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Der Pharmacia Lettre, 2016, 8 (11):60-68
(http://scholarsresearchlibrary.com/archive.html)

ISSN 0975-5071
USA CODEN: DPLEB4

Formulation and evaluation of gliclazide loaded liposomes

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ABSTRACT

Diabetes is the disease caused due to deficiency of insulin. Antidiabetic drug Gliclazide decreases blood glucose level by inducing insulin. Frequent administration of Gliclazide decreases patient compliance. The present study was aimed to formulate a sustained release formulation of Gliclazide, liposomes. Due to the sustained release property of liposomes it increases patient compliance. Ethanolic injection method without sonication was used to prepare Gliclazide liposomes. Various parameters such as 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 were evaluated. FT-IR spectral studies and differential scanning calorimetry studieswere used to perform drug excipient compatability study. Liposomes were prepared by using varying concentration of cholesterol in ethanol injection method. The results proved The formulations fulfilled all official requirements. The drug release was slow and sustained for >12 hrs. The formulations followed first order kinetics and release mechanism was non-fickian diffusion.

Keywords: Liposomes, antidiabetic drug, Gliclazide, ethanolic injection method.

INTRODUCTION

Diabetes mellitus is a disease which results due to deficiency or diminished effectiveness of endogenous insulin[1]. It is characterised by hyperglycaemia, deranged metabolism and sequelae predominantly affecting the vasculature. The main types of diabetes mellitus are Type 1 diabetes mellitus, Type 2 diabetes mellitus, Gestational diabetes, Maturity-onset diabetes of the young (MODY) and Secondary diabetes. Type 2 diabetes is associated with excess body weight and physical inactivity and may eventually need insulin treatment. Liposomes are artificial microstructure carriers with one or more concentric lipid bilayer spheres[2]. Due to their non-toxicity and biodegradability liposomes can be widely used to deliver sustained release of various drugs. They transport drugs with the help of phospholipid bilayers which increase the bioavailability of drugs, thereby decreasing drug toxicity. The particle size of liposomes varies from 30 nm to several micrometers. Liposomes(Fig 1) help in increasing the therapeutic index of the administered drugs. Without the use of surfactants and emulsifiers, in liposome formulations, water soluble and insoluble materials can be used together in liposome formulations. Gliclazide (Fig 2), a second generation drug, belonging to sulphonyl urea group is an oral hypoglycemic agent used for diabetic patients with type 2 diabetes mellitus[3].Mechanism of action of gliclazide is by binding to sulphonyl urea receptors of beta-cells of pancreas. It decreases blood sugar by activating calmodulin indirectly which causes exocytosis of insulin vesicles inducing the release of insulin. The solubility of Gliclazide in water is 0.027mg/l.Due to frequent administration of dosage forms patients feel difficult to attain 100% patient compliance. To reduce patient non compliance, sustained release formulations can be used with single daily dosing. Liposomes, the sustained released

dosage form can be formulated with the help of its pharmacokinetic parameters. The aim of the present work is to formulate and evaluate various parameters of Gliclazide loaded liposomes by ethanol- injection method.

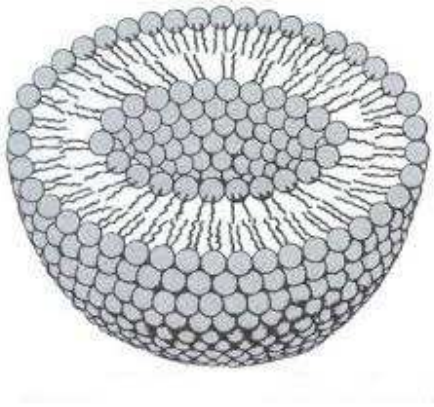

Fig. no. 1 Structure of Liposomes

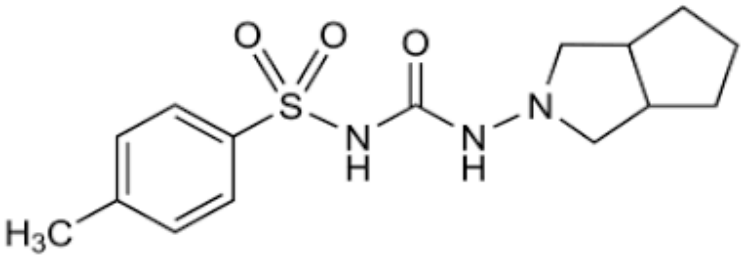

Fig. no.2 Chemical structure of Gliclazide

MATERIALS AND METHODS

Gliclazide was procured from Dr. Reddy's Laboratories, Hyderabad. Soya phospholipids, cholesterol and tween 80 were purchased from Sd fine chemical, Mumbai. All the other ingredients used are of analytical grade. The method applied for the formulation of liposomes was modified ethanol-injection method.

Construction of standard calibration curve of Gliclazide :

An accurately weighed 100 mg of Gliclazide was solubilized in pH 7.4 Phosphate buffer and the volume had made upto 100 ml. 10 ml of solution from the primary stock solution was pipette out and made up the volume upto 100 ml. The calibration curve of was constructed from the aliquots prepared with a series of concentrations 5, 10, 15, 20, 25 and 30 µg/ml and the samples were scanned using UV spectrophotometer, against the reagent blank(Table 1). The $\lambda_{max}$was found to be at 227 nm(Fig 3). The absorbance was noted at 227nm using UV spectrophotometer.

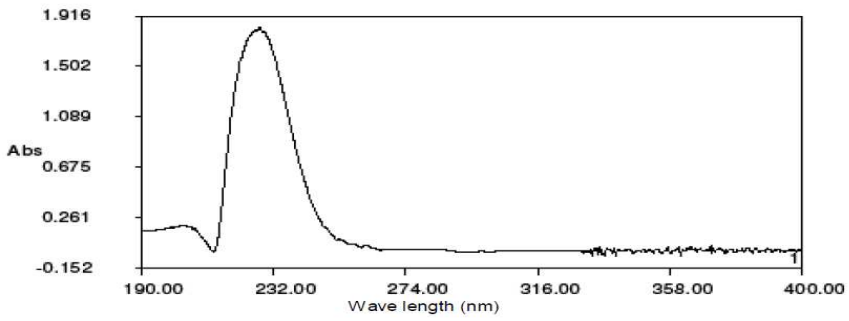

Fig. no. 3 λmax of Gliclazide

Table no. 1 Calibration data of Gliclazide at 227 nm

S. No	Concentration (µg/ml)	Absorbance (nm)
1	5	0.158
2	10	0.331
3	15	0.514
4	20	0.672
5	25	0.83
6	30	0.988

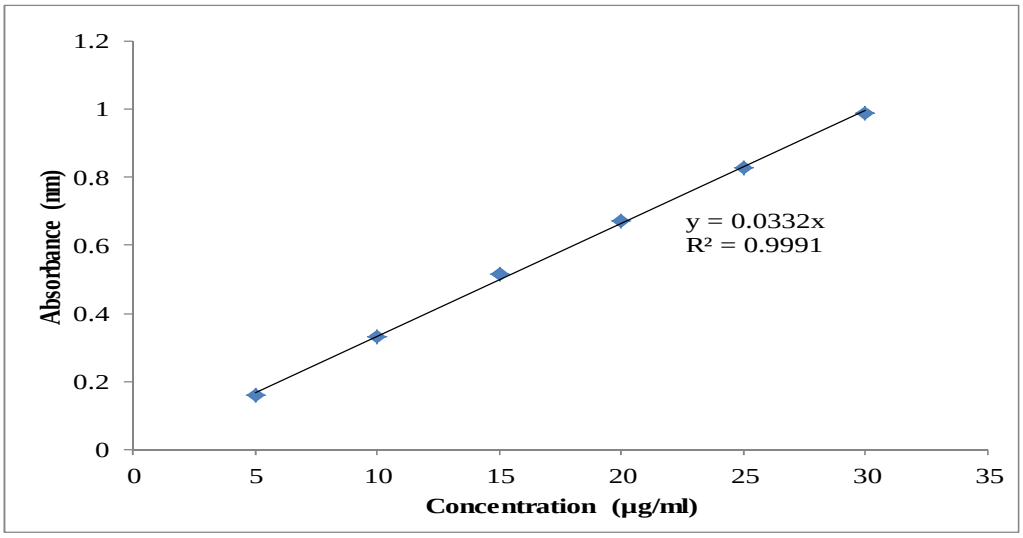

Fig no. 4 Calibration graph of Gliclazide at pH 7.4 at 227 nm

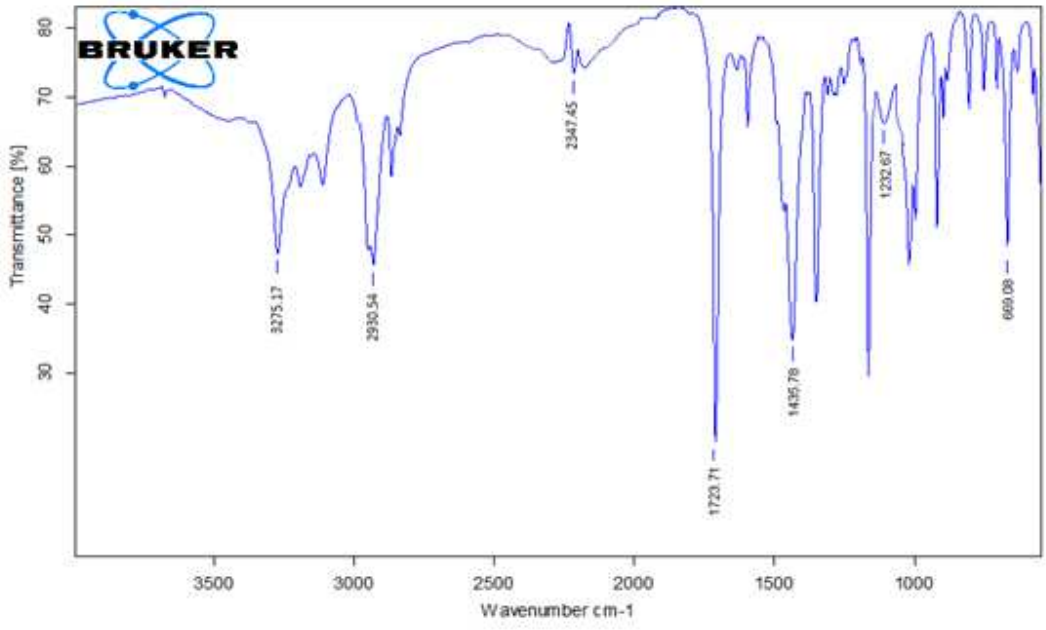

Fig no. 5 FTIR Spectra of Gliclazide with carrier

Characterisation of Drug and Excipients:

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[4]. Melting point determination of Gliclazide was determined by using the melting point apparatus. Drug polymer compatibility studies were studied using DSC and FT-IR studies(Fig 4 and 5). The results did not show any drug-polymer interaction.

Preparation of Gliclazide loaded liposomes:
As the drug is soluble in ethanol, gliclazide-loaded liposomes were prepared by modified ethanol-injection method[5]. For this 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.

Determination of drug encapsulation efficiency
The percentage of drug encapsulated was determined after lysis of the prepared liposomes with absolute alcohol and sonication for 10 minutes[6]. The concentration of drug, Gliclazide, in absolute alcohol was determined spectrophotometrically at 227 nm using a UV-visible spectrophotometer in triplicate. The encapsulation efficiency expressed as entrapment percentage was calculated through the following relationship

% Encapsulation efficiency = Total drug – free drug /Total drug × 100

Optical microscope
After dilution of the formulation with 5% mannitol, one drop of diluted Gliclazide liposomal suspension was viewed under optical microscope (Olympus BHA, Japan) to observe the shape and lamellar nature of vesicles.

Scanning electron microscopy (SEM)
The morphology (shape and surface characteristics) of Gliclazide liposomes was studied by scanning electron microscopy (SEM) using JSM-5610LV Scanning Microscope[7]. The sputtering was done for nearly 5 minutes to obtain uniform coating on the sample to enable good quality SEM images.

Transmission electron microscopy (TEM)
Gliclazide-loaded liposomes were observed by transmission electron microscopy (TEM) using a JEM 1010®, Jeol (USA). The samples were prepared by placing the diluted liposomes onto a 400-mesh grid coated with carbon film[8]. TEM images were analyzed using the soft-imaging software ImageJ®.

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 a Zeta Sizer 4 (Malvern Instruments, Worcestershire, UK) equipped with a He-Ne gas laser (k = 0.633 Am) for data acquisition and analysis. 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[9].

Turbidity measurement
The liposomes were diluted with distilled water. After rapid mixing by sonication for 5 min, the turbidity was measured as the absorbance at respective nm with a UV- visible spectrophotometer[10] (Shimadzu-1700, Japan).

***In -vitro* drug release & kinetic study**
In vitro drug release studies were done using a dynamic dialysis method. After separation of free drug from drug-loaded liposomes, the release rate of drug was evaluated by placing it in dialysis tubing (10,000 MWCO, Millipore, Boston, USA) and exhaustively dialyzed for 15 min for several times, each time against 100 mL of phosphate buffer (pH 7.4). After 1 h no further drug was detected in the solution[11]. The dialyzed suspension containing the drug or plain drug aqueous solution was sealed in a dialysis bag. The dialysis bag was immersed in 100 mL of phosphate buffer (pH 7.4) thermostatically maintained at 37.5± 2 °C at 50 rpm. The samples (1 mL) were withdrawn at various time intervals and analyzed by a UV spectrophotometer at respective nm[12]. Volumes lost by sample withdrawal were replaced with fresh medium. The data of *in vitro* release from various liposomes were evaluated kinetically using various mathematical models like zero-order, first-order, Higuchi and Koresmeyer–Peppas model equations.

Determination of stability studies

To determine the stability of Gliclazide liposomes, after washing and removal of the free drug, each formulation was stored either at 4°C or at 25°C at predetermined time intervals of 15, 30, 60 and 90 days[13].

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 2, it is obvious that Gliclazide-encapsulation efficiency had higher values in formulation FB 3.

Table no. 2 Encapsulation efficiency, zeta potential, particle size and turbidity of Gliclazide formulations

CODE	Encapsulation Efficiency (%)	Zeta potential (mV)	Particle size(nm)	Turbidity
FB 1	78.21 ± 1.52	-15.6±2.8	105.31±9.26	143 ± 2.1
FB 2	94.52 ± 1.72	-18.2±3.1	96.46±12.81	122 ± 2.4
FB 3	98.21 ± 1.04	-16.5±1.2	75.78±6.31	202 ± 3.5
FB 4	87.31 ± 2.13	-19.3±2.6	107.33±3.26	199 ± 2.3
FB 5	87.45 ± 3.33	-12.4±2.9	230.21±8.36	220 ± 3.1
FB 6	82.43 ± 1.43	-16.6±3.1	104.21±16.13	192 ± 5.2
FB 7	84.52 ± 3.12	-17.8±3.0	99.56±6.14	162 ± 3.3
FB 8	82.98 ± 2.12	-18.1±1.8	156.31±17.20	191 ± 3.2
FB 9	82.44 ± 3.33	-15.9±2.2	205.58±10.31	209 ± 3.0

Morphological study

The images confirmed that liposomes were spherical and smooth in nature(Fig6 and 7). 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(Fig 8).

Optical microscope

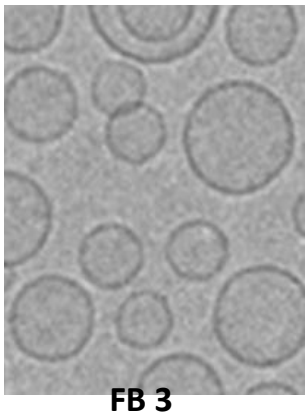

Fig no. 6 Optical microscopic image of Gliclazide formulations FB 3

Scanning electron microscopy (SEM)

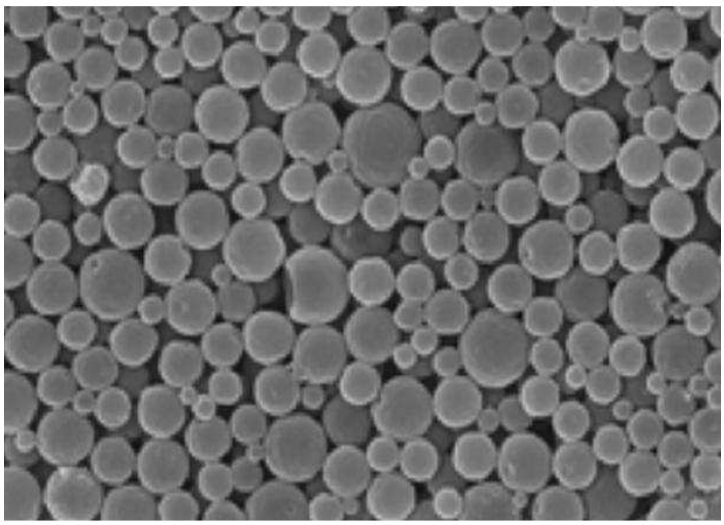

Fig no.7 SEM image of Gliclazide loaded liposomes

Transmission electron microscopy (TEM)

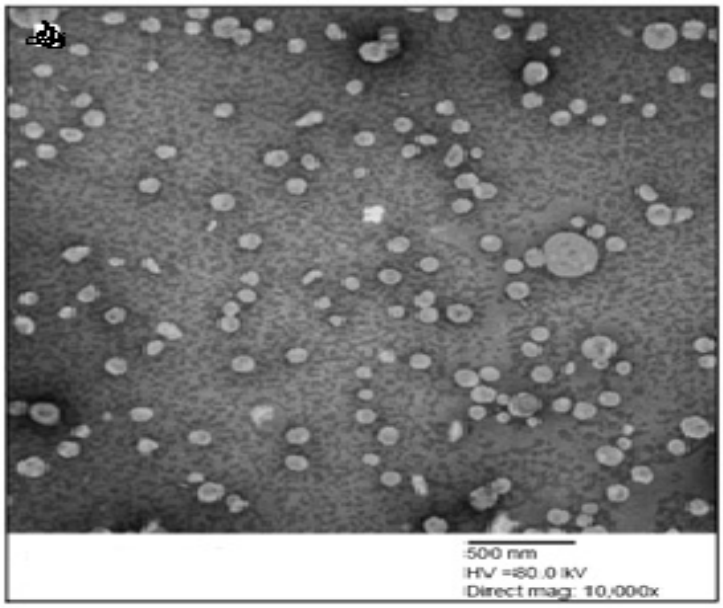

Fig no.8 TEM image of Gliclazide loaded liposomes

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 -30mV due to the electric repulsion between particles. Non-ionic 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.

***In -vitro* drug release & kinetic study**

The percent cumulative amounts of Gliclazide released as a function of time from liposomes formulated was illustrated in table 3 and in figures 9,10 and 11.

Table no. 3 Diffusion characteristics of Formulations FB 1 – FB 9

Formulation code	Correlation coefficient values (r)		Diffusion exponent value (n)
	Zero order	Higuchi's model	
FB 1	0.982	0.997	0.601
FB 2	0.968	0.991	0.532
FB 3	0.998	0.988	0.501
FB 4	0.989	0.993	0.605
FB 5	0.979	0.993	0.611
FB 6	0.983	0.990	0.509
FB 7	0.937	0.986	0.324
FB 8	0.981	0.997	0.556
FB 9	0.983	0.995	0.550

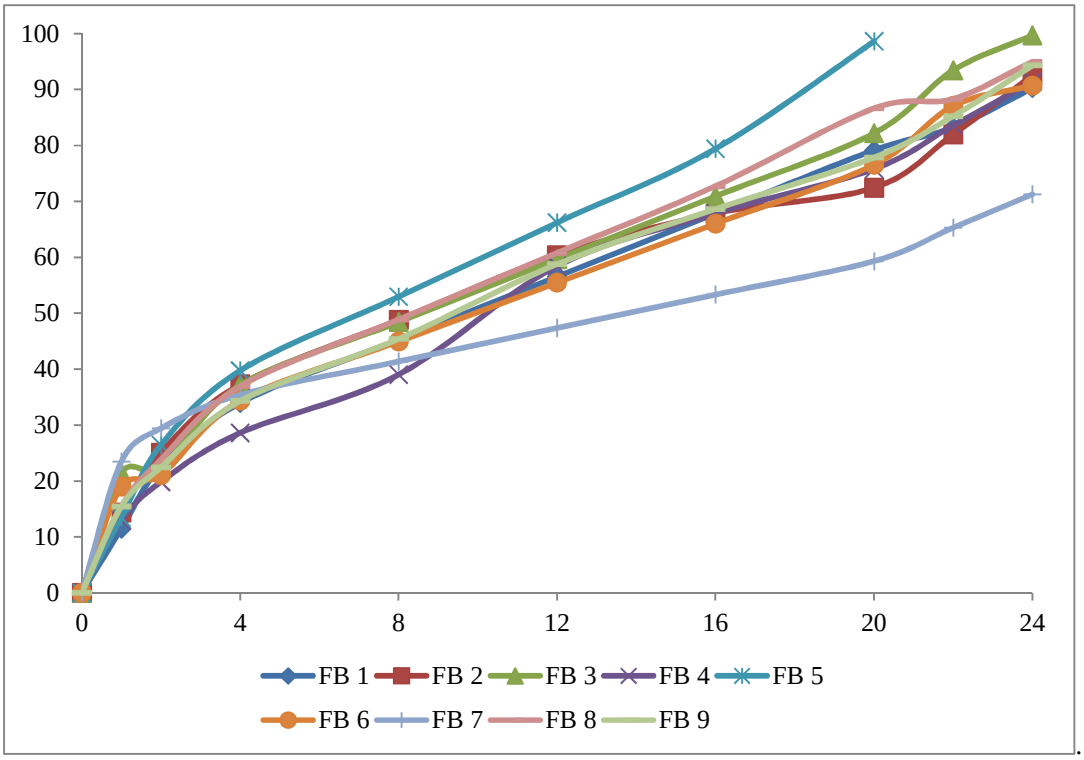

Figure no. 9*In-vitro* drug release graph FB 1 – FB 9

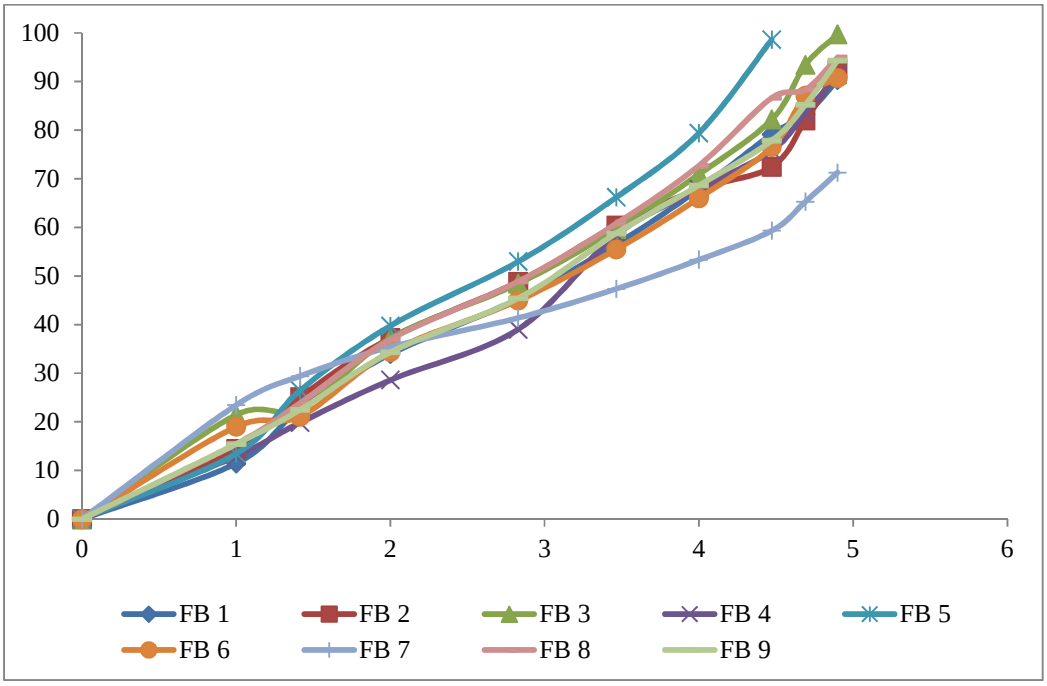

Figure no. 10 Higuchi's plot for FB 1 – FB 9

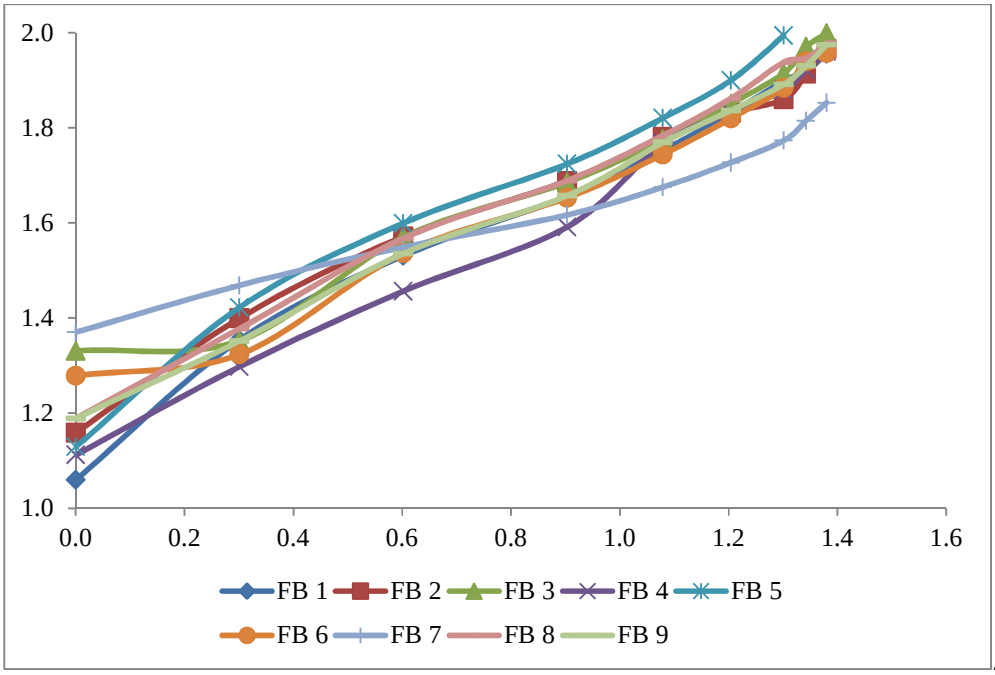

Figure no 11 Peppa's plot for FB 1 – FB 9

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(Table 4). The encapsulation efficiency of gliclazide liposomes was determined.

Table no. 4Stability studies of formulations FB 3

FB 3	Stored at 4°C (in days)				Stored at 25°C (in days)			
	15	30	60	90	15	30	60	90
Encapsulation Efficiency (%)	98.43 ± 3.2	97.61 ± 2.4	96.92 ± 1.2	96.21 ± 1.2	99.98 ± 3.2	98.14 ± 2.4	97.31 ± 1.2	97.24 ± 1.2

CONCLUSION

After checking the compatibility of Gliclazide with the polymers by DSC and FT-IR studies, Gliclazide liposomal formulations were formulated. Based on the particle size, zeta potential, entrapment efficiency, and drug release characteristics, the liposomal formulation FB3 was considered as optimized formulation. Thus, the antidiabetic drug, gliclazide when formulated showed sustained release property. So it could be successfully developed as liposomal formulation.

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