Journal of Chemical Health Risks

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JCHR (2023) 13(4), 992-1000 | ISSN:2251-6727

Formulation and Evaluation of Lamivudine Niosomes by thin Film Hydration Technique

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(Received: 02 September 2023 Revised: 14 October Accepted: 07 November)

KEYWORDS
Niosomes, ionic-surfactant, Lamivudine

ABSTRACT:
Niosomes are generated from the self-assembly of hydrated amphiphilic surfactant monomers. Various nonionic surfactants belonging to different chemical classes have been found to be useful alternatives to phospholipids in assembling vesicular carriers. The terminology does suggest that distinctions exist between niosomes and liposomes. They may differ in their chemical composition, but have similar physical properties. However, niosomes may also be prepared with ionic amphiphilic like negatively charged dicetylphosphate (DCP) or positively charged stearylamine (SA) in order to achieve a stable vesicular suspension The concept of incorporating the drug into niosomes for a better targeting of the drug at appropriate tissue destination is widely accepted by researchers and academicians. Niosomes represent a promising drug delivery module. They present a structure similar to liposome and hence they can represent alternative vesicular systems with respect to liposomes, due to the niosome ability to encapsulate different type of drugs within their multi environmental structure. Niosomes are thought to be better candidates drug delivery as compared to liposomes due to various factors like cost, stability etc. Lamivudine is one of the most effective drug in the treatment of antiretroviral.
The objective of the study is to develop lamivudine niosomes containing in different concentration of surfactant by thin Rotary Evaporator. The Lamivudine Niosomes aim to treat HIV/AIDS: Inhibits the HIV transcriptase enzyme competitively and act as a chain terminator of DNA synthesis were formulated by thin film Hydration Technique using Rotary evaporator.

INTRODUCTION

Controlled drug delivery systems have acquired a center stage in the area of pharmaceutical research and development sector. [1,2] Controlled drug delivery systems, which release the drug in continuous manner by both dissolutions controlled as well as diffusion controlled mechanisms. In recent years it has been shown that the skin a useful route for drug delivery to the systemic circulation. [3-7] Transdermal drug delivery system includes all topically administered drug formulations intended to deliver the active ingredients into the circulation. [8]. They provide controlled continuous delivery of drugs through the

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skin to the systemic circulation. Niosomes are non-ionic surfactant vesicles, capable of forming vesicles & entrapping hydrophilic and hydrophobic molecule. [9,10] 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. 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 liposome's such as higher chemical stability, intrinsic skin penetration enhancing properties and lower costs and ability to deliver the drug at the target site in a controlled or sustained manner which enhances bioavailability.[11,12] Transdermal drug delivery system is a new approach to provide prolonged action of the drug with low toxicity and better patient compliance and thus reduces the side effects caused by oral route. TDDS is one of the lying under the category of controlled drug delivery, in which the aim is to deliver the drug through skin in a predetermined in a controlled rate. [13-15] For transdermal delivery of drug, stratum corneum is main barrier layer for permeation of drug. Hence, to increase the flux through the skin membrane, different approaches of penetration enhancement are used. Topical applicability of niosomes was further enhanced by developing niosomal gel formulation using Carbomers. [16-22]

MATERIAL AND METHODS

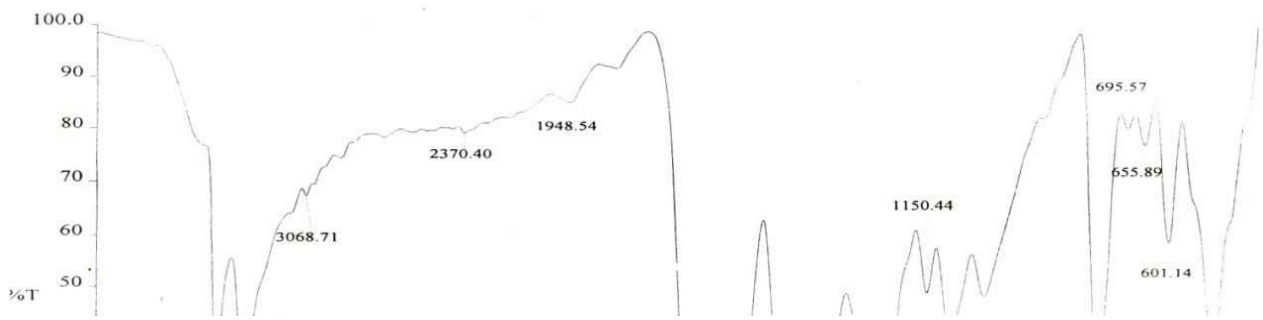
Materials

Lamivudine was obtained as a gift sample from Arco labs ltd, Bangalore. Span 60 and 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).

Identification of Pure Drug

Pure drug (Lamivudine) was identified using FTIR Technique. IR Spectra was taken for Cholesterol, Span 40, Span 60, Span 80 and for mixture of drug, cholesterol and Surfactants.

Figure 1: FTIR Spectra of Lamivudine



Preparation of Lamivudine Niosomes [23,24]

lamivudine niosomes were prepared by Thin Film Hydration Technique using Rotary Flash Evaporator. According to this method, accurately weighed quantity of cholesterol and non-ionic surfactant were dissolved in 10ml of chloroform and poured into a round bottom flask. The flask was rotated at 1.5 cm above a water bath at 60oC± 20C under reduced pressure, until all the organic phase evaporated and a thin layer was formed on the wall of a round bottom flask. Then accurately weighed quantity of drug was dissolved in 10ml of phosphate buffer saline pH 7.4. The dried non- ionic surfactant and cholesterol film was subsequently hydrated with this drug solution and the mixture was rotated by immersing in a water bath at 60^{o}C± 2^{o}C for 1 hour until a good dispersion of mixture was obtained. The niosomes vesicles containing lamivudine vesicles were subsequently formed. The suspension was then sonicated to form unilamellar vesicles.

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Table 1: Preparation of Lamivudine Niosomes

F.code	Drug	Cholesterol	Span 40	Chloroform	PBS pH 7.4	C.S Ratio
F1	10mg	30mg	30mg	10ml	10ml	1:1
F2	10mg	30mg	25mg	10ml	10ml	1:1
F3	10mg	30mg	20mg	10ml	10ml	1:1

Table 2: Formulation Code of Niosomes

S.NO	Formulation code	Span 40 (concentration)
1	F1	30mg
2	F2	25mg
3	F3	20mg

Niosomes are promising vehicle for drug delivery and being nonionic, it is less toxic and improves the therapeutic index of drug by restricting its action to target cells. Hence, in present study lamivudine was encapsulated in niosomes using non- ionic surfactants like span 40 in different concentration. The preparation of niosomes was done by Thin Film Hydration Technique using Rotary flash evaporator. The formulated niosomes were characterized for optical microscopy, SEM, particle size distribution, entrapment efficiency, *in-vitro* drug release, sterility test, stability studies were compared. [25,26]

Optical microscopy

The formulated niosomes were viewed through optical microscope. Less spherical vesicles are formed in F-I containing span 40(30mg). Numerous spherical vesicles are formed in F-II containing span 40(25mg). F-III containing span 40(20mg) showed less spherical vesicles than F-I and F-II.

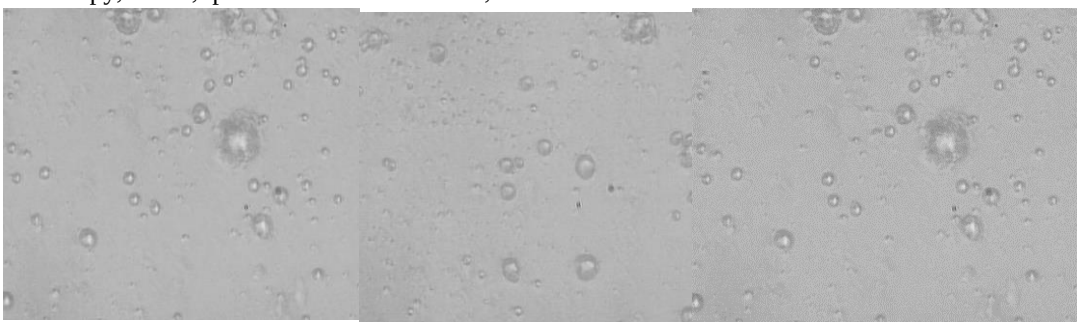

Figure 2: Optical Microscopy of F1, FII, FIII

Morphological studies

The formulated niosomes were subjected to microscopic examination by Scanning Electron Microscopy for characterizing size and shape. Microscopic examination revealed spherical small unilamellar vesicles of 110-130 nm, 150-260 nm and 250- 280 nm for F-I, F-II and F-III respectively. These results revealed that the vesicle diameter complies within the niosomal size range of 100-300nm.

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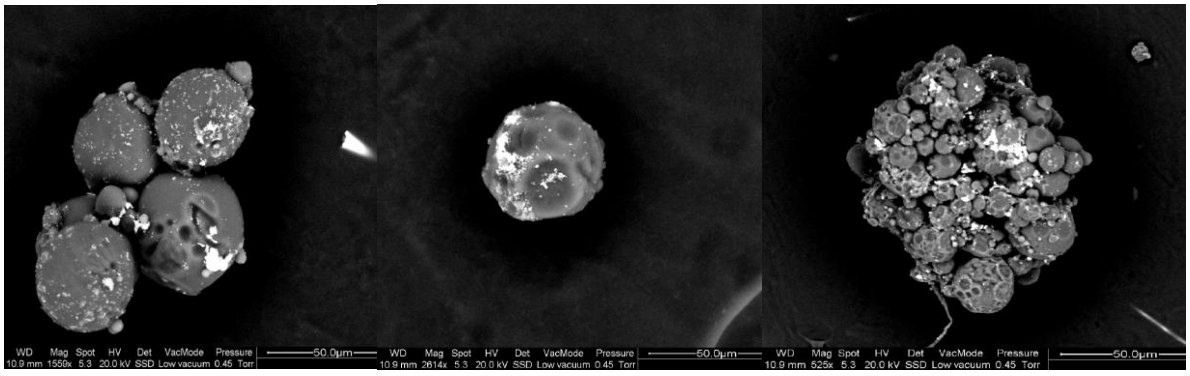

Figure 3: Scanning electron Microscopy of F1, FII, FIII

Particle Size Distribution of Niosomes

Niosomes were subjected to particle size Analyzer for characterizing size distribution of niosomes. The average mean particle size range was 120nm, 205 nm and 265nm for F-I, F-II and F-III respectively. These values revealed that the mean particle size of all the three formulations complies within the niosomal size range of 100-300nm**.**

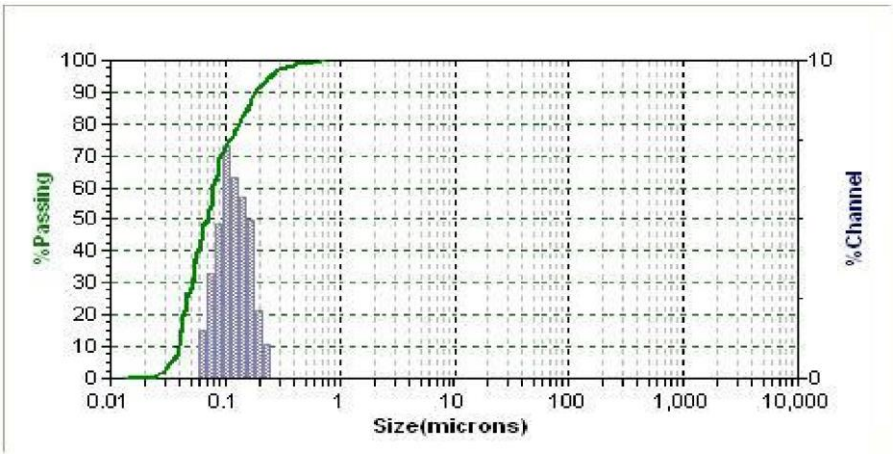

Figure 4: Particle Size Distribution of F1

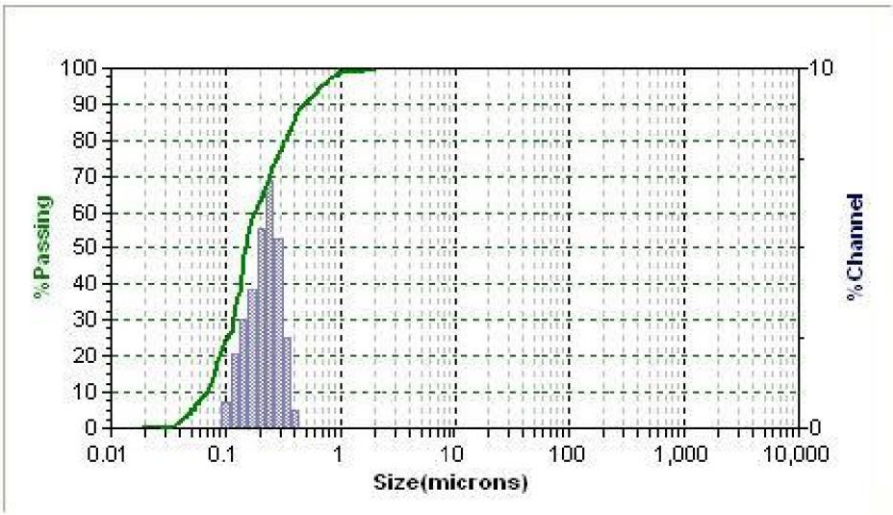

Figure 5: Particle Size Distribution of F2

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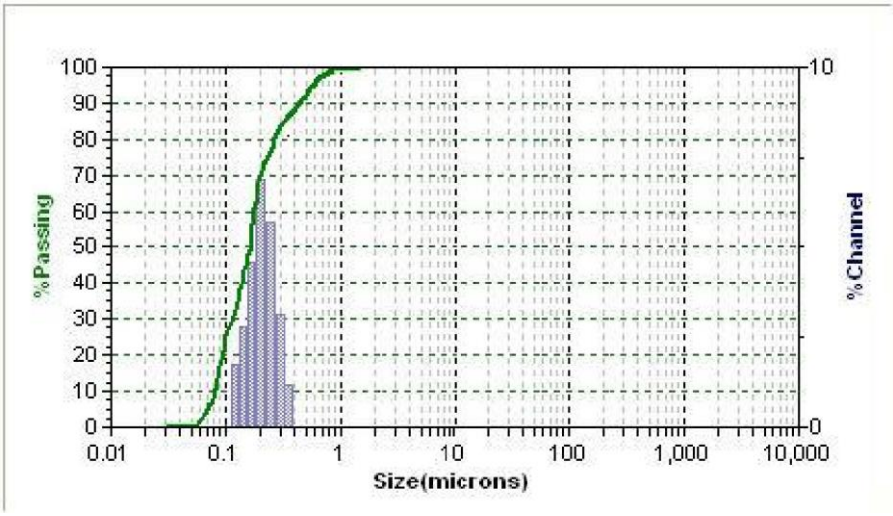

Figure 6: Particle Size Distribution of F3

Entrapment efficiency

The quantity of the drug entrapped in the niosomes is very essential to know before studying the behaviors of this entrapped drug in physical or biological system. The process and formulation variables (cholesterol and surfactant) were altered and optimized to obtain the niosomes with maximum drug entrapment. All the three formulations F-I, F-II and F-III were subjected to percentage drug entrapment. The entrapment efficiency of drug in F I containing span 40(30mg) was Found to be 76.61% which showed maximum percent drug entrapment where as those containing span 40(25mg) and span 40(20mg) (F-II and F-III) was found to encapsulate 66.42% and 58.53% respectively. This showed that span- 60 is the more suitable surfactant along with cholesterol for enhancing Maximum entrapment for the drug Lamivudine. Further, the percent drug entrapment is increased by decreasing the sonication time. Therefore, the sonication time was optimized to 15 Minutes and further reduction in the size by increasing sonication time was not attempted.

***In-vitro* release for Pure Drug Lamivudine**

10mg of pure drug was dissolved in 10ml of 0.1N HCl. 5ml of solution was taken in a dialysis tube and placed in 200ml of 0.1 N HCl. The medium was stirred by using the magnetic stirrer and the temperature was maintained at 37±20C. Periodically 5 ml of samples were withdrawn and after each withdrawal same volume of medium was replaced. Then the samples were assayed spectrophotometrically at 255nm using 0.1 N HCl as blank

Table 3: *In-vitro* release for Pure Drug Lamivudine

Time (min)	Absorbance at 255 nm	Concentration (mcg/ml)	Amount diffused (mg)	% Amount diffused
15	0.0197	3.47	0.69	13.9
30	0.0466	4.58	0.91	18.34
45	0.1031	6.92	1.38	27.68
60	0.1679	9.59	1.91	38.39

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75	0.2191	11.71	2.34	46.85
90	0.296	14.89	2.97	59.57
105	0.3746	18.14	3.62	72.56
120	0.4427	20.95	4.19	83.81
135	0.5089	23.69	4.73	94.76
150	0.5348	24.76	4.95	99.04

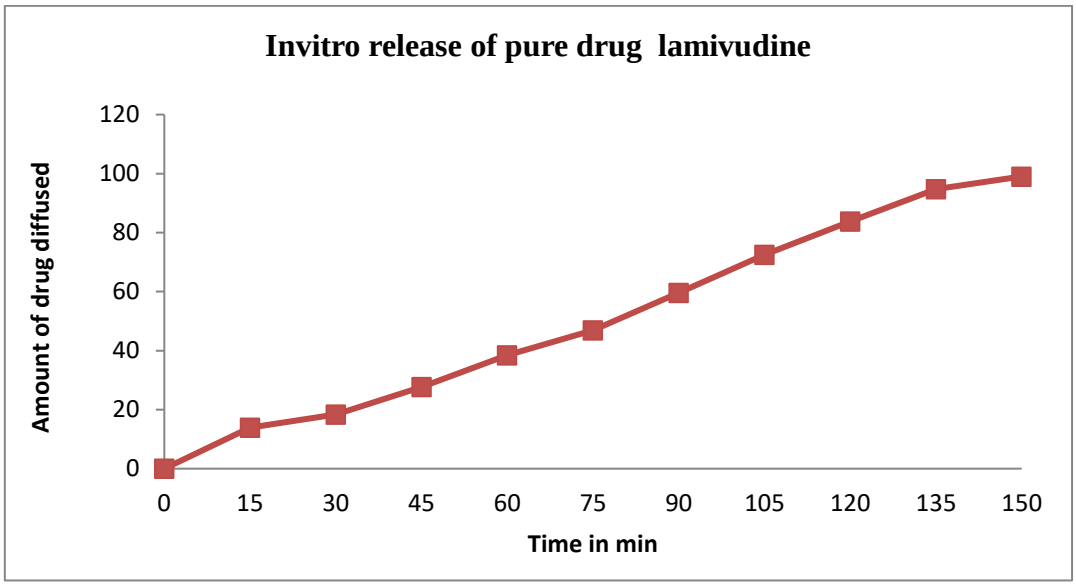

Figure 7: *In-vitro* release for Pure Drug Lamivudine

***In – vitro* drug release**

The formulated niosomes were subjected to *in vitro* drug release using 0.1N HCl as the medium in sigma dialysis membrane. The amount of Lamivudine diffused was estimated spectrophotometrically at 255nm. The percentage amount of free drug released was 99.04% within 2.5 hours. F-I showed 93.48 % of drug release within 19 hours. F-II showed 87.97% of drug release within 20 hours where F-III gave 84.99% of drug release within 19 hours. These results showed that niosomal Lamivudine has sustained release up to 20 hours whereas free Lamivudine was released within 2.5 hours. This is because the drug is released slowly for a prolonged period of time in niosomal Lamivudine. Also, F-I containing span 40(30mg) showed higher release when compared to F-II containing span 40(25mg) and F-III containing span 40(20mg). Therefore, F-I is selected for further studies like sterility test, stability

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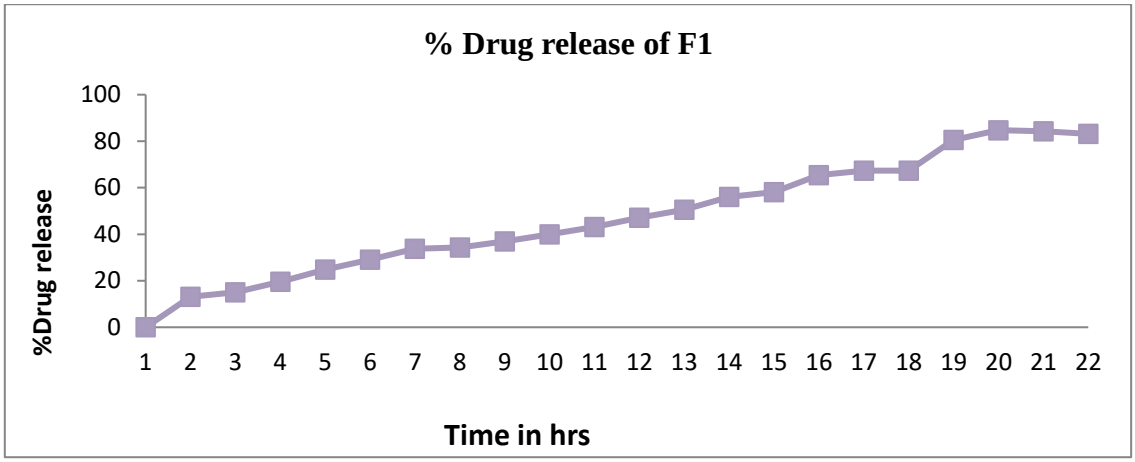

Figure 8: *In-vitro* release of Lamivudine niosomes F1

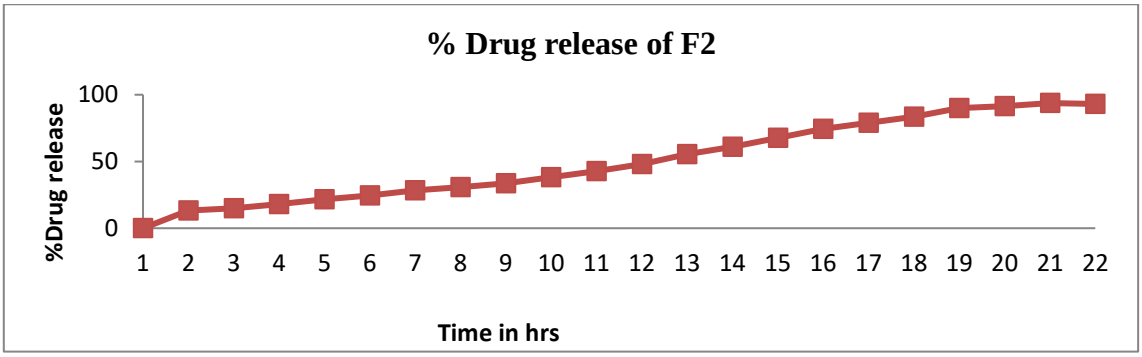

Figure 9: *In-vitro* release of Lamivudine niosomes F2

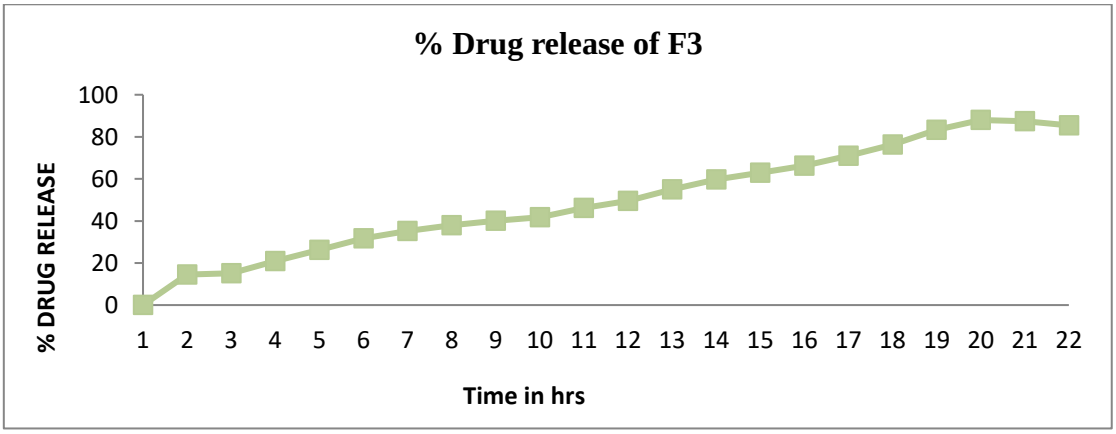

Figure 10: *In-vitro* release of Lamivudine niosomes F3

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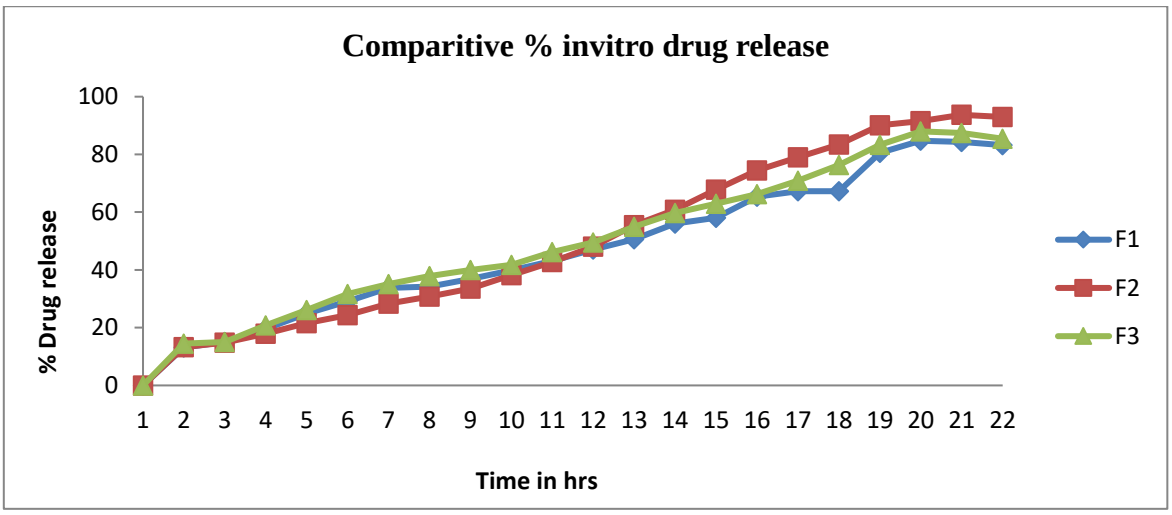

Figure 11 : comparative % Invitro drug release.

SUMMARY AND CONCLUSION

Niosomes of lamivudine containing surfactants in varying concentrations were produced using the thin film hydration technique with a rotary evaporator. Niosomes have a distinct spherical structure devoid of aggregation, as determined by SEM. The range of average mean particle sizes for F-I, F-II, and F-III was determined to be 265nm, 120nm, and 205nm, respectively. With the reduction in surfactant proportion, the particle size of the niosomes exhibited a progressive increase. Up to a certain concentration, the drug content of the niosomes increased as the concentration of the surfactant increased.

Niosomes containing Lamivudine were formulated using different concentrations of surfactants, specifically 30mg, 25mg, and 20mg. These formulations were then evaluated for various parameters. Based on the aforementioned studies, it can be concluded that the use of niosomes to encapsulate Lamivudine resulted in a prolonged release of the drug and a longer duration of action. This demonstrates the achievement of sustained release. The results showed that niosomal Lamivudine has superior anti-antiretroviral activity. The objective of minimizing the dose of Lamivudine was successfully achieved through the use of a niosomal delivery system.

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