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IN-VITRO CHARACTERIZATION OF NIOSOMAL FORMULATIONS FOR CONTROLLED DELIVERY OF ANTIRETEROVIRAL DRUG

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ABSTRACT

Objective: The present study was aimed to prepare and evaluate niosomal formulations for controlled delivery of an antiretroviral drug; tenofovir disoproxil fumarate (TDF).

Methods: Niosomes were prepared by using various non-ionic surfactants (span-20, span-60 and span-80) and cholesterol in different ratios by ether injection technique to achieve increased permeation of drug at the particular site of action which significantly reduces dosage frequency hence increase patient compliance. The prepared vesicles were evaluated for entrapment efficiency, vesicle size, zeta potential, surface morphology by transmission electron microscopy (TEM) and *in-vitro* release.

Results: TEM results confirmed that, the niosomes formed were white and spherical in shape and have a definite internal aqueous space with uniform particle size. A formulation F5 composed of span 60 and cholesterol (2:1) gave the most advantageous entrapment (92.46±1.62%) and slower release results after 8 hrs (Q8h=55.35±1.93%) as compared to other compositions.

Conclusion: Thus, the niosomes may be considered as a promising carrier for the controlled delivery of tenofovir disoproxil fumarate (TDF).

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KEYWORDS

Niosomes; Tenofovir Disoproxil Fumarate (TDF); Non-ionic Surfactant; Cholesterol; Drug Release.

INTRODUCTION

Tenofovir Disoproxil Fumarate (TDF) is antiretroviral drug, acting by blocking the enzyme reverse transcriptase, which is crucial to viral production in HIV-infected people. It has been ascertained a drug of choice in the treatment of HIV-1 infection and hepatitis-B in humans either alone or in combination with other drug molecules [1, 2]. The major problem with the therapy of TDF is its poor bioavailability (25%), which may be due to its poor and poor permeability across the biological membrane of gastrointestinal tract [3] which favours the development of niosomal vesicular system [4].

Niosomes are formed from the self-assembly of non-ionic amphiphiles in aqueous media resulting in closed bilayer structures [5], which can entrap both hydrophilic and lipophilic drugs either in an aqueous layer or in vesicular membrane [6]. Hence, in the present study an attempt was made to formulate TDF loaded niosomes by using non-ionic surfactants (span-20, span-60 and span-80) and cholesterol in different ratios by ether injection technique. This may develop a convenient means of administration to overcome the problem encountered with the conventional dosage form such as low oral bioavailability and the associated adverse effects of the drug thereby providing controlled release action.

MATERIALS AND METHODS

Tenofovir Disoproxil Fumarate (TDF) was received as a gift sample from Ranbaxy Laboratories Ltd. (Dewas, India).Sorbitan monolaurate (span 20), sorbitan monosterate (span 60), sorbitan monooleate (span 80), and cholesterol were procured from Loba Chemie Pvt. Ltd. (Mumbai, India).

Formulation of TDF loaded niosomes

TDF loaded niosomes were prepared by using non-ionic surfactants (span 20, span 60 and span 80) and cholesterol in different ratios by ether injection technique as shown in Table 1. For each ratio, non-ionic surfactant and cholesterol were weighed accurately and dissolved in 20 ml of diethyl ether. TDF (10 mg) was then dissolved in this lipid solution. The resulting solution was taken in a syringe and injected slowly through a 16-gauge needle into 10 ml of aqueous phase (phosphate buffer solution PBS; pH 7.4) held in a beaker maintained at 60^0C to 65^0C and agitated slowly. As the lipid solution was injected slowly into the aqueous phase, vaporization of diethyl ether resulted in the formation of niosomes. The prepared niosomes were separated by ultracentrifugation (Remi C-24, Mumbai, India) at 4^0C [4].

Table 1: Composition for niosomes preparation

Formulation code	Surfactant	Amount of surfactant (mg)	Cholesterol (mg)	Drug (mg)	Ratio (Surfactant: Cholesterol)
F1	Span 20	100	100	10	1:1
F2	Span 20	200	100	10	2:1
F3	Span 20	100	200	10	1:2
F4	Span 60	100	100	10	1:1

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F5	Span 60	200	100	10	2:1
F6	Span 60	100	200	10	1:2
F7	Span 80	100	100	10	1:1
F8	Span 80	200	100	10	2:1
F9	Span 80	100	200	10	1:2

EVALUATION OF TDF ENTRAPPED NIOSOMES

Drug entrapment efficiency (% EE)

The proportion of encapsulated TDF was obtained by ultracentrifugating 1 ml of the niosomal suspension at 25,000 rpm for 2 hr using a cooling centrifuge at 4°C (Remi C-24, Mumbai, India). The niosomes were separated from the supernatant and were washed twice, each time with 1 ml PBS pH 7.4, and recentrifuged again for 1 hr. The amount of entrapped zaleplon was determined by lysis of the separated vesicles with isopropanol. A 100 µl sample of niosomes was mixed with 1 ml of isopropanol; the volume was completed to 10 ml with PBS pH 7.4 and covered with parafilm to prevent evaporation. The concentration of the drug was determined by UV spectrophotometer (UV 1700 Pharm Spec, Shimadzu, Japan) at 291 nm[5]. The % drug entrapment efficiency can be calculated by using following formula:

$$\% \text{ EE} = \frac{\text{Actual drug content}}{\text{Theoretical drug content}} \text{ X } 100$$

Vesicle size and zeta potential measurements

Vesicle size of different niosomal formulations were observed under an optical microscope (Olympus Model BX 41, Japan) at suitable magnification. The zeta potential of the prepared niosomal formulations was determined by Zetasizer Nano ZS-90 (Malvern Instruments Ltd., UK) using 0.1 M KCl buffer in demineralized water at 25°C [5].

Transmission electron microscopy (TEM)

The prepared niosomal formulations were characterized for their shape using transmission electron microscope (JEM-200 CX, JEOL, Tokyo, Japan) at 80 KV, after being stained and TEM micrograph was taken at suitable magnification [6].

In-vitro drug release from niosomes

The *in-vitro* release of entrapped drug within niosomes was determined using membrane diffusion technique. The niosomal formulation equivalent to 5 mg of TDF was placed in a glass tube that was previously covered with pre-soaked cellulose membrane, which acts as a donor compartment. The glass tube was placed in a beaker containing 100 ml of PBS pH 7.4, which acted as receptor compartment. The temperature of receptor medium was maintained at 37±100^{0}C and agitated at 100 rpm speed using magnetic stirrer. Aliquots of 5 ml sample were withdrawn periodically and after each withdrawal same volume of medium was replaced. The collected samples were analysed spectrophotometrically at 291 nm using PBS pH 7.4 as blank [7].

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RESULTS AND DISCUSSION

Drug entrapment efficiency in niosomes

It was observed that the entrapment efficiency of niosomes composed of span 60 were superior as compared to those prepared from span 20 (Table 2). The formulation containing span 80 showed the lowest entrapment efficiency. This can be due to:

(a) Span 60 has highest phase transition temperature (50^0C) as compared to span 20 (16^0C) and span 80 (-12^0C) and hence high entrapment efficiency.

(b) Span 60 has a longer saturated alkyl chain (C18) compared to span 20 (C12), so it produces niosomes with higher entrapment efficiency. Span 60 and span 80 have the same head group but span 80 has an unsaturated alkyl chain which results in enhanced permeability and decreased entrapment [8, 9].

Vesicle size and zeta potential measurements

Vesicle size of all formulations was ranges between 1.14±0.16µm to 8.26±0.42µm (Table 2). These sizes are well acceptable for oral administration. It was observed that the niosomes prepared using span 60 is larger in size than those prepared using span 20 and span 80. Span 60 has a longer saturated alkyl chain and it was reported that surfactants with longer alkyl chains generally give larger vesicles [10].

The zeta values for niosomal formulations were found to be in range of -23.90±1.86 mV to -28.14±0.12 mV (Table 2).The zeta potential of the niosome under study was found to be -23.90±1.86 mV as shown in Figure 1. The results revealed that the zeta values of the vesicles increase towards negative with increasing the HLB values of the surfactants [11].

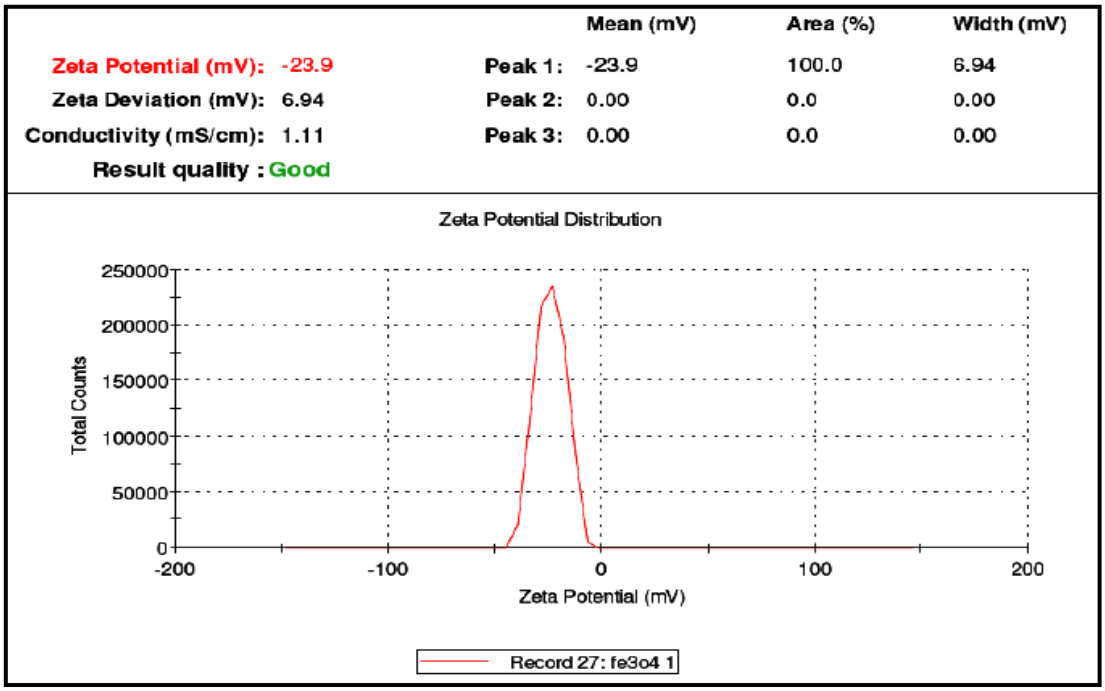

Figure 1: Zeta potential of niosomal formulation (F5).

Transmission electron microscopy

It was demonstrated that the vesicles are well identified and present in a nearly perfect sphere like shape with a smooth surface and having a definite internal aqueous space (Figure 2).

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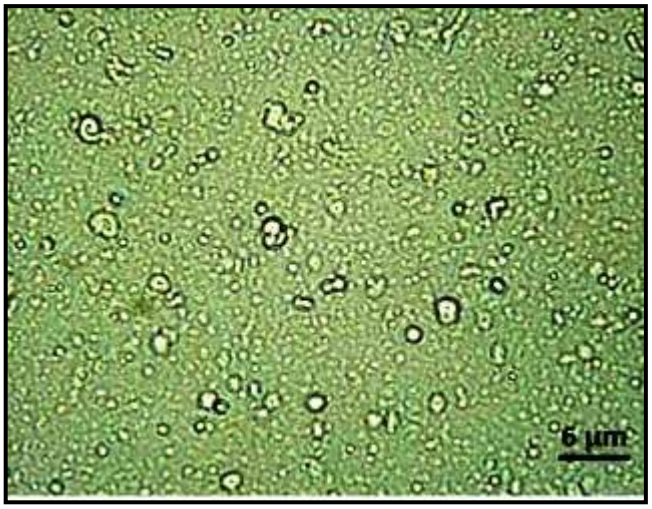

Figure 2: Transmission electron micrograph of niosomal formulation.

***In-vitro* drug release from niosomes**

The percentage of drug released after 8 hr from the prepared niosomal vesicles in PBS pH 7.4 varied from 55.35±1.93% to 78.81±1.64% (Figure 3). Niosomal formulations prepared using span 60 (2:1) yielded a lower rate of release compared to span 20 and span 80 niosomes. This can be explained by the fact that niosomes exhibit an alkyl chain length dependent release and the higher the chain length, the lower the release rate [12, 13]. It has been revealed that release after 8 hours for the niosomal formulations can be arranged in the following decreasing order: F1 > F2 > F9 > F7 > F3> F6 > F8 > F4> F5. From results, it is obvious that the increase of cholesterol molar ratio reduced the efflux of the drug from niosomal preparations, which is in accordance with its membrane stabilizing ability [14].

Table 2: Evaluation of niosomes

Formulation Code	Entrapment efficiency (%)	Vesicle size (µm)	Zeta potential (mV)	Q8h (%)
F1	77.05±1.65	1.14±0.16	-29.11±1.32	78.81±1.64
F2	82.16±1.29	2.34±0.99	-28.05±1.11	73.15±2.75
F3	76.21±0.23	1.89±1.26	-26.17±0.64	68.74±0.86
F4	91.02±2.13	5.64±1.32	-25.08±1.18	57.04±1.68
F5	92.46±1.62	6.09±2.11	-23.90±1.86	55.35±1.93
F6	90.04±3.15	5.97±1.95	-26.12±0.92	68.05±2.72
F7	88.76±2.18	7.01±0.27	-26.21±1.28	69.33±1.42
F8	81.04±0.56	8.26±0.42	-27.12±1.05	64.54±3.69
F9	76.08±2.11	7.75±1.24	-28.14±0.12	71.98±1.24

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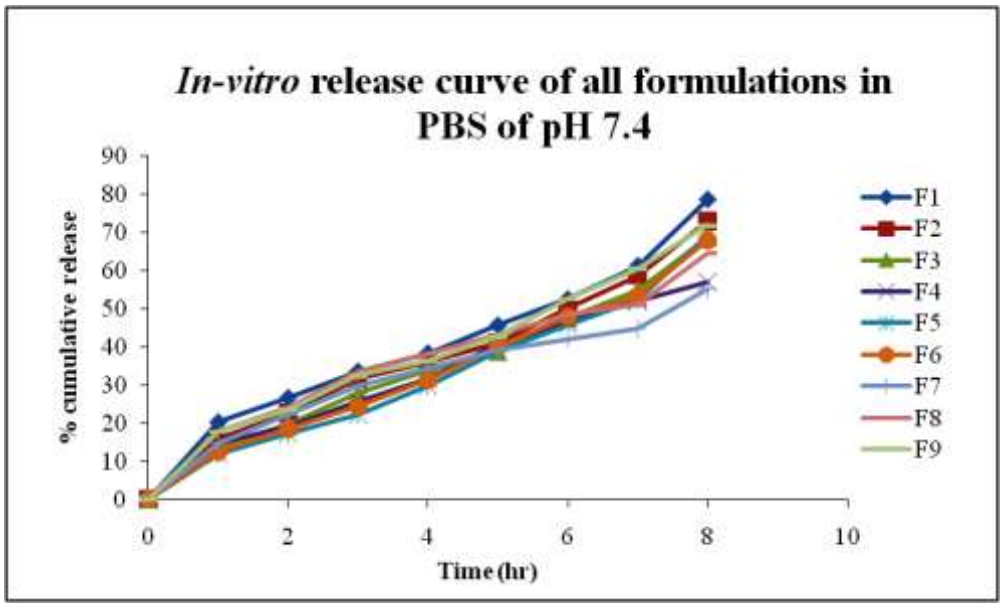

Figure 3: Drug release profile of TDF loaded niosomes.

CONCLUSION

A formulation F5 composed of span 60 and cholesterol (2:1) gave the most advantageous entrapment (92.46±1.62%) and slower release results after 8 hrs (Q8h=55.35±1.93%) as compared to other compositions. Niosomal formulations show a lower percentage of drug release and have higher stability. These results suggest that the niosomes may be considered as promising carriers for the controlled delivery of TDF.

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