ISSN:2230-7346
Available online http://WWW.JGTPS.COM
Research Article
Journal of Global Trends in Pharmaceutical Sciences
Vol.1, Issue 1, pp 7-14, October–December 2010

PREPARATION AND EVALUATION OF CATIONIC NIOSOMES ENCAPSULATED WITH MICELLAR SOLUBILIZED RIFAMPICIN

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ABSTRACT

Rifampicin is a first line drug used for the treatment of tuberculosis. Niosomes are biodegradable, biocompatible drug carriers. Encapsulation of drug in cationic niosome delivers the drug at target site, thereby reducing its toxicity by the effective uptake of drug. The encapsulation efficiency of vesicles is improved by encapsulating miceller solubilized solution of rifampicin in hydrating medium. The niosomes are prepared by Film Hydration Technique and evaluated for their morphological characteristics, zeta potential, entrapment efficiency and drug release studies.. The obtained niosomes were multi lamellar with high encapsulation efficiency without any leakage and aggregation. Results of the present study indicate that the prepared cationic niosomes showed improved encapsulation, prolonged release and capable of maintaining drug concentrations. Thus, rifampicin encapsulated cationic niosomes can be successfully used for the treatment of tuberculosis with lesser dose, minimum toxicity and improved patient compliance.

KEY WORDS: Cationic, niosomes, micellar, rifampicin, vesicles.

INTRODUCTION:

Rifampicin is an effective drug used for the treatment of tuberculosis, a widely prevalent disease, requiring high dose for 4-6 months. Rifampicin shows anti microbial activity by binding to microbial DNA dependant RNA polymerase thereby inhibits the initiation of RNA synthesis. It shows various side effects such as immunological disturbances, lupoid syndrome, jaundice and other hepatotoxic manifestations. The toxicity can be circumvented by developing a new delivery system that would release the drug at target site at controlled rate. (1, 2, 3)

Niosomes are the uni or multi lamellar vesicles made up of non-ionic surfactants capable of entrapping both hydrophilic and hydrophobic drugs (4). Rifampicin is a hydrophobic drug which can be in capsulated in lipid bilayer. The drugs encapsulated in aqueous region shows improved encapsulation efficiency, stability and does not require special condition for production and storage. (6) Mullatchiram et al., reported very poor entrapment efficiency of rifampicin in the hydrating medium of niosome (5, 8)**.** In the present study, we made an attempt to encapsulate rifampicin in internal aqueous phase by preparing the miceller solubilized solution using plantacare (Lauryl glucoside) and Tween 80 as solubilizing agents.

Cationic vesicles form complexes with negatively charged cell or endoplasmic membrane and facilitate in drug release after endocytosis. The presence of charge increases the encapsulation of drug and decreases aggregation of vesicles by electrostatic stabilization. Cationic Niosomes are considered to be the novel delivery systems for targeted delivery(7, 11).

The objective of the present work is to develop a novel formulation of cationic niosomes encapsulated with miceller solubilized rifampicin. The obtained niosomes were evaluated for entrapment efficiency, charge, and drug release studies.

MATERIALS AND METHODS:

Rifampicin was obtained from lupin pharmaceutical Pvt. Ltd; Aurangabad, Cholesterol from Qualigen fine chem. Ltd, Mumbai, Span from Koch light lab. Ltd; England, Stearyl amine from sigma chemicals, St.Louis U.S.A. and the plantacare(Lauryl Glucoside) were obtained from Henkel KGA, Germany. All the solvents used were of analytical grade.

PREPARATION OF RIFAMPICIN CATIONIC VESICLES:

Rifampicin cationic vesicles were obtained by encapsulating micellar solubilized solution in internal aqueous compartment of vesicular system. Preparation of micellar solubilized rifampicin: Tween 80 and plantacare in 1:1 proportion were dissolved in 5ml of methanol to which rifampicin is added to give a concentration of 500 µg\ml. The total volume was adjusted to 10ml and the obtained solution was subjected to evaporation under reduced pressure in a vacuum evaporator at a temperature of 55-60˚c. After the complete evaporation, 10ml of distilled water was added and rotated for 5 minutes to obtain a micellar solubilized rifampicin.

Encapsulation of solubilized rifampicin in niosome:

The cationic vesicles of rifampicin were prepared as per Rilm et.al. Span 60 and cholesterol in 1:1 molar ratio with 50% of stearyl amine giving a total lipid content of 200µmoles are dissolved in 14 ml of chloroform in a round bottom flask to which 1ml methanol was added. resultant clear solution was subjected to evaporation under reduced pressure in a rotary vacuum evaporator at a temperature of 60ºc and the obtained thin dry film was hydrated by incorporating 5ml of micellar solubilized rifampicin solution.

Characterization of rifampicin cationic vesicles:

Morphological characterization:

Freshly prepared niosomal dispersion was scanned and imaged using on optical microscope attached to video camera. Particle size distribution of vesicular dispersion was observed for aggregation and appearance.

Entrapment Efficiency:

Encapsulation efficiency of vesicular system was determined using Centrisart tubes (solitorius, Germany) with a molecular weight cut off of 20kDa. The prepared cationic niosomes were separated from unencapsulated rifampicin by ultra filtration. The niosomal dispersion is taken in centrisort tubes and rotated at 10,000 r.p.m for 15 min from the obtained supernatant 0.5 ml was taken and made up to 10 ml with solvent mixture (20:80) of chloroform and methanol. The absorbance of resultant solution was determined at 334nm using UV spectrophotometer.

Zeta potential determination:

The surface charge of the niosomes was determined by the electrophoretic mobility of prepared niosomal particle towards electrode using zeta meter 3.0 (zeta meter Inc, USA). Freshly prepared 1ml of cationic niosomal dispersion was diluted to 50 ml with double distilled water ,then placed in sample cells and measured for the zeta potential (10).

Drug release studies:

The release pattern of rifampicin from niosomal dispersion was carried out in dialysis bag method. Accurately measured 2 ml of cationic niosomal dispersion was taken in dialysis bag and the bag was suspended in 100 ml of 7.4 phosphate buffer. The samples were withdrawn in different intervals and simultaneously sink condition was maintained. The drug content was estimated at 334nm using UV spectrophotometer (Shimadzu, Japan) (5, 12)

RESULTS & DISCUSSION: Morphological characterization:

The optimized dispersions of cationic niosome were examined for vesicle formation under optical microscope attached with video camer, **Fig. 1** revealed that vesicles are multilamellar and large unilamellar in nature. Incorporation of cholesterol and stearyl amine assumed to be responsible for the formation of large vesicular structure.

The obtained vesicles were spherical, discrete, abundant and uniformly distributed. Vesicles are observed with out any aggregation tendency.

Entrapment efficiency:

Encapsulation of rifampicin in cationic vesicular dispersion was determined by ultra filtration using centrisort tubes was found to be 58.42% with a capture volume of 14.92µl/mol. Formulation of large unilamellar vesicles are most appropriate type to achieve higher capture volume, This is because large internal core is available for the efficient entrapment of rifampicin solution(13).

Zeta potential determination:

Zeta potential is a significant parameter in governing the degree of repulsion between charged particles. The remarkable increase in positive charge was observed with increased concentrations of stearyl amine in niosomal formulation. The zeta potential of optimized niosome was found to be +56.80 ± 9.812 mV which indicates that attractive forces exceed the repulsive forces. The presence of positive charge indicates the formation of cationic vesicles and the obtained higher zeta potential may overcome flocculation & aggregation of vesicles.

Drug release studies:

As the cholesterol molar ratio increases in niosome particle size, entrapment increases. The stearyl amine might be influencing the entrapment efficiency of the drug molecule in the vesicle. The incorporation of stearyl amine in bilayers of niosomes enhances the vesicle size which could be due to electrostatic stabilization, resulting in increase in size and entrapment efficiency. The incorporation of different molar concentrations of cholesterol and stearyl amine showed entrapment efficiency from 37.63 to 62.58%.

The cumulative drug release profile of different formulations is graphically illustrated that the percentage drug released increases with increase in cholesterol and stearyl amine. The optimized niosomal dispersion does not show any leakage and aggregation on storage. It was assumed that the incorporation of rifampicin in aqueous region and the concentration of stearyl amine are responsible for improved stability.

CONCLUSION:

Currently, niosomes are drug delivery system with greater potential for targeted and controlled release. The treatment of tuberculosis requires continuous administrations of rifampicin by conventional dosage forms, which lead to toxicity .To overcome toxicity, the effort was made to prepare rifampicin cationic niosome with sustained release. The data revealed that niosomes are cationic, multilamellar with high encapsulation efficiency and showed release profile for 24 hr. The zeta potential value confirms the stability of niosomes. In conclusion, niosomal formulation could be a promising delivery system for rifampicin with prolonged drug release profile and extention of this research work may be useful in the targeted delivery of rifampicin.

Fig: 1 PhotoMicroscopy of cationic Niosomes Encapsulated with Micellar solubilized Rifampicin.

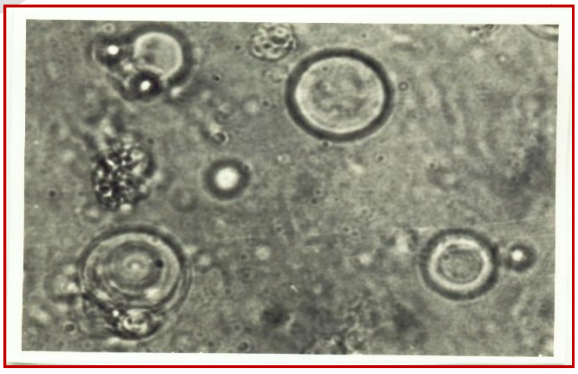

Table 1: Characterization parameters of rifampicin cationic niosomes

S.no	Formulation	Molar ratio of Span-60, Cholesterol, Stearyl Amine.	Encapsulation Efficiency	Particle Size (microns)	Zeta Potential(mv)
1	R1	50:50:20	37.63%	2.78±0.12	+32.46±5.678
2	R2	75:75:25	45.34%	4.56±0.2	+41.57±6.065
3	R3	75:75:50	58.42%	5.45±0.16	+56.80±9.812
4	R4	85:85:30	54.34%	5.04±0.11	+45.78±5.466

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