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Formulation and *in-vitro* evaluation of Rifampicin-Loaded Niosomes

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ABSTRACT

To prepare niosomes containing rifampicin were prepared using various nonionic surfactants of sorbitan ester class and cholesterol in 50:60 (1:1.2) percent mol fraction ratios for sustain release. To improve the dissolution rate of noisome are prepare handshaking method using Surfactants and cholesterol (150 µmol) in 50:60 (1:1.2) percent mol fraction ratio. The percent of drug estimated to be entrapped was noted to decrease progressively for various sorbitan esters used in the order of Span-85>Span-80>Span-60>Span-40>Span-20. In vitro release rate studies revealed that the cumulative percent rifampicin released was maximum for Span-20-based niosomes and minimum for Span-85-based niosomes. The handshaking method is a simple and efficient technique for designing functional niosomes for hydrophobic or amphiphilic drugs.

Keywords: Niosomes, Liposomes, Rifampicin, Span-80, Nonionic surfactant.

INTRODUCTION

Niosomes are nonionic surfactant vesicles that have potential applications in the delivery of hydrophobic or amphiphilic drugs. 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 lipophilic drugs that can partition into the lipid phase and unilamellar vesicles can be used to entrap water-soluble drugs in the interior aqueous space [1]. Non-ionic surfactant vesicles (niosomes) have shown promise as cheap, chemically stable alternatives to liposomes. Niosomes of spans (Sorbitan monoesters) have shown promise of commercial exploitation. Improvement in therapeutic effectiveness of conventionally well established drugs by controlled and sustained delivery upon encapsulation in liposomes has

gained greater momentum with the launch in the market of some liposomal formulations [2]. Niosomes are surfactant vesicles which are used to entrap several pharmaceutical drugs to enhance their sustainability [3]. Rifampicin is frequently used in the treatment of tuberculosis, a disease widely prevalent, especially in Third World countries, and requiring high dose treatment over a period of 4-6 months. The causative organism is known to develop resistance if drug blood levels remain below the minimum effective concentration, leading to clinical failure. Rifampicin also has various side effects, such as immunological disturbances, rheumatoid or lupoid syndromes, allergic rashes, eosinophilia, leucopoenia, jaundice and other hepatotoxic manifestations [4]. Niosomes are prepared by employing different techniques namely, thin film hydration, hand shaking, ether injection, lipid layer hydration and trans membrane pH gradient method [5]. Niosomes, a vesicular formulation, has been explored extensively for topical application to enhance skin penetration as well as to improve skin retention of drugs [6]. Nonionic surfactant vesicles (niosomes) to improve poor and variable oral bioavailability [7, 8]. In ophthalmic treatment the site of action may be any ocular tissue, depending on where the disorder is located. Hence the drug should be targeted to many different sites within the eye. Poor bioavailability of drugs from ocular dosage form is mainly due to the tear production, non-productive absorption, transient residence time, and impermeability of corneal epithelium [9]. Niosomes or nonionic surfactant-based vesicles, formed when a mixture of cholesterol and surfactant is hydrated, can entrap solutes, are osmotically active and stable and are similar in terms of their physical properties to liposomes (lipid-based vesicles). Niosomes may overcome the problems associated with liposomes, one of which relates to the chemical instability of the constituent phospholipids. Due to their predisposition to oxidative degradation, phospholipids must be stored and handled in nitrogen atmosphere. The cost and variable purity of natural phospholipids also militate against adoption of liposomes as drug delivery vesicles [10, 11, 12]. Niosomes have been used for improving the stability of entrapped drug[13]; for detection of tumors [14]; and to modify the tissue distribution of entrapped harmine [15], proteins and biologicals products [16] terbinafine hydrochloride [17], colchicines[18], Acyclovir[19] methotrexate[10], salbutamol sulphate [5], propranolol HCL [20] methotrexate complexed with beta-Cyclodextrin [21], Aceclofenac [6] Erythromycin [7] griseofulvin [8] doxorubicicin [11] and ketoprofen [3].

MATERIALS AND METHODS

Rifampicin was procured from Cadila health care Ltd., Zydus tower, Ahemdabad. Triton X-100 was procured from Va Sudha Chemicals Pvt. Ltd., Mumbai. Span-20, Span-40, Span-60, Span-80 and Span-85 were procured from Jiansu Haian Petrochemical Plant, China. Cholesterol was procured from Otto Kemi, Mumbai. Diethyl ether and methanol procured from CDH, Dehli of analytical grade were used.

2.1 Preparation of Niosomes:

The reported methods of hand shaking by Baillie et al.[11] Briefly procedures followed were as hand shaking method in this the liquid mixture was dissolved in 10ml diethyl ether in a round bottom flask. The solvent was evaporated using a rotary evaporator (Buchi Model, Yorco, New Delhi) until complete evaporation of solvent was ensured and the surfactant film deposited on the wall of the flask was hydrated with 5ml of phosphate buffer saline (PBS, pH 7.4) containing drug (10mg/ml) at 60 ± 2^{o}C for 1h to obtain a niosomal dispersion.

The niosomes were prepared by the method reported by Azmin et al. [10] Surfactants and cholesterol (150 µmol) in 50:60 (1:1.2) percent mol fraction ratio were dissolved in 10 ml diethyl ether in a 50 ml round-bottom flask, and ether was removed at an ambient temperature (37±1°C) under reduced pressure in a rotary flash evaporator (Buchi Model, Yorco, New Delhi). The dried film of surfactant was hydrated with occasional shaking for 15 min at 70°C on water bath with 5 ml aqueous phase (phosphate buffer saline, pH 7.4) containing 25 mg rifampicin. This suspension was then sonicated to form unilamellar niosomes.

The resultant aqueous dispersions of rifampicin-bearing niosomes were dialysed exhaustively in Cuprophane dialysis tubing against phosphate buffer saline (pH 7.4) to separate the unentrapped rifampicin from the niosome-entrapped rifampicin.

2.2 *In vitro* Characterization of Niosomes:

The shape and size of the niosomes was studied by an optical microscope using a pre-calibrated ocular eye piece. The entrapment efficiencies were determined by complete dissolution of vesicles using Triton X-100. The entrapped rifampicin was estimated by digesting a definite quantity of the niosomal suspension with 10% Triton X-100 for 5 min and centrifuging the resulting solution to get clear supernatant. The supernatant was suitably diluted using phosphate buffer saline and rifampicin estimated using HPLC method reported by Peh et al. [22].

The *in vitro* release rate was determined using glass tube of diameter 2.5cm with an effective length of 8cm that was previously covered with cellophane membrane. Measured amount of niosomes were placed in the cylinder. The cylinder was placed in 100 ml of phosphate buffer saline, pH 7.4, which acted as receptor compartment. The temperature of receptor medium was maintained at 37±1°C and agitated at 100rpm speed using magnetic stirrer. Aliquots of 5ml sample were withdrawn at intervals of 24 h for 3 days. At each sampling time, the volume of receptor compartment was maintained with an equal volume of phosphate buffer saline, pH 7.4. The drug in withdrawn samples was estimated by the reported HPLC method (Acme 9000 Model, Younglin, Korea).

2.3 Determination of Entrapment Efficiency:

An aliquot of the freshly purified niosomal dispersion (5 mg lipid mL^{-1}) was diluted with 10% Triton X-100 in a ratio of 1:99 vol/vol. The detergent dissolved the niosomes and yielded a clear solution. The resultant solution was analyzed for rifampicin concentration using the described high-performance liquid chromatography (HPLC) method to calculate the amount of entrapped rifampicin. The percentage of entrapped rifampicin was calculated by applying the following equation:

$$\% \text{ Entrapment} = (A_E \times 100) / (A_I) \quad (1)$$

where, A_E is the amount of entrapped drug, and A_I is the initial amount of drug in the aqueous phase.

RESULTS AND DISCUSSION

In this study the niosomes were observed as spherical vesicles with smooth surface. The vesicles were discrete and separate with no aggregation or agglomeration. The size of the vesicles was

uniform and independent of surfactant, as vesicles of all the surfactants were sonicated to same size. The average size of the vesicles is reported in [Table - 1].

The percent of drug estimated to be entrapped was noted to decrease progressively for various sorbitan esters used in the order of Span-85>Span-80>Span-60>Span-40>Span-20 [Table - 1]. This may be explained on the basis of chemical nature of the surfactants. The corresponding HLB values for these surfactants are 1.8, 4.3, 4.7, 6.7 and 8.6 respectively. The lower the HLB number, the more lipophilic is the compound. Thus Span-85 has the highest lipophilicity; therefore, the maximum drug was entrapped in Span-85. Yoshioka et al [23] found that the release rate of carboxyfluorescein, a water-soluble compound, from niosomes prepared with span 60 was slower than the release rate from other span formulations (span 20, 80, and 85). This result is because at 25ºC, the molecules of span 60 are in the ordered gel state, but those of other spans are in the disordered liquid crystalline state.

Table 1 : Characteristics of rifampicin loaded niosomes.

Composition of niosomes (Surfactant used)	Mean size of niosomes (µm)	% rifampicin entrapped	% rifampicin released in 72 h
Span - 85	2.0	34.4	30
Span – 80	1.8	30.3	33
Span – 60	1.6	25.8	39
Span – 40	2.0	23.5	42
Span – 20	1.9	2.3	45

CONCLUSION

The handshaking method is a simple and efficient technique for designing functional niosomes for hydrophobic or amphiphilic drugs. In vitro release rate studies revealed that the cumulative percent rifampicin released was maximum for Span-20-based niosomes and minimum for Span-85-based niosomes [Table - 1]. The difference in release rate is assumed to be based on lipophilicity of the surfactant. The Span-20, being least lipophilic, would provide easy access to the release media (aqueous phase) to the drug; whereas Span-85, being relatively lipophilic, impedes the easy permeation to the aqueous phase.

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