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Research Article

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Preparation and Evaluation of Proliposomes Containing Glibenclamide

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Abstract The aim of the present study was to investigate the proliposome as potential carriers for efficient oral delivery of GIibenclamide in order to improve its bioavailability. The Glibenclamide loaded proliposomes were prepared using hydrogenated soyphosphatidylcholine (HSPC), cholesterol and using sorbitol as the carrier in varying ratios and characterized for entrapment efficiency, vesicle size, The formation of liposomes and surface morphology of optimized proliposome formulation was studied by optical and Electron transmission microscopy, respectively. *In vitro* release and dissolution study carried out provide an insight on the stability and enhanced dissolution of Glibenclamide from proliposome formulations. Several factors, level of lipid, content of cholesterol were investigated and optimized.

Keywords Proliposome, *in-vitro*, HSPC, Surface morphology

Introduction

The effectiveness of several drugs is often limited due to their potential to reach the site of therapeutic action. In most cases (conventional dosage forms), only a small amount of administered dose reaches the target site, while the majority of the drug distributes throughout the rest of the body in accordance with its physicochemical and biochemical properties. Thus, the fundamental goal of drug therapy is to provide therapeutic amount of drug to targeted site in the body to promptly achieve and maintain optimum plasma drug concentration in order to produce desired effect [1]. In recent years, significant efforts have been devoted for the development of new drug delivery system to improve the therapeutic efficacy and safety of the existing drugs by altering the bio-distribution pattern of the drug, by reducing the amount and frequency of dosing. Drug delivery system using colloidal particulate carriers such as liposomes [2], niosomes [3]or proliposomes proved to have distinct advantage over conventional dosage with an increasingly important role in drug delivery, on account of their small size, better drug targeting, delivery and release, with their additional potential to combine diagnosis with therapy [4]. Liposomes and niosomes have attracted an immense attention in the delivery of drugs because of their numerous advantages *i.e.* biodegradablity, non-toxicity, amphiphilic nature, penetration enhancer property and modulation of drug release properties [5]. Although application of liposomes for improved drug delivery is encouraging, they exhibits some difficulties including the instability of aqueous dispersions on storage and the leakage of the encapsulated drugs, the high cost of synthetic phospholipids and variable purity of natural phospholipids have raised concerns over the adoption of liposomal drug delivery systems. An alternative approach involves formation of liposome-like vesicles from non-ionic surfactants, commonly referred as to as niosomes that overcomes several of these problems associated with liposomes [6]. Niosomes are hydrated mixtures of cholesterol and non-ionic surfactant which are quite stable and require no unique conditions such as low temperature or inert atmosphere for fabrication or storage and the relatively low cost of the materials make niosomes more attractive than liposomes for industrial manufacturing. Inspite of several advantages over liposomes, aqueous suspensions of niosomes may exhibit problems of physical

instability such as aggregation, fusion, leaking of entrapped drug or hydrolysis of encapsulated drug thus limiting their shelf life. The most recent advancement in the field of vesicular delivery is to formulate a dry product (proliposmes) which could be hydrated immediately before use and would avoid many of the problems associated with aqueous niosome dispersions and problem of physical stability (aggregation, fusion, leaking) could be minimized with potential for drug delivery via oral route. The additional convenience of the transportation, distribution, storage, and dosing would make 'dry proliposomes' a promising pharmaceutical product.

Glibenclamide (GLB) is a second–generation orally administered sulphonylurea derivative with potent hypoglycemic activity, is a poorly water-soluble BCS Class II drug and is usually available as conventional oral dosage form containing 5 mg of the active ingredient. However, existing formulations exhibit poor solubility in gastrointestinal fluids, which can give rise to variation in its dissolution rate and incomplete and/or unpredictable bioavailability. Because GLB is characterized by a short half- life time and requires chronic administration, controlled release formulations, as a result of improved therapeutic effect and patient compliance by reducing dosing frequency, a more constant prolonged therapeutic effect and possible enhanced bioavailability. An appropriately designed extended release drug delivery system can be a major advance towards solving such problems [7].

Materials and Methods

Materials

Glibenclamide was obtained as gift from Cipla, Mumbai (India). Methanol and Chloroform absolute were obtained from Merck KgaA, (Germany). Hydrogenatedsoyphosphatidylcholine was kindly provided by Lipoid, (Germany). Cholesterol was purchased from Sigma Aldrich, (USA), Sorbitol was supplied by S. D. Fine Chem Ltd, Mumbai (India), Disodium hydrogen phosphate, Sodium chloride and Potassium dihydrogen phosphate was purchased from Qualigens Fine Chemicals Ltd. Mumbai, (India).

Development Powder proliposoms

Proliposomes containing Glibenclamide were prepared by film deposition carrier method [8], and the composition was represented in Table 1. In brief, accurately weighed amounts of lipid mixture comprising of HSPC and cholesterol at various molar ratios (1:2, 1:4, 1:6, and 1:8 respectively) and drug (10 mg) were dissolved in 20 mL of solvent mixture containing chloroform and methanol (9:1). The resultant solution was transferred into a 250 mL round bottomed flask, and spray dried sorbitol (10 mg) was added to form slurry. The flask was attached to a rotary flash evaporator (perfit, India), and the organic solvent was evaporated under reduced pressure at a temperature of 45 ± 2 ºC. After ensuring the complete removal of solvent, the resultant powders were further dried overnight in a vacuum oven at room temperature so as to obtain dry, free-flowing product. The obtained proliposome powders were sieved with a 60 mesh screen and stored in a tightly closed container at cool condition for further evaluation.

Table 1: Composition of different proliposomal formulations

S. No.	Formulation Code	Drug: HPSC:Sorbitol (µmol)	Drug of (mg)	HPSC (mg)	Cholesterol (mg)	Sorbitol (mg)
1	F1	1:02:01	10	20	-	10
2	F2	1:04:01	10	40	-	10
3	F3	1:06:01	10	60	-	10
4	F4	1:08:01	10	80	-	10
5	F5	1:4:1:1	10	40	10	10
6	F6	1:4:2:1	10	40	20	10
7	F7	1:4:3:1	10	40	30	10
8	F8	1:4:4:1	10	40	40	10

Characterization of proliposomes

Light microscopy A thin layer of 0.2 g proliposome was spread in a cavity slide, and then a cover slip was placed. Slide was observed under ordinary light microscope (BA-310, Motic, USA) at 100x magnification. A drop of water was added through the side cover slip into the cavity slide while under microscope and observed again. Photomicrographs was taken at suitable magnification after addition of water.

Vesicle size analysis

The size and size distribution of the liposomes formed from the hydration of the proliposomeswas determined using zetasizer (Malvern Instruments, UK) at 25°C using disposable sizing cuvettes. 1 ml sample was taken for particle size analysis and three replicates were taken for each sample. Polystyrene beads were used as a standard to check instrument performance. The polydispersity index (PI) was determined as a measure of homogeneity. Small values of PI (<1) indicate a homogeneous population.

Glibenclamide Entrapment efficiency (EE%)

The Glibenclamide content was determined by dissolving the proliposome powder (10mg) in 10 mL of solvent mixture comprising of methanol:water (60:40 v/v) An aliquot of sample was taken in microcentrifuge tubes and followed by centrifugation at 10,000 rpm for 20 min. The supernatant was separated, suitably diluted with methanol, and analyzed by UV-Visible spectrophotometer.

The entrapment efficiency of the liposomal formulation was determined by measuring the concentration of drug in the dispersion medium. The experiment was performed in triplicate, and percentage entrapment of Glibenclamide in liposomes was calculated from the following equation:

$$\text{Entrapment efficiency (EE\%)} = \frac{\text{Theortical drug content}}{\text{Practical drug content}} \times 100$$

Transmission electron microscopy

The outer surface morphology of optimized drug loaded Proliposomes were studied by TEM. For TEM study, one drop of diluted liposome suspension was deposited on a copper grid and then negatively stained with one drop of 2 % (w/v) aqueous solution of phosphotungstic acid (PTA) for contrast enhancement. The excess staining solution was removed with filter paper within 60 seconds and the sample was allowed to dry before examined under the TEM (QingzhiLva).

Differential scanning calorimetry (DSC)

To investigate the possible interaction between Glibenclamide and vesicle ingredients, samples of 6 mg of each of Glibenclamide, Cholesterol, and drug loaded proliposome derived liposomes was submitted to DSC analysis using differential scanning calorimeter (Perkin Elmer, DSC – 4000, USA). Each sample was sealed in a standard aluminum pan and scanned between 40 °C and 350 °C while another empty pan was used as a reference. The thermograms was obtained at a scanning rate of 10 °C/min.

***In vitro* release study**

In vitro dissolution study of proliposomal powders and control (pure drug) was performed using USP type II (paddle) apparatus (Labindia DS 8000, Mumbai, India) in phosphate buffer (0.1 N HCl). The volume of dissolution medium used was 900 ml and maintained at a temperature of 37 ± 0.5 °C with paddle speed set at 50 rpm throughout the experiment. An aliquot of 5 ml was collected at predetermined time intervals up to 8 hours and replaced with fresh dissolution medium to maintain constant volume. Samples were centrifuged for 15 minute in cooling centrifuge at 15000 rpm (REMI, India) and analyzed by UV.

Results and Discussions

Characterization of proliposomal Powder

Light microscopy

The prepared vesicles were studied under 100x magnification to observe the formation of vesicles. The liposomes are unilamellar spherical vesicles with smooth surface. The vesicles obtained are discrete and separate with no aggregation or agglomeration (fig.1).

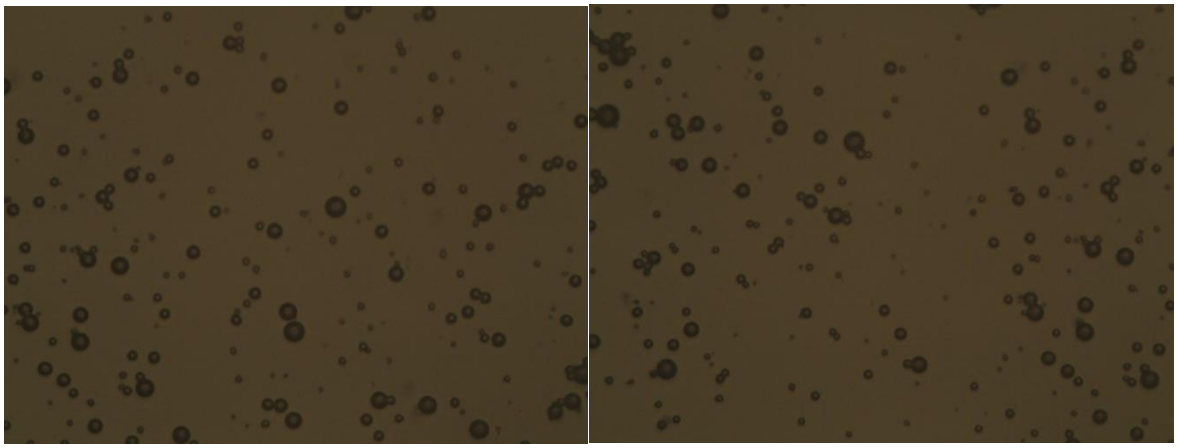

Figure 1: Microscopic images of optimized formulation

Particle size analysis

Vesicle size and size distribution is an important consideration for the vesicular systems. The particle size and polydispersity index of the prepared optimized proliposome formulation was found to be 232.4 nm and 0.506 respectively. The size and surface charge of the vesicles mainly depends on the cholesterol concentration. The small value of the polydispersity index (PDI) (<1) indicates a homogenous population. The PDI, used as a measure of a unimodal size distribution, was within the acceptable limits for all the proliposomal formulations.

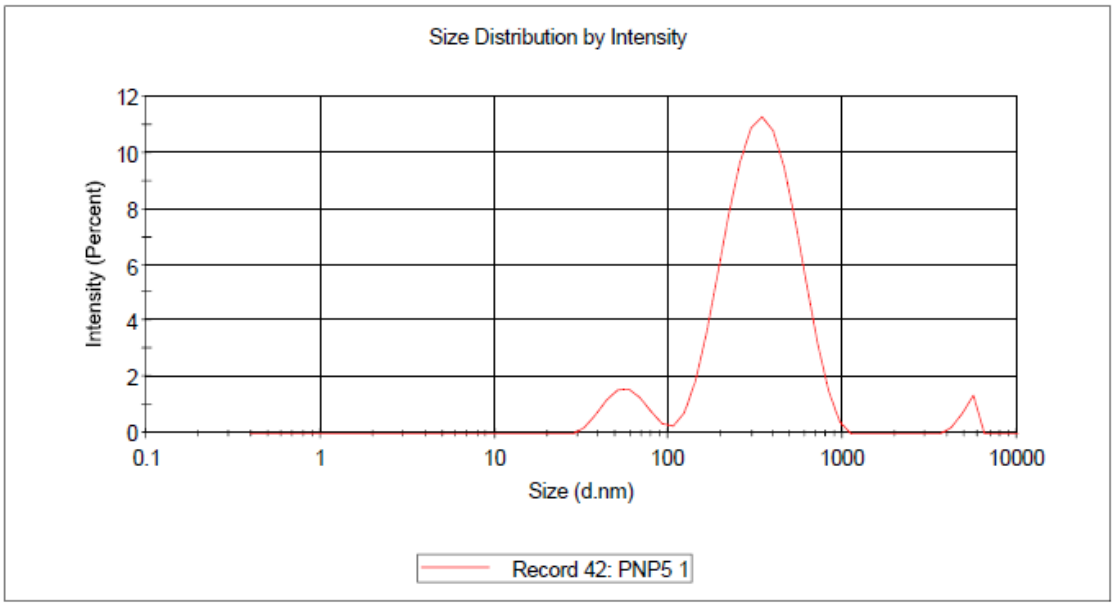

Figure 2: Particle size and size distribution of optimized formulation F7

Glibenclamide Entrapment efficiency (EE %)

The entrapment efficiency of proliposome formulations was between 66.32% and 83.79%. The entrapment efficiency of Glibenclamide is dependent on the composition of liposomes. Increasing the cholesterol the EE% increased However, further increase cholesterol resulted in EE% significant decrease. This is because cholesterol molecules accommodate itself as ‘‘vesicular cement’’ in the to optimize molecular cavities formed when surfactant monomers are assembled into bilayers to form liposoml membranes [8]. This space filling action was responsible for the increased rigidity, decreased permeability of cholesterol-containing membranes compared to cholesterol-free membranes and the improved entrapment efficiency. In contrast, on increasing cholesterol beyond a certain concentration it may compete with the drug for the space within the bilayers, hence excluding the drug and can disrupt the regular linear structure of vesicular membranes [9].

Table 2: Entrapment Efficiency of different Proliposomal formulations

S. No.	Formulation code	% Drug Entraplment Efficiency± SD
1	F1	66.32±1.23
2	F2	68.97±1.47
3	F3	65.27±1.67
4	F4	61.78±2.04
5	F5	76.38±1.89
6	F6	79.86±1.72
7	F7	83.79±1.08
8	F8	78.48±1.98

Transmission electron microscopy

Transmission electron micrographs revealed that most of the vesicles are well identified, spherical and discreet with sharp boundaries having large internal aqueous space after hydration of proliposomes.

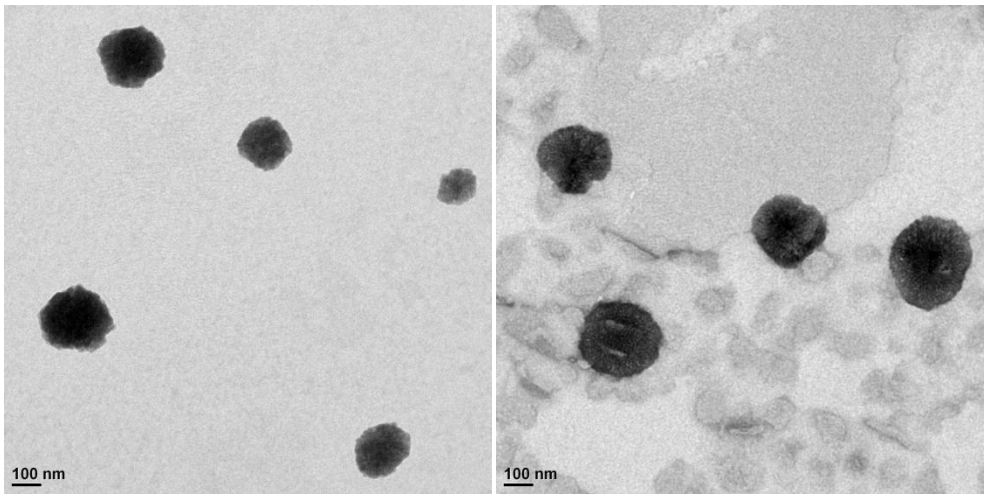

Figure 3: Transmission electron micrographs of liposomes formed after hydration of formulation F7

Differential scanning calorimetry

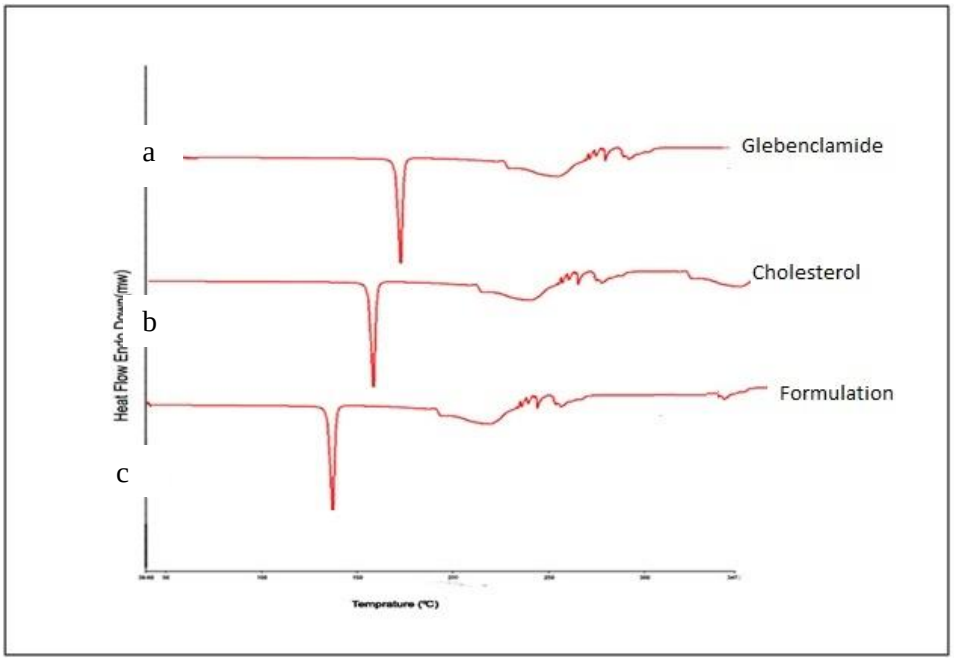

Figure 4: DSC thermograms of (a) Glibenclamide (b) Cholesterol (c) Drug loaded proliposome, F7

DSC thermograms of Glibenclamide, Cholesterol and drug loaded liposomes are illustrated in Figure. Glibenclamide, Cholesterol showed endotherms at 178 °C and 156 °C respectively, resultant to their melting temperatures., thermogram of Glibenclamide loaded liposomes revealed a disappearance of the characteristic endothermic Glibenclamide peak, and the endotherm of the liposomal bilayer was shifted to peak at 84.96 °C. These results propose the dispersion and entrapment of Glibenclamide in to the bilayers of liposomal vesicles.

***In vitro* release studies**

The dissolution profile of proliposomes containing Glibenclamide in 0.1 N HCl (pH 1.2) is shown in the Figure. The dissolution of Glibenclamide from proliposomes was found to be 3.0–3.5 times higher than the pure drug (control). The percent drug release and percent dissolution efficiency was significantly higher for optimized proliposome formulations, compared to control (Table 4). This indicates that the presence of phospholipid in the proliposome formulation enhanced the solubility of Glibenclamide, which might be the result of improved surface area of drug molecules and also a change in the physical nature of the drug from the crystalline to the amorphous state.

Table 3: *In-Vitro* drug release study of Optimized formulation F7 and Pure drug suspension

Time (hr.)	Formulation F7	Pure drug suspension
0	0±0	0±0
0.5	11.23±0.94	2.89±0.54
1	18.96±1.26	5.78±0.69
2	29.78±1.98	9.89±0.74
3	44.98±1.85	15.59±1.23
4	61.78±2.04	18.74±1.04
5	72.08±2.11	21.74±1.17
6	76.89±2.45	24.89±1.19
7	80.98±3.12	26.55±1.42
8	83.59±3.47	27.11±1.47
10	84.78±3.57	28.46±1.96
12	85.69±3.41	28.94±1.78

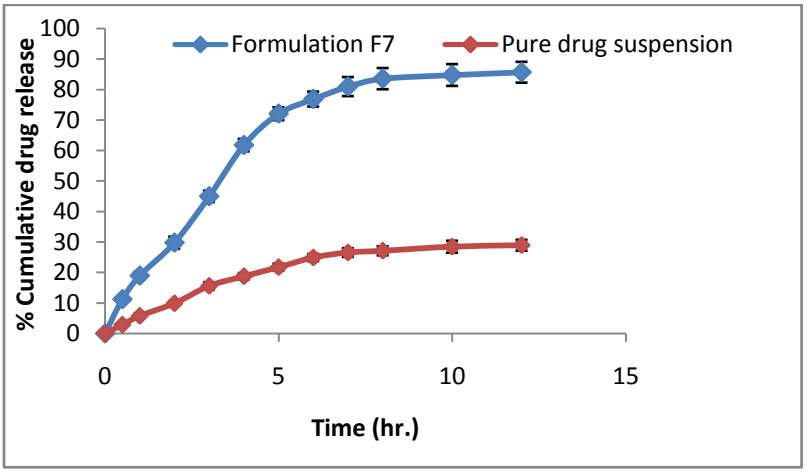

Figure 5: In vitro drug release for formulation F7

Conclusions

The proliposome drug carriers could be successfully developed for the oral delivery of Glibenclamide. The proliposome powders did obviate the physicochemical problems of the liposomes. The formulation containing HSPC and cholesterol in the ratio of 4:3 seems to be more stable with desired physicochemical and intestinal

permeability characteristics. The optimized Glibenclamide proliposomes improved the solubility of Glibenclamide, when compared to pure drug suspension.

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