Ghule and Bhoyar, J Develop Drugs 2018, 7:1
DOI: 10.4172/2329-6631.1000186

Journal of Developing Drugs

Research Article Open Access

Formulation and Evaluation of Modified Liposome for Transdermal Drug

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Rec date: June 01, 2018; **Acc date:** June 13, 2018; **Pub date:** June 20, 2018

Abstract

Objective: Pramipexole is widely used in the treatment of anti-Parkinson's, however it is associated with several side effects so the objective was to develop liposomal drug delivery of Pramipexole and thus reduce its side effect and toxicity and improve bioavaibility, efficacy and therapeutic index. Further study to modify drug delivery and to determine effect of stearylamine and sodium cholate content on the liposomal characteristics was investigated.

Methods: In the present study the modified liposomes were prepared by using Pramipexole, phosphotidylcholine, cholesterol, stearylamine and sodium cholate in different ratio. These liposomes were prepared using thin film hydration method and characterized for vesicle size, drug entrapment studies, *in-vitro* release, and zeta potential, *ex-vivo* study.

Results: The preparation of pramipexole loaded liposome was initiated by studying the influencing of drug lipid ratio on drug entrapment in vesicles. The drug bearing capacity of liposome was found to be invariably dependent on drug lipid ratio employed in liposomal composition, then formulation addition of stearylamine and sodium cholate to determine penetration enhancer. Modified liposome of optimized liposomal batches was formulated. Further modified liposomes were evaluated for entrapment efficiency, *in-vitro* release, zeta potential, and vesicle size. Thus, positive charged liposome seen to be promising as carrier for pramipexole drug thought transdermal drug delivery system.

Conclusion: Incorporation of stearylamine enhanced the percent entrapment of pramipexole owing to rigidizaton effect on the membrane packing. Modified Liposomes of pramipexole can be promising carriers for the effective treatment of Parkinson's.

Keywords: Liposome; Pramipexole; Phosphotidylcholine; Thin layer hydration method

Introduction

The stratum corneum of the skin contains a high ratio of negatively charged lipids, which are expected to interact with cationic liposomes. Transfer of some of the bilayer components of the liposomes to the skin is then induced. Several studies have demonstrated that positively charged liposomes have a remarkable effect in enhancing the penetration of drugs across the skin as the latter is negatively charged and favors the electrostatic attraction for positively charged liposomes. For this reason, Stearylamine (SA), a positively charged lipid, has been included in the formulation of the different liposomes in this work. Moreover, these vesicles as drug delivery systems have the potential to provide controlled release of the administered drug and to increase the stability of the labile drugs. The limited stability of liposomes during storage and administration restricts their application and development (*in-vitro* and *in-vivo*), although some attempts have been made to improve stability. The addition of charged agent the overall structure of the lipid vesicles constitutes an interesting strategy to improve the stability of formulations. The use of stearylamine and sodium cholate is more reduces size are obtained of charge inducer on the encapsulation efficiency of drug, results evidenced that the percentage of drug encapsulated increased in stearylamine formulations can be attributed to the inclusion of a charge inducer in liposomes, which modified the spacing between the adjacent bilayers. The negatively charged lipid electrostatically attracts the drug cation, which may be expected to push phospholipids head groups apart, hence increasing the particle diameter. The presence of the positive charge in the lipid bilayer produces a change in the lateral packing of the liposome bilayer causing a decrease in the entrapment efficiency [1,2].

Materials and Methods

Pramipexole dihydrochloride hydrate (Torrent research center), phospholipid (KGN Ingredient), cholesterol (Loba chemicals), sodium chloride (Chemsworth), sodium dihydrogen phosphate (Himedia), potassium hydrogen phosphate (Fischer Scientific), stearylamine (Himedia), sodium cholate (Merck).

Formulation of modified liposome

In this formulation (M1 and M6) was denoted as modified liposome formulation. Modified Liposomes (Table 1) were prepared using thin film hydration technique with the phospholipid namely soya lecithin and pramipexole, cholesterol and phospholipid and stearylamine and sodium cholate were added in the different ratios. They were accurately weighed and carefully transferred to round bottom flask and requisite amount of chloroform was added to it the contents were subjected to evaporation in a rata evaporate at 700°C for 45 min at a speed 95 rpm and reduced pressure of 25 rpm Hg for solvent removal. The resulting

film was hydrated with required quantity of phosphate buffer saline pH 7.4. The obtained colloidal dispersion was sonicated using bath sonicator for 45 min [3,4].

Ingredient	M1 (mg)	M2 (mg)	M3 (mg)	M4 (mg)	M5 (mg)	M6 (mg)
Drug	0.25	0.25	0.25	0.25	0.25	0.25
Phospholipid	1	1	1	1	1	1
Cholesterol	1	1	1	1	1	1
Sodiumcholate	-	-	-	0.25	0.5	1
Stearylamine	0.25	0.5	1	-	-	-

Table 1: Formulations of Modified Liposome.

Evaluation of modified liposomes

Particle size determination: The particle size of liposomes was determined by using motic microscope. All the prepared batches of the liposomes were viewed under motic microscope to study their size [4]. Size of liposomal vesicles from each batch was measured by taking a small drop of liposomal dispersion on glass slide and average size of the liposomal vesicles was determined.

Drug entrapment efficiency: Entrapment efficiency of liposomes was determined by centrifugation method. Aliquote (1 mL) of liposomal dispersion was subjected to centrifugation on a laboratory centrifuge at 5000 rpm for a period of 35 min at controlled temperature of 4°C. The clear supernatant was removed carefully to separate the unentrapped drug and absorbance was recorded at 262 nm. The sediment in the centrifugation tube was washed with acetonitrile thrice and it was diluted to 5 mL with acetonitrile and the absorbance was recorded at 262 nm. A calibration curve was produced by making different concentration from (1 µg/mL-10 µ/10 mL) with acetonitrile. Amount of the drug in supernatant and sediment gave a total amount of drug in 1 mL dispersion [5].

%Entrapment of drug was calculated by the follow formula:

%Drug Entrapment=Amount of the drug in the sediment/Total amount of drug

***In-vitro* release study:** An *in-vitro* drug release study was performed using modified Franz diffusion cell. Dialysis membrane was placed between the lower cell reservoir and the glass cell top containing the sample and secured in place with a pinch clamp. Liposomal formulation 1 mL was placed in donor compartment and the receptor compartment was filled with phosphate buffer pH 7.4 (17 mL). The diffusion cell was maintained at 37+0.5°C with stirring at 500 rpm throughout the experiment. 1 mL of receptor fluid were withdrawn from the receiving compartment at 30 min for the period of 5 hours and replaced with 1 mL fresh phosphate buffer pH 7.4 solution and after the suitable dilution analyzed by spectrophotometer at λ_{max} 262 nm.

***Ex-vivo* skin permeation study of goat ear skin:** An *in-vitro* drug release study was performed using modified Franz diffusion cell. The excised Goat Ear skin was mounted between the compartments of the diffusion cell with stratum corneum facing the donor compartment and clamped into position. Liposome formulation 1 mL was placed in donor compartment and the receptor compartment was filled with phosphate buffer pH 7.4 (17 mL). The diffusion cell was maintained at 37+0.5°C with stirring at 500 rpm throughout the experiment. 1 mL of receptor fluid were withdrawn from the receiving compartment at 30 min for the period of 5 hours and replaced with 1 mL fresh phosphate buffer pH 7.4 solution and after the suitable dilution analyzed by spectrophotometer at λ_{max} 262 nm against blank.

Zeta potential: Particle size and size distribution measurements of the liposomal dispersions were performed using photon correlation spectroscopy (PCS). The average particle sizes (z-average size) were measured by photon correlation spectroscopy Malvern Zetasizer at 25°C under a fixed angle of 90°C in disposable polystyrene cuvettes. Zeta potential was measured by using Zetasizer. Samples were placed in clear zeta cells and results were recorded. Before putting the fresh sample, cuvettes were washed with the methanol and rinsed using the sample.

Results

Vesicle size by microscopy: In optical microscopy, spherical lamellar vesicles with drug are observed under 100X oil immersion lens the average particle size of liposomes (Figure 1).

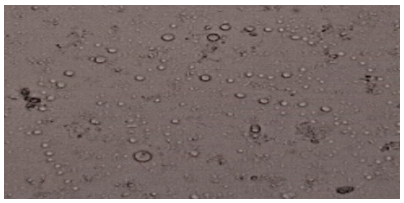

Figure 1: Morphology of modified liposome.

Particle size: The effect of varying concentration of stearylamine on particle size was studied. With increase in the stearylamine concentration the particle size increased the particle size. It may attribute the fact the presence of high stearylamine to the inclusion of a charge inducer in liposomes, which modified the spacing between the adjacent bilayers [6-8]. The effect of varying concentration of sodium cholate on particle size was determined. With increase in the sodium cholate concentration the particle size decreased. The negatively charged lipid electrostatically attracts the drug action, which may be expected to push phospholipids head groups apart, hence increasing the particle size [4].

Drug entrapment efficiency: The pramipexole entrapment efficiency of modified liposome varied with different concentration of stearylamine. The highest encapsulation efficiency was observed with the positively charged unilamellar liposome. This increase in the entrapment efficiency occurs because SA may affect specific characteristics such as the membrane permeability or the electric charge in fact lower concentrations of SA produce higher encapsulation efficiency [9]. The pramipexole entrapment efficiency of modified liposome varied with different concentration of sodium cholate. The highest encapsulation of sodium cholate by this reason, sodium cholate charge does not generate important repulsive interactions in the lipid bilayer. Indeed, with higher concentrations of sodium cholate, the entrapment efficacy was increased [10-12].

Zeta potential: The Stearylamine increased in the formulation, the zeta potential should be increased. However, results indicated a possible asymmetrical distribution of SA in the bilayer because a

nonlinear relationship between the SA content and the zeta potential values was obtained. In the SA is organized in micelles, undergoing a rapid aggregation into the medium, which range the surface charge [13]. The effect of increasing the sodium cholate concentration had no significant impact on the zeta potential [14].

***In-vitro* drug study:** The *in-vitro* release study for formulation of stearylamine showed that with increase concentration the percent release increased. Because of The lipid lamellae of the stratum corneum of the skin contain a high ratio of negatively charged lipids, which are expected to interact with cationic liposomes. Transfer of some of the bilayer components of the liposomes to the skin is then induced. Several studies have demonstrated that positively charged liposomes have a remarkable effect in enhancing the penetration of drugs across the skin as the latter is negatively charged and favors the electrostatic attraction for positively charged liposome [15]. The *in-vitro* release study for formulation of sodium cholate concentration was showed that increased concentration the percent release decreased [16,17]

***Ex-vivo* study:** The *in-vitro* skin permeation study was performed in Franz diffusion cell using ear goat membranes maintain at 37 ± 1°C. Here also increasing release with the increased in concentration of phospholipid ratio. After that further increased in cholesterol concentration showed decline in release. *In-vitro* skin permeation study of liposomes using phospholipids. They observed that increased alkyl chain of lipid slower or sustained the percent drug release stearylamine effect in enhancing the penetration of drugs across the skin as the latter is negatively charged and favors the electrostatic attraction for positively charged liposome

Stability study of optimized formulation: The liposome was unstable at room temperature and 40°C. Stability studies revealed that the physical appearance, rheological properties, drug release in the prepared liposome remained unchanged upon storage at 2-8°C for 3 months.

Conclusion

Modified liposome is used in transdermal drug delivery system (TDDS) which means transport of therapeutic substance through the skin for systemic effect. Topically administrated drug formulation intended to the controlled and continuous delivery. The quantity of cholesterol is increased then vesicle size is reduced. Also lipid concentration show that retention of drug can be enhanced by employed phospholipids increases entrapment efficiency show increase solubility and enhance permeation through bilayer membrane. Modified liposomes of optimized liposomal batches were formulated. Further modified liposomes were evaluated for entrapment efficiency, *in-vitro* release, zeta potential, and vesicle size, *ex-vivo* study. Thus, positive charged liposome seen to be promising as carrier for pramipexole drug thought transdermal drug delivery system.

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