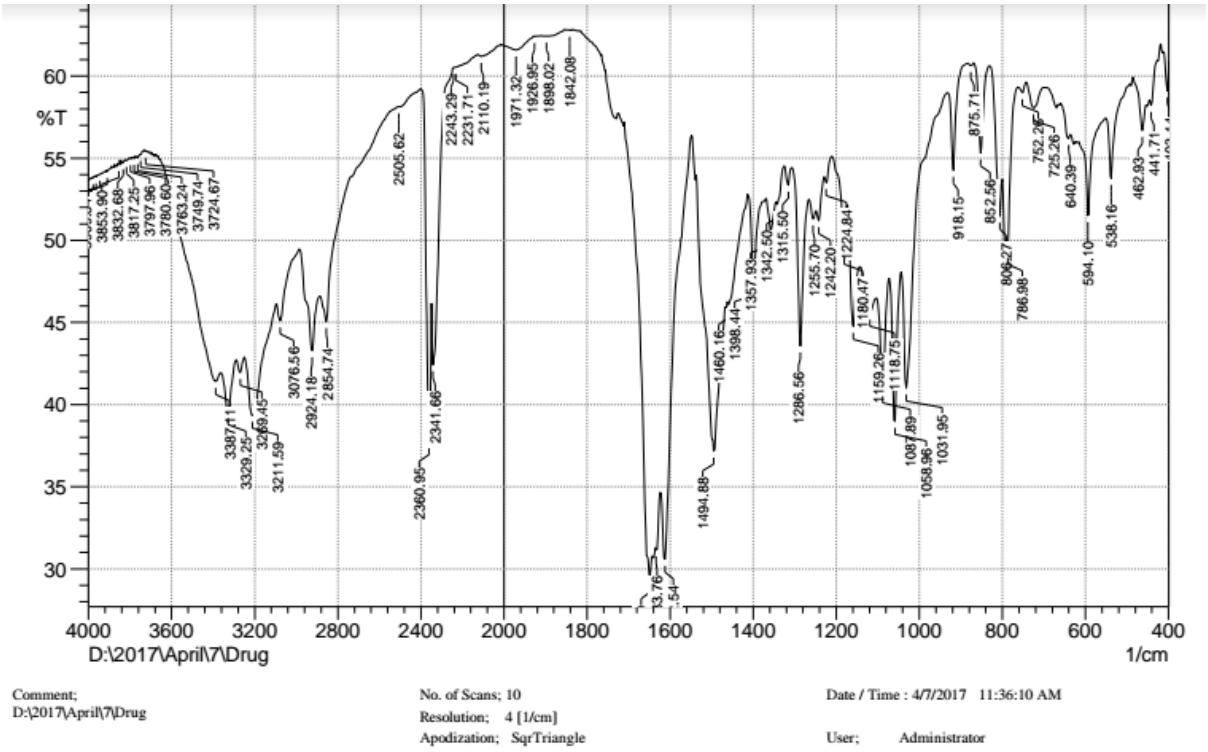

Figure 3. FTIR spectra of Lamivudine

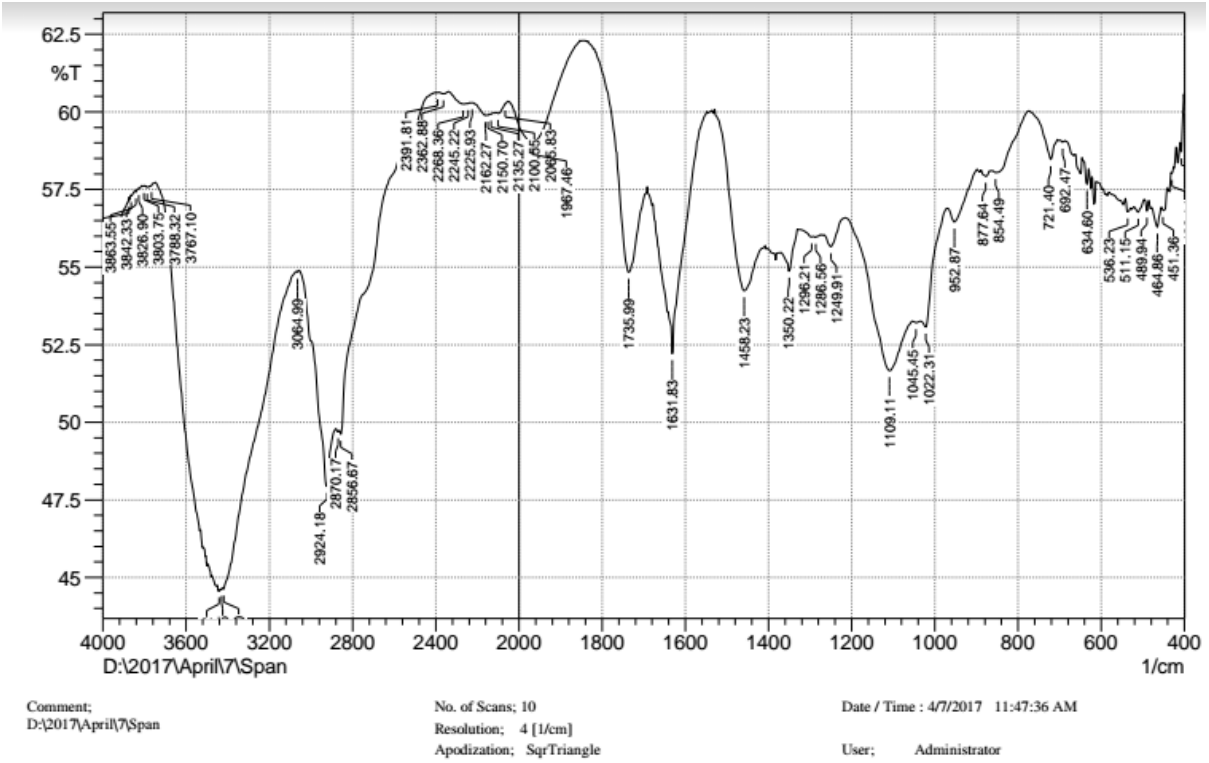

Figure 4.FTIR spectra of span 80

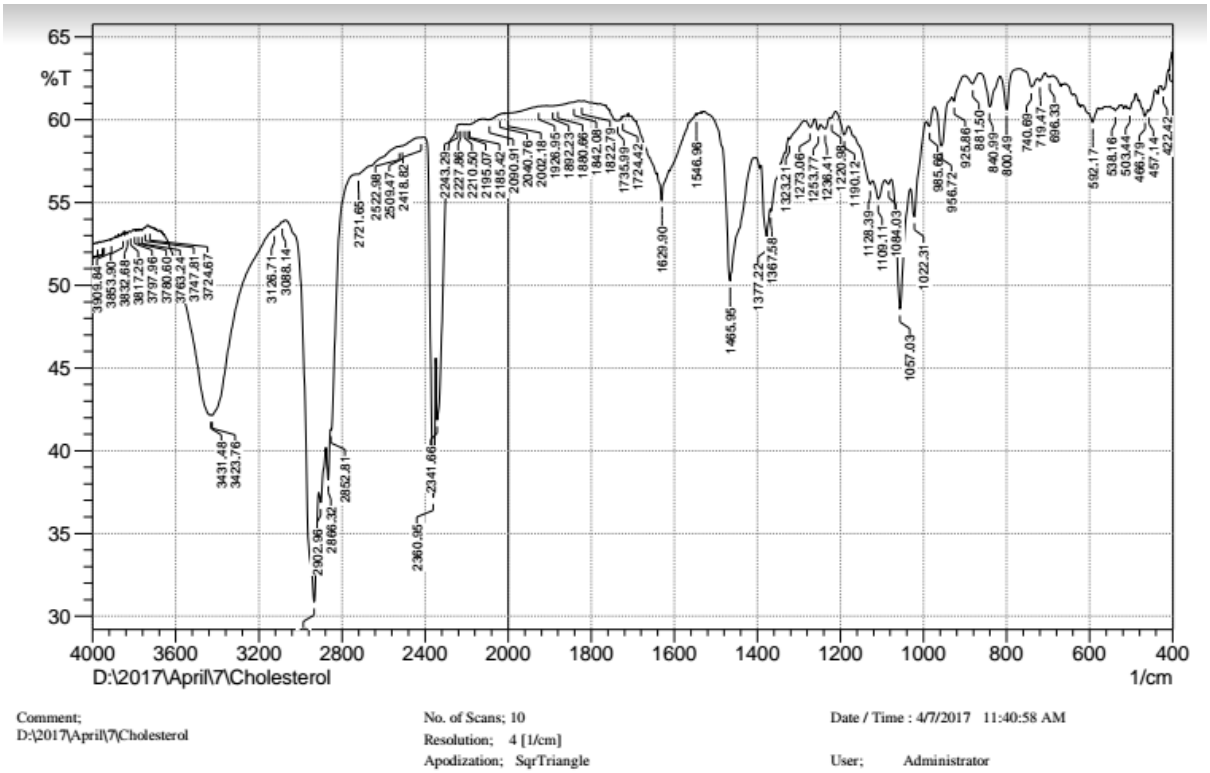

Figure 4 FTIR spectra of Cholesterol

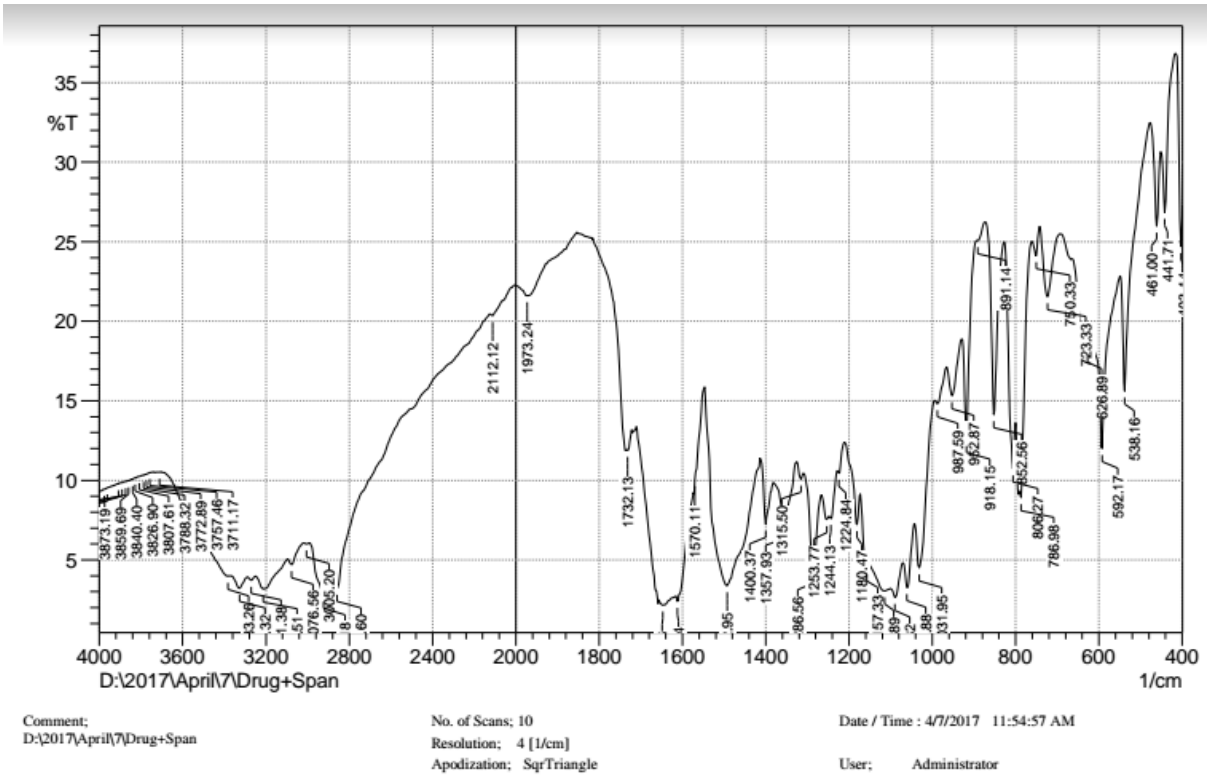

Figure 5 FTIR spectra of Lamivudine and span 80

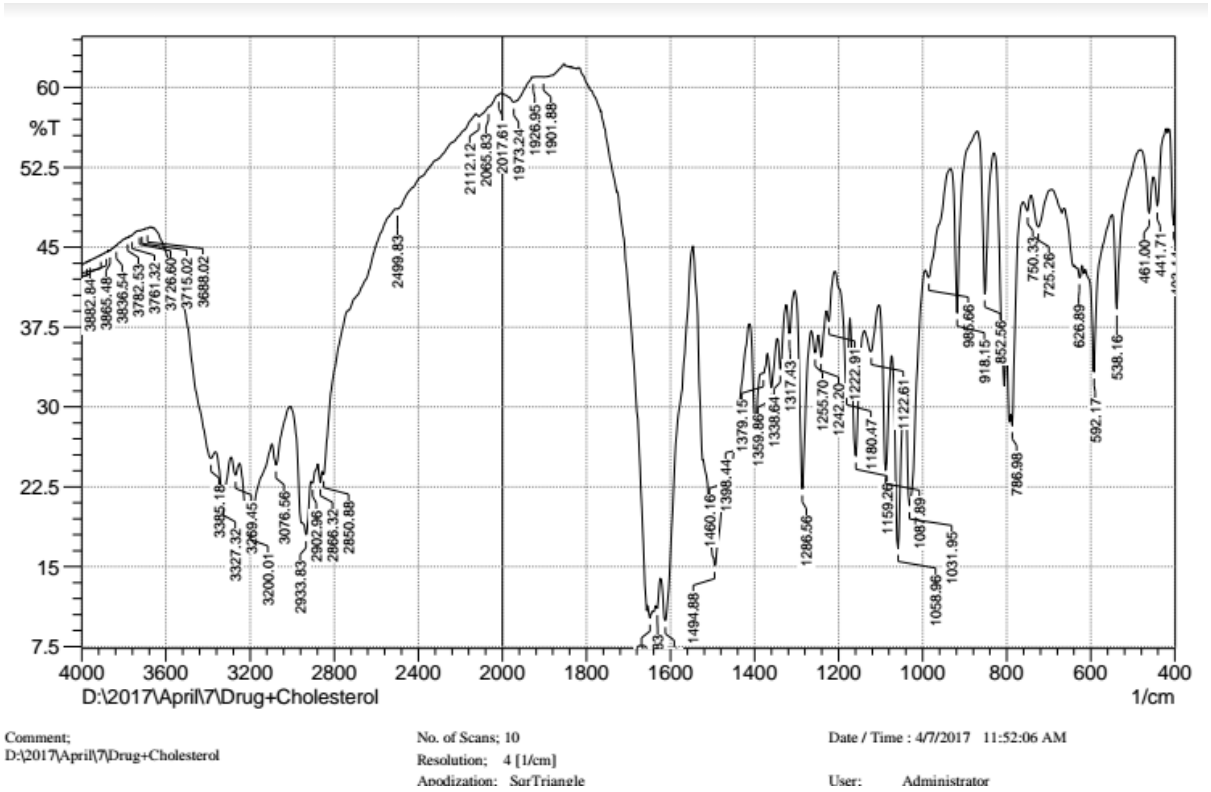

Figure 6.FTIR spectra of Lamivudine and Cholesterol

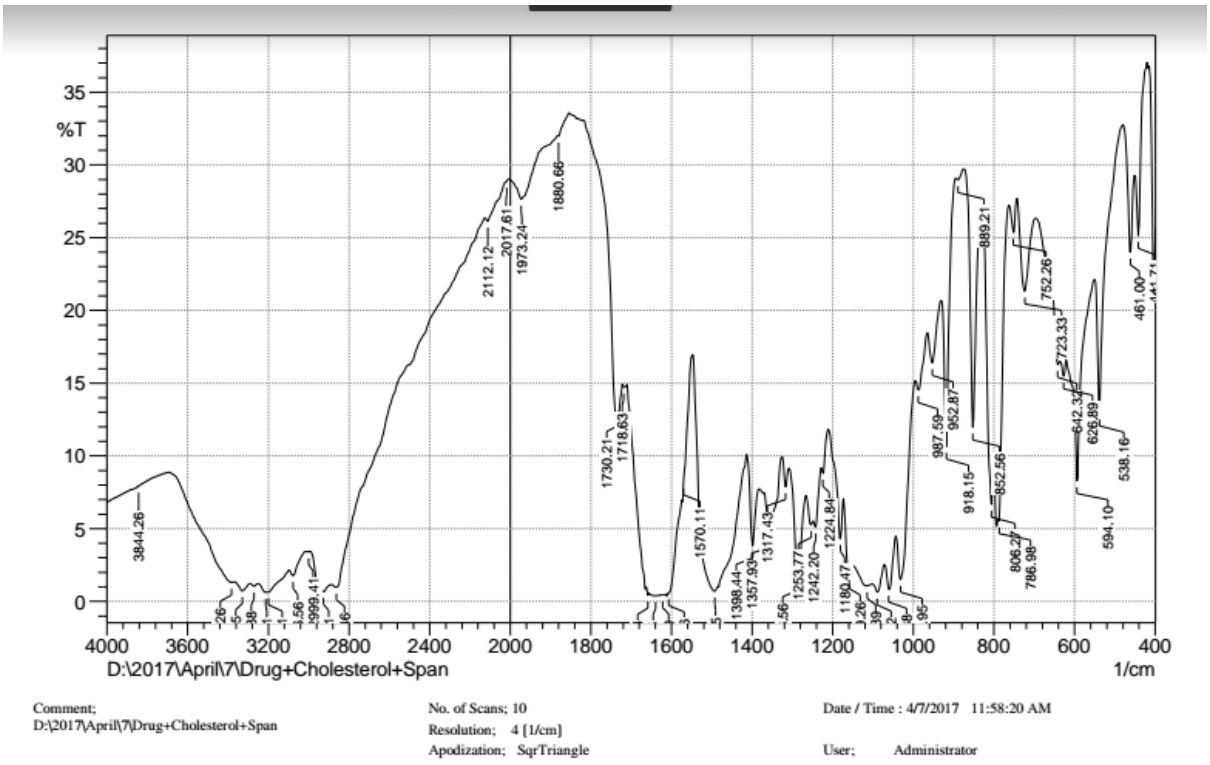

Figure 7 FTIR spectra of Lamivudine, Cholesterol and span 80

Morphology

The optical microscope of the prepared niosomes reveals that they are discrete and spherical /round shape and some vesicles are slightly elongated. The vesicles are slightly yellow in color. There is a thickening around the inner comportment of the vesicles prepared by hand shaking method. In ether injection method, thinning around the inner comportment of the vesicles. Individual vesicles could be seen under high power as shown in the photograph (fig 3-7)

Vesicle size determination

By optical microscopy

The formed vesicles from all batches of formulation were viewed under the high power objective in the optical microscope. It was also observed that niosomes prepared by hand shaking method was comparatively larger (fig-13) of size range (0.1-5μm) with an average size of 3.5 μm than niosomes prepared by ether injection method of size range (0.5-2.5μm) with an average size 1.5μm (fig-14). The reason for reduced size of the vesicles may be because in ether injection method, Span 80and cholesterol mixture is injected in to drug solution by forcing it through a needle orifice, so that smaller vesicles were formed.

Table. 3 Size Distribution of Niosomes by Hand Shaking Method

Size range (μm)	Number of Niosomes		
	1:1:1 Ratio	2:1:1 Ratio	3:1:1 Ratio

Below 0.1	19-21	15-17	7-8
0.1-5	69-70	73-74	84-88
Above 5	13-18	8-10	5-9

Table – 4 Size Distributions of Niosomes by Ether Injection Method

Size range (μm)	**Number of Niosomes**		
	1:1:1 Ratio	**2:1:1 Ratio**	**3:1:1 Ratio**
Below 0.5	19-21	14-16	5-7
0.5-2.5	66-71	66-72	80- 84
Above 2.5	4-6	4-5	3-5

By scanning electron microscopy (SEM)

The prepared niosomes vesicles sizes were performed by SEM. The size range was observed to be 0.5-5μm for niosomes prepared by hand shaking method and 0.5-2.5μ in ether injection method. This is shown in the photographs. (Fig-8).

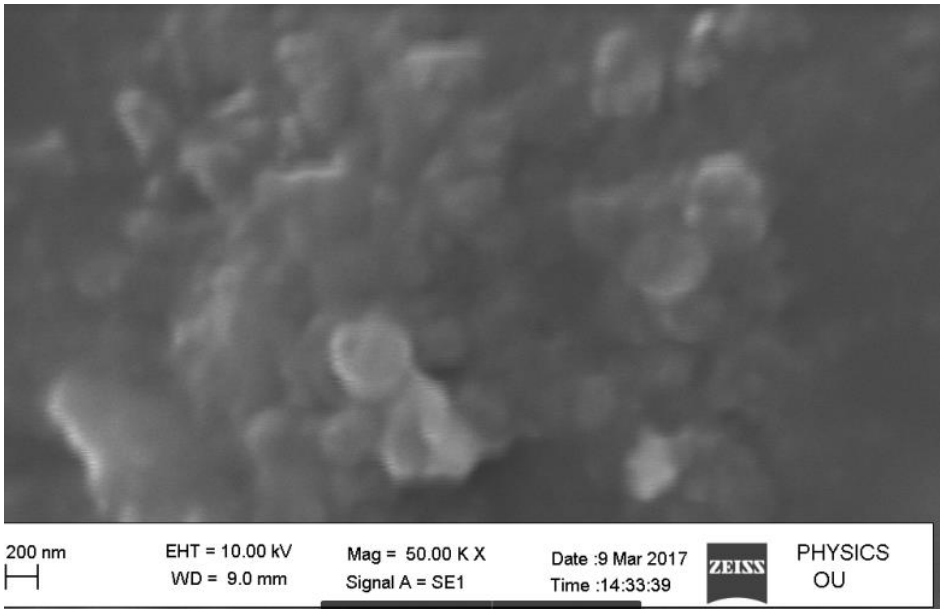

Figure 8.Scanning Electron Microscopic view of Lamivudine loaded Niosomes by Hand Shaking Method For formulation HS 3 (400 X)

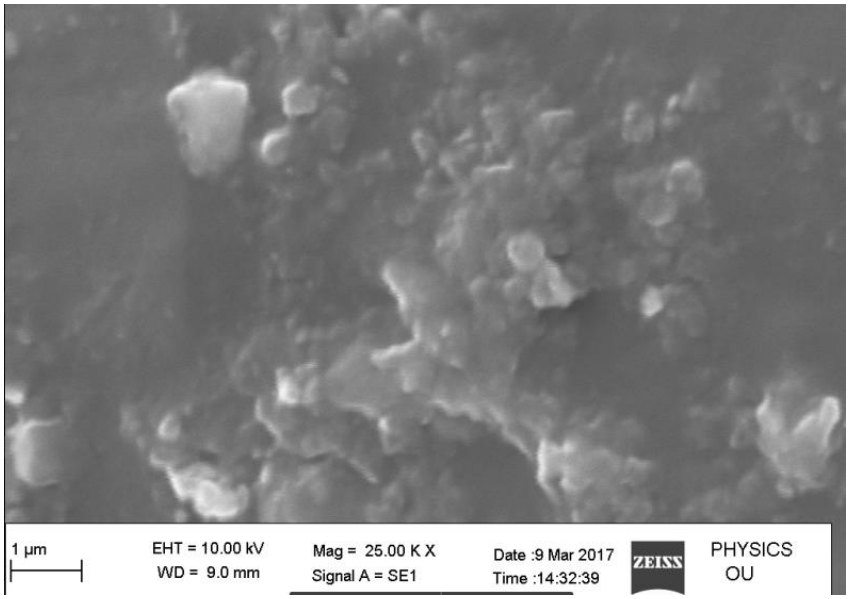

Figure 9.Scanning Electron Microscopic view of Lamivudine loaded Niosomes by Hand Shaking Method For formulation HS 3 (900 X)

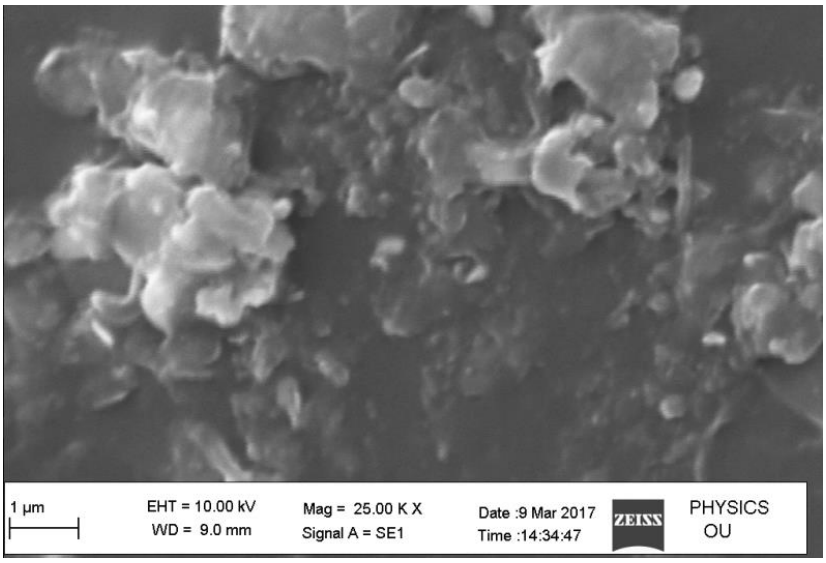

Figure 10. Scanning Electron Microscopic view of Lamivudine loaded Niosomes by Ether Injection Method For formulation ES 3 (300 X)

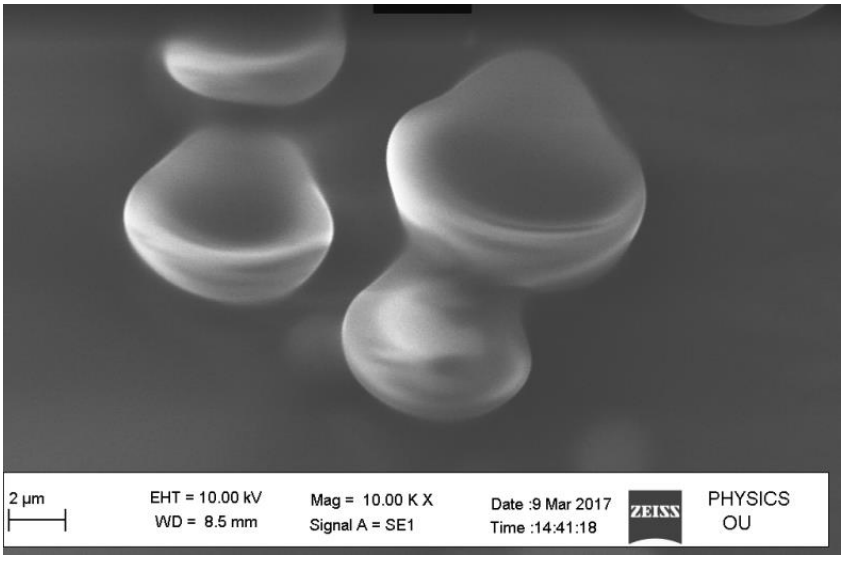

Figure 11 Scanning Electron Microscopic view of Lamivudine loaded Niosomes by Ether Injection Method For formulation ES 3 (1200 X)

Osmotic shock

Effect of osmotic shock was studied by incubating the vesicular suspension in media of different tonicity for a period of 3 h. The vesicular suspension incubated with hypertonic media (1.5 % w/v NaCl) for a 3 h, shrinked completely (Tab-9). This was observed with all the formulations. Similarly, incubation of vesicular suspension in a hypotonic media (0.5 % w/v NaCl) recorded an increase in their vesicle size (Tab-9). This was a significant increase as compared to the vesicular dispersion in PBS (pH 7.4) (Tab-9). However, no significant change in vesicle size was observed with vesicles incubated in normal saline (0.9 % w/v NaCl).

Table – 5 Average vesicle size for Lamivudine encapsulated niosomal formulation

Formulation Code	Average vesicle size (µm) after incubation with			
	PBS (pH 7.4)	1.5 % w/v NaCl	0.9 % w/v NaCl	0.5 % w/v NaCl
HS 1	3.84	Shrinked	3.96	6.24
HS 2	4.21	Shrinked	4.31	6.58
HS 3	4.54	Shrinked	4.62	6.97
ES 1	0.61	Shrinked	0.64	0.92
ES 2	0.73	Shrinked	0.74	1.51
ES 3	0.75	Shrinked	0.77	1.68

Drug leakage studies from vesicles

The bar diagram between percent drug remaining in niosomes at 7,14,21, &28 days at refrigeration temperature, room temperature and high temperature has been represented in (Tab 10). Niosomes have shown a fairly high retention of the drug inside the vesicles at a refrigeration temperature up to a period of one month (≈90%). While, storage at room temperature lead to a substantial loss (≈20 %) and storage at high temperature lead to a substantial loss (≈ 40 %) of the drug from the niosomes at the end of one-month period The drug leakage at elevated temperature may be related to the degradation of lipids in bilayers resulting in defects in membrane packing making them leaky.

Table –6.Percentage of drug retention in Lamivudine niosomal formulation

Formulation Code	Percentage of drug retention in niosomes											
	REFRIGERATION Temp. ($4^0 \pm 1$ C)				Room Temp ($25^0 \pm 1$ C)				High Temp. ($37^0 \pm 1$ C)			
	Days				Days				Days			
	7	14	21	28	7	14	21	28	7	14	21	28
HS1	100	95	90	86	99	88	84	77	94	85	73	69
HS2	100	96	95	87	99	90	84	79	95	84	75	70
HS3	100	97	95	90	100	94	87	80	95	85	76	70
ES1	100	95	93	85	98	93	84	77	93	84	73	68
ES2	100	97	94	86	99	95	85	78	94	85	74	69
ES3	100	98	95	90	100	95	88	80	96	86	75	72

Percentage of Drug Encapsulation

The percentages of drug encapsulated in niosomes are given in (Tab-11). Percentage of drug encapsulation varies when the Span 80 proportion was varied. This explained that encapsulation was increased with increase in Span 80 content while cholesterol content was maintained at constant value. Niosomes prepared by hand shaking method have good encapsulation efficiency. This may be due to the good vortexing and more over the vesicles were larger. In the case of ether injection method, the vesicles were smaller; this may cause a decrease in encapsulation. A good percentage of drug encapsulation was achieved from batches HS3& ES 3. Least percentage of drug encapsulation was achieved from batches HS 1 and ES 1.

Table—7 Encapsulation efficiency for Lamivudine Niosomal Formulations

Formulation Code	Amount of drug used in (mgs)	Percentage of drug encapsulated
HS 1	50 mg	54
HS 2	50 mg	63
HS 3	50 mg	82
ES 1	50 mg	42
ES 2	50 mg	55
ES 3	50 mg	71

In vitro drug release studies

In vitro drug release was studied for all the batches of niosomes. The studies were performed up to 24 h for all the batches. The cumulative % release of Lamivudine from various formulations by hand shaking method at 24 h was found to be 68.57%, 73.84% and 85.56% for HS 1, HS 2 and HS 3 respectively. The above result indicates that though the entrapment efficiency was increased with HS 2 and HS 3 formulation, the release was little fast. But in the HS 1 formulation though entrapment was less, the drug was release for a longer duration. Niosomes prepared by ether injection method showed little less entrapment efficiency than niosomes prepared by hand shaking method. The release pattern of batch ES 1 and ES 2 did not show so much difference. This may be due to the formulation of similar size vesicles in both the batches. But in the case of batch ES 3, the release rate is more. An almost constant drug release was observed in all formulations indicating zero order release pattern (fig-22-27). This is indicated by the linearity in regression value when plotted with cumulative percentage release vs time curve.

Table – 8 In vitro release data for Lamivudine Niosomes for formulation Hs and ES

	CUMULATIVE % DRUG RELEASE						
Time	**PURE DRUG**	**HS-1**	**HS-2**	**HS-3**	**ES-1**	**ES-2**	**ES-3**
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.5	23.61	6.10	5.49	6.31	7.86	5.19	5.55
1	34.39	10.14	8.12	8.07	11.76	8.05	7.09
2	43.28	16.43	14.10	12.17	15.50	11.96	11.04
4	56.64	21.58	17.41	21.58	17.67	20.35	19.13
6	72.75	25.17	22.70	23.60	22.28	29.19	30.00
8	84.66	33.96	34.70	34.10	27.13	32.97	37.00
10	95.85	39.99	35.88	45.78	36.56	43.44	45.52
12	--------	41.42	47.72	54.90	38.90	46.54	49.35
16	--------	50.42	56.40	63.53	46.76	53.87	56.58
20	-------	59.58	66.13	75.87	52.48	57.65	64.56
24	---------	68.57	73.84	85.56	55.77	63.68	71.69

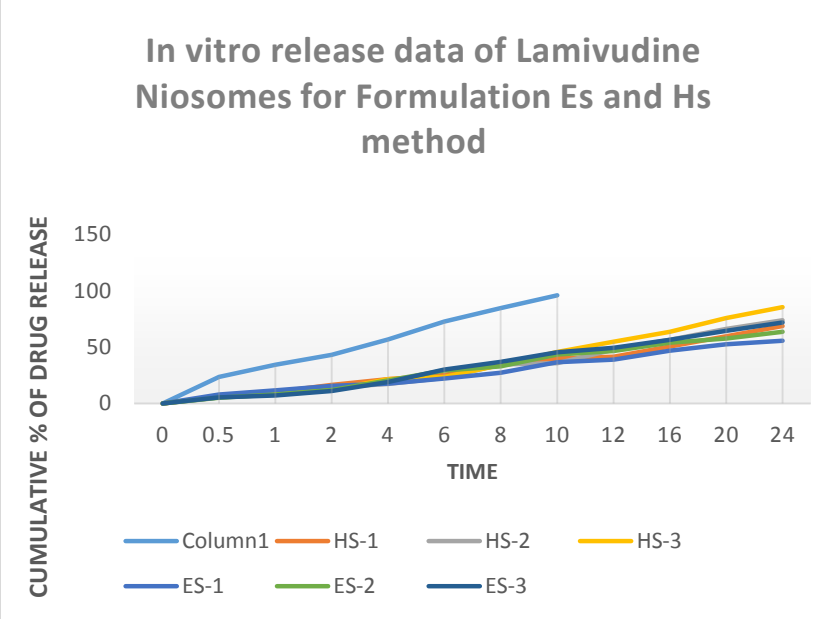

Fig 12 IN VITRO RELEASE DATA OF LAMIVUDINE NIOSOMES FOR FORMULATION BY HS AND ES METHOD.

SUMMARY AND CONCLUSION

Stable Lamivudine loaded Niosomes can be prepared by hand shaking method and ether injection method with Span 80 and cholesterol in the ratio of 1:1, 2:1, and 3:1.Preformulation study and drug excipients compatibility study was done initially and results directed the further course of formulation. Most of the vesicles are spherical in shape, the size range of the vesicles, fall in the narrow size range of 0.5-5µ and 0.5-2.5 µ by hand shaking method and ether injection method respectively. A high % of Lamivudine can be encapsulated in the vesicles (74-82%) prepared by hand shaking method. Concentration of non-ionic surfactant such as Span 80 might influence the drug release pattern of all formulation.In vitro release of Lamivudine from niosomes was very slow when compared to the release from pure Lamivudine solution. Drug release studies showed that the niosomal preparation was stable at refrigeration temperature (4^0 C).The vesicles prepared by hand

shaking method were found to be larger in size as compared to vesicles prepared by ether injection method.Almost constant drug release was observed in all formulations indicating zero order release pattern.Osmotic shock studies on vesicles showed that no significant change in the niosomal preparation was stored at normal saline. From above these studies it was concluded that Lamivudine was successfully encapsulated into niosomes, Span 80 (1:1:3) vesicles prepared by hand shaking method showed best result in terms of encapsulation efficiency, in vitro drug release and to enhance the therapeutic effectiveness of Lamivudine, producing prolonged activity and simultaneously minimizing the side effects.

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