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# Design and Characterization of Lamivudine Niosomal Drug Delivery System

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**ABSTRACT**
*The purpose of this research was to formulate Lamivudine Niosome drug delivery system by thin film hydration technique using non-ionic surfactants (Span 40) in different ratio. Optimization of formulation variables are done by Statistical design. Three independent variables such as Span, RPM, Sonication time; and three dependent variables such as particle size, EE%, drug release %. Total of 15 formulations were run at three level design. FT-IR spectra of pure drug and its polymer used in formulations indicate that there were no structural changes caused by excipients. Using scanning electron microscopy, the synthesised niosomes were examined microscopically to determined. Microscopically, F-I, F-II, and F-III were identified as spherical tiny unilamellar vesicles with diameters of 110–130 nm, 150–260 nm, and 250–280 nm, respectively. The percentage of drug entrapment in F I, which included span 40(30mg), was found to be 76.61%, whereas that of F-II and F-III, which contained span 40(25mg) and span 40(20mg), was found to be 66.42% and 58.53%, respectively. Particle size analyzer was used to characterise the size distribution of niosomes. For F-I, F-II, and F-III, respectively, the typical mean particle size range was 120 nm, 205 nm, and 265 nm. In-vitro release studies of F-I indicated a 19-hour drug release of 93.48%. In contrast to F-III, which exhibited an 84.99% drug release within 19 hours, F-II demonstrated an 87.97% drug release within 20 hours. Additionally, F-I containing span 40(30mg) demonstrated higher release compared to F-II and F-III containing span 40(25mg) and 20mg, respectively. Niosomal Lamivudine tubes were clear, indicating that they are sterile. The formulated niosomes passed the Sterility test.*
***Keywords:*** *Niosomes, Lamivudine, Non-ionic surfactants, Drug release, Sterility test.*

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## INTRODUCTION

Controlled drug delivery systems have acquired a centre stage in the area of pharmaceutical research and development sector. Controlled drug delivery systems, which release the drug in continuous manner by both dissolution-controlled as well as diffusion-controlled mechanisms [1].

In recent years it has been shown that the skin a useful route for drug delivery to the systemic circulation. Transdermal drug delivery system includes all topically administered drug formulations intended to deliver the active ingredients into the circulation. They provide controlled continuous delivery of drugs through the skin to the systemic circulation.

Niosomes are non-ionic surfactant vesicles, capable of forming vesicles & entrapping hydrophilic and hydrophobic molecule.[2] Niosomes possess an infrastructure consisting of hydrophilic and hydrophobic moieties together, and as a result, can accommodate drug molecules with a wide range of solubilities. Non-ionic surfactants are comprised of polar and non- polar segments and possess high interfacial activity [3,4]. The formation of bilayer vesicles instead of micelles is dependent on the hydrophilic–lipophilic balance (HLB) of the surfactant. They have longer shelf life, stability and ability to deliver the drug at the target site in a controlled or sustained manner which enhances bioavailability and they offer several advantages over liposomes such as higher chemical stability, lower costs and ability to deliver the drug at the target site in a controlled or sustained manner which enhances bioavailability [5,6].

## MATERIAL AND METHODS

### Materials

Lamivudine was obtained as a gift sample from Micro Labs, Hosur. Span 40 and cholesterol, Chloroform were purchased from SD fine chemicals ltd, (Mumbai, India).

## MATERIAL AND METHOD

### FORMULATION OF LAMIVUDINE NIOSOMES

Lamivudine niosomes were produced utilising a Rotary flash evaporator and the Thin Film Hydration Technique. In this technique, non-ionic surfactant and cholesterol were weighed out exactly and then added in 10ml of chloroform [7,8]. Once all of the organic phase had evaporated and a thin film had formed on the wall, the flask with a circular bottom was rotated at a height of 1.5 cm above a water bath heated to 60°C±20°C under reduced pressure. The medication was precisely weighed and then dissolved in 10ml of pH 7.4 phosphate buffered saline. The dried non-ionic surfactant and cholesterol film was rotated for an hour in a water bath set at 60°C±2°C after the medication solution had been added to ensure that the mixture was well dispersed. Subsequently, the solution was sonicated to produce unilamellar vesicles.

### Optimization of Niosomes

Optimization of formulation variables are done by Statistical design. [9]. Three independent variables such as Span, RPM, Sonication time; and three dependent variables such as particle size, EE%, drug release %. Total of 15 formulations were run at three level design.

### CHARACTERIZATION OF NIOSOMES

### OPTICAL MICROSCOPY

An optical microscope was used to view the created niosomes. Vesicular dispersion is properly diluted, wet straddling on a hemocytometer, and phase contrast microscopy images are taken.

### SCANNING ELECTRON MICROSCOPY

A sample stub was used to transfer a formulation containing niosomes from a cover glass to a container. Using a sputter coater, platinum alloy was applied to dried samples to coat them to a thickness of 100 A. After coating, the shape and size were checked using scanning [10].

### ENTRAPMENT EFFICIENCY

Using a Remi centrifuge, 1 gm of the sample is centrifuged at 13000 RPM for 90 minutes at 40 ºC. The free drug in the supernatant layer was then diluted with PBS pH 7.4 and examined with a UV spectrophotometer [11].

$$\text{Percentage drug entrapment} = \frac{\text{Amount of drug entrapped}}{\text{Initial amount of drug}} \times 100$$

### VESICLE SIZE, POLYDISPERSITY INDEX AND ZETA POTENTIAL

For all the batches of Niosomes vesicle size analysis was carried out using Malvern Zetasizer Nano ZS (Malvern Instruments, UK). Polydispersity Index (PDI) was also determined as a measure of homogeneity [12]. Zeta potential of the niosome formulations was determined to estimate the stability of the formulations.

### IN VITRO RELEASE STUDIES

Release for Pure Drug in vitro 10ml of 0.1N HCl were used to dissolve 10mg of pure lamivudine. 200ml of 0.1 N HCl were added to a dialysis tube containing 5ml of the solution. The medium was swirled with a magnetic stirrer while being kept at a constant 37°±20°C. 5 ml of samples was periodically removed, and the same volume of medium was reintroduced. After that, the samples were spectrophotometrically analysed at 255 nm with 0.1 N HCl as a blank [13].

### STERILITY TEST FOR LAMIVUDINE:

After Lamivudine niosomes were sterilised, a sterility test was performed on the formulations. The goal of the sterility test is to find any living bacteria, fungus, or yeast in the preparations. To prevent unintentional product contamination during the test, the tests were conducted in an aseptic environment [14].

### Culture Media

### A. For aerobic Bacteria and Fungi

Fluid thioglycollate medium: This medium can be cast-off for the finding of aerobic bacteria and fungi.

### For Anaerobic Bacteria

**Chopped meat (CM) medium:** It can be used for the detection of anaerobic microbes.

### PROCEDURE

The fluid thioglycollate medium was supplemented with the Lamivudine niosomal formulation, which was then incubated at 20-250C for at least seven days. It takes at least fifteen days to incubate the chopped meat medium. Throughout the incubation period, the media were periodically visually inspected for microbial growth. A positive control and a negative control test were also conducted in demand to sustenance the previously performed test [15]. A positive control test was conducted to ensure that the media and incubation atmosphere were appropriate for the development of microorganisms. Hemolytic Streptococci and Clostridium tetani were introduced into a fluid thioglycollate medium and a chopped meat medium, respectively, and a growth promotion was seen.

## RESULTS AND DISCUSSION

## FORMULATION OF LAMIVUDINE NIOSOMES

**Table 1: Preparation of Lamivudine Niosomes**

| F.Code | Drug | Cholesterol | Span 40 | Chloroform | PBS pH 7.4 | C.S ratio |
|---|---|---|---|---|---|---|
| F1 | 10.0 mg | 30.0 mg | 30.0 mg | 10.0 ml | 10.0 ml | 1:1 |
| F2 | 10.0 mg | 30.0 mg | 25.0 mg | 10.0 ml | 10.0 ml | 1:1 |
| F3 | 10.0 mg | 30.0 mg | 20.0 mg | 10.0 ml | 10.0 ml | 1:1 |
| F4 | 10.0 mg | 30.0 mg | 15.0 mg | 10.0 ml | 10.0 ml | 1:1 |
| F5 | 10.0 mg | 30.0 mg | 10.0 mg | 10.0 ml | 10.0 ml | 1:1 |
| F6 | 10.0 mg | 30.0 mg | 5.0 mg | 10.0 ml | 10.0 ml | 1:1 |

**Table 2: Optimization of Niosomes**

| Std | Run | X1- Span 40 (mg) | X2- RPM | X3- Sonication time (mins) | Y1-Particle Size (nm) | Y2- EE (%) | Y3- Drug Release (%) |
|---|---|---|---|---|---|---|---|
| 7 | F1 | 20 | 100 | 30 | 257 | 70.12 | 79.25 |
| 9 | F2 | 25 | 80 | 10 | 224 | 79.63 | 89.23 |
| 13 | F3 | 25 | 100 | 15 | 205 | 85.23 | 93.01 |
| 2 | F4 | 30 | 80 | 15 | 215 | 80.68 | 90.98 |
| 11 | F5 | 25 | 80 | 30 | 246 | 73.21 | 80.87 |
| 15 | F6 | 25 | 100 | 15 | 205 | 85.23 | 93.01 |
| 12 | F7 | 25 | 120 | 30 | 256 | 70.86 | 78.93 |
| 14 | F8 | 25 | 100 | 15 | 205 | 85.23 | 93.01 |
| 6 | F9 | 30 | 100 | 10 | 227 | 78.6 | 88.63 |
| 4 | F10 | 30 | 120 | 15 | 213 | 82.06 | 91.23 |
| 3 | F11 | 20 | 120 | 15 | 265 | 69.54 | 77.63 |
| 5 | F12 | 20 | 100 | 10 | 242 | 74.68 | 85.52 |
| 8 | F13 | 30 | 100 | 15 | 227 | 76.61 | 84.23 |
| 10 | F14 | 25 | 120 | 10 | 253 | 71.54 | 79.53 |
| 1 | F15 | 20 | 80 | 15 | 237 | 76.23 | 87.36 |

### Response 1: Particle Size

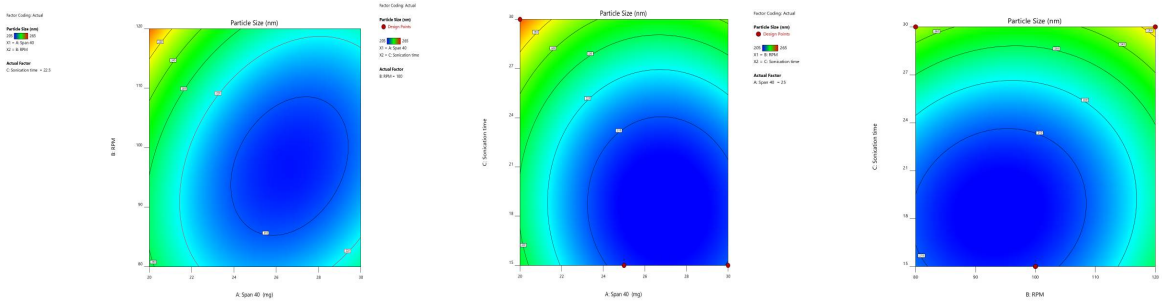


**Fig. 1: Contour plots of formulation variables on Y1-Particle Size**

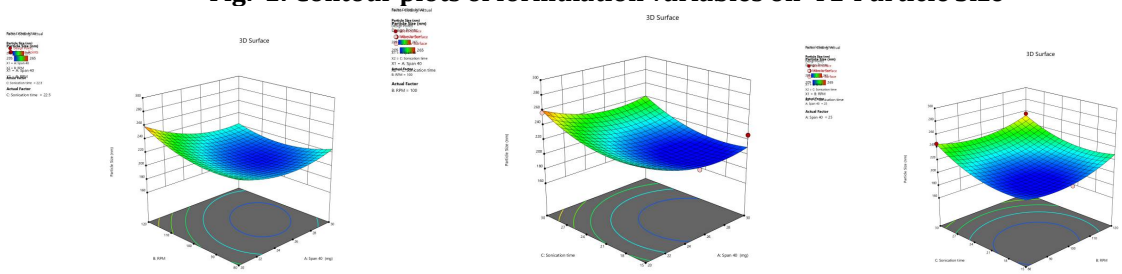


**Fig. 2: 3-D surface plots of formulation variables on Y1-Particle Size**

## Response 2: EE

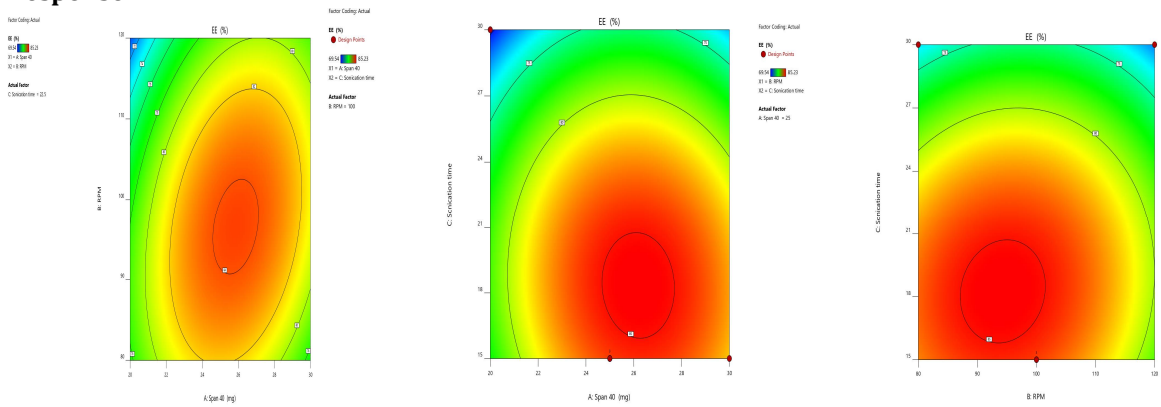


**Fig. 3: Contour plots of formulation variables on Y2-Entrapment Efficiency**

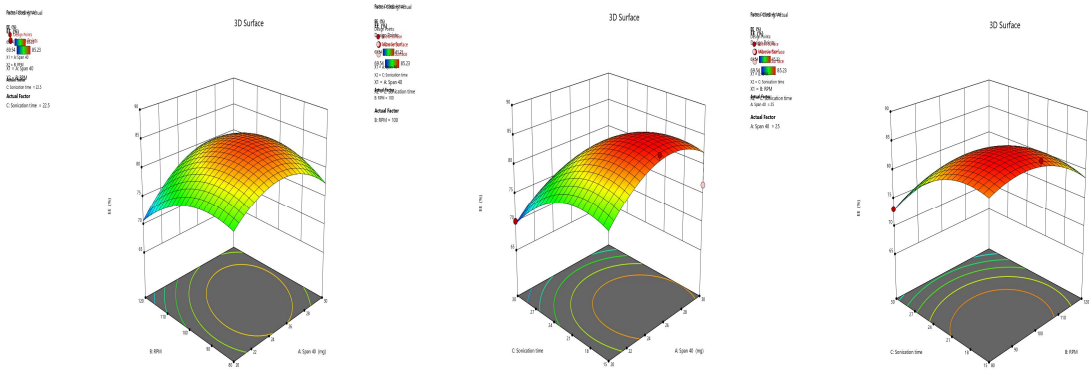


**Fig. 4: 3-D surface plots of formulation variables on Y2-Entrapment Efficiency**

## Response 3: Drug Release

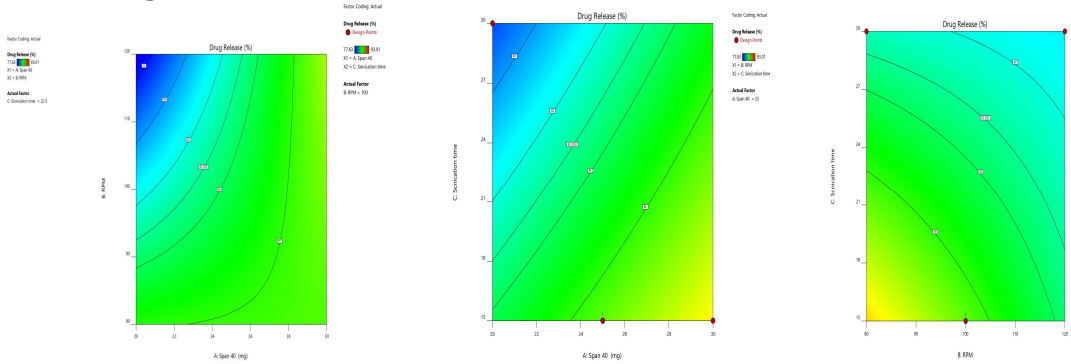


**Fig. 5: Contour plots of formulation variables on Y3-Drug Release**

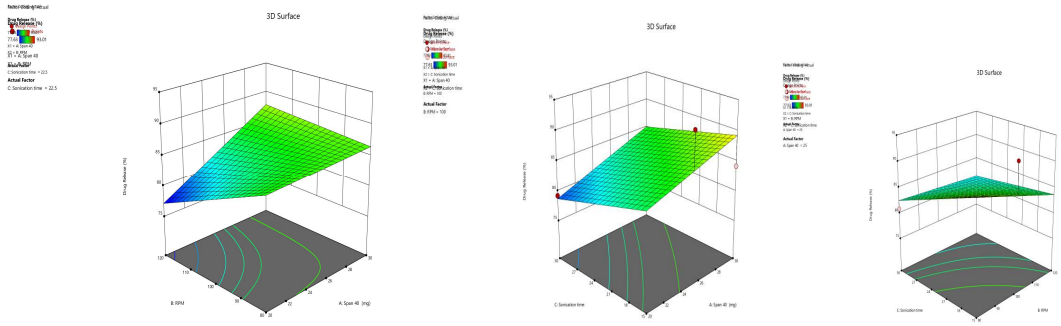


**Fig. 6: 3-D surface plots of formulation variables on Y3-Drug Release**

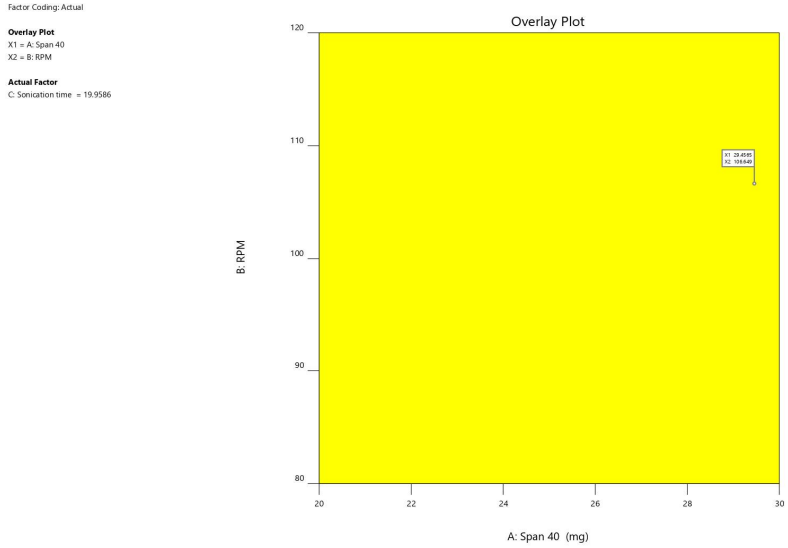


**Fig. 7: Overlay plot**

**Point prediction**

**Table 3: Point prediction**

| Factor | Name | Level |
|---|---|---|
| A | Span 40 | 29.46 |
| B | RPM | 106.65 |
| C | Sonication time | 19.96 |

**CHARACTERIZATION OF NIOSOMES**

**OPTICAL MICROSCOPY**

An optical microscope was used to view the formed niosomes. The vesicles that develop in the F-I containing span 40(30mg) are less spherical. In F-II containing spherical vesicles 40(25mg), many spherical vesicles develop. Matched to F-I and F-II, F-III with span 40(20mg) displayed less spherical vesicles.

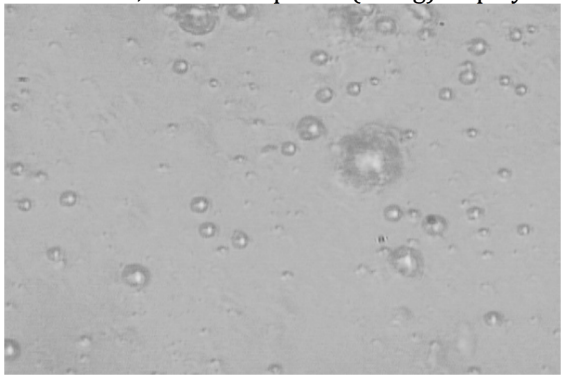

**Fig. 8: Formulation 1**

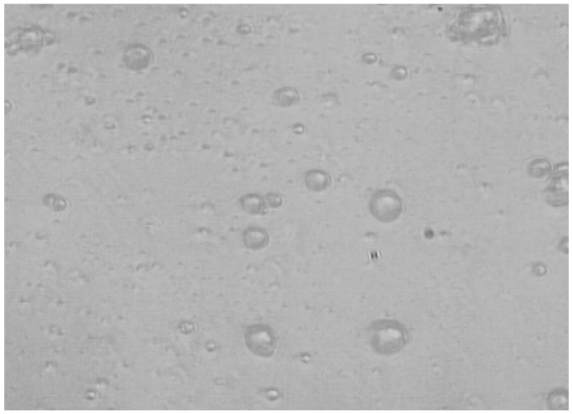

**Fig. 9: Formulation 2**

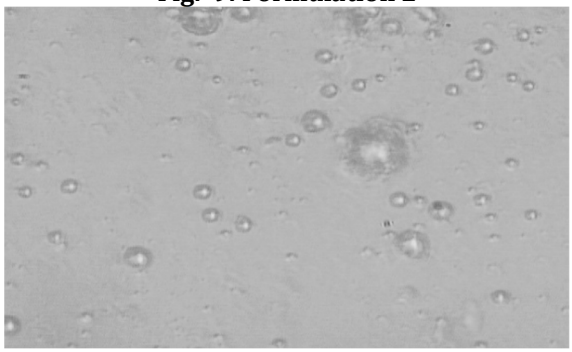

**Fig. 10: Formulation 3**

**MORPHOLOGICAL STUDIES**

Using scanning electron microscopy, the synthesised niosomes were examined microscopically to determine their size and shape. Microscopically, F-I, F-II, and F-III were identified as spherical tiny unilamellar vesicles with diameters of 110–130 nm, 150–260 nm, and 250–280 nm, respectively. These findings showed that the vesicle diameter is inside the range of niosomal sizes (100–300 nm).

**Table 4: Vesicle Diameter of niosomes**

| Sr. No | Type of formulation | Size (nm) |
|---|---|---|
| 1 | F1 | 110-130 |
| 2 | F2 | 150-260 |
| 3 | F3 | 250-280 |

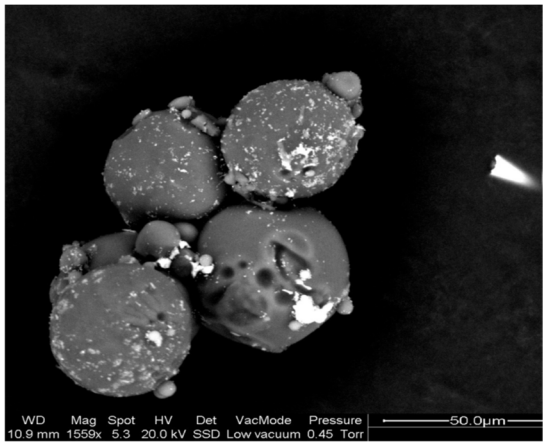


**Fig. 11: SEM PHOTOGRAPH FOR F1**

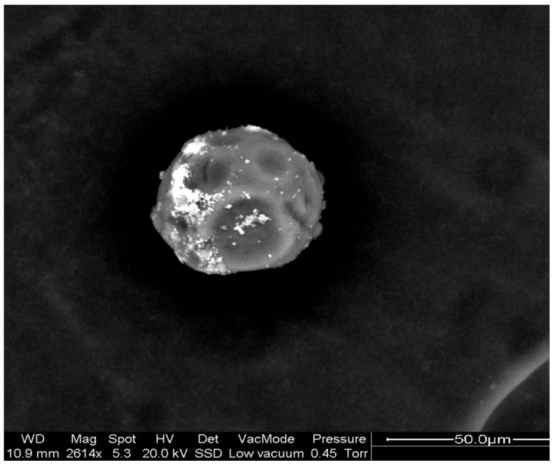


**Fig. 12: SEM PHOTOGRAPH FOR F2**

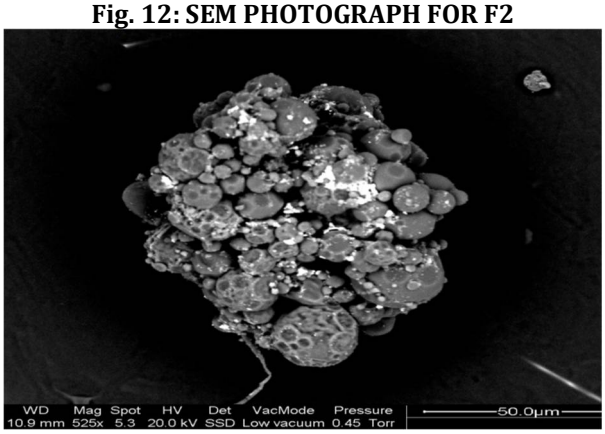


**Fig. 13: SEM PHOTOGRAPH FOR F3**

**DETERMINATION OF DRUG ENTRAPMENT EFFICIENCY**

**Table 5: % Drug Entrapment of the formulated Niosomes**

| Formulation code | % Drug Entrapment |
|---|---|
| F1 (Span 40)30mg | 76.61 % |
| F2 (Span 40)25mg | 66.42 % |
| F3 (Span 40)20mg | 58.53 % |

To produce the niosomes with the greatest amount of drug entrapment, the formulation and process factors (surfactant and cholesterol) were changed and optimised. The percentage of drug entrapment in F I, which included span 40(30mg), was found to be 76.61%, whereas that of F-II and F-III, which contained span 40(25mg) and span 40(20mg), was found to be 66.42% and 58.53%, respectively. This demonstrated that span- 60, along with cholesterol, is the better surfactant for augmenting supreme entrapment for the drug lamivudine. Decreased sonication duration further increases the percentage of drug entrapment. The sonication time was therefore optimised to 15 minutes, and additional size reduction by increasing sonication time was not tried.

**PARTICLE SIZE DISTRUBUTION OF NIOSOMES**

Particle size analyzer was cast-off to characterise the size distribution of niosomes. For F-I, F-II, and F-III, respectively, the typical mean particle size range was 120 nm, 205 nm, and 265 nm. These numbers showed that all three formulations' mean particle sizes fall within the 100-300 nm range for niosomes.

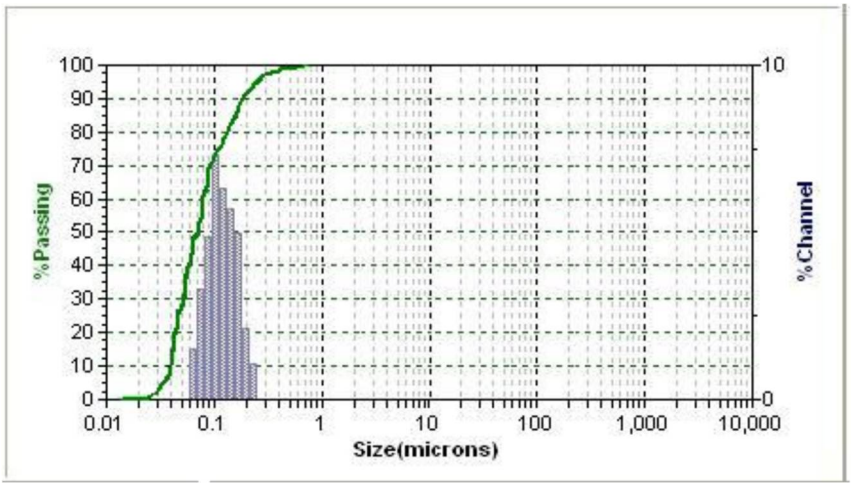


**Fig. 14: Particle Size Distribution of F1**

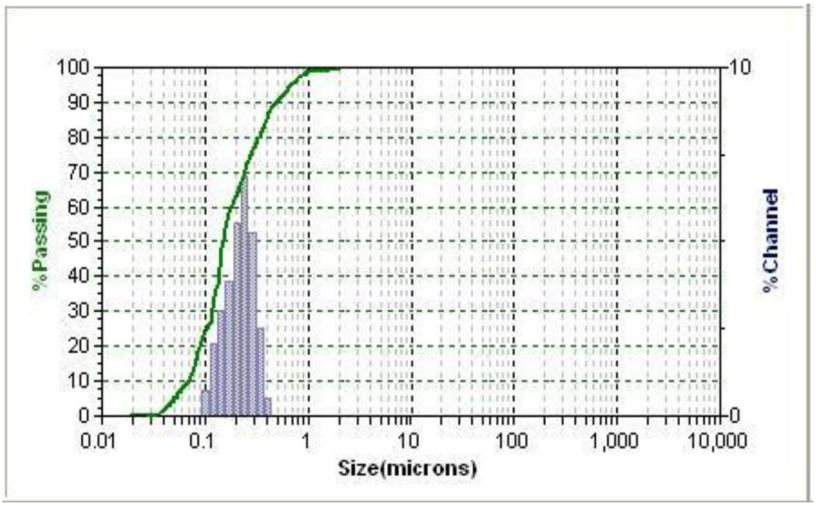


**Fig. 15: Particle Size Distribution of F2**

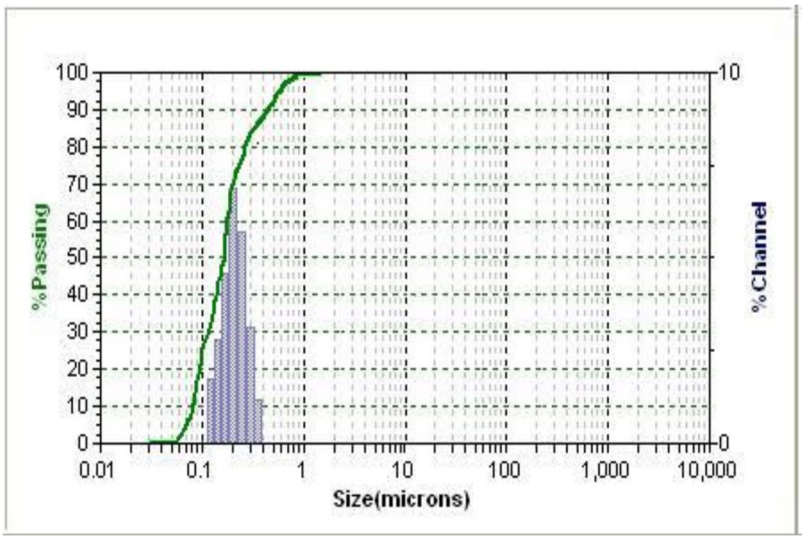


**Fig. 16: Particle Size Distribution of F3**

**Table 6: Particle Size Distribution of Lamivudine Niosomes**

| S.No | Lamivudine formulations | Average particle size of Niosomes (nm) |
|---|---|---|
| 1 | F1 | 120 |
| 2 | F2 | 205 |
| 3 | F3 | 265 |

**IN VITRO RELEASE STUDIES**

Utilising a diffusion cell apparatus, the in vitro releases of niosomes were investigated. A dialysis tube containing niosomes corresponding to 5 mg of lamivudine was filled with 200 ml of 0.1 N HCl. The medium was swirled with a magnetic stirrer while being kept at a constant 37°±2°C. 5 ml of samples were periodically removed, and the same volume of medium was reintroduced. The samples were then measured spectrophotometrically at 255 nm with a blank of 0.1 N HCl

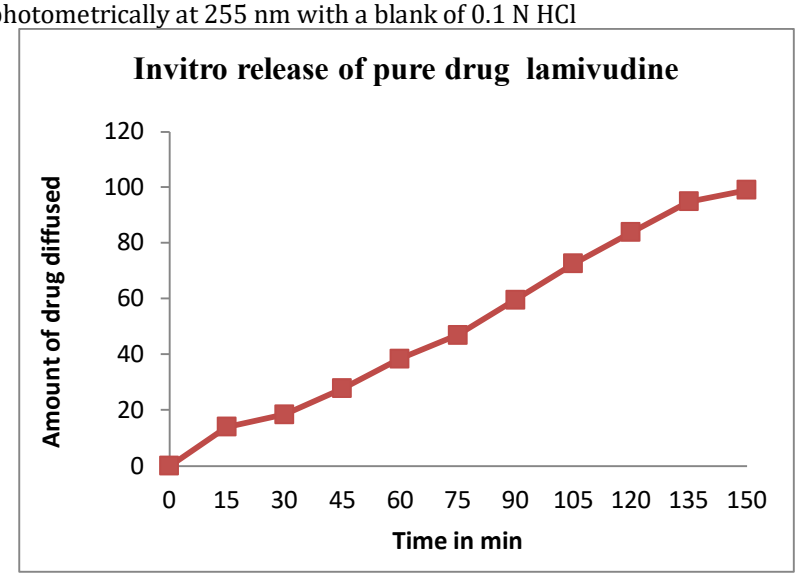


**Fig. 17: *In-vitro* release for Pure Drug**

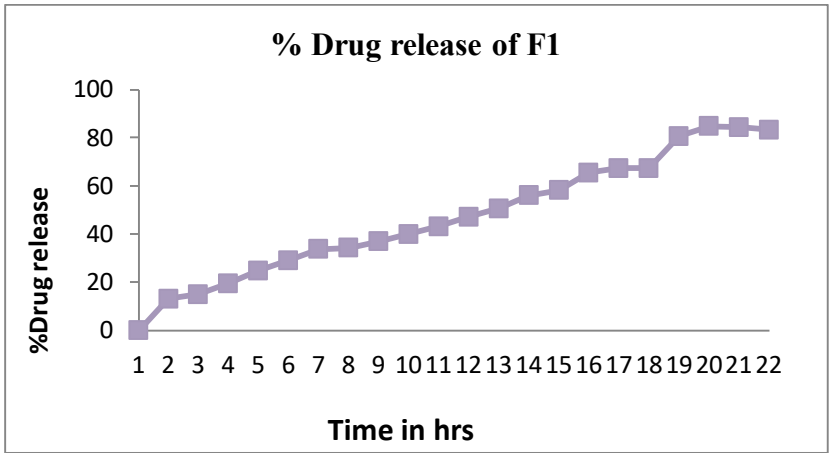


**Fig. 18: *In-vitro* release of F1**

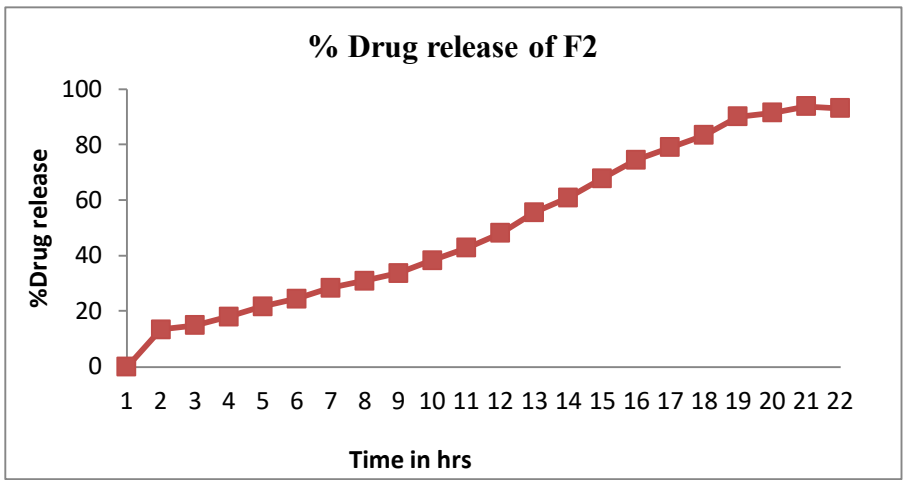


**Fig. 19: *In-vitro* release of F2**

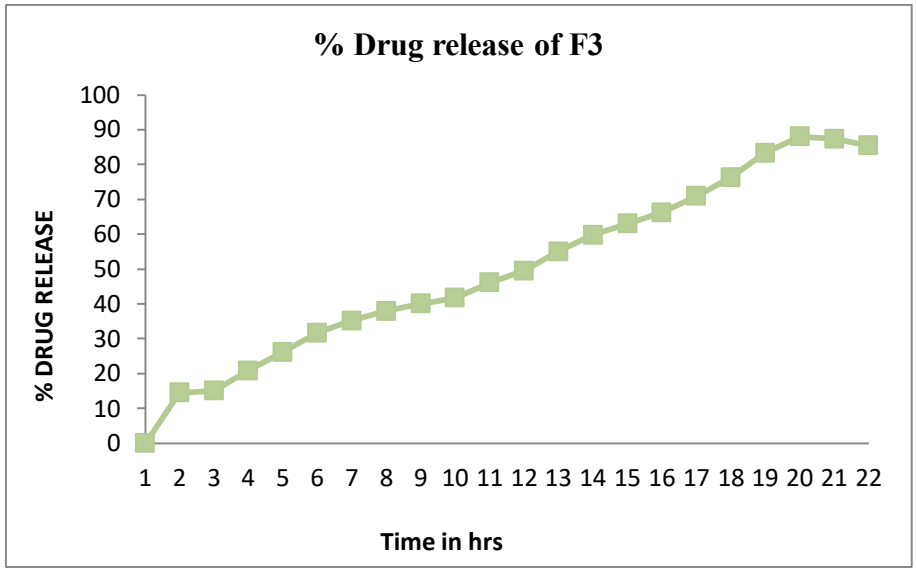


**Fig. 20: *In-vitro* release of F3**

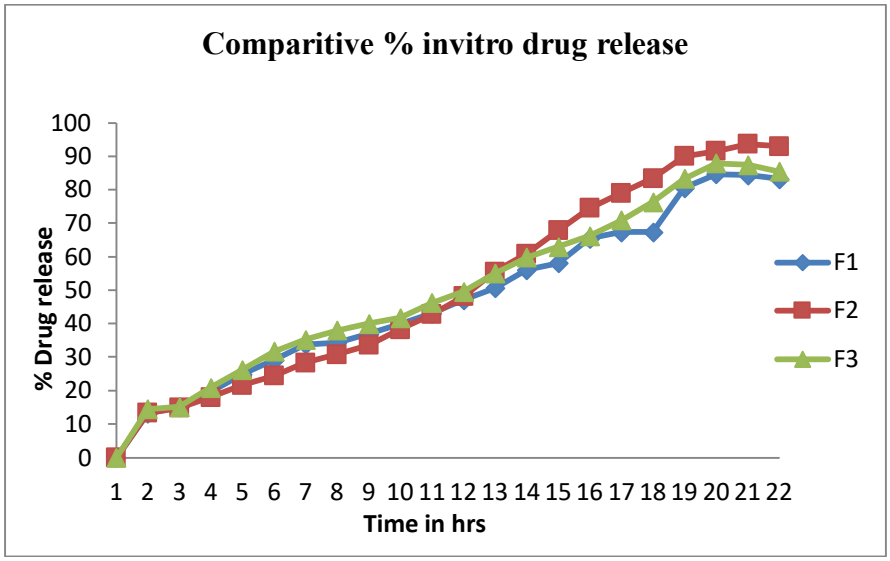


**Fig. 21: Comparative % invitro drug release**

The formed niosomes were put through in vitro drug release employing a sigma dialysis membrane and 0.1N HCl as the medium. Within 2.5 hours, 99.04% of the drug was released free of inhibition. F-I indicated

a 19-hour drug release of 93.48%. In contrast to F-III, which exhibited an 84.99% drug release within 19 hours, F-II demonstrated an 87.97% drug release within 20 hours. These findings demonstrated that while free Lamivudine was released within 2.5 hours, niosomal Lamivudine had a continuous release up to 20 hours. This is due to the fact that niosomal Lamivudine releases the medication slowly over an extended period of time. Additionally, F-I containing span 40(30mg) demonstrated higher release compared to F-II and F-III containing span 40(25mg) and 20mg, respectively. Consequently, F-I is chosen for additional research.

**STERILITY TEST**


**Fig. 23: Sterility Test**

Turbidity was seen in the positive control tube while there was none in the negative control tube. The negative control tube was transparent, indicating that there were no microorganisms whatsoever in the media, location, apparatus, or other accessories utilised for the sterility test. The growth of the bacteria that were put into the positive control demonstrates that the media and surroundings favourable for microbial evolution. Niosomal Lamivudine tubes were clear, indicating that they are sterile. The formulated niosomes passed the Sterility test.

**CONCLUSION**

The purpose of this research was to formulate Lamivudine Niosome drug delivery system by thin film hydration technique using non-ionic surfactants (Span 40). FT-IR spectra of pure drug and its polymer used in formulations indicate that there were no structural changes caused by excipients. Using scanning electron microscopy, the synthesised niosomes were examined microscopically to determined. Microscopically, F-I, F-II, and F-III were identified as spherical tiny unilamellar vesicles with diameters of 110–130 nm, 150–260 nm, and 250–280 nm, respectively. The percentage of drug entrapment in F I was found to be 76.61%, whereas that of F-II and F-III was found to be 66.42% and 58.53%, respectively. Particle size for F-I, F-II, and F-III, respectively, the typical mean particle size range was 120 nm, 205 nm, and 265 nm. Additionally, F-I containing span 40(30mg) demonstrated higher release compared to F-II and F-III, respectively. Consequently, F-I is chosen for additional research. Niosomal Lamivudine tubes were clear, indicating that they are sterile. The formulated niosomes passed the Sterility test.

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