



## Formulation and evaluation of glimepiride loaded liposomes

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### ABSTRACT

The present study reveals the formulation and evaluation method of Glimepiride loaded liposomes. Glimepiride, a third generation sulfonylureas class drug, for treatment of type 2 diabetes was selected. Rotary film evaporator was employed for the formulation of liposomes by using thin film hydration method. The liposomal formulations of Glimepiride were prepared by using phosphotidyl choline (lecithin) and cholesterol. The prepared liposomal formulations were evaluated for different parameters including refractive index and pH measurement. By the FT-IR and DSC studies revealed that the raw materials used for preparation of liposomes were showing no interaction with the drug Glimepiride. The remarkable variations in the results revealed the dependence on ratio of lecithin to cholesterol. The drug releases at a targeted site can be achieved via liposomes followed by diffusion mechanism.

**Keywords:** Antidiabetic; Drug entrapment efficiency; Glimepiride; *In-vitro* release studies; Liposomal formulations; Refractive index; Thin film hydration method.

### INTRODUCTION

A remarkable quantity of research is done by researchers in various oral and parenteral formulations to formulate drugs in controlled and sustained release dosage forms. To achieve greater efficacy, minimize toxic effects, effective site specific drug delivery controlled dosage forms plays a greater role when compared to conventional ones (Sahoo SK et al, 2003). Liposomes are microscopic multilamellar structures. They are primed by the mixture of soya lecithin and cholesterol with ensuing hydration using aqueous media (Gabizon A et al, 1998). Liposomes are mostly targeted for delivering drugs in a controlled form for treating various diseases and viral infections. With their slow releasing vehicles, liposomes are known to be used for application of topical drugs (Allen TM, 1997).

For treating patients with type II diabetes mellitus, an oral antidiabetic drug, Glimepiride is commonly prescribed. Glimepiride is a sulfonylureas class drug with molecular formula C<sub>24</sub>H<sub>34</sub>N<sub>4</sub>O<sub>5</sub>S. The chemical structure of Glimepiride is displayed in the figure 1. The weak acidic drug, Glimepiride, has its pKa value as 6.2. According to the Biopharmaceutical classification System (BCS) Glimepiride has no practical solubility in water and acidic environment but has high permeability (class II) (Hindustan Abdul Ahad et al, 2010). For its oral bioavailability, its poor aqueous solubility is the main

constraint. The main aim of this study was to improve the solubility, dissolution property and controlled releasing property of the drug. Using thin film hydration method in a rotary film evaporator liposomes were formulated. The liposomal formulations of Glimepiride were prepared by using phosphotidyl choline (lecithin) and cholesterol.

### MATERIAL AND METHODS

Glimepiride was procured from KrebZ Healthcare Pvt Ltd (Chennai, India); Lecithin was procured from Mumbai HiMedia Laboratories Pvt Ltd; Cholesterol was procured from Mumbai Thomas Bakers Chemical Ltd. Rest analytical grade ingredients were used. Thin film hydration method was employed for the formulation of Glimepiride liposomes.

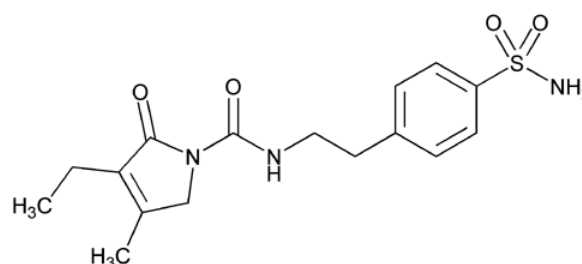


Figure 1: Chemical structure of Glimepiride

### Drug-polymer compatibility studies

The drug-polymer compatibility studies were performed using DSC and FT-IR studies. The results showed no drug-polymer interaction.

### Preparation of Glimepiride liposomes

Liposomal formulations of Glimepiride were formulated by the technique, thin film hydration method

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(Chanda H et al, 2011). Lecithin and cholesterol were dissolved at different ratios in a round bottom flask with organic solvent ether (Sipai ABM et al, 2012). The resultant solution was fitted with rotary film evaporator kept at an angle of 45°. The flasks were rotated for 30 to 40 mins at 100 rpm by passing atmospheric inert air nitrogen. On the wall of round bottom flask, thin dried lipid film was formed. Required quantities of Glimepiride (100 mg) were dissolved in pH 7.4 phosphate buffered saline. The round bottom flask was turned and liquid phase having drug to be entrapped was moved through the side of flask and was slowly returned to upright position. For complete swelling the fluid was passed slowly over non-aqueous layer and flask was left aside for 2h at 37°C. The vesicles are harvested to yield milky white suspension by swirling the contents of the flask followed by centrifugation in a centrifuge. Suitable formula was selected from the different batches of liposomal formulations. By using the above explained common method, the six batches of liposomal formulations were prepared. The various lipid compositions for the preparation of liposomes has been tabulated in Table 1.

#### Zeta potential and particle size distribution

The globules size in liposomes was found out by using photon correlation spectroscopy. The fluctuations in scattering of light due to Brownian motion of the particles were analyzed using a Zeta sizer (Nano ZS-90, UK). 0.1ml formulation was dispersed in 50 mL distilled water and subsequently mixed and scattering of light was monitored. Zeta potential measurement was done by using a disposable zeta cuvette. The mean diameter/zeta potential  $\pm$  standard deviation of all determinations was calculated for each sample by multimodal analysis (Behfar Moghaddam et al, 2011).

#### Liposomal drug entrapment efficiency

Liposomal drug entrapment efficiency of liposomes was found out done by using centrifugation method (Bhalerao SS et al, 2003). The liposomal dispersion (1.0ml) was subjected to centrifugation at 3500 rpm for 90 min. The clear supernatants and non-entrapped Glimepiride were separated and absorbance was noted at 228 nm. With phosphate buffer pH 7.4(100ml), the sediment in the centrifugation tube was diluted and the absorbance of this solution was noted at 228 nm. The additive quantity of Glimepiride in supernatant liquid and sediment gave a total quantity of Glimepiride in liposomal dispersion.

The % of drug entrapped was determined by the formula:

$$\% \text{ drug entrapped} = \frac{\text{Amount of drug in sediment}}{\text{Total amount of drug loaded}} \times 100$$

#### Viscosity determination

Brookfield viscometer was used for viscosity determination. The angular velocity of the viscometer was in-

creased gradually from 2 to 50 rpm. Viscosity was obtained by calculating the mean of the two readings. The pH of the formulation was raised to 7.4 by using 0.5 M NaOH by hallipath spindle. By using Brookfield viscometer the rheology of the resultant formulation was studied (Bergman M et al, 1998).

#### Refractive index and pH measurement

Abbe type refractrometer was used in studying the refractive index of selected formulations. The obvious pH of liposomal formulation was measured by digital pH meter in triplicate at 25 $\pm$ 1°C.

#### In-vitro release study

*In-vitro* release studies of liposomal formulations of Glimepiride were carried out using standard Franz diffusion cells. An area of 0.75 cm<sup>2</sup> was selected as diffusion area and receptor volume was selected as 5.0 ml. After filling the receptor chambers with of PBS (5ml) at pH 7.4, by using small magnetic bars it is stirred continuously. Stir the receptor fluid with a magnetic rotor at 35 $\pm$ 0.5°C, which is ocular surface temperature. Separate the both chambers by means of activated dialysis membrane bag with molecular weight 12,000 Da. One milliliters of each formulation were filled into the donor compartment and the chamber was occluded with Paraffin. The withdrawn samples subjected to filtration by using 0.45  $\mu$ m membrane filter. The drug content analysis was done by UV spectrophotometer at 228 nm (Zhang JA et al, 2005).

#### In-vitro drug release kinetic studies

To determine the release kinetics of Glimepiride from the liposomal formulations various data from *in-vitro* release were fixed into different equations and kinetic models. Zero-order equation, Higuchi's model and Peppas's models were used as kinetic models. By using the results received in these formulations various model treatment were plotted. i.e. (Higuchi's) Cumulative percentage release of drug Vs Square root of time and (Peppas) Log cumulative percentage release Vs Log time. The mechanism of drug release of Glimepiride from the liposomal formulation was studied by fitting the drug release data into various mathematical kinetic models (Peschka R et al, 1998).

Determination of mechanism of drug release from liposomal formulations, the *in-vitro* dissolution data of each liposomal formulation were done with different kinetic drug release equations. Zero order:  $Q=K_0t$ ; Higuchi's square rate at time:  $Q=K_Ht^{1/2}$  and Peppas:  $F=K_m t^n$ , where Q - drug release amount at time t, F - drug release fraction at time t,  $K_0$  - zero order kinetic drug release constant,  $K_H$  - Higuchi's square root of time kinetic drug release constant,  $K_m$  - constant incorporating geometric and structural characteristic of the tablets and n - the diffusion exponent indicative of the release mechanism. The correlation coefficient values (r) from Higuchi's model point out the kinetic of drug

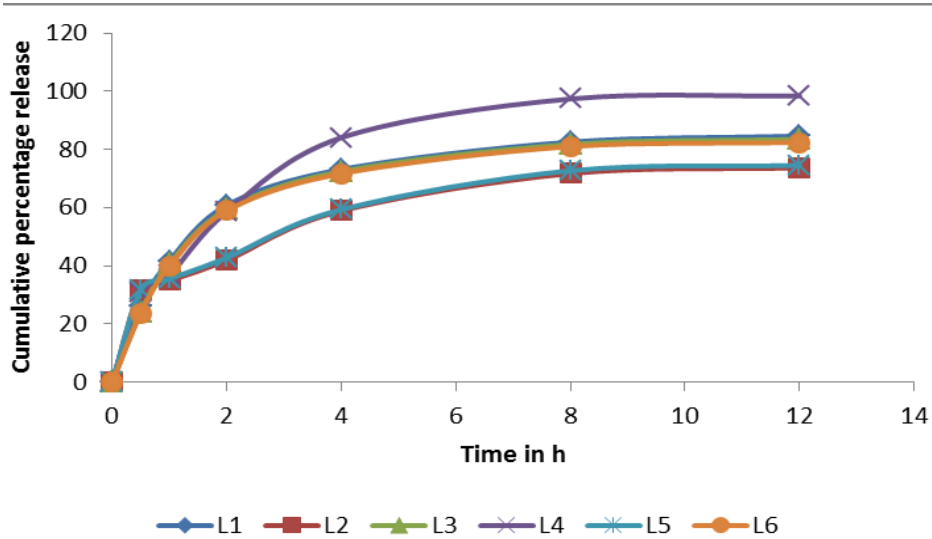


Figure 2: In-vitro drug release plot of L1-L6

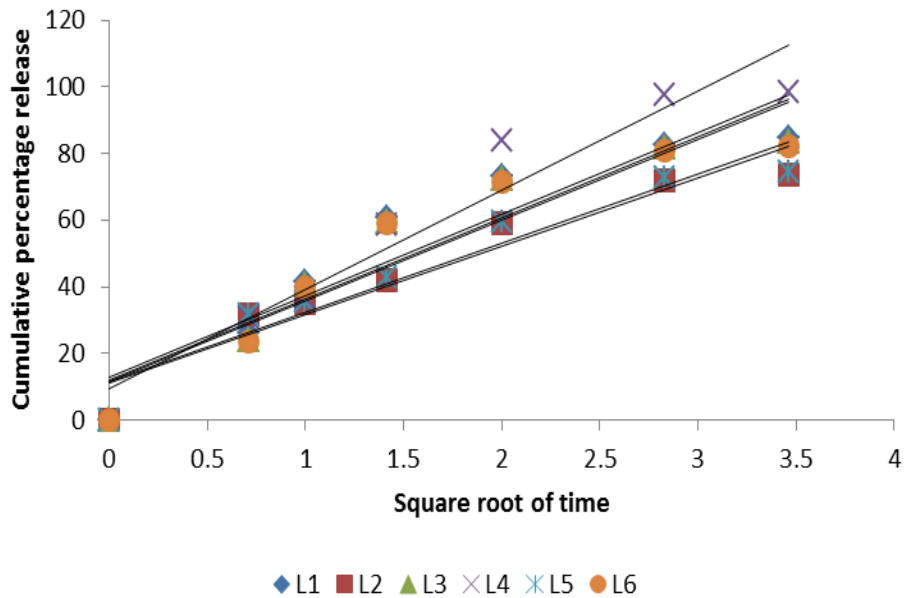


Figure 3: Higuchi's plot of L1-L6

Table 1: Composition of lipids

Formulation code	Lecithin in mg	Cholesterol in mg
L1	540	60
L2	480	120
L3	420	180
L4	360	240
L5	300	300
L6	240	360

release and diffusion exponent values (n) from Peppas model point out the mechanism of drug release.

**RESULTS AND DISCUSSION**

From the various methods for the formulation of liposomes, thin film hydration method was chosen for the production of Glimepiride liposomes. The liposomal formulations of Glimepiride were prepared by using

phosphotidyl choline (lecithin) and cholesterol with different ratios.

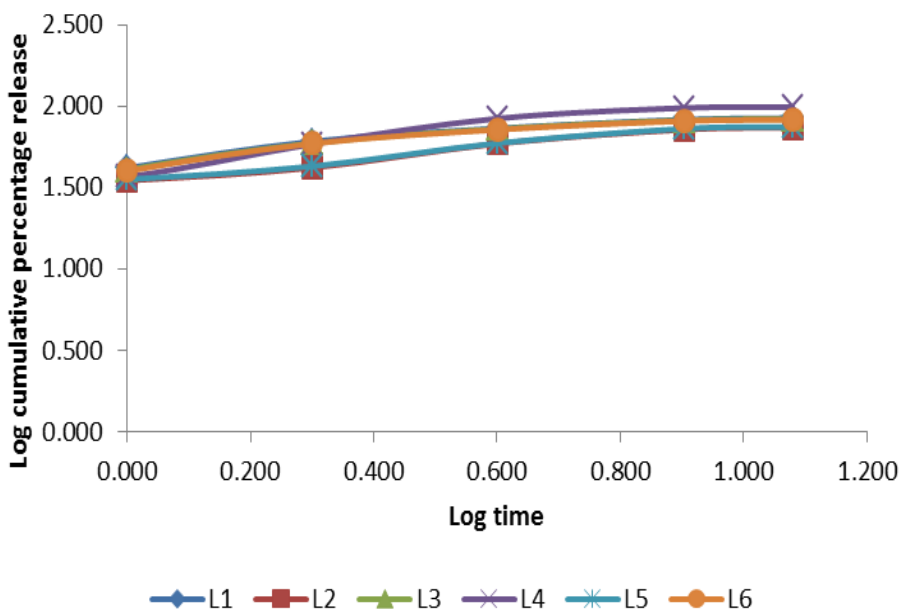
The spectra obtained from the FT-IR and DSC studies proved the lipids and API were compatible. The size of globules in liposomes was calculated by photon correlation spectroscopy using a Zeta sizer. The zeta potential and particle sizes of liposomes are ideal. Based on

**Table 2: Mean droplet size and zetapotential measurement**

Formulation code	Mean droplet size (nm±SD)	Zeta potential (mV±SD)
L1	24.82±2.324	-21.33±0.106
L2	37.64±2.387	-41.32±0.442
L3	23.21±3.783	-28.77±0.602
L4	10.08±1.132	-37.83±0.934
L5	27.82±2.126	-40.13±0.231
L6	23.64±1.642	-37.37±1.154

**Table 3: Viscosity, refractive index, pH measurement and Drug entrapment efficiency**

Formulation code	Viscosity (cps)	Refractive index	pH	Drug entrapment efficiency (%)
L1	8.18±1.01	1.471±0.001	6.6±0.04	98.24
L2	8.14±0.96	1.469±0.003	6.7±0.02	97.56
L3	8.31±0.99	1.465±0.003	6.6±0.03	98.87
L4	8.23±0.91	1.472±0.002	6.6±0.06	98.98
L5	7.84±1.21	1.469±0.002	6.6±0.04	87.64
L6	7.96±1.18	1.467±0.001	6.7±0.05	87.21



**Figure 4: Peppas's plot of L1-L6**

the particle size analysis the formulation F4 showing more SUVs among the other liposomal formulations and the results were tabulated in the table 2.

From the drug entrapment efficiency results, it is clear that drug entrapment efficiency of liposomal formulations is reduced with the lowering of concentration of lecithin. This is because of the fact that low lecithin content provides less drug entrapment efficiency. The ability of formulation to withstand drug molecules in the bilayer membrane of the vesicles was proves the encapsulation efficiency of liposomal formulations. Cholesterol increases the fluidity property of the bilayer membrane and improves the retainability of bilayer membrane in the presence of biological fluids. From the percentage of drug entrapped results it was found that with the increase in the percentage of cho-

lesterol the stability and rigidity of liposomes was successively increased but at the same time percentage drug entrapment reduced with the reduction in Lecithin. The prepared liposomal formulations also evaluated for viscosity refractive index and pH measurement. The obtained results were tabulated in the table 3.

*In-vitro* dissolution study performed was by using standard Franz diffusion cells. The release profile of the all prepared nanoemulsions formulations are presented in the figures 2 to 5. The maximum percentage of Glimepiride release was observed in the formulation L4. As expected for the liposomes, fast drug release behavior was observed due to the enhanced dissolution and forming of the lipid vesicles as much as smaller in size of the vesicles. Distinguishable differences

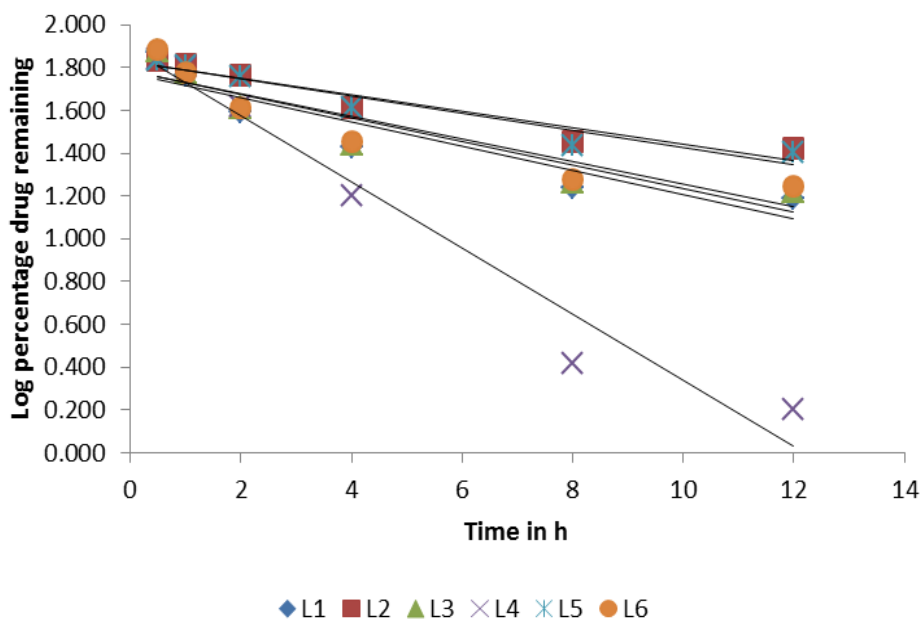


Figure 5: First order plot of L1-L6

Table 4: Diffusion characteristics of formulations L1-L6

Formulation code	Correlation coefficient values (r)			Diffusion exponent value (n)
	Zero Order	Higuchi's Model	First order	
L1	0.816	0.942	0.931	0.362
L2	0.856	0.962	0.962	0.295
L3	0.819	0.943	0.925	0.378
L4	0.862	0.965	0.982	0.404
L5	0.856	0.963	0.962	0.297
L6	0.818	0.942	0.92	0.381

were observed in the other liposomal formulations. The *in-vitro* dissolution data of each formulation were calculated with different kinetic drug release equations to determine the mechanism of drug release from hydrophilic matrices. The kinetics of drug release were by the correlation coefficient values (r) from Higuchi's mode and diffusion exponent values (n) from Peppas's model indicate the mechanism of drug release. Different release mechanisms and the diffusion characteristics were characterised by using the n value of all formulations and were tabulated in the table 4.

## CONCLUSION

Glimepiride liposomes were successfully prepared by using different ratios of lecithin and cholesterol. The method employed for the preparation of liposomal formulations is lipid thin film hydration method and the equipment used was rotary film evaporator. Liposomes are somehow stable system for the targeted drug delivery. The size and size distribution analysis was carried out on selected formulations. The viscosities, refractive index, pH and drug entrapment efficiency of the optimized formulations were determined. *In-vitro* dissolution study was performed by using standard Franz diffusion cells. Distinguishable differences were observed in all liposomal formulations. The maximum percentage of Glimepiride release was observed

in the formulation L4. From the research it was concluded that the Glimepiride is one of the good candidate for the successful development of liposomes for its therapeutic activity.

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