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Rifampicin Proniosomes- An Approach to Improve the Stability of Niosomes

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ABSTRACT

The aim of the work was to prepare a Rifampicin niosomal formulation and to compare the entrapment efficacy and release having Cholesterol and Myristyl alcohol as membrane stabilizers and to convert the niosome formulation into proniosome for improved stability. Six formulations of Rifampicin niosomes were prepared with three different surfactants by thin film hydration method using cholesterol and myristyl alcohol as membrane stabilizers. The niosomal suspensions were evaluated for size analysis, drug entrapment and invitro release. Lyophilization was also performed for niosomal formulation. Rifampicin proniosome were prepared by thin film hydration method using mannitol as carrier with surfactant Span20. The formulations of span 40 with myristyl alcohol and span 20 with cholesterol showed higher entrapment efficiency. Invitro drug release from span 20 formulations with cholesterol was found to be slower than with the formulation containing span 40 with myristyl alcohol. The study proves that the membrane stabilizing activity of cholesterol shows the feasibility of storing niosomal suspension in the form of proniosomes for a longer duration.

Key words: Rifampicin; Niosomes; Cholesterol;

INTRODUCTION

Many active compounds have limited aqueous solubility, so there is great need for delivery systems suitable for hydrophobic and amphiphilic drugs. One approach to this problem has been to use lipid- based vesicles as drug carriers. Multilamellar liposomes can be used for hydrophobic or lipophillic drugs that can partition into the lipid phase and unilamellar vesicles can be used to entrap water – soluble drugs in the interior aqueous phase. Niosomes are non-ionic surfactant vesicles obtained on hydration of synthetic nonionic surfactants, with or without incorporation of cholesterol or other lipids. They are vesicular systems similar to liposomes that can be used as carriers of amphiphilic and lipophillic drugs. In niosomes, the vesicle forming amphiphile is a nonionic surfactant such as Span 60 which is usually stabilized by addition of

cholesterol and small amount of anionic surfactant such as diacetyl phosphate. Niosomes has better stability than liposomes. It can prolong the circulation of the entrapped drugs. Because of the presence of nonionic surfactant with the lipid, there is better targeting of drugs to tumour, liver and brain. Niosomes are promising vehicle for drug delivery and being non-ionic, it is less toxic and improves the therapeutic index of drug by restricting its action to target cells.Niosomes have been investigated for drug delivery through the most common routes of administration, such as intramuscular, intravenous, subcutaneous, ocular, oral and transdermal [1-4]. There are certain factors that affect vesicle size, entrapment efficiency and release characteristics are drug, amount and type of surfactant, cholesterol content and charge, and resistance to osmotic stress.Rifampicin is a broad -spectrum antibiotic. It is active against Mycobacterium tuberculosis and leprosy. It has several side effects such as anemia, hypersensitivity reactions, and renal, hepatic and reproductive side effects. The half life of Rifampicin is about 2 to 5 hours. Rifampicin is slightly soluble in water and fairly soluble in other solvents [5].These factors necessitated niosomal formulation for Rifampicin .As this dosage form would reduce the dosing frequency, hence better patient compliance. The present study was aimed with the formulation of niosomes of Rifampicin followed by evaluating parameters such as drug content, entrapment efficiency and particle size and also to convert niosome to proniosome.

MATERIALS AND METHOD

The materials used were Rifampicin (Tablets India Pvt Ltd, Chennai), Chloroform(Nave, Mumbai),Surfactants : Span 20, 40, 80 (Loba Chem. Pvt. Ltd., Mumbai.) , Cholesterol (Loba Chem Pvt. Ltd., Mumbai.) , Myristyl Alcohol (Himedia Laboratories Pvt Ltd, Mumbai and phosphate buffer pH 7.4 was prepared freshly for each studies as per IP 1996.

2.1 Preparation of Niosomes

Niosomal formulations were prepared by thin hydration film method using cholesterol and myristyl alcohol as membrane stabilizers [6]. In this process, Rifampicin was mixed along with surfactant, cholesterol and then it was dissolved in 25ml of chloroform, vortexed in Rotary flash evaporator. Thin film was formed. It was then hydrated with 5ml of phosphate Buffer pH 7.4.

2.2. Size evaluation of niosomes

Size evaluation was carried out using optical microscope at a magnification of 40x by means of a fitted camera (Olympus Digital Camera, 8 Mega pixel) [7, 8].

2.2. Percentage drug entrapment determination

Three milliliter of the niosomal formulation was taken and centrifuge at 12,000 rpm for 15 mins. The supernatant was diluted with 5 ml of phosphate buffer pH 7.4. From the above solution, 1 ml was taken and transferred to 10 ml standard flask and made up to 10 ml with phosphate buffer pH 7.4. Absorbance was taken at 475nm using phosphate buffer pH 7.4 as blank. The percentage of drug entrapped was calculated [9, 10].

2.3. Lyophilization

Two milliliter of the sample were prefrozen at -20^{o}c for 1hour and then frozen at -70^{o}c for 2 hours. In the freeze dryer, vaccum manifolds were closed and the temperature was brought down to -40^{o}c. Vaccum was applied to 0.01M Pascal. The frozen samples were now attached to vaccum manifold and the process was continued for 8 hours. At the end of operation, vaccum was reduced and the freeze dried samples were taken out. The samples were found to be sticky even after storage in desiccator overnight.

2.4. *In vitro* release studies

The formulation was centrifuged and the pellet was taken for *invitro* release studies .This was carried by using Himedia dialysis membrane. This study was carried out for 10 hours using phosphate buffer solution pH 7.4 in the receptor compartment and the pellet containing niosomes in the donor compartment at specified time intervals, buffer solution from the receptor compartment was taken and replaced with the same quantity. The absorbance was measured at 475nm and cumulative % release was determined.

2.5 Formulation of proniosomes

4.5 g of mannitol was sieved in 100 mesh and placed in a rotary flash under vaccum condition for 30 minutes at 80-90 rpm in water bath at 90^{o}c to dry the mannitol powder. Rifampicin niosomes formulated with span 20 (0.5 ml) was added to this mannitol powder at 37^{o}c. Flask was rotated until the 3 ml of the Rifampicin suspension was fully loaded in the dry mannitol powder. This resulted proniosome powder was checked for the presence of vesicles after adding warm phosphate buffer pH 7.4.

RESULTS

Stable Rifampicin niosome vesicles were prepared using cholesterol and myristyl alcohol as membrane stabilizers with the non- ionic surfactants Span 20, 40, 80. Unsonicated vesicles were found to be spherical and it exists in the size range of 6.6µm to 26.88µm. Span 20 formulation with cholesterol has large vesicle size when compared to the formulation Span 40 with myristyl alcohol (Table 1). Drug entrapment was high in Span 40 formulation with myristyl alcohol (90.55%) and Span 20 formulation with cholesterol (72.2%). The order of entrapment is decreasing with respect to the membrane stabilizer myristyl alcohol in the sequence of Span 40 > Span 80 > Span 20 (Table 2). The order of entrapment is decreasing with respect to the membrane stabilizer cholesterol in the sequence of Span 20 > Span 80 > Span 40 (Table 2). In vitro drug release from Span 20 formulation with cholesterol was found to be slow. About 7.46% of drug was only released in 10 hours when compared with Span 40 with myristyl alcohol (16.62%). Span 40 formulations with myristyl alcohol and Span 20 formulation with cholesterol were lyophilized for improving the stability during storage. But after Lyophilization the powder was found to be sticky and not flowing. Span 20 formulations were converted into proniosomes using mannitol as the carrier for stability purpose. The presence of vesicles after storage for 90 days at room temperature was confirmed after reconstitution with hot water.

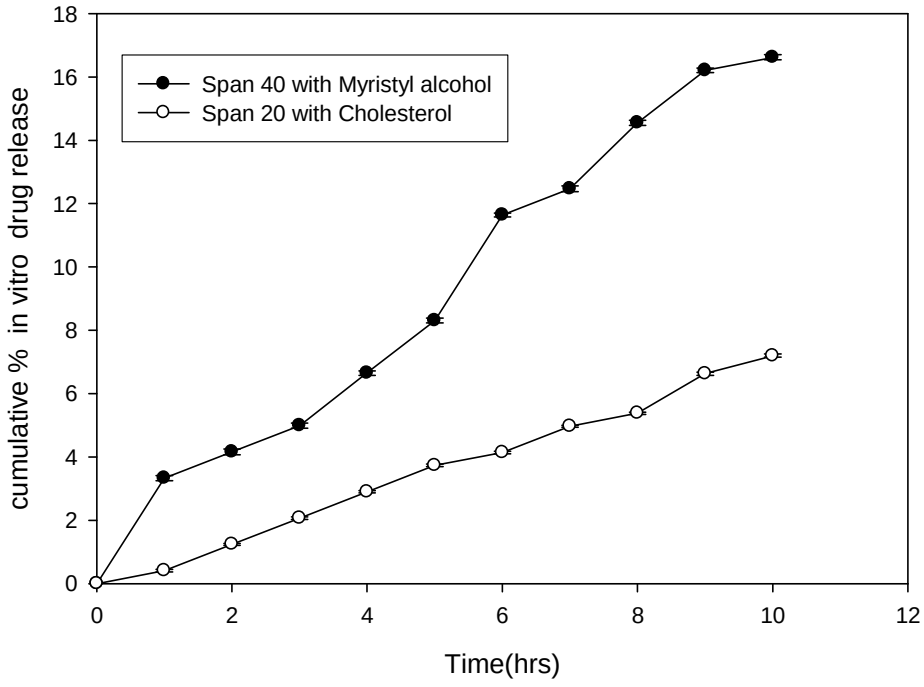
Table -I: Rifampicin Niosome Formulation with 3 Different Surfactants

Formulations	Rifampicin (mg)	Chloroform (ml)	Phosphate buffer pH 7.4 (ml)	Span 20 (mg)	Span 40 (mg)	Span 80 (mg)	Cholesterol (mg)	Myristyl alcohol (mg)
1	60	25	5	28	-	-	28	-
2	60	25	5	-	28		28	-
3	60	25	5	-	-	28	28	-
4	60	25	5	28	-	-	-	28
5	60	25	5	-	28	-	-	28
6	60	25	5	-	-	28	-	28

Table 2: Average Mean Diameter of Vesicles for The Formulations With 2 Different Membrane Stabilizers

FORMULATIONS	AVERAGE MEAN DIAMETER OF THE VESICLES IN (µm)		PERCENTAGE AMOUNT OF DRUG ENTRAPPED
	UNSONICATED	SONICATED	
FORMULATION -1 (Span 20 + Cholesterol)	26.88	6.7	72.2
FORMULATION – 2 (Span 40 + Cholesterol)	24.6	6.5	69.15
FORMULATION – 3 (Span 80 + Cholesterol)	26.12	6.4	70.2
FORMULATION – 4 (Span 20 + Myristyl alcohol)	6.6	0.8	75.25
FORMULATION – 5 (Span 40 + Myristyl alcohol)	8.8	1.2	90.55
FORMULATION – 6 (Span 80 + Myristyl alcohol)	9.14	1.9	77.3

Fig 1. *In vitro* drug release of rifampicin from different niosomes



DISCUSSION

Entrapment of drug in niosomes increases vesicle size, probably by interaction of solute with surfactant head groups, increasing the charge and mutual repulsion of the surfactant bilayers, thereby increasing vesicle size. Span 20 formulation with cholesterol has large vesicle size when compared to the formulation Span 40 with myristyl alcohol. This shows formulation with myristyl alcohol as membrane stabilizer forms smaller average mean diameter vesicles. The order of entrapment is decreasing with respect to the membrane stabilizer myristyl alcohol in the sequence of Span 40 > Span 80 > Span 20 .The order of entrapment is decreasing with respect to the membrane stabilizer cholesterol in the sequence of Span 20 > Span 80 > Span 40. The entrapment efficiency increases with increase in the concentration and lipophilicity of surfactant. In vitro drug release from Span 20 formulation with cholesterol was found to be slow.

CONCLUSION

The present study demonstrated the successful preparation of Rifampicin Niosomes and their evaluation. This study also proves that the membrane stabilizing activity of cholesterol shows the feasibility of storing niosomal suspension in the form of proniosomes for a longer duration.

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