ENCAPSULATION OF MICELLAR SOLUBILIZED RIFAMPICIN IN CATIONIC NIOSOMES

Raju Jukanti*, Srinivas Ampati, K.S. Nataraj

SRR College of Pharmaceutical Sciences, Valbhapur, Elkathurthy, Karimnagar. 505476

ABSTRACT

New generation dosage forms mainly comprise three dimensional colloidal drug carrier systems for the drugs, which have been designed to control the release of the drug according to the need or can be shooted at the target site to pool the target site with the drug. Niosomes have proved to be the better alternatives for liposomes because of their low cost, greater stability and ease of storage. Cationic vesicles have proved to be very useful since they can readily interact with negatively charged cell membranes. Hydrophobic drugs are entrapped into the hydrophobic matrix of bilayers of niosomes to a limited extent with less encapsulation efficiency. Henceforth, we put forward a novel approach to improve encapsulation of highly water insoluble drugs into niosomes by loading drugs into micelles which can be conveniently encapsulated into aqueous region of bilayers. Cationic vesicles were formulated and optimized the formulation by varying the ratios of Span 60, Cholesterol and charge inducer, Stearylamine. Micellar solubilized Rifampicin was loaded into the aqueous compartments of cationic vesicles without any change in the shape and charge of the vesicles. Further the vesicles were subjected to release study to understand the permeability characteristics of the bilayer. The approach definitely provides a means of encapsulating insoluble drugs into the aqueous compartment with enhanced drug load.

Key words: Cationic vesicles, Niosomes, Micellar solubilization, Rifampicin

1. INTRODUCTION

Targeting of the drugs through carrier system has been the central theme of research in therapeutics. One of the primary objectives in the design of novel drug delivery systems is controlled delivery of the pharmacological agent to its site of action at a therapeutically optimal rate and dose regimen (Kreuter, 1992). This site specific or targeted delivery combined with delivery at an optimal rate would not only improve the efficacy of a drug but would also reduce the possibility of unwanted toxic side effects of the drug, thus improving the therapeutic index (Youssef et al., 1988). New generation dosage forms mainly comprise three dimensional colloidal drug carrier systems for the drugs, which have been designed to control the release of the drug according to the need or can be shooted at the target site to pool the target site with the drug.

The success achieved with liposomal systems stimulated the search for other vesicle forming amphiphiles. Non-ionic surfactants were among the first alternative materials studied and a large number of surfactants have since been found to self assemble into closed bilayer vesicles which may be used for drug delivery (Handjani Vila et al., 1979).

Niosomes have proved to be the better alternatives for liposomes because of their low cost, greater stability and ease of storage. They are now established as useful model membrane systems and have demonstrated potential for delivering molecules as large as DNA to the intracellular compartment. Not only DNA, they have been exploited as potential carriers for a variety of drugs (Azmin et al., 1985; Baillie et al., 1986; Uchegbu et al., 1995), therapeutic proteins (Hassan et al., 1996) and diagnostic agents (Erdogan et al., 1996).

Much of the literature cited regarding niosomes has dealt with preparation methods and their use for delivering anticancer, anti-infective agents etc. Current research in cationic liposomes is pointing out in the direction of targeting of drugs and DNA into the cellular compartment. For this purpose cationic vesicles have proved to be very useful since they can readily interact with negatively charged cell membranes. They are useful in delivering DNA vaccines and in Gene therapy.

* Address for Correspondence

Dr. Raju Jukanti
Assistant Professor
Phone: 9849333431
email: jukantiraj@gmail.com, jukantiraj@yahoo.co.in

Hydrophobic drugs are entrapped into the hydrophobic matrix of bilayers of niosomes to a limited extent with less encapsulation efficiency. Moreover, there is every possibility that the drugs so encapsulated may perturb the bilayer and interfere with the microstructure of the bilayer resulting in destabilisation of the system. Hence there is a need to develop methods to incorporate higher amounts of hydrophobic drugs keeping the integrity of bilayer of the niosome. We put forward a novel approach to improve encapsulation of highly water insoluble drugs into niosomes by loading drugs into micelles which can be conveniently encapsulated into aqueous region of bilayers.

2. MATERIALS & METHODS

Span 60, Cholesterol, Stearylamine were purchased from Sigma chemicals, St.Louis, USA. Rifampicin was a kind gift sample from Lupin, India. Lauryl glucoside (Planta care G) was purchased from Henkel KGAa, Germany. Dialysis membrane was purchased from Hi media, Mumbai, India. All other solvents used were of HPLC grade.

Preparation of Vesicles by Film Hydration method

Vesicles were prepared by using film hydration method [Bangham et al., 1965]. Stock solutions of Span 60, cholesterol and stearylamine were prepared in chloroform, so as to minimize the variations in the amount while weighing. Aliquots of Span 60, cholesterol and stearylamine were taken from the stock solutions representing the required concentrations in a round bottom flask and the volume is made upto 14 mL with chloroform and 1 mL of methanol. The solvent was evaporated under reduced pressure in rotary vacuum evaporator (Heidolph, Germany). Temperature of the water bath was maintained between 55 - 60°C and the flask was rotated at 150 rpm. After complete evaporation of the organic solvent, dry thin lipid film formed along the inside wall of flask was hydrated by adding 5 mL of double distilled water heated to the same temperature and the flask was subjected to rotation at a low speed in water bath for about 30 min and the final preparation was diluted to 10 mL. The traces of solvent in the preparation was removed by prolonged exposure to vacuum. Levels of solvent in the final preparation was monitored by HPLC.

Preparation of Vesicles by varying cholesterol concentration

The molar ratio between Span-60 and cholesterol was varied keeping charge inducer at 25% of the total lipid. The niosomal preparations with these mixtures were prepared as per the above procedure.

Preparation of Vesicles by varying Stearylamine concentration

Vesicles with Span 60 and Cholesterol in 1:1 molar ratio and charge inducer stearylamine in varying concentrations (10, 12.5, 15, 20, 25, 30 mole percent) were prepared as per the above procedure.

Preparation of micellar solubilized Rifampicin

Rifampicin and Plantacare (lauryl glucoside) were taken in a small beaker. 250 mg of Polysorbate 80 followed by 10 ml of methanol was added, so that a clear solution is obtained. After complete evaporation of the organic solvent under reduced pressure in rotary evaporator (Heidolph, Germany), the film formed along the inside wall of the flask was hydrated with double distilled water.

Encapsulation of Micellar Solubilized Rifampicin

The required concentration of rifampicin was taken from the above solution and was entrapped into the selected cationic niosome formulation as per the film hydration method.

Characterization
Microscopy

The vesicles prepared were suitably diluted and a drop of niosomal dispersion was mounted on a slide with cover slip and was observed at a magnification of 450X using light microscope to confirm the formation of vesicles, shape, agglomeration and type of vesicles.

Measurement of Electrokinetic Potential

The Electrokinetic potentials were measured (1 in 50 dilution) using Zeta Meter (Zeta Meter Inc., USA).

Total drug content

One mL of the preparation was digested with chloroform and methanol mixture (1:4) and a suitable dilution was used to estimate total rifampicin content in the niosome preparation.

Percent encapsulation using Ultra filtration

Centrisart (Saltorius, Germany) with molecular weight cut-off of 20 KD was used for ultrafiltration.

Diluted niosome dispersion was taken in a centrisart tube and centrifuged for 10 min at 5000 rpm. The clear solution in the inner tube was withdrawn immediately and diluted suitably with methanol and the absorbance was measured at 334 nm using UV spectrophotometer (Elico, India). The percent encapsulation was calculated by substracting the unentrapped drug from the total drug content.

Release Study

Niosome dispersion (2 mL) was placed in the dialysis pouch made up of cellulose membrane. Pouch was suspended in 100 mL beaker containing 90 mL of 7.4 pH phosphate buffered saline. The total dialysis medium was replaced with equal volume of fresh medium at an interval of 15 min. The absorbance of dialysis samples was measured at 334 nm using a UV spectrophotometer (Sanya SP55, gallenkamp, UK). The concentration in samples was obtained from standard plot and the dialysis was stopped when drug concentration reached a limiting value which gives the unentrapped Rifampicin in the dispersion.

The dialysed niosome dispersion which is almost devoid of unentrapped rifampicin was used for release studies. The pouch containing this preparation was placed in 100 mL beaker containing 90 mL of release medium. The aliquots of 5 mL were withdrawn and replaced with same amount of 7.4 pH Phosphate buffer saline at pre-set intervals for 24 hours. The samples were analyzed for Rifampicin by UV spectrophotometer (Sanya SP55, gallenkamp, UK) at 334 nm. All experiments were performed in triplicate and values are reported as mean ± SD.

3. RESULTS AND DISCUSSION

The vesicles were formed in all the systems but the abundancy and uniformity of the vesicle shape was mainly dependent on the charge inducer, stearylamine and the ratio of cholesterol. Reducing Span-60 : Cholesterol ratio the hydration was not good moreover it resulted in formation of vesicles with improper shapes and with poor stability, which can be attributed to non rigidity of the bilayers. In accordance with the reports, the systems containing cholesterol in equimolar proportion with vesicle former, Span 60 resulted in better formulation (Bangham et al., 1965).

At 10 and 12.5 mole % stearylamine hydration was incomplete and hence few vesicles were seen in the preparation. From 15 mole% of stearylamine onwards hydration improved gradually resulting in the increase of abundancy of vesicles. At 25 mole% hydration was quick and complete. In this preparation large numbers of spherical vesicles were observed. Further increase in SA concentration of 30 mole% though hydration was good, vesicle shape distorted. The observations made from different preparations varying stearylamine and cholesterol ratios clearly indicated that the abundancy of vesicles was more in Span 60, cholesterol, and stearylamine (37.5 : 37.5 : 25 mole%) respectively (Table 1 & 2).

Normally with increase in stearylamine positive zeta potential of vesicles should increase, when the ratio of other components is constant. However from 10 to 15 mole % stearylamine postive charge decreased, this can be attributed to improper hydration and incomplete vesicle formation, wherein charge inducer is not distributed evenly into bilayers. Interestingly when hydration started improving from 15 to 30 mole% number of vesicles with uniform spherical shape increased and zeta potential also has gradually increased. However, at 30 mole% again vesicle shape distorted.

The light microscopic examination of the vesicles revealed that vesicles are of large unilamellar and multilamellar type (Fig. 1). The micellar solubilized rifampicin has been encapsulated into the cationic niosomes without any significant change in the charge and shape of the vesicles (Table 3).

The zeta potential measurements showed that the vesicles carry positive charge on their surfaces. The zeta potential values of control vesicles and Rifampicin encapsulated vesicles are +55.71 ± 9.3 and +53.55 ± 6.7 respectively. The entrapment efficiency of Rifampicin loaded vesicles was 35.43% (Table 3).

The average Rifampicin release profiles from formulation along with control (micellar solubilized Rifampicin) is shown in Fig. 2. The faster release rate was observed for drug solution with 90% release within 2 hrs indicating permeability of the membrane to the drug. The formulations exhibited a typical biphasic pattern of release. Initially up to 2 hrs, a faster release and later on the release was slowed down. The formulation exhibited a sustained release when compared to control.

The initial rapid phase is because of the burst release of the drug present in the bilayer which is due to the saturation of the bilayer with the drug during the exhaustive dialysis (Muhlen et al., 1998).

Table - 1: Zeta Potential Measurement for the preparations containing varying Stearylamine concentration (1 in 50 dilution)

Composition (mole%) Span60 : Cholesterol : Stearylamine	Zeta Potential (mV) Avg $\pm$ SD	Remarks
45 : 45 : 10	+58.37 $\pm$ 5.65	Poor hydration, few vesicles and not uniform in shape
43.75 : 43.75 : 12.5	+51.25 $\pm$ 8.74	Few vesicles, shapes are different and hydration to certain extent.
42.5 : 42.5 : 15	+41.57 $\pm$ 6.062	Vesicles no. and hydration is increased with some spherical vesicles.
40 : 40 : 20	+53.0 $\pm$ 7.968	Vesicles are present & hydration is good
37.5 : 37.5 : 25	+56.80 $\pm$ 9.812	Complete hydration, abundant and spherical vesicles
35 : 35 : 30	+58.9 $\pm$ 7.46	Complete hydration but the vesicles shape disturbed.

Table - 2: Zeta Potential Measurement for the preparations containing varying Cholesterol concentration (1 in 50 dilution)

Composition (mole%) Span60 : Cholesterol : Stearylamine	Zeta Potential (mV) Avg. $\pm$ S.D	Remarks
37.5 : 37.5 : 25	+64.6 $\pm$ 11.6	Complete hydration, abundant and spherical vesicles.
40.5 : 32.5 : 27	+51.64 $\pm$ 7.82	Hydration is good, with spherical vesicles, less in number.
43.5 : 27.5 : 29	+59.34 $\pm$ 5.46	Partial hydration with vesicles few in number.
46.5 : 22.5 : 31	+59.36 $\pm$ 8.5	Vesicles few in no, hydration is incomplete
52.5 : 12.5 : 35	+51.77 $\pm$ 6.65	Poor hydration, vesicles with poor stability

Table - 3: Zeta Potential and Percent encapsulation for the Micellar solubilized Rifampicin entrapped vesicles

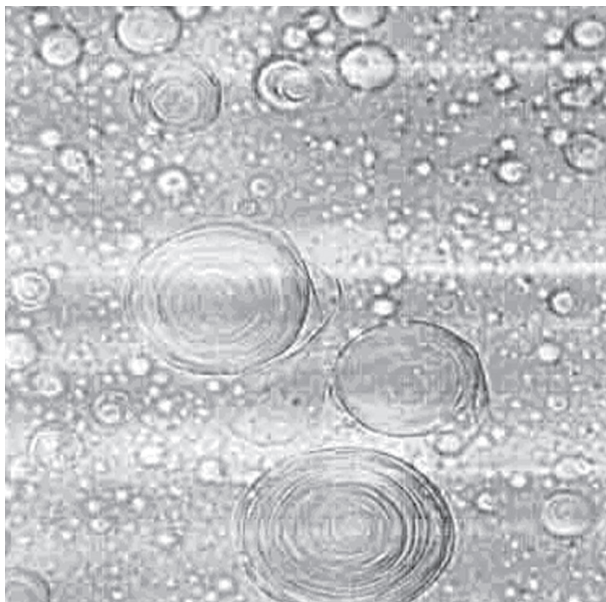| Preparation	Zeta Potential (mV) Avg. $\pm$ S.D	Percent Encapsulation
Control	+55.7 $\pm$ 9.3	--
Rifampicin encapsulated cationic noisome dispersion	+53.5 $\pm$ 6.7	35.43

Fig. 1 Photomicrograph of Cationic niosomes dispersion loaded with micellar solubilized Rifampicin (Under 450X magnification)

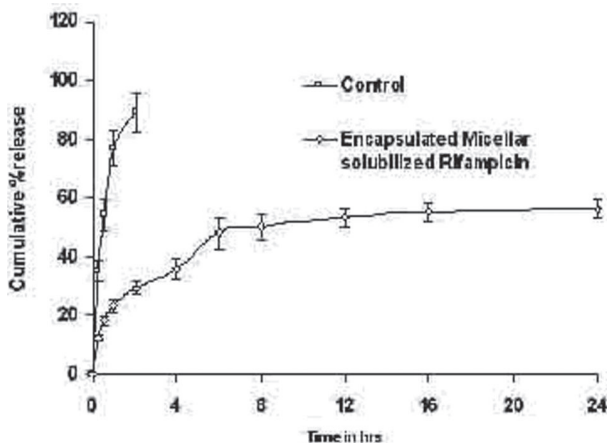
Fig.2: Release profiles of Cationic niosomes encapsulated with micellar solubilized Rifampicin

4. CONCLUSIONS

Many times loading water insoluble drugs into bilayer may result in drug leakage and interfere in formation of vesicles due to perturbation of the bilayer. Micellar solubilized drugs can be loaded with ease into aqueous compartment. The novel approach may resolve the issue to a great extent and definitely proves to be worthwhile to explore the micellar solubilized loaded vesicles for drug load enhancement and targeting to the specific site.

REFERENCES

Azmin MN, Florence AT, Handjani-vila RM, Stuart JFB, Vanlerberghe G, Whittaker JS. The effect of non-ionic surfactant vesicle (niosome) entrapment on the absorption and distribution of methotrexate in mice, J Pharm Pharmacol, 37, 1985, 237-242.

Baillie AJ, Coombs GH, Dolan TF, Laurie J. Non-ionic surfactant vesicles, niosomes, as a delivery system for the anti-leishmanial drug, sodium stibogluconate, J Pharm Pharmacol, 38, 1986, 502-505.

Bangham AD, Standish MM, Watkins JC. Diffusion of univalent ions across the lamellae of swollen phospholipids, J Mol Biol, 13, 1965, 238-252.

Erdogan S, Ozer AY, Ercan MT, Erylmaz M, Hincal AA. *In vivo* studies on iopromide radio opaque niosomes, STP Pharma Sci, 6, 1996, 87-93.

Handjani-Vila RM, Ribier A, Rondot B, Vanlerberghe G. Dispersion of lamellar phases of non-ionic lipids in cosmetic products, Int J Cosmetic Sci, 1, 1979, 303-314.

Hassan Y, Brewer JM, Alexander J, Jennings R. Immuno-responses in mice induced by HSV-1 glycoproteins presented with ISCOMS or NISV delivery systems, Vaccine, 14, 1996, 1581-1589.

Kreuter J. Nanoparticles – preparation and applications. Microcapsules and Nanoparticles in Medicine and Pharmacy (M.Doubrow, ed.) BocaRaton, Fla: CRC Press, 1992, 125.

Muhlen AZ, Schwarz C, Mehnert W. Solid Lipid Nanoparticles (SLN) for controlled release drug delivery – drug release and release mechanism, E J Pharm Biopharm, 45, 1998, 149-155.

Uchegbu IF, Double FA, Turton JA, Florence AT. Distribution, metabolism and tumoricidal activity of doxorubicin administered in Span 60 niosomes in the mouse, Pharm Res, 12, 1995, 1019-24.

Youssef M, Fattal E, Alonso MJ, Roblot, TL. Effectiveness of nanoparticle-bound ampicillin in the treatment of *Listeria monocytogenes* infection in athymic nude mice, Antimicrob Agents Chemother, 32, 1988, 1204.