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Research Article

FORMULATION AND EVALUATION OF *IN-SITU* FORMING LIPOSOMES OF GLICLAZIDE

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ABSTRACT

An approach was used to enhance the solubility of BCS Class II drug, Gliclazide. The objective of this study was to formulate liposomal drug delivery system and to show the potential of Soya lecithin, tween 80 in enhancing the solubility and bioavailability of Gliclazide. Initial preparations were done by mixing the drug and carrier. Formulations were prepared by heat fusion method as it is considered as the mechanism for the enhancement of solubility and dissolution of the drug. The *in-vitro* releases of the different formulations were studied based on the effect of surfactant and oil including their thermodynamic stability. Formulated drug and adjuvants were characterized by spectrophotometry (UV, FTIR and photon correlation). Dissolution studies showed that F3 had the smallest particle size of 127.6 nm, with values for other formulations ranging from 176.8-248.8 nm. The cumulative percentage release for all formulations ranged from 21.20 ± 1.68% to 80.92 ± 3.82%; with F3 having the highest value of 80.92 ± 3.82%. Soya lecithin, soybean oil and tween 80 showed no significant influence on formulation's stability. These results confirm that the potential of liposomal drug delivery containing oil and surfactants as an adjuvant are expected to increase the oral bioavailability as confirmed by the increased *in-vitro* release.

Key words: Bioavailability, Gliclazide, Liposomal drug delivery system, Solubility, Soya lecithin, Soybean oil.

INTRODUCTION

Gliclazide (GLZ) is a Biopharmaceutics Classification System (BCS) Class II drug which follows dissolution dependent pharmacokinetics[1]. The absorption and bioavailability of these types of drugs are dependent on their solubility and dissolution rates. Various researchers have made different formulation approaches available for dissolution rate enhancement like co-melt dispersion, co-precipitation with hydrophilic polymers, hydrotropic techniques, particle size reduction, crystal engineering techniques, solid dispersion technique, inclusion complexation, micellization, cryogenic techniques, emulsification, drug lipid carriers and self-emulsifying drug delivery systems. All these techniques have their own advantages and disadvantages[2-9].

In recent years, a great deal of interest has been focused on lipid-based carrier systems. The most popular approach is the incorporation of the active poorly water-soluble component into inert lipid vehicles such as oils, surfactant dispersions[10], solid dispersions, solid lipid nanoparticles, emulsions, micro emulsions, nanoemulsions, self-emulsifying formulations (SEF), micro/nano emulsifying formulations[11], liposomes[12], Niosomes etc.

BCS is a guidance tool for predicting the intestinal drug absorption provided by the U.S. Food and Drug Administration [1]. According to BCS, the drugs have been differentiated on the basis of their solubility and permeability[13] and are divided into four categories, i.e. Class I drugs having high solubility and high permeability, Class II drugs having low solubility and high permeability, Class III drugs having high solubility and low permeability and Class IV drugs having low solubility and low permeability[13-17]. The BCS is a useful tool for decision making in the discovery and early development of new drug.

Out of above-mentioned techniques liposomal drug delivery system, here, are of primary choice because of its one of the amphiphilic polar lipid component Soya Lecithin (SL) that might be advantageous when combined with oral hypoglycemic agents like GLZ.

Current researches proposed that a lecithin supplemented diet can alter the cholesterol homeostasis and lipoprotein metabolism. This diet may adjust the cholesterol homeostasis in hepatic system, extending the development of cholesterol-7-alpha-hydroxylase and HMG-CoA (β-hydroxy β-methylglutaryl-CoA) reductase, and lessening the microsomal ACAT (Acyl-CoA cholesterol acyltransferase) movement[18,19]. Moreover, it was also reported that at neutral pH, crude phosphatidylcholine from egg yolk or soybean increased the stability of the emulsion and lowered the interfacial tension more effectively. SL has low solubility in water but upon hydration at effective temperature its phospholipids can form either liposomes or bilayer sheets or micelles or lamellar structures[18,19].

In contrast, it is not possible to form lecithin based micro emulsions using water and the same oil without the addition of a co-surfactant [20]. Hence, Tween 80 was selected as another component of surfactant mix. Tween 80 is non-ionic and non-toxic in nature and it is also considered to meet the solubility requirements[21]. Apart from this, the addition of non-ionic surfactant in the emulsion-based formulation increase the stability of such formulations by imparting steric repulsion between the formed globules[22].

Another essential component of the liposomes is non-polar lipid component that is added to stabilize the liposomal structure. The mostly used such a component is cholesterol[23]. Cholesterol being not good for the cardiac problems associated with diabetes i.e. hyperlipidemia and hypercholesterolemia[20,24] is here replaced by soybean oil for its various benefits. It is an edible and digestible surfactant and emulsifier of natural origin. Soybean oil is very popular with rich value of omega-3 and omega-6 fatty acids[25]. Soybean oil is also a rich source of vitamin E. Vitamin E is essential to protect the body fat from oxidation and to scavenge the free radicals and therefore helps to prevent their potential effect upon chronic diseases such as coronary heart diseases and cancer[25].

As far as the selection of drug is concerned, GLZ was selected as model BCS Class II drug as it is considered as representative of this class of drug and was easily available. GLZ, a white or almost white powder is practically insoluble in water, slightly soluble in alcohol, sparingly soluble in acetone, freely soluble in dichloromethane and acetonitrile is a second generation sulphonyl urea oral anti hyperglycaemic agent used in the treatment of non-insulin-dependent diabetes mellitus by binds to the β cell sulphonyl urea receptor-1 and subsequently blocks the ATP sensitive potassium channels[26].

MATERIAL AND METHODS

GLZ was obtained as a gift sample from Alkem Laboratories Ltd. Mumbai. SL, tween 80 were purchased from Central Drug House, New Delhi and all other chemicals and ingredients used in the experiment were of analytical grade.

Drug identification and excipient interaction studies

Drug identification was carried out by using Fourier-transform infrared (FTIR) Spectrophotometry. Samples were ground in dehumidified FTIR chamber using an agate mortar and pestle. Where, GLZ 1 mg was mixed with 99 mg of anhydrous KBr and was transferred into Evocable Pellet Dieto form a pellet. A load of 2 tons was needed to make the 7-13 mm diameter KBr pellet, which required the 15T Manual Hydraulic Press. This pellet was used for determination of FTIR spectra of pure drug. Drug-Excipient (D-E) interaction was also determined by FTIR spectrophotometry[27]. Soybean oil, SL, tween 80 and drug were taken in 1:1:1:1 ratio, respectively and mixed with appropriate amount of anhydrous KBr. Similarly, this pellet was used for determination of FTIR spectra. The obtained spectrum was then compared with the reference spectrum[28] for the presence of peaks in the functional group region and the fingerprint region of the spectra.

Determination of drug solubility

The solubility of GLZ in various buffers over a wide range of pH (1.2, 2, 4, 5, 6.8, 7.4 and 7.8) was determined by using UV Spectrophotometer (Shimadzu 1800)[29]. Briefly, 100 mg drug was dissolved in 20 ml of buffer at room temperature using magnetic stirrer and the solubility was determined by the taking the absorbance of the samples at λmax 225 nm. The study was performed in triplicate.

Formulation Design

Eight formulations were developed, which comprised of various ratios of surfactant mixtures and soybean oil (Table 1). The different ratios were to screen for mixture with the best drug delivery. The amount of drug in the formulations was kept constant at 2%.

Table 1: Formulations and composition of gliclazide and excipients

S. No	Formulation	**Ingredients** (value in %)			
	Code	Soya lecithin	Soybean Oil	Tween 80	Gliclazide
1	F_1	19	60	19	2
2	F_2	24	50	24	2
3	F_3	29	40	29	2
4	F_4	42	42	14	2
5	F_5	44.8	39.2	14	2
6	F_6	51.33	32.67	14	2
7	F_7	37	60	1	2
8	F_8	57	40	1	2

Preparation of formulation

Heat fusion Method

Heat fusion method was the method of choice for formulation of liposomal drug delivery system (LDDS) in this study. In this method the formulation was prepared by mixing the Surfactant mixture in a porcelain dish at 70°C followed by the addition of soybean oil at same temperature. The mixture was homogenously mixed on a magnetic stirrer and GLZ was added at 40°C and stirred constantly until homogenous dispersion was obtained. This final dispersion mixture was then rapidly solidified at room temperature and stored in desiccators for further use.

Characterization of formulations

Particle size Analysis

Particle size and particle size distribution are the most essential characteristics through the point of stability of formulation and drug delivery at the appropriate target site in physiological system. Particle size parameters have been characterized immediately after the production of lipid based nanostructured carriers. The particle size was determined by photon correlation spectroscopy (PCS) (Zetasizer Nano ZS, Malvern Instruments, UK). PCS measures the mean particle size and the poly dispersity index (PDI) which is a measure of the width of the size distribution. The sample was diluted with bi-distilled water to have a suitable scattering intensity, and then the particle size measurement was carried out immediately. The average particle size and PDI values were obtained by averaging of 3 measurements at 25°C, each measurement consists of 13-15 runs.

Viscosity Determination

The viscosity of the prepared lipid-based mixtures was determined by using Brookfield viscometer (DV-III RV Cone and Plate rheometer, Brookfield Engineering Laboratories Inc., USA) equipped with spindle 40 at 25 ± 0.5°C.

Refractive Index

Refractive index of prepared formulations was determined using Abbe's type refractometer (Nirmal International, New Delhi, India) at 25 ± 0.5°C[30].

Thermodynamic Stability Studies

To assess the thermodynamic stability of drug loaded formulations, clarity, phase separation, droplet size and drug content were evaluated before and after subjecting to following stress tests as previously reported[31].

a) Centrifugation

Formulations were centrifuged 15,000 rpm for 15 min or 3500 rpm for 30 minutes[32]. These formulations that did not show any sign of instability were taken for the Heating cooling cycle.

b) Heating cooling cycle

Six cycles of heating and cooling were performed at 45°C and 4°C, respectively, with storage at each temperature for not less than 48 hours. Those formulations, which were stable at these temperatures, were subjected to freeze thaw testing.

c) Freeze thaw cycle (accelerated aging)

Three freeze thaw cycles between -21°C and +25°C with storage at each temperature for not less than 48 hours was done for the formulations. Prepared formulations were kept in deep freezer (at -21°C) for 24 hours. After 24 hours, the formulations were removed and kept at room temperature. The thermodynamically stable formulations returned to their original form within 2–3 minutes. Such cycles were repeated 2–3 times.

***In-vitro* dissolution studies**

In-vitro drug release studies were performed using USP dissolution rate test apparatus Type II (paddle type) in a dialysis tubing (molecular cut-off 1000 nominal molecular weight). An amount of the prepared formulation equivalent to 40 mg GLZ was accurately weighed and transferred to the dialysis tubing previously activated by keeping in phosphate buffer pH 7.4 ± 0.1 at room temperature for 24 hr. The tubing's were then tightened at both the ends and transferred to the dissolution baskets (6 basket) of 8 station unit USP dissolution rate test apparatus (Electrolab TDT-08L Dissolution tester) each containing 900 ml dissolution medium i.e. phosphate buffer pH 7.4 ± 0.1 maintaining at 37 ± 0.5°C temperature. The dissolution test apparatus was then operated at 50 rpm stirring speed. Aliquots of the samples (5 ml) were withdrawn at predetermined time intervals of 0, 5, 10, 15, 30, 45, 60, 90 and 120 min by maintaining the sink conditions every time by replacing each withdrawal with same volume of fresh phosphate buffer pH 7.4 ± 0.1. Each aliquot was then assessed for drug release by UV-spectrophotometrically at 225 nm after suitable dilution and the cumulative percent drug release (% CDR) was calculated using MS Excel software. % CDR was then plotted against time to get the dissolution profiles of the formulations[33].

Drug content estimation

Each formulation's weight is taken and then added into acetonitrile in a 100 ml capacity volumetric flask. These volumetric flasks containing formulation and acetonitrile were then sonicated for 15 min to ensure complete dissolution of GLZ from formulation. The sonicated solutions were then assessed for drug content using UV spectrophotometrically at λmax 225 nm. The study was performed in triplicate.

RESULTS AND DISCUSSION

In LDDS, surfactant and co-surfactant get specially adsorbed at the interface in order to reduce the interfacial tension and thus reduces the free energy of the system. This reduced free energy of the system is ultimately responsible for the thermodynamic stability of emulsion or LDDS.

Drug identification and excipient interaction studies

The GLZ drug samples exhibited their characteristic peaks similar to the peaks exhibited by their respective standards in fingerprint region of FTIR spectrum as shown in Figure 1, 2 and Table 2 respectively. Thus, the GLZ drug had been identified. Also, there was no observed caking, discoloration and liquefaction on physical observation. Odor formation was also not found in the mixture. All these observations primarily indicate no signs of incompatibility between drug and excipients. This was further proved by the analysis of IR spectra of the mixture at 0 Time and after 3 week of storage Figure 3.

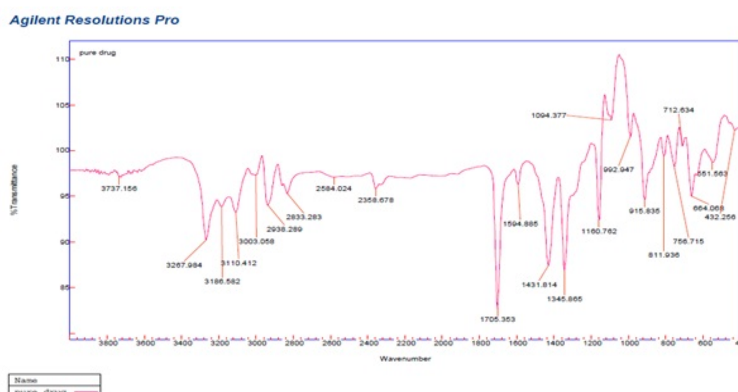

Figure 1: FTIR spectrum of pure gliclazide

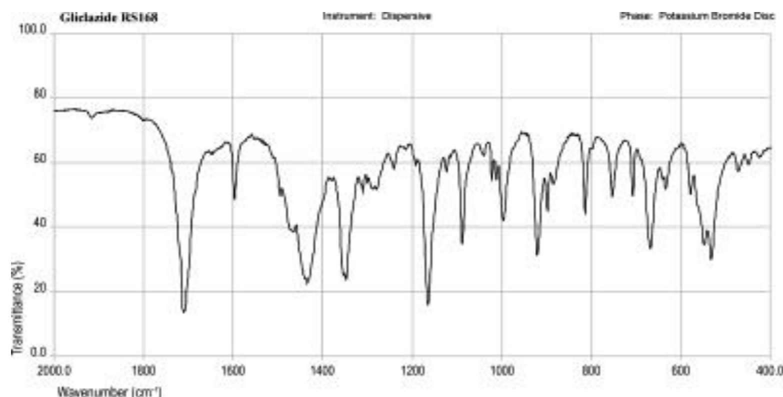

Figure 2: Reference FTIR spectrum of gliclazide

Table 2: FTIR spectra absorption of gliclazide

Wave Number		Characteristic functional group/ vibration
Recorded (cm^{-1})	**Absorption frequency band (Reference (cm^{-1})**	
3267.984 1594.88	3350-3250 1650-1580	Secondary amine N-H - Stretching - Bending
3003.058	3100-2990	Alkane =CH stretching
1705.35	1870-1540	Acyclic ketone carbonyl C=O Stretching
1345.86 1160.762	1350-1300 1160-1120	Sulphonyl S=O stretching - Asymmetric - Symmetric
811.936	900-675	Aromatic C-H bending (out of plane)

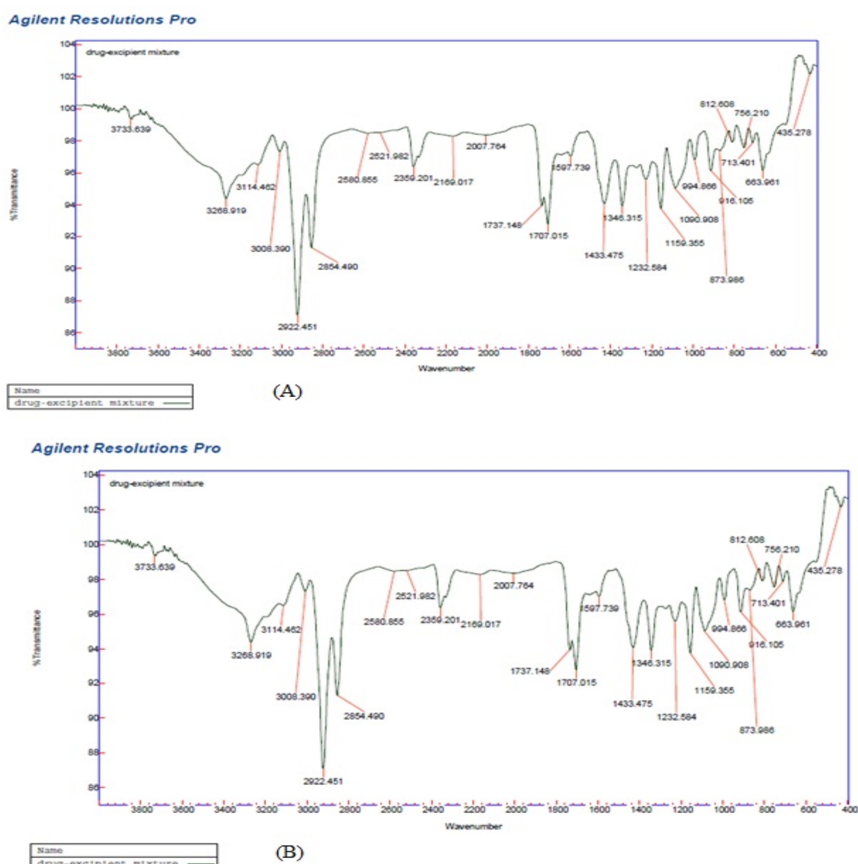

Figure 3: FTIR spectra of D-E mixture at 0 time (A), and after 3rd week (B) of storage at 55°C with 5% moisture

Determination of drug solubility

The solubility of GLZ in the pH range of 1.2 to 7.8 was presented in Table 3 and it was found to be poorly soluble drug as indicated by the fact that the highest dose of the drug GLZ (i.e. 240 mg) requires 16985 ml to 37558 ml aqueous buffer medium for complete dissolution. Thus, the results indicate that GLZ complies with the criteria of a poor water-soluble drug.

Table 3: Solubility of drugs at different buffer pH ranges

pH	Solubility* (µg/ml)	Volume of solvent required to dissolve highest dose (240 mg) of gliclazide (ml)
1.2	11.27 ± 0.22	21,295
2.0	06.39 ± 0.08	37,558
4.0	07.00 ± 0.11	34,285
5.0	06.79 ± 0.10	35,346
6.8	13.64 ± 0.27	17,595
7.0	13.76 ± 0.27	17,441
7.4	13.87 ± 0.28	17,303
7.8	14.13 ± 0.31	16,985

* Mean ± S. D. of triplicate determination.

Physico-chemical characterization of formulations

All prepared formulations were brownish-yellow and well homogenized, Figure 4(a). The formation of particles by formulation F3 after its dispersion in the phosphate buffer pH 7.4 ± 0.1 using compound microscope under 40X magnification is shown in Figure 4(b). The Figure shows the formation of particles by formulations after dispersion.

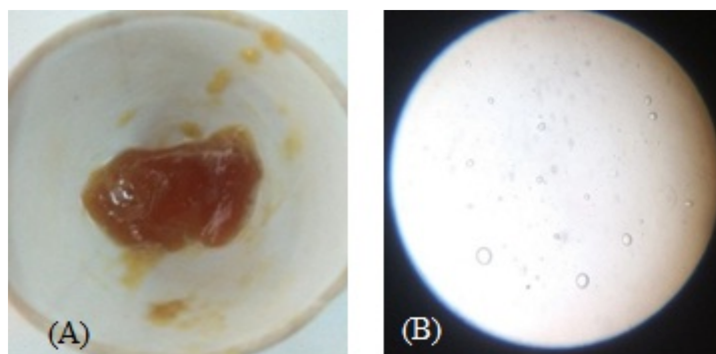

Figure 4: Photograph of formulation F3 (A) for physical appearance and (B) for the formation of globules/ particles formed after dispersion of the formulation in the phosphate buffer pH 7.4 ± 0.1 using compound microscope under 40X magnification

Particle size Analysis

Particle size of the prepared formulations is presented in Table 4. On decreasing Surfactant mixture to soybean oil ratio from formulation F5 (1.5:1) through formulation F4 (1.45:1) to formulation F3 (1.33:1) and simultaneously increasing the SL to Tween 80 ratio from 1:1 to 3.2:1 particle size after dispersion of the prepared formulations into the phosphate buffer pH 7.4 ± 0.1 has become decreased from 223.9 nm through 176.8 nm to 127.6 nm respectively. However, on further increasing the Surfactant mixture to soybean oil ratio to 2:1 and the SL to Tween 80 ratio to 3.66:1, particle size became decreased to 205.0 nm. Also, on fixing the amount of Tween 80 ratio to 1%, in the formulation F8 having Surfactant mixture to soybean oil ratio of 1.45:1, particle size had increased from 176.8 nm to 248.8 nm; so, formulations F3, F4, F5, F6 and F8 were selected for further characterization. Particle size of F1, F2, and F7 were not determined due to unequal distribution of adjuvants.

Table 4: Physico-chemical properties of formulations

Formulation Code	Particle Size* (nm)	Zeta Potential*	PDI*	Viscosity* (mP)	Refractive Index*
F3	127.6 ± 3.38	-33.7 ± 2.92	0.449 ± 0.12	78.59 ± 3.53	1.399 ± 0.4
F4	176.8 ± 5.24	-37.2 ± 1.96	0.475 ± 0.03	115.16 ± 5.26	1.406 ± 0.6
F5	223.9 ± 4.92	-39.0 ± 3.38	0.521 ± 0.10	151.10 ± 5.70	1.416 ± 0.5
F6	205.0 ± 8.17	-51.1 ± 1.68	0.510 ± 0.23	156.30 ± 4.03	1.408 ± 0.3
F8	248.8 ± 3.34	-48.8 ± 5.55	0.650 ± 0.09	189.60 ± 2.80	1.418 ± 0.6

* Mean ± S. D. of triplicate determinations

Viscosity Determination

Viscosity of formulations was depicted in Table 4. When formulations with different Surfactant mixture ratios were compared, the minimum viscosity values were obtained for all formulations ranging from 78.59 ± 3.53 mP for F3 to 189.60 ± 2.80 mP for F8 through 115.16 ± 5.26 mP for F4, 151.10 ± 5.70 mP for F5 and 156.30 ± 4.03 mP for F6. As a result of viscosity measurements, a similar behavior, as for particle size was obtained. Viscosity of all the prepared formulations was very low as expected for o/w preparation. The low viscosity can be due to presence of low amount of Tween-80.

Refractive Index

Refractive index is the net value of the components of LDDS and indicates isotropic nature of formulations. The data is depicted in Table 4 which indicates that the mean value of the refractive index for all the formulations were almost same.

Thermodynamic Stability Studies

Stress test consists of centrifugation, heat-cooling cycle, and freeze thaw cycles point out that all formulations had a decent physical stability. Following three weeks, GLZ was seen as steady with recuperation 96±3 % for all the selected formulations. No huge change in the mean estimations of the refractive index of the formulations was seen for 3 weeks (information not appeared). Consequently, it very well may be presumed that the LDDS formulations were truly physically and chemically stable.

In-vitro **drug release studies for formulations**

Results of release studies are presented in Figure 5 for formulations in phosphate buffer pH 7.4 ± 0.1. It can be understood clearly that GLZ which is a highly lipophilic drug released at considerably faster rate from the prepared LDDS formulations compared to another pure drug. The % CDR of all the formulation was found to be 21.20% ± 1.68% for F1, 28.04% ± 1.26% in F2, 80.92% ± 3.82% for F3, 68.69% ± 2.12% for F4, 41.32% ± 1.78% for F5, 46.53% ± 2.54% for F6, 24.98% ± 1.19% for F7 and 32.50% ± 1.44% for F8. This could be suggested that LDDS formulation forms micro emulsion with a small droplet size spontaneously upon its introduction into the dissolution medium of phosphate buffer of 7.4 ± 0.1 pH that permit faster rate of drug release. Hence, this greater availability of dissolved form of GLZ from the LDDS formulations could ultimately lead to higher intestinal absorption and thus higher oral bioavailability.

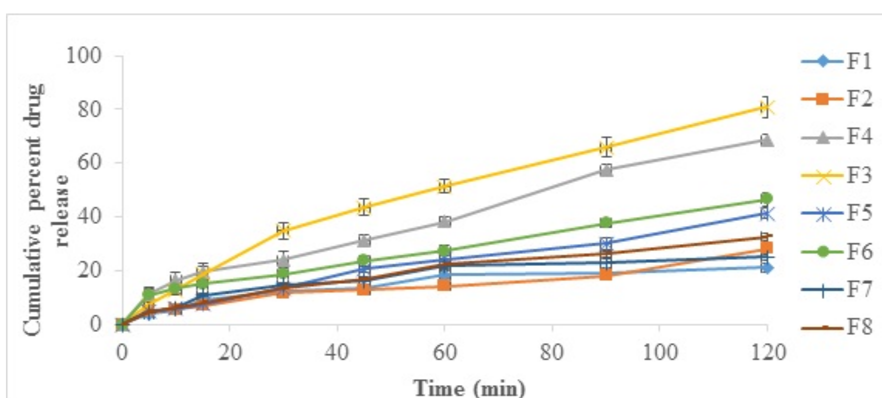

Figure 5: ***In-vitro*** **drug release profile of the prepared formulations in phosphate buffer pH 7.4 ± 0.1**

Effect of surfactant mixtures to soybean oil ratio on ***in-vitro*** **drug release**

Figure 6 showed the effect of surfactant mixture to soybean oil, with amount of drug kept constant at 2% over a period of 120 minutes. It is clear from the results that on increasing the Surfactant mixture to soybean oil ratio from 0.63 in F1 to 1.33 in F3 through 0.96 in F2, release was found to increase from 21.20% ± 1.68% in F1, to 80.92% ± 3.82% in F3 through 28.04% ± 1.26% in F2. On further increasing the ratio from 1.33 in F3 to 1.50 in F5 through 1.45 in F4, there was a little fall in F4, whereas a great fall in F5 drug release and the release was found to be 68.69% ± 2.12% in F4 and 41.32% ± 1.78% in F5. This fall in drug release on further increasing the amount of SL in the formulation with respect to soybean oil might be possibly due to increase in viscosity of the formulation with increased amount of SL. After increasing the ratio from 1.50 to 1.99, there was found almost no change in drug release with 41.32% ± 1.78% in F5 to F6 46.53% ± 2.54% at 120 min.

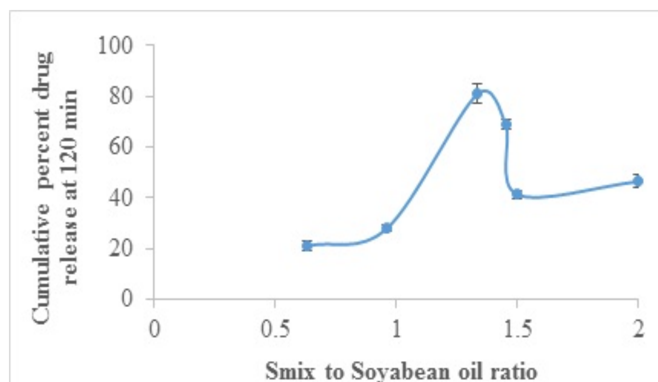

Figure 6: Effect of surfactant mixtures to soybean oil ratio on ***in-vitro*** **drug release**

Effect of Soya lecithin to Tween 80 ratio on *in-vitro* drug release

The results are presented in Figure7; as on comparing the drug release at 120 min for formulations F3, F5 and F6 with SL to Tween 80 ratio 3:1, 3.22:1 and 3.66:1, respectively. It was observed that on increasing the ratio (i.e. decreasing amount of Tween 80, with respect to SL) from 3:1 in F1 to 3.66:1 in F6 through 3.2:1 in F5, a fall in drug release was observed i.e. from 80.92% ± 3.82% in F3 to 46.53% ± 2.54% in F6, through 41.32% ± 1.78% in F5. This fall in drug release might be due to decrease in solubilizing capacity of Surfactant mixture because of decrease in amount of Tween 80, a non-ionic surfactant.

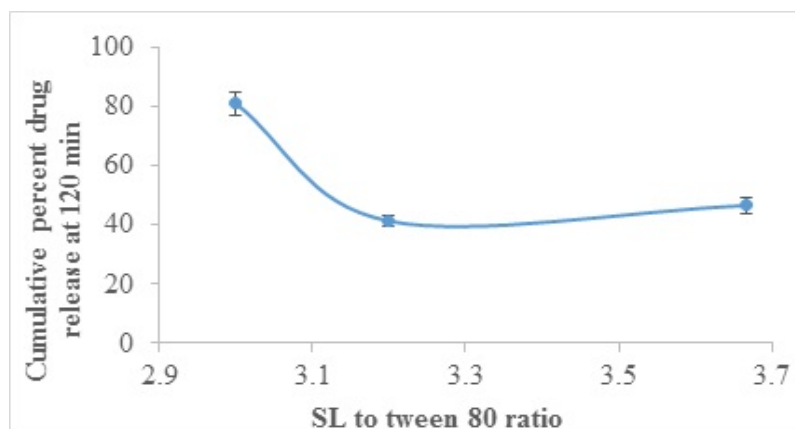

Figure 7: Effect of soya-lecithin to Tween 80 ratio on *in-vitro* drug release

Estimation of drug content in formulations

As it is clear from the Table 6, amount of drug in the prepared formulations were found to be in the range of 90.02% ± 01.40%-96.97% ± 00.93%. This indicates that almost all the drug was entrapped in the formulation.

Table 6: Drug content in prepared formulations

Formulation Code	Amount of drug* (%)
F1	94.80 ± 01.49
F2	95.10 ± 01.30
F3	93.80 ± 01.47
F4	95.85 ± 01.11
F5	90.02 ± 01.40
F6	91.45 ± 01.22
F7	93.80 ± 01.47
F8	96.97 ± 00.93

* Mean ± S. D. of triplicate determinations

CONCLUSION

LDDS of GLZ were prepared by the hot fusion method. This strategy for assembling was observed to be straight forward, did not require particular types of gear. The prepared formulations displayed all the attractive characteristics of a perfect LDDS and were likewise observed to be steady over a time of three months at room temperature conditions. After evaluation of the prepared formulation batches for parameters like particle size and *in-vitro* dissolution studies, the formulation F3 having a composition of SL, Tween 80 and soybean oil at the levels of 3, 1 and 3 respectively with amount of drug in the formulation at the level of 2% of total formulation weight can be said to be optimized on the basis of the fact that the smallest particle size (127.6 nm) when compared to that of rest formulations (particle size ranging from 176.8 nm to 248.8 nm) and the highest % CDR (80.92% ± 3.82%), when compared with that of other formulation's % CDR 21.20% ± 1.68% for F1, 24.98% ± 1.19% for F7, 28.04% ± 1.26% for F2, 32.50% ± 1.44% for F8, 41.32% ± 1.78% for F5, 46.53% ± 2.54% for F6 and 68.69% ± 2.12% for F4) at 120 min, was achieved at this composition.

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