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Research Article

FORMULATION AND EVALUATION OF GLIMEPIRIDE-LOADED LIPOSOMES BY ETHANOL-INJECTION METHOD

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ABSTRACT

Objective: The objective of present study deals with an attempt to formulate Glimepiride in liposomal drug delivery system, in order to maintain steady state plasma concentration; hence it has to be administered frequently to the patients, because of its short plasma half life. To maintain steady plasma concentration for longer period of time formulation of controlled drug release system is essential. Liposomal drug delivery system provides sustained release of Glimepiride.

Methods: Glimepiride liposomes were prepared by ethanol injection method using varying concentration of cholesterol and lecithin. Drug excipient compatibility study was performed by FT-IR spectral studies and differential scanning calorimetry studies. Liposomes were evaluated for drug encapsulation efficiency, morphological study by optical microscope, SEM and TEM, vesicle size and zeta potential determination, *In -vitro* drug release & kinetic study and stability studies.

Results: The formulations fulfilled all official requirements. The drug release was slow and sustained for >12 hrs. The formulations followed zero order kinetics. Zeta potential and stability study for 90 days demonstrated that the formulation was stable at 25¬¬¬¬oC than when refrigerated.

Conclusion: Glimepiride was formulated as liposomal formulation after checking the compatibility by DSC and FT-IR studies. The liposomes preparation FA3 was optimized based on the particle size, zeta potential, entrapment efficiency, and drug release characteristics.

Keywords: Liposomes, Antidiabetic, Glimepiride, Ethanol-injection method.

INTRODUCTION

Liposomes are small artificial vesicles of spherical shape that can be created from cholesterol and natural nontoxic phospholipids [1-3]. Depending on the nature of the drugs, liposomes have one or more phospholipid bilayers, to transport drug materials [4]. The particle size of liposomes varies from 30 nm to several micrometers [5,6]. Liposomes help in increasing the therapeutic index of the administered drugs [7]. In liposome formulations, water soluble and insoluble materials can be used together without the use of surfactants and emulsifiers.

Glimepiride, a third generation sulfonyl urea drug is commonly prescribed for diabetic patients with type 2 diabetes mellitus. It lowers blood sugar by stimulating the release of insulin by pancreatic beta cells and by inducing increased activity of intracellular insulin receptors. Considering the solubility, it has no practical solubility in water (<0.004 mg/mL). The main factor which affects the bioavailability of drugs is patient noncompliance due to frequent administration of dosage forms [8-12]. Sustained release formulation scan helps in overcoming this problem by its one daily dosing. The dosage schedule and pharmacokinetics help in the formulation of sustained release dosage form, liposomes. Hence, the study has been focused on the formulation and *in vitro* evaluation of glimepiride-loaded liposomes by modified ethanol injection method.

METHODS

Glimepiride was procured from Krebs Healthcare Pvt. Ltd. Soya phospholipids and Tween-80 were purchased from HiMedia Laboratories Pvt. Ltd. Cholesterol was purchased from Mumbai Thomas Bakers Chemical Ltd. All the other ingredients used are of analytical grade. The method applied for the formulation of liposomes was modified ethanol injection method.

Preformulation studies

Preformulation studies were performed for physical appearance, solubility, melting point, and drug-polymer compatibility studies. The physical appearance of the drug was noted by visual observation. Solubility studies were performed in thoroughly cleaned and dried volumetric flask using different investigative solvents and the drug concentration was determined spectrophotometrically [1]. Melting point determination of glimepiride was determined by using the melting point apparatus. Drug-polymer compatibility studies were studied using differential scanning calorimetry (DSC) and Fourier transform-infrared spectroscopy (FT-IR) studies. The results did not show any drug-polymer interaction.

Preparation of glimepiride-loaded liposomes

As the drug is soluble in ethanol, a modified ethanol injection is used to prepare glimepiride-loaded liposomes. The required amounts of soybean phospholipids, Tween-80 and cholesterol were dissolved in ethanol. The resulting organic phase was gently injected to the 55±2°C aqueous phase under magnetic stirring. Spontaneous liposome formation occurred as soon as ethanolic solution was in contact with the aqueous phase (phosphate buffered saline, pH 7.0). The liposome suspension was then kept under stirring for at room temperature to remove the traces of solvent [13].

Characterization of liposomal formulations

Drug encapsulation efficiency

The percentage of drug encapsulated was determined after lysis of the prepared liposomes with absolute alcohol and sonication for 10 minutes. The concentration of drug, glimepiride, in absolute alcohol was determined spectrophotometrically at 227 nm using an ultraviolet (UV)-visible spectrophotometer (model UV-1700 (E); Shimadzu, Kyoto, Japan) in triplicate. The encapsulation efficiency expressed

as entrapment percentage was calculated through the following relationship [14-16].

% Encapsulation efficiency total drug free drug/total drug×100.

Dynamic light scattering: Vesicle size and zeta potential determination

The average diameter of liposomes was determined by dynamic light scattering using the photon correlation spectroscopy (PCS) technique. The measurements were performed at 25°C using either a Zeta Sizer 4 (Malvern Instruments, Worcestershire, UK) equipped with a He-Ne gas laser (k=0.633 Am) or a Zeta Plus (Brookhaven Instrument Corporation, USA). Brookhaven or Malvern PCS software (version 1.52), depending on the system, was used for data acquisition and analysis. Polystyrene size standards 220T6 nm (Duke scientific corp., Duke, NC) was used to verify the performance of the instrument. For viscosity and refractive index, the values of pure water were used (1.0). The samples were diluted with 10 mM Tris-buffer at pH 7.4 to achieve the optimal vesicle concentration. Surface charge on the vesicles was measured indirectly via analysis of zeta potential at 25°C using a ZetaPlus instrument (Brookhaven Instrument Corporation, USA) in a 1/10 solution of 10 mM Tris-buffer [17].

Turbidity measurement

The liposomes were diluted with distilled water to give a total lipid concentration of 0.312 mM. After rapid mixing by sonication for 5 minutes, the turbidity was measured as the absorbance at respective nm with a UV-visible spectrophotometer (Shimadzu-1700, Japan) [18,19].

Morphological study

Optical microscope

Multilamellar vesicles after dilution with 5% mannitol were viewed under optical microscope (Olympus BHA, Japan) to observe the shape and lamellar nature of vesicles. Photomicrographs were prepared by a camera attached to the optical microscope in 10 × 100 magnifications [20].

Scanning electron microscopy (SEM)

The morphology (shape and surface characteristics) of liposomes was studied by SEM (model JSM-5610LV scanning microscope; Jeol, Tokyo, Japan). The sputtering was done for nearly 5 minutes to obtain uniform coating on the sample to enable good quality SEM images. The SEM was operated at low accelerating voltage of about 15 kV with load current of about 80 mA. The condenser lens position was maintained between 4.4 and 5.1. The objective lens aperture has a diameter of 240 μ and the working distance=39 mM [20].

Transmission electron microscopy (TEM)

Drug-loaded liposomes were observed by TEM using a JEM 1010®, Jeol (USA). Liposomal dispersion (500 μL) was diluted with phosphate buffer and stabilized with glutaraldehyde 2.75%. The samples were prepared by placing the diluted liposomes onto a 400-mesh grid coated with carbon film. TEM images were analyzed using the soft-imaging software ImageJ® (measurement included 100 liposomes) [21].

In vitro drug release and kinetic study

In vitro release of the prepared formulations was evaluated using a dynamic dialysis method. The release rate of drug was determined after separation of free drug from drug-loaded liposomes by placing it in dialysis tubing (10,000 MWCO, Millipore, Boston, USA) and exhaustively dialyzed for 15 minutes for several times, each time against 100 mL of phosphate buffer (pH 7.4). The dialysis of free drugs was completed after 1 hr after which no further drug could be detected in the solution. The dialyzed suspension containing the drug or plain drug aqueous solution was sealed in a dialysis bag (10,000 MWCO, Millipore, Boston, USA). The dialysis bag was then immersed in 100 mL of phosphate buffer (pH 7.4) thermostatically maintained at 37±0.5°C and magnetically stirred at 50 rpm. The samples (1 mL) were withdrawn at various time intervals and analyzed by a UV spectrophotometer at respective nm. Volumes lost by sample withdrawal were replaced with fresh medium. The experiments were conducted in triplicate [22].

The data of *in vitro* release from various liposomes were evaluated kinetically using various mathematical models like zero-order, first-order, Higuchi and Korsmeyer–Peppas model equations.

Stability studies

Physical stability study of the prepared liposomes was carried out to determine the comparative leakage of the drug from liposomes stored at different conditions compared to each other. After washing and removal of the free drug, each liposomal formulation was stored either at 4°C or at 25°C. At predetermined time intervals of 15, 30, 60 and 90 days. The entrapment efficiency of glimepiride-liposomes was determined [23].

RESULTS AND DISCUSSION

Drug encapsulation efficiency

Entrapment efficiency was studied for all the 9 formulations to find the best, in terms of entrapment efficiency. The encapsulation efficacy was obtained as the mass ratio between the amount of the drug incorporated in liposomes and this ratio was used in the liposome preparation. There are reports that entrapment efficiency was increased, with increasing cholesterol content and by the usage of Tween-80 which has higher phase transition temperature. By inspection of Table 1, it is obvious that glimepiride-encapsulation efficiency had higher values in formulation formative assessment (FA) 3.

Dynamic light scattering: Vesicle size and zeta potential determination

Zeta potential is a key factor to evaluate the stability of colloidal dispersion. In general, particles could be dispersed stably when absolute value of zeta potential was above −30 mV due to the electric repulsion between particles. Nonionic surfactant could not ionize into charging group like ionic, but demonstrated its zeta potential, the reason might be due to molecular polarization and the adsorption of emulsifier molecule on the charge in water, it was absorbed to the emulsifier layer of particle/water interface and electric double layer similar to ionic was formed. The results have been mentioned in Table 1.

Morphological study

The images confirmed that liposomes were spherical and smooth in nature. Therefore, it seems that encapsulation of drug did not affect the morphology of liposomes. The TEM study demonstrated that the particles had almost spherical and uniform shapes and did not stick to each other TEM confirmed the formation of liposomes. The morphological images have been shown in Figs. 1-3 of optical microscopy, SEM and TEM.

Table 1: Results of various characterizations of prepared liposomes

Code	Encapsulation efficiency	Zeta potential (mV)	Particle size (nm)	Turbidity
FA 1	82.42±2.45	−19.6±3.8	215.24±8.32	123±2.3
FA 2	92.62±2.37	−17.8±2.6	320.66±10.18	183±2.2
FA 3	97.55±2.59	−17.6±3.2	115.78±9.31	219±3.2
FA 4	89.71±2.31	−16.6±2.8	105.82±3.26	194±2.4
FA 5	88.54±2.32	−15.4±3.8	103.02±6.26	230±5.2
FA 6	79.60±2.19	−14.6±2.4	140.12±12.18	202±3.2
FA 7	89.25±2.21	−15.6±2.6	99.56±9.31	192±4.2
FA 8	85.35±2.52	−15.4±2.8	205.12±9.27	169±2.5
FA 9	89.77±2.43	−17.6±3.2	295.78±9.31	188±3.9

Fig. 1: Optical microscopic image of the optimized formulations formative assessment 3

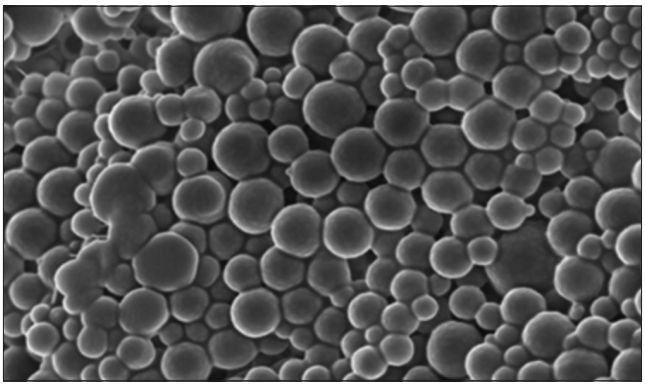

Fig. 2: Scanning electron microscopic image of the optimized formulations formative assessment 3

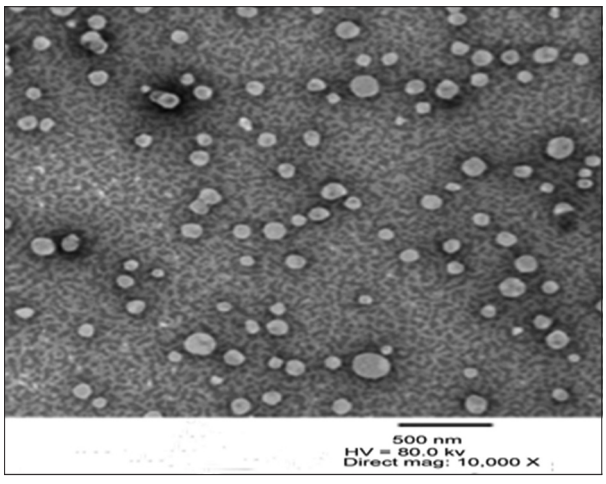

Fig. 3: Scanning electron microscopic image of the optimized formulations formative assessment 3

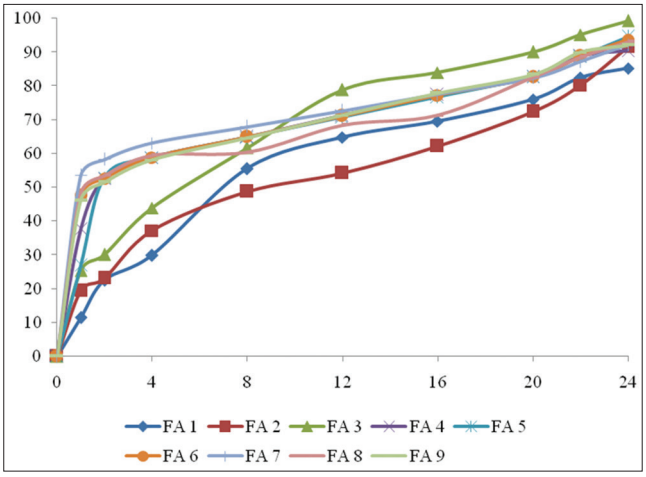

Fig. 4: *In vitro* drug release graph formative assessment (FA) 1-FA 9

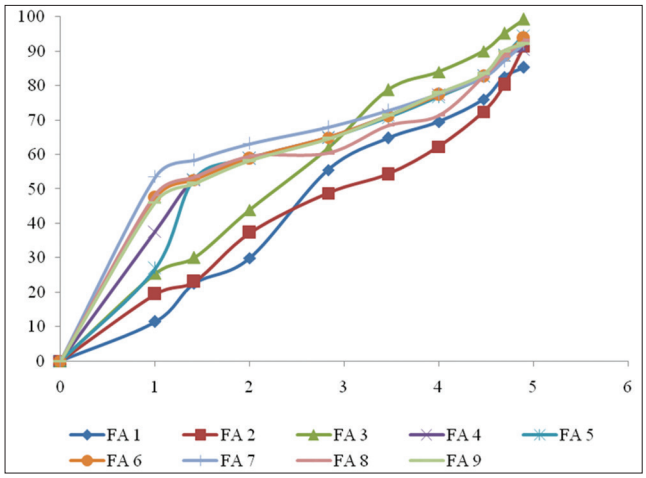

Fig. 5: Higuchi's plot for formative assessment (FA) 1-FA 9

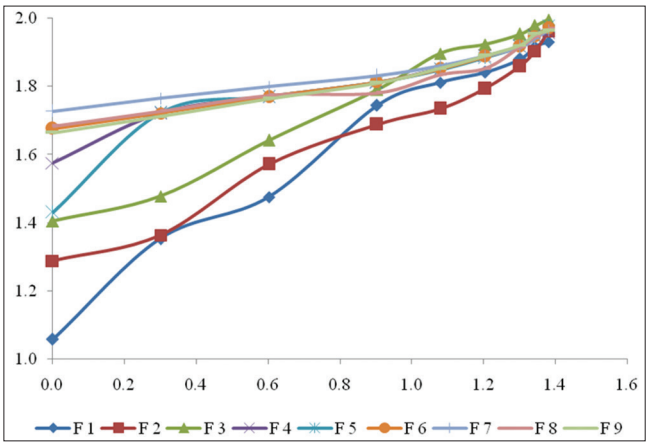

Fig. 6: Peppa's plot for formative assessment (FA) 1-FA 9

Table 2: Stability studies results at various time intervals

FA 3	Stored at 4°C (in days)				Stored at 25°C (in days)			
	15	30	60	90	15	30	60	90
Encapsulation efficiency (%)	95.23±3.2	92.31±2.4	89.24±1.2	86.23±1.2	97.23±3.2	97.31±2.4	96.24±1.2	95.23±1.2

Optical microscope

The drug release from glimepiride-liposomes, however, did not show significant burst release. The drug was released slower and controlled from liposomes. The release of glimepiride from loaded liposomes was in accordance with the Zero-order kinetics, Higuchi's and Peppa's plot which forecast the release amount and mechanism of drug release from the liposomes. *In vitro* drug release studies of the glimepiride-liposomes revealed that the glimepiride diffusion drug release from all the formulations followed zero-order kinetics (r^2-0.952) and ascertained by Higuchi's and Peppas mechanism which reveals that it governed by erosion (r^2-0.996) and Fickian type (n=0.450) of drug diffusion release, respectively. Based on the % drug entrapment efficiency and% drug release, formulation FA 3 was considered to be the optimized formulations. The percent cumulative amounts of glimepiride released as a function of time from liposomes formulated was illustrated as zero-order, Higuchi's and Peppa's plot in Figs.4-6, respectively.

Stability studies

Physical stability study of the prepared liposomes was carried out to determine the comparative leakage of the drug from liposomes stored at different conditions compared to each other. After washing and removal of the free drug, each liposomal formulation was stored either at 4°C or at 25°C. At predetermined time intervals of 15, 30, 60 and 90 days has shown in Table 2. The encapsulation efficiency of glimepiride-liposomes was determined.

CONCLUSION

Antidiabetic drug, glimepiride was formulated as liposomal formulation after checking the compatibility by DSC and FT-IR studies. The liposomes preparation FA 3 was optimized based on the particle size, zeta potential, entrapment efficiency, and drug release characteristics. Thus, glimepiride when formulated showed sustained release property which could be successfully developed as liposomes.

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