Formulation and Development Study of Primaquine Phosphate Niosome and its Evaluation

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ABSTRACT

Primaquine phosphate (PQP) is the most preferred drug in treatment of malaria, which cause the complete eradication of parasites and prevention of relapse by destruction of the exo-erythrocytic liver stages of *Plasmodium vivax* and *P. ovale*. However, wider use of the PQP in the prophylactic therapy is limited by toxic side effects. REV method was selected which entraps higher aqueous phase containing the drug. Among different blends of solvents tried diethyl ether and chloroform in a 1:1 ratio provided stable emulsion in presence of drug and niosomes were formed in size ranges between 200-250 nm approximately.

**Keywords:** Primaquine phosphate, niosomes, dihydrocholate, Reverse evaporation technique.

INTRODUCTION

Vesicular systems not only help in providing prolonged and controlled action at the specific organ but also help in providing controlled delivery by preventing metabolism of the drug from the enzymes. Vesicular drug delivery systems used in such an diseases broadly include liposomes and niosomes. Despite liposomes being a potentially useful system for Parenteral delivery they are not very popular because of their short shelf life, limited drug loading capacity, and problems in sterilization. Further, vesicles consisting of one or more surfactant bilayers enclosing aqueous phase called niosomes have been considered of particular interest as they offer several advantages over liposomes with respect to chemical stability, lower cost and availability of materials.

EXPERIMENTAL WORK

Primaquine phosphate was received as a gift sample from IPCA Ltd. Mumbai, India. Dihydrocholate 30 was received as a gift sample from Nikko Laboratories chemicals, Japan. Materials like surfactant and other chemicals were provided by Finechem Ltd. India.

- **Preformulation**

  Different lipids like cholesterol, dihydrocholate (DHC-30), soysteol (BPS-5) and non-ionic surfactants like sorbitan laurate (span 20), sorbitan palmitate (span 40), sorbitan stearate(span60), sorbitan olate(span 80), and polysorbate-20(tween-20) were screened on the basis of niosome formation, appearance and drug entrapment efficiency. Reverse evaporation technique (REV), Film hydration (FH), Sonication and Ether injection were the four methods by which niosomes were prepared. Chloroform, diethyl ether, methanol, acetone, dichloromethane were screened as the solvents.

- **Formulation of Primaquine phosphate niosomes**

  Niosomal formulations of PQP containing cholesterol, dihydrocholate, containing were prepared using non-ionic surfactants mainly sorbitan laurate (Span-20), sorbitan palmitate (Span 40), sorbitan stearate (Span60), sorbitan oleate (Span 80) and polysorbate-20(Tween-20) along with DMPC and DCP. They were formulated by the reverse evaporation technique using diethyl ether and chloroform as the solvent blend in a 1:1 ratio as reported by Szoka and Papahadjiopoulos. Aqueous phase containing PQP was added such that the organic to aqueous phase ratio was 10:1. The mixture was then sonicated for 15mins using bath sonicator. Emulsion so formed was dried down to a semisolid gel in a rotary evaporator at 600C and the resultant viscous dispersion was diluted with phosphate buffer. Niosomes containing Primaquine phosphate were also prepared by adopting the procedure described by Azmin et al i.e. the film hydration method wherein Tween-20 and cholesterol in a molar ratio of 1:1 were dissolved in ether: chloroform (1:1). The solvent was evaporated using a rotavapor apparatus and the dry film thus formed was hydrated with a 0.2 % w/v solution of PQP in phosphate buffer saline pH 7.4 at 60 0C. Niosomes were formulated with different molar ratios of lipid and non-ionic surfactants ranging...
from 1:1 to 2:2 and the formulations were optimized on the basis of drug entrapment.

- **Optimization of formulation: (By factorial design)**
  2$^3$ factorial design was used for the optimization of niosomal formulation. The eight experiments were designed with independent variable (amount of aqueous phase, sonication time, cholesterol concentration) and dependent variables (particle size and entrapment efficiency) in two levels in order to optimized the formulation.

- **Characterization of the niosomal formulation**
  a) **Transmission electron microscopy**
  The surface morphology and topography of different prepared niosomes was studied using TEM (JEM-2000 FX electron microscope, Jeol Ltd, Tokyo, Japan) for large unilamellar vesicles (LUV). Niosomal samples were loaded on carbon coated grid and photographed.

  b) **Vesicle size**
  Niosomes prepared using cholesterol; dihydrocholate and soysterol were characterized for vesicle size by photon correlation spectroscopy (Beckman Coulter Inc, Miami, USA) before and after lyophilisation.

  c) **Drug entrapment**
  The entrapment of drug in the niosomal formulation was determined indirectly by estimating the drug content in the supernatant after centrifugation of niosomal formulation. The niosomes were centrifuged using a cooling centrifuge (Remi C-30BL). The supernatant of the dispersion was appropriately diluted with phosphate buffer saline 7.4 and analysed on a UV spectrophotometry at 259nm. Drug entrapments of niosomal formulation were calculated before and after lyophilisation.

  d) **Stability studies**
  Freeze dried niosomal formulation were evaluated for the every week for three month for the particle size and entrapment efficiency. Niosomal dispersion of 0.2%w/v Primaquine phosphate concentration were packed in vials and kept at 4°C ± 2°C, 25°C ± 2°C conditions for stability studies as per ICH guidelines. The samples were analysed at periodic time intervals for vesicle size and drug entrapment.

e) **Haemolytic study**
  The haemolytic study of the formulation carried out in triplicate for the safety of the formulation. The triton X-100 is used as a positive control in the study.

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\% \text{ Hemolysis} = \frac{\text{Absorbance of test sample} \times 100}{\text{Absorbance at 100% lysis}}
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**RESULT AND DISCUSSION**
Reverse evaporation technique (REV) , Film hydration (FH) , Sonication and Ether injection techniques were tried using cholesterol, dihydrocholate, soysterol with non-ionic surfactants mainly span 20, 40, 60, 80 and Tween 20, 40, 60, 80. A validated UV spectrophotometric technique (λmax = 259 nm) (fig.1) was used for routine analysis of PQP was validated in terms of linearity and accuracy. Standard curve of PQP was prepared in phosphate buffer saline pH 7.4 over a concentration range of 3 μg/ml - 18 μg/ml at 259nm. Standard equation y = 0.0573x-0.0147(r²=0.999) with %RSD less than the prescribed value was obtained.

REV method provided niosomes with higher drug entrapment efficiency (59.70-61%) compared to film hydration (10.49%) and Ether injection method. REV method was selected which entrapped higher aqueous phase containing the drug. The niosomes were opalescent with no aggregates or precipitate when observed under light microscope. Niosomes prepared with cholesterol and Tween -20 were found to give higher entrapment efficiency than other lipids and surfactants. Among different blends of solvents tried ,diethyl ether and chloroform in a 1:1 ratio provided stable emulsion in presence of drug.

Transmission Electron Micrograph the Chol: Tween-20, Chol:Tweeent-20 without stabilizer, Chol:Tweeent-20 with stabilizer and Chol:Tweeent-20 with stabilizer and liver targeting agent have been shown in the Fig 2, fig 3, Fig 4 and Fig 5. TEM images showed that niosomes have a distinct solid dense structure with spherical shape. Discrete structure of the niosomes could be attributed to negative surface charge of the niosomes. TEM images showed the niosomes were in size ranges between 200-250 nm approximately.

The % hemolysis of the formulation along with the individual components were shown graphically in fig.6. From the graph the niosomes containing PQP (N$\text{p}$) showed 2.05 % hemolysis, whereas the blank niosomes (N$\text{b}$) showed more hemolysis i.e. 2.28 %. The individual components of the formulation Tweeent-20, DCP and DMPC showed hemolysis 1.92%, 1.33% and 1.19% respectively.
Hemolysis could occur either by absorption of surfactant molecules on cellular surface or penetration of surfactant molecules into cell membrane or induction of alterations within cellular membrane or increasing permeability of cellular membrane followed by gradual increase of osmotic phenomenon resulting in destruction of cellular membrane. In case of surfactants the observed higher hemolysis could be due to the first by increasing cellular membrane permeability followed by cellular lysis. The in vitro hemolytic study results revealed less hemolysis in the niosomal formulation containing PQP as compared to the blank niosomal formulation. This could be attributed to the interaction/entrapment of PQP in surfactant vesicle. It was reported that the polyoxyethylene chain of Tween 20 was responsible for the hemolysis. In the PQP loaded niosomes PQP got entrapped in the polyoxyethylene chain, thus free chain was not available for hemolysis. Also the formulation accumulate in the liver i.e. targeted to liver cells so there were very less contact time between formulation and cell membrane, thus caused less hemolysis.

CONCLUSION
Niosomes were formulated with different molar ratios of lipid and non-ionic surfactants ranging from 1:1 to 2:2 and the formulations were optimized on the basis of drug entrapment. The blank niosomes exhibited negative values of the zeta potential which might be attributed to the adsorption of counter ions or the preferential adsorption of hydroxyl ions at the vesicle surface. Scavenger receptors in the kuffer of liver were responsible for liver uptake of niosomes containing negative charge on their surface. The cells were the binding site for negatively charge niosomal formulation. Incorporation of PQP into the niosomes helped to increase the stability of PQP by preventing it from chemical and enzymatic degradation which further helped to increase residence time. DMPC and DCP containing PQP loaded niosomal formulation helped in the prolonge released and liver targeting and hence ultimately increased the efficacy of the developed formulation as compared to conventional formulation. Therefore effect of DCP on aggregation of niosomes was evaluated. Also PQP niosomes prepared by adding DMPC as liver targeting agent. Using reverse evaporation technique, niosomes of Cholesterol: Tween 20: DCP molar ratio (1:1: 0.05 ) with the chloroform: ether (1:1) as an solvent was selected, which gave the particle size of 220 nm (PI 0.61) and entrapment efficiency 60.7%.

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Fig. 2: TEM of PQP niosomes containing DCP and DMPC

Fig. 3: TEM of PQP niosomes containing DMPC without DCP

Fig. 4: TEM of PQP niosomes

Fig. 5: TEM of PQP niosomes containing DCP

Fig. 6: % Hemolysis Vs formulation

REFERENCES


3. Krishna S, Bustamante L, Haynes RK, Staines HM. Artemisinins: their growing treatment failure in severe